Pharmacoepigenetics

Translational Epigenetics Series

Volume 10

Edited by

Ramón Cacabelos

PHARMACOEPIGENETICS

Translational Epigenetics Series

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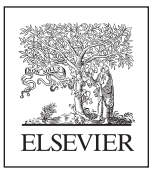

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Preface

Pharmacoepigenetics: A Long Way Ahead

In 1936 Hans Selye (1907–82) postulated the "general adaptation syndrome," describing how different types of environmental stressors might affect physiological functions and promote disease development. Then, 20 years later Conrad Waddington (1905–75) introduced the concept of transgenerational inheritance and epigenetics. Since the pioneering ideas of Waddington in 1956 regarding the inheritance of a characteristic acquired in response to an environmental stimulus the field of epigenetics has undergone explosive growth, especially during the last decade. Over 900 papers were published in 2010. These figures tripled in 2015 and quadrupled in 2017. Over 26,000 papers have been published during the 1958–2018 period. Epigenetics is conceived as a natural progression of genetics to explain gene expression and the interaction of the genome with the external milieu. However, the conceptual scope of epigenetics has greatly expanded in parallel with our current understanding of the mechanisms configuring the epigenetic phenomenon. Some genuine features of epigenetics include the following: (i) transgenerational inheritance of particular phenotypes without apparent structural changes in the genetic code associated with subtle chemical changes in DNA and RNA; (ii) transcriptional and posttranscriptional regulation of gene expression under the promiscuous control of the epigenetic machinery (DNA methylation, chromatin/histone modifications, miRNA regulation); and (iii) the reversibility of epigenetic marks by exogenous intervention.

Considering the plurality of actions in which epigenetics is involved, it is hard to imagine any cellular or biological function/reaction in which epigenetic regulation is absent. Therefore, this emerging field will transform our conception of health and disease. Development, maturation, aging, stem cell-dependent differentiation/regeneration, and disease are largely controlled by the epigenetic status of cells. The impact of early-life environmental exposures on epigenetic mechanisms may determine the long-term health of an individual and his/her progeny. This postulate has led to formulation of the "Developmental Origins of Health and Disease" theory.

Medical epigenetics will revolutionize medical practice in three main areas: etiology, diagnosis, and treatment. Only about 10% of human pathology is reasonably understood by the scientific community in pathogenic terms. It is very likely in the coming decades that genomics, epigenetics, transcriptomics, proteomics, and metabolomics will help us to understand the molecular basis of disease, facilitating early (preventive) intervention. In fact, there is clear evidence that epigenetic aberrations can contribute to the pathogenesis of major problems of health (cardiovascular disorders, cancer, brain disorders, metabolic disorders). Conceptually, conventional medicine associates a disease with symptoms (phenotype); without symptoms there is no disease. This is obviously erroneous, as someone may be dying without any apparent symptomatology. The combination of genomic and epigenetic signatures will allow the identification of risk many years before the onset of a particular disease. This predictive (presymptomatic) diagnosis is essential for the implementation of preventive programs, because without prediction prevention is impossible. Likewise, epigenetic marks will help to define differential diagnosis, disease status and progression, and therapeutic monitorization. Finally, epigenetics

will contribute to establishing effective personalized treatments based on the experience of pharmacogenetics accumulated over the past 70 years. The discipline of pharmacogenetics was introduced by Vogel in 1959, after the pioneering studies of Bönicke and Reif, Carson, Kalow and Staron, and Motulsky in the 1950s. Pharmacogenetics accounts for 50%–80% of the variability in pharmacokinetics and pharmacodynamics; however, it does not explain drug efficacy and safety in full. The pharmacogenetic outcome results from the bidirectional interaction of drugs with by-products of a cluster of genes, be they pathogenic, mechanistic, metabolic, transporter, or pleiotropic. The expression of pharmacogenetics-related genes is regulated by the epigenetic machinery, and the assembly of pharmacogenetics-related effectors with components of the epigenetic machinery, which regulates the expression of genes involved in the pharmacogenetic network, configures the functional structure of the pharmacoepigenetic apparatus. This complex system may well be responsible for over 95% of the variability in drug efficacy, toxicity, and resistance.

The pharmacoepigenetic landscape has to be built on the foundations of pharmacogenetics, integrating genomics, epigenetics, and pharmacology. Genetic defects in genes associated with the pharmacogenetic network, mutations in epigenes, and epigenetic aberrations in different components of the epigenetic machinery may all contribute to individual responses to conventional drugs.

The emerging discipline of pharmacoepigenetics is still in its early infancy with fewer than 50 papers published during the period 2007–18. However, over 3000 papers have been published on epigenetic drugs (1981–2018). Epidrugs are generally recognized as chemicals (or bioproducts) whose main targets are components of the epigenetic machinery (DNA methyltransferases, DNA demethylases, histone deacetylases, histone methyltransferases, histone demethylases, and chromatin-associated proteins). The genes encoding these targets are the mechanistic genes in the pharmacoepigenetic network. Over 90% of epidrugs are focused on cancer, and some have been approved by the US Food and Drug Administration for the treatment of different forms of neoplastic processes. According to their primary targets the classification of epidrugs currently includes the following categories: DNA methyltransferase inhibitors, DNA demethylase modulators, histone deacetylase inhibitors, histone acetyltransferase inhibitors, histone methyltransferase inhibitors, histone demethylase inhibitors, ATP-dependent chromatin remodelers, polycomb repressive complex inhibitors, bromodomain inhibitors, and chromodomain inhibitors.

The great variety of epidrugs highlights just how heterogeneous their pharmacokinetic and pharmacodynamic properties are. Most synthetic compounds are not devoid of side effects and their hydrophilic profile does not allow some of them to cross physiological barriers (i.e., the blood-brain barrier). This circumstance may limit their applicability for the treatment of brain disorders, although novel drug delivery systems can probably overcome this drawback. Despite abundant black holes in the universe of epidrugs, the potential reversibility of epigenetic aberrations with efficient pharmacological intervention is a promising area of future development in which pharmacoepigenetics holds a privileged position.

This book is the first attempt to establish the foundations of pharmacoepigenetics. It is the culmination of over 5000 studies reported by many researchers during the past two decades. I would like to thank the contributing authors from all over the world for their excellent chapters and their attempts at harmonizing still uneven information to construct a preliminary doctrine on pharmacoepigenetics. I would also like to thank Megan Ashdown and the Elsevier staff involved in this book for their professional excellence and exquisite care with details.

In science nothing is definitive, and I hope that these initial ideas and postulates on pharmacoepigenetics will be of help in the construction of a solid discipline in the coming years. Perhaps, the Decalogue of Golden Rules put forward in this book can be summarized by making the following points: (i) epigenetics is a biological language for fluent communication between the environment and the genome of living creatures; (ii) mutations in genes encoding components of the epigenetic machinery can lead to epigenetic Mendelian disorders; (iii) prenatal exposure to xenobiotic influences may affect individual health later in life and its consequences may be transmitted to progeny via transgenerational inheritance; (iv) epigenetic changes are potentially reversible by means of appropriate endoxenobiotic intervention; (v) any xenobiotic agent (drugs, nutrients, environmental toxicants) undergoes epigenetic processing via the pharmacoepigenetic apparatus, yielding positive or negative effects on health conditions; (vi) epigenetic drugs may open up new horizons for the treatment of complex disorders in which epigenetic aberrations influence pathogenesis; (vii) drug efficacy and safety depend to a great extent on the functional integrity of the pharmacoepigenetic apparatus and/or the intrinsic properties of each drug; (viii) in some instances drug resistance may be the result of malfunctioning in by-products of pathogenic, mechanistic, metabolic, transporter, and pleiotropic genes associated with the pharmacoepigenetic network; (ix) epigenetic drugs, like any other xenobiotic compound, are subject to global pharmacoepigenetic processing; and (x) all treatments should be personalized according to the pharmacoepigenetic profile of each patient for therapeutic optimization.

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CHAPTER

1

The Epigenetic Machinery in the Life Cycle and Pharmacoepigenetics

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1.1 INTRODUCTION

Epigenetics is a discipline that studies heritable changes in gene expression without structural changes in the DNA sequence. Epigenetics is one of the most rapidly developing fields in the history of biology. The concept of epigenetics has evolved since Waddington defined it in the late 1930s, becoming a contextual multifaceted discipline with influence in evolution, speciation, functional genomics, transcriptomics, proteomics, metabolomics, and obviously in species-specific health and disease.[1] Epigenetics plays an important role in phenotypic variation in different species of the animal and plant kingdoms.[2] Epigenetic memory can persist across generations. A stress-induced signal can be transmitted across multiple unexposed generations leading to persistent changes in epigenetic gene regulation.[3] Epigenetic mechanisms contribute to phenotypic variation and disparities in morbidity and mortality.[4] Epigenetics acts as an interface between the genome and the environment, and the mechanistic changes associated with the epigenetic phenomena can also be considered a sophisticated form of intracellular and intercellular communication.[5] Epigenetics is an adaptive mechanism of developmental plasticity, a phenomenon of relevance in evolutionary biology and human health and disease, which enables organisms to respond to their environment based on previous experience without changes to the underlying nucleotide sequence.[6]

Genetic variation correlates with phenotypes depending on allele-specific genetic changes linked to gene expression, DNA methylation, histone marks, and miRNA regulation of proteomic and metabolomic processes.[7]

Epigenomic modifications are involved in a great variety of pathological conditions; of major importance are those related with age and with major problems of health such as cardiovascular disorders, obesity, cancer, inflammatory disorders, asthma, allergy, and brain disorders. Pharmaceuticals, pesticides, air pollutants, industrial chemicals, heavy metals, hormones, nutrition, and behavior can change gene expression through a broad array of gene regulatory mechanisms which include regulation of gene translocation, histone modifications, DNA methylation, DNA repair, transcription, RNA stability, alternative RNA splicing, protein degradation, gene copy number, and transposon activation.[8] Epigenetic modifications are reversible and can potentially be targeted by pharmacological and dietary interventions.[9]

The effects of drugs (pharmacokinetics and pharmacodynamics) and their therapeutic outcome in the treatment of a given disease are the result of a network of metabolomic events (genomics-transcriptomics-proteomics) associated with the binomial interaction of a chemical or biological molecule with a living organism. The clusters of genes currently involved in a pharmacogenomic process include pathogenic, mechanistic, metabolic, transporter, and pleiotropic genes.[10] In practice, the expression of these genes is potentially modifiable (transcriptionally and/or posttranscriptionally) by epigenetic mechanisms which may alter (i) pathogenic events, (ii) receptor-drug interactions, (iii) drug metabolism (phase I and II enzymatic reactions), (iv) drug transport (influx-efflux across membranes and cellular barriers), and (v) pleiotropic events leading to unexpected therapeutic outcomes. Understanding these mechanisms is the main focus of pharmacoepigenetics to optimize therapeutics and advance toward a personalized medicine.[11–13]

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Unfortunately, the molecular mechanisms underlying the assembly, function, and regulation of the epigenetic machinery are poorly understood, and most information in this regard is fragmented. This restrictive knowledge on epigenetic mechanisms represents an important limitation for defining the fundamentals of pharmacoepigenetics. Furthermore, the number of studies on pharmacogenetics and pharmacoepigenetics of current drugs for the treatment of common pathologies is still very limited; however, the available information is shedding light on the benefits that these complementary disciplines can provide to physicians and patients for the implementation of an efficient personalized medicine.[13–15]

1.2 THE EPIGENETIC MACHINERY

The epigenetic machinery is integrated by a cluster of interconnected elements that in a coordinated manner contribute to regulate gene expression at the transcriptional and posttranscriptional level. Classical epigenetic mechanisms include DNA methylation, histone modifications, and microRNA (miRNA) regulation; however, the execution of these basic mechanisms compromises a pleiad of subsidiary biochemical effectors which contribute to correctly express or repress gene expression, protein synthesis, and protein degradation. Not only nuclear DNA (nDNA), but also mitochondrial DNA (mtDNA) may be subjected to epigenetic modifications.[16] Therefore, conventional epigenetics can be divided into two major areas: (i) epigenetics of nuclear-encoded DNA, and (ii) epigenetics of mitochondrial-encoded DNA. DNA methylation, chromatin remodeling and histone modification, and noncoding RNAs (ncRNAs) are the principal regulators in epigenetics of nuclear-encoded DNA. Mitochondrial epigenetics uses DNA methylation and ncRNAs in a similar fashion, but it differs to some extent in the role of components coiling DNA. Nuclear DNA is coiled around histones; in contrast, mtDNA is located in nucleoids, a set of mitochondrial pseudocompartments. Mitochondrial epigenetics influences cell fate, transcription regulation, cell division, cell cycle, physiological homeostasis, bioenergetics, and diverse conditions of health and disease.[17] Methylation of nuclear genes encoding mitochondrial proteins is involved in the regulation of mitochondria function. There is debate concerning the existence of cytosine methylation in the mitochondrial genome and it has been suggested that cytosine methylation is virtually absent in mtDNA.[18, 19] mtDNA is differentially methylated in various diseases. The activity of the mitochondrial transcription factor A (TFAM), a protein involved in mtDNA packaging, influences gene expression. Several mechanisms have been suggested to explain mtDNA methylation and epigenetic-like modifications of TFAM, including methylation within the noncoding D-loop, methylation at gene start sites (GSS), and posttranslational modifications (PTMs) of TFAM.[20]

Many new concepts on epigenetics-related gene expression have emerged over the past few years. This vertiginous progression of epigenetics permanently modifies our perception of genetic regulation and opens new avenues for a better understanding of health and disease.

1.2.1 DNA Methylation

DNA methylation is a process by which methyl groups are incorporated into cytosine molecules by DNA methyltransferases (DNMTs), forming 5-methylcytosine and contributing to the suppression of transcription. Approximately 70% of CpG dinucleotides within the human genome are methylated. CpG islands in promoter regions of genes are defined as 200-bp regions of DNA where the GC content is greater than 60%. The human genome contains ~30,000 CpG islands (CGIs). CGIs associated with promoters nearly always remain unmethylated, and most of the ~9000 CGIs lying within gene bodies become methylated during development and differentiation. Both promoter and intragenic CGIs may become abnormally methylated as a result of genome rearrangements and in cancer. Transcription running across CGIs, associated with specific chromatin modifications, is required for DNA methyltransferase 3B (DNMT3B)-mediated DNA methylation of many naturally occurring intragenic CGIs.[21] Of the ~2.2 million CpGs tested for allele-specific methylation (ASM), nonallelic methylation (mQTL), and genotype-independent effects, approximately 32% are genetically regulated (ASM or mQTL) and 14% are putatively epigenetically regulated. Epigenetically driven effects are strongly enriched in repressed regions and near transcription start sites, whereas genetically regulated CpGs are enriched in enhancers. Imprinted regions are enriched among epigenetically regulated loci.[7]

DNA methylation inhibits transcription by interfering with the binding of transcription factors to recognition sites on promoters or by recruiting and binding transcriptional repressors, methyl-CpG-binding proteins (MBDs), and altering the chromatin structure into an active state.

5-Methylcytosines (5mC) can also be oxidized to form 5-hydroxymethylcytosine (5hmC) to reduce the interaction of DNA with DNA-binding proteins.[22] CpG methylation may also cause a dual effect on transcription, repressing transcription when CpG methylation occurs at the promotor level or promoting transcription when CpG methylation affects the gene sequence.[23] The transfer of methyl groups from S-adenosyl-methionine (SAM) to cytosine in CpGs is catalyzed by a family of DNA methyltransferases (DNMTs), which in mammals are represented by two de novo DNMts (DNMT3A, DNMT3B) and a maintenance DNMT (DNMT1) that is expressed in neurons. DNMT2 methylates aspartic acid tRNA, and does not methylate DNA[24, 25] (Table 1.1). The modification of DNA bases is a classic hallmark of epigenetics. Four forms of modified cytosine (5-methylcytosine, 5-hydroxymethylcytosine, 5-formylcytosine, and

TABLE 1.1 DNA Methyltransferases

Gene	Name	Locus	Other names	MIM number	Phenotype
DNMT1	DNA methyltransferase 1	19p13.2	CXXC9, MCMT, HSN1E, ADCADN	126375	Cerebellar ataxia, deafness, and narcolepsy, autosomal dominant; neuropathy, hereditary sensory, type IE
DNMT3A	DNA methyltransferase 3A	2p23.3	TBRS	602769	Acute myeloid leukemia, somatic; Tatton-Brown-Rahman syndrome
DNMT3B	DNA methyltransferase 3B	20q11.21	ICF1, ICF	602900	Immunodeficiency-centromeric instability-facial anomalies syndrome 1
DNMT3L	DNA methyltransferase 3-like protein	21q22.3	MGC1090	606588	Embryonal carcinoma
DMAP1	DNMT1-associated protein 1	1p34.1	DNMAP1, DNMTAP1, EAF2, FLJ11543, KIAA1425, MEAF2, SWC4	605077	
MBD1	Methyl-CpG-binding domain protein 1 (protein containing methyl-CpG-binding domain 1)	18q21.1	PCM1	156535	Colorectal cancer; lung cancer
MBD2	Methyl-CpG-binding domain protein 2	18q21.2		603547	Colorectal cancer; lung cancer; primary immune thrombocytopenia; systemic lupus erythematosus
MBD3	Methyl-CpG-binding domain protein 3	19p13.3		603573	Glioblastoma
MBD4	Methyl-CpG-binding domain protein 4	3q21.3	MED1	603574	Primary immune thrombocytopenia; systemic lupus erythematosus
MBD5	Methyl-CpG-binding domain protein 5	2q23.1	KIAA1461, MRD1	611472	Mental retardation, autosomal dominant 1
MBD3L1	Methyl-CpG-binding domain protein 3-like 1	19p13.2	MBD3L	607963	
MBD3L2	Methyl-CpG-binding domain protein 3-like 2	19p13.2		607964	
MECP2	Methyl-CpG-binding protein-2	Xq28	PPMX, MRX16, MRX79, AUTSX3, MRXSL, MRXS13	300005	Autism susceptibility, X linked 3; encephalopathy, neonatal severe; mental retardation, X linked syndromic, lubs type; mental retardation, X linked, syndromic 13; Rett syndrome; Rett syndrome, atypical; Rett syndrome, preserved speech variant
MGMT	Methylguanine-DNA methyltransferase	10q26.3		156569	Neoplasms
N6AMT1	N6 adenine-specific DNA methyltransferase 1, putative	21q21.3		614553	
SMCHD1	Structural maintenance of chromosomes flexible hinge domain-containing protein 1	18p11.32	FSHD2, KIAA0650, BAMS	614982	Bosma arhinia microphthalmia syndrome; Fascioscapulohumeral muscular dystrophy 2, digenic

5-carboxylcytosine) have been discovered in eukaryotic DNA. In addition to cytosine carbon-5 modifications, cytosine and adenine methylated in exocyclic amine-N4-methylcytosine and N6-methyladenine are other modified DNA bases discovered even earlier. Each modified base can be considered a distinct epigenetic signal with broader biological implications beyond simple chemical changes. A structural synopsis of writers, readers, and erasers of the modified bases from prokaryotes and eukaryotes has been proposed, suggesting that base flipping is a common structural framework broadly applied by distinct classes of proteins and enzymes across phyla for epigenetic regulation of DNA.[26]

Currently four regulatory, noncanonical bases with a methyl (CH3)-, a hydroxymethyl (CH2 OH)-, a formyl (CHO)-, and a carboxyl (COOH)-group are known. 5-Methylcytidine is a classic regulatory base in the genome, while the other three bases and their enzymatic apparatus have been recently discovered.[27] 5-Hydroxymethyl-, 5-formyl-, and 5-carboxy-2′-deoxycytidine are new epigenetic bases (hmdC, fdC, cadC) that were discovered in the DNA of higher eukaryotes. The same bases (5-hydroxymethylcytidine, 5-formylcytidine, and 5-carboxycytidine, hmC, fC, and caC) have now also been detected in mammalian RNA with a high abundance in mRNA. While DNA phosphoramidites (PAs) that allow the synthesis of xdC-containing oligonucleotides for deeper biological studies are available, the corresponding silyl-protected RNA PAs for fC and caC have not been disclosed until recently.[28]

The epigenetic modification of cytosine and its continuous oxidative products are called the "new four bases of DNA" (5mC, 5hmC, 5fC, 5caC).[29] The synthesis of 2′-*O*-methyl-5-hydroxymethylcytidine (hm5 Cm), 5-hydroxymethylcytidine (hm5 C), and 5-formylcytidine (f5 C) phosphoramidite monomers has been elucidated.[30]

5′-Hydroxymethylcytosine (5hmC) is a variant of the common covalent epigenetic modification of DNA 5′-methylcytosine (5mC). The role of 5hmC remains elusive. It was proposed that 5hmC is a variant of the 5mC epigenetic signal involved in the epigenetic regulation of gene function and in the activation of lineage-specific enhancers.[31] 5-Formylcytosine (5fC) is an endogenous DNA modification frequently found within regulatory elements of mammalian genes. Although 5fC is an oxidation product of 5-methylcytosine (5mC), the two epigenetic marks show distinct genome-wide distributions and protein affinities, suggesting that they perform different functions in epigenetic signaling. A unique feature of 5fC is the presence of a potentially reactive aldehyde group in its structure. 5fC bases in DNA readily form Schiff-base conjugates with Lys side chains of nuclear proteins. These covalent protein-DNA complexes are reversible, suggesting that they contribute to transcriptional regulation and chromatin remodeling. 5fC-mediated DNA-protein cross-links, if present at replication forks or actively transcribed regions, may interfere with DNA replication and transcription.[32]

Recently discovered cytosine derivatives in the human genome are recognized by specific DNA-binding proteins.[33] Cytosine methylation is a well-characterized epigenetic mark that occurs at both CG and non-CG sites in DNA. Covalent modification of DNA via deposition of a methyl group at the 5′ position on cytosine residues alters the chemical groups available for interaction in the major groove of DNA. The information content inherent in this modification alters the affinity and the specificity of DNA binding. Some proteins favor interaction with methylated DNA, and others disfavor it. Both methylated CG (mCG)- and mCH (H = A, C, or T)-containing DNAs, especially mCAC-containing DNAs, are recognized by methyl-CpG-binding protein 2 (MeCP2) to regulate gene expression in neuron development.

Molecular recognition of cytosine methylation by proteins often initiates sequential regulatory events which impact gene expression and chromatin structure. Methyl-CpG-binding proteins play an essential role in translating DNA methylation marks into a downstream transcriptional response. A detailed mechanism explaining this molecular process remains to be elucidated. ZBTB38 is an undercharacterized member of the zinc finger (ZF) family of methyl-CpG-binding proteins. A subset of the C-terminal ZBTB38 ZFs exhibits high-affinity DNA interactions, and preferential targeting of the consensus DNA site is methyl specific. The C-terminal ZFs of ZBTB38 can directly occupy promoters harboring the newly identified sequence motif in a methyl-dependent manner and, depending on the gene context, contribute to modulating transcriptional response.[34]

The known methyl-DNA-binding proteins have unique domains responsible for DNA methylation recognition, including (i) the methyl-CpG-binding domain (MBD), (ii) the C2H2 zinc finger domain, and (iii) the SET- and RING finger-associated (SRA) domain. Each domain has a characteristic methylated DNA-binding pattern, and this difference in the recognition mechanism renders the DNA methylation mark able to transmit complicated biological information.[35] DNA methylation is associated with gene silencing in eukaryotic organisms. Several MBDs participate in this process. The methyl-CpG-binding domain 7 (MBD7) complex suppresses DNA methylation-mediated gene silencing.[36] Methyl-CpG-binding domains (MBD) of MeCP2 and MBD1–4 bind mCG-containing DNAs independently of the sequence outside the mCG dinucleotide. Some MBDs bind to both methylated and unmethylated CA dinucleotide-containing DNAs, with a preference for the CAC sequence motif. In addition to mCG sites, unmethylated CA or TG sites also serve as DNA-binding sites for MeCP2 and other MBD-containing proteins.[37]

Through a genome-wide analysis of DNA methylation across 19 cell types with T-47D as reference,[38] 106,252 cell type-specific differentially methylated CpGs categorized into 7537 differentially (46.6% hyper- and 53.4% hypo-) methylated regions have been identified; 44% of promoter regions and 75% of CpG islands were T-47D cell type-specific methylated. DMRs overlapped with 1145 known tumor suppressor genes. Integration of DNA methylation and transcription factor information revealed interplay patterns between regulators. This integrative analysis shows cell type-specific and genomic region-dependent regulatory patterns.

Symmetric CpG methylation can be mitotically propagated over many generations with high fidelity. Heterogeneous methylation largely reflects asynchronous proliferation, but it is intrinsic to actively engaged *cis*-regulatory elements and cancer.[39] Differentially methylated or hydroxymethylated regions (DMRs) in mammalian DNA are often associated with tissue-specific gene expression.[40]

DNA methylation downregulates transcription. However, a large number of genes that are unmethylated in the promoter region are inactive. A large group of unmethylated promoters is regulated by DNMT1 through DNA methylation-dependent silencing of upstream regulators such as transcription factor HNF4A.[41] DNA methylation in repetitive elements (RE) suppresses their mobility and maintains genomic stability.[42]

Our perception of DNA methylation as a dynamic process is strongly moving as a result of daily new findings on this epigenetic phenomenon.[43] Initially, it was generally admitted that DNMT3A and DNMT3B are associated with de novo methylation and DNMT1 is associated with inheritance DNA methylation. Several partners of DNMTs have been involved in both the regulation of DNA methylation activity and DNMT recruitment on DNA. The DNMT3L/DNMT3A complex is mainly related to de novo DNA methylation in embryonic states, whereas the DNMT1/PCNA/UHRF1 complex is required for maintaining global DNA methylation following DNA replication. Some recently identified DNMT-including complexes are recruited on specific DNA sequences. The coexistence of both types of DNA methylation suggests close cooperation and orchestration between these systems to maintain genome and epigenome integrities. According to Hervouet et al.[43] deregulation of these systems can lead to pathologic disorders.

In mammals three DNA methyltransferases (DNM1, DNMT3A, and DNMT3B) have been identified. DNMT3A and DNMT3B are responsible for establishing DNA methylation patterns produced through their de novo-type DNA methylation activity in implantation stage embryos and during germ cell differentiation. DNMT3-like (DNMT3L), which is a member of the DNMT3 family but does not possess DNA methylation activity, was reported to be indispensable for global methylation in germ cells. Once the DNA methylation patterns are established, maintenance-type DNA methyltransferase DNMT1 faithfully propagates them to the next generation via replication. All DNMTs possess multiple domains.[44]

The functions of the canonical DNMT enzymes (DNMT1, DNMT3A, DNMT3B) go beyond their traditional roles of establishing and maintaining DNA methylation patterns. Molecular interactions and changes in gene copy numbers modulate the activity of DNMTs in diverse gene regulatory functions, including transcriptional silencing, transcriptional activation, and posttranscriptional regulation by DNMT2-dependent tRNA methylation.[45]

Cytosine methylation is both ubiquitous and a stable regulatory modification, which may potentially stabilize triple-helix formation. Methylation on both the Hoogsteen and Crick strands yields the largest favorable free energy. Methylation increases cytosine protonation by shifting the apparent p*K*a value to a higher pH.[46]

5meC can be maintained through DNA replication by the activity of "maintenance" DNA methyltransferases. In evolutive terms DNA methylation coevolved with the DNA alkylation repair enzyme ALKB2 across eukaryotes. Alkylation damage is intrinsically associated with DNMT activity, and this may promote the loss of DNA methylation in many species.[47]

DNA methylation in promoter regions represses gene expression and is copied over mitotic divisions by DNMT1. DNMT1 activity is regulated by its replication foci targeting sequence (RFTS) domain which masks the catalytic pocket. DNMT1 activity on unmethylated DNA is inhibited in nucleosome cores. DNMT1 fully methylates naked linker DNA in dinucleosomes, whereas maintenance methylation is repressed at all CpG sites in nucleosome core particles. Histone H3 tail peptides inhibit DNMT1 in an RFTS-dependent manner and repression is modulated by acetylation or methylation. Mishima et al.[48] propose a novel function for RFTS in the regulation of DNMT1 activity in nucleosomes.

DNA methylation at promoters is largely correlated with inhibition of gene expression. DNA methylation at enhancers requires a crosstalk with chromatin marks. Studies on the relationship between DNA methylation and active chromatin marks through genome-wide correlations show an anticorrelation between H3K4me1 and H3K4me3 enrichment at low and intermediate DNA methylation loci. DNA methylation discriminates between enhancers and promoters, marked by H3K4me1 and H3K4me3, respectively. Low-methylated regions are H3K4me3 enriched, while those with intermediate DNA methylation levels are progressively H3K4me1 enriched.

The decrease in DNA methylation smoothly switches the state of the enhancers from a primed to an active state. According to Sharifi-Zarchi et al.,[49] "In each genomic region only one out of these three methylation marks (DNA methylation, H3K4me1, H3K4me3) is high. If it is the DNA methylation, the region is inactive. If it is H3K4me1, the region is an enhancer, and if it is H3K4me3, the region is a promoter."

DNA methylation can affect tissue-specific gene transcription and is not merely the consequence of changes in gene expression, but is often an active agent for fine-tuning transcription in association with development. In some tissues promoter region hypermethylation of defined genes is associated with gene repression, and DNA hypermethylation is absent in many other repressed cells. In other genes DNA hypermethylation overlaps cryptic enhancers or superenhancers and correlates with downmodulated, but not silenced, gene expression. Methylation is absent in both nonexpressing genes and highly expressing genes, suggesting that some genes need DMR hypermethylation to help repress cryptic enhancer chromatin only when they are actively transcribed.[50]

Loss-of-function mutations of the maintenance methyltransferase DNMT1 in normal human cells is lethal, mainly affecting promoters and gene bodies in four gene classes: (i) protocadherins, which are key to neural cell identity; (ii) genes involved in fat homeostasis/body mass determination; (iii) olfactory receptors; and (iv) cancer/testis antigen (CTA) genes. Hypomethylated regions are associated with Polycomb repression and are derepressed on addition of an EZH2 inhibitor.[51]

1.2.2 DNA Demethylation

1.2.2.1 Ten-Eleven Translocation (TET) Proteins

DNA demethylation can be produced by at least three enzyme families: (i) the ten-eleven translocation (TET) family, mediating the conversion of 5mC into 5hmC; (ii) the AID/APOBEC family, acting as mediators of 5mC or 5hmC deamination; and (iii) the BER (base excision repair) glycosylase family involved in DNA repair[22] (Table 1.2). The DNA demethylation pathway plays a significant role in DNA epigenetics. This pathway removes the methyl group from cytosine, which is involved in the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine (5-hmC) by ten-eleven translocation (TET) proteins (Table 1.2). Then 5-hmC can be iteratively oxidized to generate 5-formylcytosine and 5-carboxylcytosine.[52]

TABLE 1.2 DNA Demethylases

Gene	Name	Locus	Other names	MIM number	Phenotype
AICDA	Activation-induced cytidine deaminase	12p13.31	AID, ARP2, CDA2, HIGM2	605257	Follicular lymphoma; immunodeficiency with hyper-IgM, type 2; leukemia; non-Hodgkin lymphoma; skin cancer
APEX1	APEX nuclease (multifunctional DNA repair enzyme)	14q11.2	APE, APE-1, APEN, APX, HAP1, REF-1, REF1	107748	Age-related macular degeneration and other neovascular diseases; pancreatic cancer
APEX1	Apurinic/apyrimidinic endodeoxyribonuclease 2	Xp11.21	APE2, APEXL2, XTH2, ZGRF2	300773	
APOBEC1	Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide 1	12p13.31	APOBEC-1, BEDP, CDAR1, HEPR	600130	HIV infectivity
APOBEC2	Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide 2	6p21.1	ARCD1, ARP1	604797	Hepatoblastoma; myopathy
APOBEC3A	Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3A (phorbolin 1)	22q13.1	PHRBN, ARP3	607109	Acute myelogenous leukemia (AML); cancer; human papillomavirus (HPV) infection
APOBEC3B	Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3B	22q13.1	FLJ21201, PHRBNL	607110	Breast cancer; chondrosarcoma; HIV infectivity; lymphoma; ovarian cancer

TABLE 1.2 DNA Demethylases—cont'd

Gene	Name	Locus	Other names	MIM number	Phenotype
APOBEC3D	Apolipoprotein B mRNA-editing enzyme catalytic subunit 3D	22q13.1	APOBEC3E, APOBEC3DE, ARP6	609900	HIV infectivity
APOBEC3F	Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3F	22q13.1	ARP8, BK150C2.4. MRNA, KA6	608993	Hepatitis B virus infection; hepatocellular carcinoma; HIV infectivity; lung cancer
APOBEC3G	Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G	22q13.1	MDS019, CEM15, FLJ12740	607113	Hepatitis B virus infection; Hepatocellular carcinoma; HIV infectivity; Uterine cancer
APOBEC3H	Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3H	22q13.1	ARP10	610976	HIV infectivity; lung cancer
APOBEC4	Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 4	1q25.3	FLJ25691, MGC26594, RP1-127C7.4	609908	HIV infectivity
MBD4	Methyl-CpG-binding domain protein 4	3q21.3	MED1	603574	
PARP1	Poly(ADP-ribose) polymerase 1	1q42.12	PARP	173870	Breast cancer; Lymphoma; Parkinson disease
RNF4	RING finger protein-4	4p16.3	RES4-26, SLX5, SNURF	602850	Breast cancer; Epstein-Barr virus (EBV) infection; lung cancer; Wilms tumor
TET1	Tet oncogene 1	10q21.3	CXXC6, LCX, KIAA1676	607790	Acute myeloid leukemia; Alzheimer disease
TET1P1	Tet methylcytosine dioxygenase 1 pseudogene 1	13q31.2	CXXC6P1		
TET2	TET oncogene family, member 2	4q24	KIAA1546, MDS	612839	Acute myeloid leukemia; Angioimmunoblastic T cell lymphoma; chronic myelomonocytic leukemia; Myelodysplastic syndrome, somatic
TET3	TET oncogene family, member 3	2p13.1	KIAA0401	613555	Head and neck cancer; renal cell carcinoma; systemic lupus erythematosus (SLE); ovarian cancer
TDG	Thymine-DNA glycosylase	12q23.3	TNG, TPG, hTDG	601423	Intestinal cancer
XRCC1	X-ray-repair, complementing defective, repair in Chinese hamster cells-1	19q13.31	SCAR26	617633	Breast cancer; gastric cancer; primary open-angle glaucoma; spinocerebellar ataxia, autosomal recessive 26; squamous cell carcinoma; testicular cancer

The oxidation of 5-methylcytosine can result in three chemically distinct species: 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxycytosine. While the base excision repair machinery processes 5-formylcytosine and 5-carboxycytosine rapidly, 5-hydroxymethylcytosine is stable under physiological conditions. As a stable modification 5-hydroxymethylcytosine has a broad range of functions, from stem cell pluripotency to tumorigenesis. The subsequent oxidation products, 5-formylcytosine and 5-carboxycytosine, are involved in an active DNA demethylation pathway.[53]

The full-length TET1 isoform (TET1e) is restricted to early embryos, embryonic stem cells (ESCs), and primordial germ cells (PGCs). A short isoform (TET1s) is preferentially expressed in somatic cells, which lacks the N-terminus including the CXXC domain, a DNA-binding module that often recognizes CpG islands (CGIs). TET1s can bind CGIs despite the fact that its global chromatin binding is significantly reduced. Global chromatin binding correlates with TET1-mediated demethylation. Mice with exclusive expression of Tet1s fail to erase imprints in PGCs and display developmental defects in progeny, suggesting that isoform switch of TET1 regulates epigenetic memory erasure and mouse development.[54] The biological roles of TETs/oxi-mCs may differ among species.[55]

Studies on the interplay of the 5-methylcytosine reader Mbd1 and modifier Tet1 revealed that Mbd1 enhances Tet1-mediated 5-methylcytosine oxidation due to enhancing the localization of Tet1, but not of Tet2 and Tet3 at heterochromatic DNA. The recruitment of Tet1 leads to the displacement of Mbd1 from methylated DNA. Increased Tet1 heterochromatin localization and 5-methylcytosine oxidation are dependent on the CXXC3 domain of Mbd1, which recognizes unmethylated CpG dinucleotides. The Mbd1 CXXC3 domain deletion isoform, which retains only binding to methylated CpGs, blocks Tet1-mediated 5-methylcytosine to 5-hydroxymethylcytosine conversion, indicating opposite biological effects of Mbd1 isoforms.[56]

Aberrant DNA methylation and demethylation are associated with developmental defects and cancer.[57, 58] TET1 is a novel target of miR-21-5p in colorectal cancer.[59]

1.2.2.2 AID/APOBEC Family Cytidine Deaminases

Cytidine deaminases of the AID/APOBEC family (Table 1.2) catalyze C-to-U nucleotide transitions in mRNA or DNA. APOBEC3 is involved in antiviral defense and AID contributes to diversification of antibody repertoires in jawed vertebrates via somatic hypermutation, gene conversion, and class switch recombination. In lampreys, an extant jawless vertebrate, two members of the AID/APOBEC family are implicated in the generation of somatic diversity of the variable lymphocyte receptors (VLRs).[60]

Activation-induced cytidine deaminase (AID) triggers antibody diversification in B cells by catalyzing deamination and subsequently mutating immunoglobulin (Ig) genes. The association of AID with RNA Pol II and the occurrence of epigenetic changes during Ig gene diversification suggest participation of AID in epigenetic regulation. AID is mutated in hyper-IgM type 2 (HIGM2) syndrome. AID binding to the IgH locus promotes an increase in H4K20me3. In 293F cells Rodríguez-Cortez et al.[61] demonstrated the interaction between cotransfected AID and the three SUV4-20 histone H4K20 methyltransferases. SUV4-20H1.2, bound to the IgH switch (S) mu site, is replaced by SUV4-20H2 upon AID binding. The AID truncated form W68X is impaired to interact with SUV4-20H1.2 and SUV4-20H2 and is also unable to bind and target H4K20me3 to the Smu site. AID deficiency associates with decreased H4K20me3 levels at the Smu site.[61]

1.2.2.3 BER (Base Excision Repair) Glycosylases

DNA glycosylases represent a new category of base excision repair proteins for hydrolytic deamination of DNA bases with exocyclic amino groups. Hydrolytic deamination of 5-methylcytosine leads to the formation of G/T mismatches which are corrected to G/C basepairs by a mismatch-specific DNA-binding glycosylase (Table 1.2).

1.2.3 Chromatin Remodeling and Histone Modifications

1.2.3.1 Chromatin

Genomic DNA is compacted in chromatin, which suppresses transcription, replication, repair, and recombination. The fundamental unit of chromatin is the nucleosome. Nucleosomes containing histone variants often have subtle but clear differences in their structural and functional characteristics, as compared to the canonical nucleosome. The overlapping dinucleosome is a new structural unit of chromatin.[62] Communication between distantly spaced genomic regions is one of the key features of gene regulation in eukaryotes. Chromatin can stimulate efficient enhancer-promoter communication (EPC). Nucleosome spacing and the presence of nucleosome-free DNA regions can modulate chromatin structure/dynamics and affect the rate of EPC.[63] Chromatin is composed of DNA (with genetic instructions for cell phenotype), and histone proteins (Table 1.3), responsible for providing the scaffold for chromatin folding and a portion of the epigenetic inheritance. Histone writers/erasers flag chromatin regions by catalyzing/removing covalent histone posttranslational modifications (PTMs). Histone PTMs contribute to chromatin relaxation or compaction and recruit histone readers to modulate DNA readout.[64]

CpG islands (CGIs) are critical genomic regulatory elements that support transcriptional initiation and are associated with the promoters of most human genes. CGIs are distinguished from the bulk genome by their high CpG density, lack of DNA methylation, and euchromatic features. While CGIs are canonically known as strong promoters, thousands of "orphan" CGIs lie far from any known transcript, leaving their function an open question. Most orphan CGIs display the chromatin features of active enhancers (H3K4me1, H3K27Ac) in at least one cell type. Relative to classical enhancers these enhancer CGIs (ECGIs) are stronger, as gauged by chromatin state, and are more broadly expressed and more highly conserved. ECGIs engage in more genomic contacts and are enriched for transcription factor binding relative to classical enhancers. ECGIs define a class of highly active enhancers, strengthened by the broad transcriptional activity, CpG density, hypomethylation, and chromatin features they share with promoter CGIs.[65]

TABLE 1.3 Histone Proteins

Gene	Name	Locus	Other names	MIM number
H1F0	H1 histone family, member 0	22q13.1	H1FV, H10, H1.0, H1(0), H1-0	142708
H1FX	H1 histone family, member X	3q21.3	H1X, MGC15959, MGC8350	602785
H2AFB	H2A histone family, member B	Xq28	H2ABBD	300445
H2AFX	H2A histone family, member X	11q23.3	H2AX	601772
H2AFY	H2A histone family, member Y	5q31.1	MH2A1, macroH2A1.2	610054
H2AFY2	H2A histone family, member Y2	10q22.1	macroH2A2	616141
H2AFZ	H2A histone family, member Z	4q23	H2AZ	142763
H2BFWT	H2B histone family, member W, testis-specific	Xq22.2		300507
H3F3A	H3 histone, family 3A	1q42.12	H3F3	601128
H3F3B	H3 histone, family 3B (H3.3B)	17q25.1	H3.3B	601058
H3F3C	H3 histone, family 3C	12p11.21	H3.5	616134
HIST1H1A	Histone 1, H1a	6p22.2	H1F1, H1.1, H1a	142709
HIST1H1B	Histone 1, H1b	6p22.1	H1F5, H1.5, H1b, H1s-3	142711
HIST1H1C	Histone 1, H1c	6p22.2	H1F2, H1.2, H1c, H1s-1	142710
HIST1H1D	Histone 1, H1d	6p22.2	H1F3	142210
HIST1H1E	Histone 1, H1e	6p22.2	H1F4, RMNS	142220
HIST1H1T	Histone 1, H1t	6p22.2	H1FT, H1t	142712
HIST1H2AA	Histone gene cluster 1, H2A histone family, member A	6p22.2	H2AFR, H2AA, bA317E16.2	613499
HIST1H2AB	Histone 1, H2ab	6p22.2	H2AFM	602795
HIST1H2AC	Histone 1, H2ac	6p22.2	H2AFL	602794
HIST1H2AD	Histone 1, H2ad	6p22.2	H2AFG	602792
HIST1H2AE	Histone 1, H2ae	6p22.2	H2AFA, H2A.1, H2A/a	602786
HIST1H2AG	Histone gene cluster 1, H2A histone family, member 6	6p22.1	H2AG, H2AFP, H2A.1b, H2A/p, pH2A/f	615012
HIST1H2AH	Histone gene cluster 1, H2A histone family, member H	6p22.1	H2AH, H2A/S, H2AFALii	615013
HIST1H2AI	Histone 1, H2ai	6p22.1	H2AFC, H2A/c	602787
HIST1H2AJ	Histone 1, H2aj	6p22.1	HIST1H2AK, H2AFE	602791
HIST1H2AK	Histone 1, H2ak	6p22.1	HIST1H2AI, H2AFD	602788
HIST1H2AL	Histone 1, H2al	6p22.1	H2AFI, H2A/i	602793
HIST1H2AM	Histone 1, H2am	6p22.1	H2AFN, H2A.1, H2A/n	602796
HIST1H2BA	Histone 1, H2ba	6p22.2	TSH2B, H2BFU, STBP	609904
HIST1H2BB	Histone 1, H2bb	6p22.2	H2BFF	602803
HIST1H2BD	Histone 1, H2bd	6p22.2	H2BFB, H2B/b	602799
HIST1H2BE	Histone 1, H2be	6p22.2	H2BFH, H2B.h, H2B/h	602805
HIST1H2BF	Histone 1, H2bf	6p22.2	H2BFG, H2B/g	602804
HIST1H2BG	Histone 1, H2bg	6p22.2	H2BFA	602798
HIST1H2BJ	Histone gene cluster 1, H2B histone family, member J	6p22.1	H2BJ, H2B/r	615044
HIST1H2BH	Histone 1, H2bh	6p22.2	H2BFJ	602806
HIST1H2BI	Histone 1, H2bi	6p22.2	H2BFK	602807
HIST1H2BK	Histone gene cluster 1, H2B histone family, member K	6p22.1	H2BK, H2BFAiii	615045

Continued

TABLE 1.3 Histone Proteins—cont'd

Gene	Name	Locus	Other names	MIM number
HIST1H2BL	Histone 1, H2bl	6p22.1	H2BFC	602800
HIST1H2BM	Histone 1, H2bm	6p22.1	H2BFE	602802
HIST1H2BN	Histone 1, H2bn	6p22.1	H2BFD, H2B/d	602801
HIST1H2BO	Histone 1, H2bo	6p22.1	H2BFN	602808
HIST1H3A	Histone 1, H3a	6p22.2	H3FA, H3/A	602810
HIST1H3B	Histone 1, H3fl	6p22.2	H3FL	602819
HIST1H3C	Histone 1, H3c	6p22.2	H3FC, H3.1, H3/c	602812
HIST1H3D	Histone 1, H3d	6p22.2	H3FB, H3/b	602811
HIST1H3E	Histone 1, H3e	6p22.2	H3FD, H3.1	602813
HIST1H3F	Histone 1, H3f	6p22.2	H3/i	602816
HIST1H3G	Histone 1, H3g	6p22.2	H3FH	602815
HIST1H3H	Histone 1, H3h	6p22.1	H3FK	602818
HIST1H3I	Histone 1, H3i	6p22.1	H3FF	602814
HIST1H3J	Histone 1, H3j	6p22.1	H3FJ	602817
HIST1H4A	Histone 1, H4a	6p22.2	H4FA	602822
HIST1H4B	Histone 1, H4b	6p22.2	H4FI	602829
HIST1H4C	Histone 1, H4c	6p22.2	H4FG, H4/g	602827
HIST1H4D	Histone 1, H4d	6p22.2	H4FB	602823
HIST1H4E	Histone 1, H4e	6p22.2	H4FJ	602830
HIST1H4F	Histone 1, H4f	6p22.2	H4FC, H4, H4/c	602824
HIST1H4G	Histone 1, H4g	6p22.2	H4FL, H4/l	602832
HIST1H4H	Histone 1, H4h	6p22.2	H4FH, H4/h	602828
HIST1H4J	Histone 1, H4j	6p22.1	H4FE, H4/e, H4F2iv	602826
HIST1H4K	Histone 1, H4k	6p22.1	H4FD	602825
HIST1H4L	Histone 1, H4l	6p22.1	H4FK, H4.k, H4/k	602831
HIST2H2AA	Histone 2, H2aa	1q21.2	H2AFO, H2A	142720
HIST2H2AB	Histone gene cluster 2, H2A histone family, member B	1q21.2	H2AB	615014
HIST2H2AC	Histone 2, H2ac	1q21.2	H2AFQ, H2A/q	602797
HIST2H2BE	Histone 2, H2be	1q21.2	H2B, H2B.1, H2B/q	601831
HIST2H3C	Histone 2, H3c	1q21.2	H3F2, H3, H3.2, H3/m, H3/M, MGC9629	142780
HIST2H4A	H4 histone, family 2	1q21.2	H4FN, H4F2, HIST2H4	142750
HIST3H2A	Histone gene cluster 3, H2A histone	1q42.13	MGC3165	615015
HIST3H2BB	Histone gene cluster 3, H2B histone family, member B	1q42.13	H2Bb	615046
HIST3H3	Histone 3, H3	1q42.13	H3FT, H3T	602820
HIST4H4	Histone gene cluster 4, H4 histone	12p12.3	MGC24116	615069

To date, four methods to change chromatin structure and regulate gene expression have been well documented: (i) histone modification, (ii) histone exchange, (iii) ATP-dependent chromatin remodeling, and (iv) histone tail cleavage.[66] Chromatin regulators (CRs) can dynamically modulate chromatin architecture to epigenetically regulate gene expression in response to intrinsic and extrinsic signaling cues. Somatic alterations or misexpression of CRs might

reprogram the epigenomic landscape of chromatin, which in turn leads to a wide range of common diseases.[67] Chromatin regulators, which are indispensable in epigenetics, mediate HMs to adjust chromatin structures and functions. Stable heterochromatin is necessary to silence transposable elements (TEs) and maintain genome integrity. ATP-dependent chromatin-remodeling complexes use ATP hydrolysis to move, destabilize, eject, or restructure nucleosomes, allowing the accessibility of transcription factors to DNA. These complexes can be classified into four families: (i) the SWI/SNF (switching defective/sucrose nonfermenting) family (Table 1.4); (ii) the ISWI (imitation SWI) family; (iii) the CHD (chromodomain, helicase, DNA binding) family (Table 1.5); and (iv) the INO (inositol requiring 80 family) (Table 1.6).[68] Their transcriptional effects (activation or repression) depend on the recruitment of coactivators or corepressors.[22]

TABLE 1.4 ATP-Dependent Chromatin-Remodeling Complexes (SWI/SNF Family)

Gene	Name	Locus	Other names	OMIM	Phenotype
SMARCA1	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 1	Xq25-q26	SNF2L1, hSNF2L, ISWI, NURF140, SNF2LB, SWI	300012	Smith-Fineman-Myers syndrome (SFMS); Schizophrenia; X-linked mental retardation
SMARCA2	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 2	9p24.3	SNF2L2, NCBRS, BRM, hBRM, hSNF2a, SNF2, SNF2LA, Sth1p, SWI2	600014	Gastric cancer; Nicolaides-Baraitser síndrome; Schizophrenia; lung cancer
SMARCA3	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 3	3q24	SNF2L3, HIP116, HLTF, HIP116A, HLTF1, RNF80	603257	Colorectal cancer; gastric cancer; uterine cancer
SMARCA4	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 4	19p13.2	BRG1, RTPS2, MRD16, CSS4	603254	Coffin-Siris syndrome 4; hypertrophic cardiomyopathy; lung cancer; prostate cancer; rhabdoid tumor predisposition syndrome 2; rhabdoid tumors (RTPS2)
SMARCA5	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 5	4q31.21	SNF2H	603375	Breast cancer; gastric cancer
SMARCAD1	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, DEAD/H box-containing, 1	4q22.3	KIAA1122, ETL1, HEL1, ADERM, BASNS	612761	Adermatoglyphia; Basan síndrome; breast cancer
SMARCAL1	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A-like	2q35	HARP, SIOD	606622	Schimke immunoosseous dysplasia
SMARCB1	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily b, member 1	22q11.23	SNF5, INI1, RDT, RTPS1, MRD15, SWNTS1, CSS3	601607	Chromic myeloid leukemia; epithelioid sarcoma; familial schwannomatosis; meningioma; Coffin-Siris syndrome 3; rhabdoid tumors, somatic; rhabdoid tumor predisposition syndrome 1; Schwannomatosis-1, susceptibility to
SMARCC1	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily C, member 1	3p21.31	BAF155, CRACC1, Rsc8, SRG3	601732	Cancer; prostate cancer
SMARCC2	SW1/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily c, member 2	12q13.2	BAF170, CRACC2, Rsc8	601734	Colorectal cancer; gastric cancer
SMARCD1	SW1/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily d, member 1	12q13.12	BAF60A, CRACD1, Rsc6p	601735	Gastric cancer; ovarian cancer
SMARCD2	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily d, member 2	17q23.3	BAF60B, SGD2	601736	Specific granule deficiency 2

Continued

TABLE 1.4 ATP-Dependent Chromatin-Remodeling Complexes (SWI/SNF Family)—cont'd

Gene	Name	Locus	Other names	OMIM	Phenotype
SMARCD3	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily D, member 3	7q36.1	BAF60C	601737	Inflammation
SMARCE1	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily E, member 1	17q21.2	BAF57, CSS5	603111	Coffin-Siris syndrome 5; meningioma, familial, susceptibility to; prostate cancer

TABLE 1.5 ATP-Dependent Chromatin-Remodeling Complexes (CHD Family)

Gene	Name	Locus	Other names	OMIM	Phenotype
CHD1	Chromodomain helicase DNA-binding protein-1	5q15-q21	PILBOS	602118	Pilarowski-Bjornsson syndrome
CHD1L	Chromodomain helicase DNA-binding protein 1-like	1q21.1	ALC1	613039	Breast cancer; hepatocellular carcinoma; ovarian cancer
CHD2	Chromodomain helicase DNA-binding protein-2	15q26.1	DKFZp547I1315, DKFZp686E01200, DKFZp781D1727, EEOC, FLJ38614	602119	Developmental delay; epileptic encephalopathy, childhood onset
CHD3	Chromodomain helicase DNA-binding protein-3	17p13.1	Mi-2a, Mi2-ALPHA, ZFH	602120	Dermatomyositis
CHD4	Chromodomain helicase DNA-binding protein-4	12p13.31	Mi-2b, Mi2-BETA, SIHIWES	603277	Sifrim-Hitz-Weiss syndrome
CHD5	Chromodomain helicase DNA-binding protein 5	1p36.31	KIAA0444, MI2R	610771	Neuroblastoma
CHD6	Chromodomain helicase DNA-binding protein 6	20q12	CHD5, RIGB	616114	Acute myeloid leukemia; Influenza virus infection
CHD7	Chromodomain helicase DNA binding protein 7	8q12.2	HH5	608892	CHARGE syndrome; Hypogonadotropic hypogonadism 5 with or without anosmia
CHD8	Chromodomain helicase DNA-binding protein 8	610528	DUPLIN, KIAA1564, AUTS18	610,528	Autism, susceptibility to
CHD9	Chromodomain helicase DNA-binding protein 9	16q12.2	BC022889, CREMM, FLJ12178, PRIC320, KIAA0308	616936	

TABLE 1.6 ATP-Dependent Chromatin-Remodeling Complexes (INO Family)

Gene	Name	Locus	Other names	OMIM	Phenotype
INO80A	INO80 complex subunit A	15q15.1	INOC1, INO80A, KIAA1259	610169	Colorectal cancer; thyroid cancer
INO80B	INO80 complex, subunit B	2p13.1	PAPA1, HMGA1L4	616456	Preeclampsia; retinitis pigmentosa
INO80C	INO80 complex subunit C	18q12.2	FLJ38183, hIes6, IES6		
INO80D	INO80 complex subunit D	2q33.3	FLJ20309		
INO80E	INO80 complex subunit E	16p11.2	CCDC95, FLJ90652		

Controlled modulation of nucleosomal DNA accessibility via posttranslational modifications (PTMs) is a critical component to many cellular functions. Charge-altering PTMs in the globular histone core (including acetylation, phosphorylation, crotonylation, propionylation, butyrylation, formylation, and citrullination) can alter the strong electrostatic interactions between oppositely charged nucleosomal DNA and histone proteins and thus modulate accessibility of the nucleosomal DNA, affecting processes that depend on access to genetic information, such as transcription.[69]

Novel nuclear structures such as 10 to 100-nm nuclear lipid islets (NLIs), are rich in phosphatidylinositol 4,5-bisphosphate. NLI periphery is associated with RNA polymerase II (Pol II) transcription machinery, including the Pol II largest subunit, transcription factors, and NM1. The PtdIns(4,5)P2-NM1 interaction is important for Pol II transcription.[70]

Pioneer transcription factors have the unique role of unmasking chromatin domains during development to allow the implementation of new cellular programs. Pioneer factors can recognize their target DNA sequences in so-called compacted or "closed" heterochromatin and can trigger remodeling of the adjoining chromatin landscape to provide accessibility to nonpioneer transcription factors. The several steps of pioneer action include rapid but weak initial binding to heterochromatin, stabilization of binding, followed by chromatin opening and loss of CpG methylation that provides epigenetic memory. Whereas CpG demethylation is dependent on replication, chromatin opening is not.[71]

Inheritance of the DNA sequence and its organization into chromatin is fundamental for genome stability and function, cell fate and self-renewal. Propagation of genetic information and chromatin-based information in cycling cells requires genome-wide disruption and restoration of chromatin, coupled to strict DNA replication. Specialized replication-coupled mechanisms assemble newly synthesized DNA into nucleosomes, and the complete restoration of chromatin organization with histone marks is a continuous process during the cell cycle. Failure to reassemble nucleosomes at replication forks blocks DNA replication progression and leads to genomic instability.[72]

Cells need to coordinate the expression of their genes to maintain homeostasis. DNA methylation and posttranslational modifications of histones affect the architecture of chromatin and create "docking platforms" for multiple binding proteins. According to Nieborak and Schneider[73] these modifications can be dynamically set and removed by various enzymes that depend on the availability of key metabolites derived from different intracellular pathways. Therefore, small metabolites generated in anabolic and catabolic processes can integrate multiple external and internal stimuli and transfer information on the energetic state of a cell to the transcriptional machinery by regulating the activity of chromatin-modifying enzymes. Many chromatin-modifying enzymes respond to alterations in the levels of their cofactors, cosubstrates, and inhibitors; however, the detailed molecular mechanisms and functional consequences of such processes are largely unresolved.

Chromatin is traditionally viewed as a nuclear entity that regulates gene expression and silencing. However, Dou et al.[74] discovered the presence of cytoplasmic chromatin fragments that pinch off from intact nuclei of primary cells during senescence, a form of terminal cell cycle arrest associated with proinflammatory responses. Cytoplasmic chromatin activates the innate immunity cytosolic DNA-sensing cyclic GMP-AMP synthase linked to stimulator of interferon genes (cGAS-STING) pathway, leading both to short-term inflammation to restrain activated oncogenes and to chronic inflammation that associates with tissue destruction and cancer. The cytoplasmic chromatin/cGAS-STING pathway promotes the senescence-associated secretory phenotype in primary human cells; this pathway is activated in cancer cells, and correlates with proinflammatory gene expression in human cancers.[74]

1.2.3.1.1 Genome-Wide Chromatin Conformation, Spatial Organization, and 3D Genomics

The genome is segmented into hierarchically organized spatial compartments. There is a dynamic coupling between chromatin organization and epigenetic regulation. It has been postulated that chromosome folding may contribute to the maintenance of a robust epigenomic identity via the formation of spatial compartments like topologically associating domains.[75] Three-dimensional (3D) compartmentalization leads to the spatial colocalization of epigenome regulators, contributing to increasing their local concentration and enhancing their ability to spread an epigenomic signal at long range in different species.[75] The dynamic three-dimensional chromatin architecture of genomes and its coevolutionary connection to its function (storage, expression, and replication of genetic information) is still one of the central issues in biology. The genome is compacted into a chromatin quasifibre with $\sim 5 \pm 1$ nucleosomes/11 nm, folded into stable ~30 to 100-kbp loops forming stable loop aggregates/rosettes connected by similar-sized linkers. Minor variations in the architecture are seen between cell types and functional states. The architecture and the DNA sequence show very similar fine-structured multiscaling behavior confirming their coevolution.[76] Eukaryotic genomes are spatially organized within the nucleus by chromosome folding, interchromosomal contacts, and interaction with nuclear structures. The arrangement of the genome within the nucleus has been shaped and conserved through evolutionary processes, probably playing an adaptive function. The spatial organization of the genome is likely to be genetically encoded by binding sites for DNA-binding proteins and might also involve changes in chromatin structure, potentially through nongenetic mechanisms.[77] Mammalian development depends on the linear genome sequence that embeds millions of *cis*-regulatory elements and on the three-dimensional (3D) chromatin architecture that orchestrates the interplay between *cis*-regulatory elements and their target genes.[78] Sequence-based profiling technologies such as high-throughput sequencing to detect fragment nucleotide sequence (Hi-C) and chromatin interaction analysis by paired end tag sequencing (ChIA-PET) have revolutionized the field of three-dimensional (3D) chromatin architecture.

The human genome functions as folded 3D chromatin units and the looping paradigm is the basic principle of gene regulation.[79] The DNA microstates that regulate transcription include sequence-specific transcription factors (TFs), coregulatory complexes, nucleosomes, histone modifications, DNA methylation, and parts of the three-dimensional architecture of genomes, which could create an enormous combinatorial complexity across the genome. The complexity of TFs, coregulators, and epigenetic marks at eukaryotic genes is highly redundant and the information present can be compressed onto a much smaller subset of marks.[80] Topologically associating domains (TADs), CTCF loop domains, and A/B compartments have been identified as important structural and functional components of 3D chromatin organization. Chromatin is organized into compartmental domains that correspond with A/B compartments at high resolution. The transcriptional state is a major predictor of Hi-C contact maps in several eukaryotes. Architectural proteins insulate compartmental domains by reducing interaction frequencies between neighboring regions. In mammals compartmental domains exist alongside CTCF loop domains to form topological domains. Compartmental domains are responsible for domain structure in all eukaryotes, with CTCF playing an important role in domain formation.[81] The 3D structure of the genome plays a key role in regulatory control of the cell. High-throughput chromosome conformation capture (Hi-C) has been developed to probe the 3D structure of the genome. However, it is important to differentiate chromosome regions that are colocalized and coregulated. With integrative approaches it might be possible to identify functional interchromosomal interactions. Intermingling regions generally fall into either active or inactive clusters based on the enrichment for RNA polymerase II (RNAPII) and H3K9me3, respectively. Active clusters are hotspots for transcription factor binding sites.[82] Mammalian genomes are folded into unique topological structures that undergo precise spatiotemporal restructuring during healthy development. These folding patterns are miswired during the onset and progression of disease states.[83]

1.2.3.2 Histones

Histones (Table 1.3) are nucleosomic proteins integrated in the nuclear chromatin. Nucleosomes are formed by 147 DNA basepairs wrapped around an octamer of histones, assembled by two copies of each of the four core histones, H2A, H2B, H3, and H4 (Table 1.3). Histone H1 is the linker binding DNA between the nucleosomal core particles to stabilize chromatin structures. Histones are formed by a central globular domain and an N-terminal tail with multiple sites for modification of nucleosomal organization, leading to ATP-dependent chromatin-remodeling complexes (Tables 1.4–1.6) and posttranslation aminoacid modifications on histone tails (histone acetylation, methylation, phosphorylation, sumoylation, ubiquitylation, glycosylation, ADP ribosylation, biotinylation).[22, 84] Histone modifications (HMs) are essential epigenetic features, with fundamental roles in biological processes such as transcription, DNA repair, and DNA replication. Histone acetylation is achieved by the action of histone acetyltransferase (HAT), which adds an acetyl group to a lysine residue, resulting in chromatin/transcriptional activation; histone deacetylation is produced by histone deacetylases (HDACs) that remove the acetyl groups, and is related to chromatin inactivation and transcriptional repression.[84, 85]

Histones organize DNA into chromatin through a variety of processes,[86] and histone modifications play a vital role in gene regulation and cell identity.[87] By regulating the accessibility of the genome, epigenetic regulators (i.e., histone proteins, chromatin-modifying enzymes) control gene expression. Proper regulation of this "histone code" is essential for the precise control of transcriptional networks to establish and maintain cell fate and identity.[88] Histone modifications are associated with transcriptional regulation by diverse transcription factors. The presence of broad H3K27me3 domains at transcriptionally active genes reflects the heterogeneous expression of major cell identity regulators.[89]

Histones (Table 1.3) are evolutionarily conserved DNA-binding proteins that, as scaffolding molecules, regulate DNA packaging into the nucleus of all eukaryotic cells. Canonical histones H3.1 and H3.2 are synthetized and loaded during DNA replication. The histone variant H3.3 is expressed and deposited into the chromatin throughout the cell cycle. H3.3 replaces the majority of canonical H3 in nondividing cells, reaching almost saturation levels in a time-dependent manner. H3.3 plays an important function in age-related processes throughout evolution and is required for proper neuronal function and brain plasticity.[90]

Histone H1 consists of a family of related proteins, including five replication-dependent (H1.1–H1.5) and two replication-independent (H1.10 and H1.0) subtypes, all expressed in somatic cells. Dynamic changes in H1 subtype expression and localization are tightly linked with chromatin remodeling and might be crucial for transitions in chromatin structure during reprogramming. Somatic H1 subtypes can distinguish male and female chromatin upon sex differentiation in developing germ cells.[91]

Histone H3.Y is conserved among primates. Exogenous H3.Y accumulates in transcription start sites, suggesting its potential involvement in transcription regulation. The H3.Y nucleosome forms a relaxed chromatin conformation with flexible DNA ends. The H3.Y-specific Lys42 residue is partly responsible for enhancing the

flexibility of nucleosomal DNA. H3.Y stably associates with chromatin and nucleosomes. The H3.Y C-terminal region including amino acid residues 124–135 is responsible for its stable association with DNA. Among the H3.Y C-terminal residues the H3.Y Met124 residue contributes to stable DNA association with the H3.Y-H4 tetramer. The H3.Y M124I mutation reduces the H3.Y-H4 association in the nucleosome, and the H3.Y K42R mutation affects nucleosome stability and contributes to the flexible DNA ends of the nucleosome.[92]

Histone variants are chromatin components that replace replication-coupled histones in a fraction of nucleosomes and confer particular characteristics to chromatin. H2A variants are the most frequent among histone protein families. H2A–H2B dimers are removed and exchanged more easily than the stable H3–H4 core. The unstructured N-terminal histone tails and the C-terminal tails of H2A histones protrude out of the compact structure of the nucleosome core where they are the preferential target sites for posttranslational modifications (PTMs). Some PTMs are shared between replication-coupled H2A and H2A variants, and many modifications are limited to a specific histone variant. H2A.Z primarily acts as an oncogene and macroH2A and H2A.X as tumor suppressors.[93]

The centromere is the specialized chromatin region that directs chromosome segregation. The kinetochore assembles on the centromere, attaching chromosomes to microtubules in mitosis. The centromere position is usually maintained through cell cycles and generations. Neocentromeres can occasionally form on ectopic regions when the original centromere is inactivated or lost due to chromosomal rearrangements. Centromere repositioning can occur during evolution. De novo centromeres can form on exogenously transformed DNA in human cells at a low frequency, which then segregates faithfully as human artificial chromosomes (HACs). A conserved histone H3 variant, CENP-A, epigenetically marks functional centromeres, interspersing with H3. Several histone modifications enriched at centromeres are required for centromere function. Acetylated histones on chromatin and transcription can create an open chromatin environment, enhancing nucleosome disassembly and assembly, and potentially contribute to centromere establishment. Alternatively, acetylation of soluble H4 may stimulate the initial deposition of CENP-AHCP-3-H4 nucleosomes.[94]

CENP-A (centromere protein A) is a histone H3 variant that epigenetically determines the centromere position. CENP-A ubiquitylation, which is inherited through dimerization between rounds of cell division, is a candidate for the epigenetic mark of centromere identity.[95] Phosphorylation at Ser68 was proposed to have an essential role in CENP-A deposition at centromeres. Blockage of ubiquitination at Lys124 was proposed to abrogate localization of CENP-A to the centromere. CENP-A mutants that cannot be phosphorylated at Ser68 or ubiquitinated at Lys124 assemble efficiently at centromeres during G1, mediate early events in centromere establishment at an ectopic chromosomal locus, and maintain centromere function indefinitely.[96]

1.2.3.3 Posttranslational Modifications

Chromatin is the physiological template of genetic information, integrating a highly organized complex of DNA and histone proteins for regulating gene expression and genome organization. A great number of histone posttranslational modifications (PTMs) have been identified with synthetic and chemical biology techniques, including genetic code expansion, histone semisynthesis, and posttranslational chemical mutagenesis.[97] Posttranslational modifications of histones and the dynamic DNA methylation cycle are finely regulated by a myriad of chromatin-binding factors and chromatin-modifying enzymes. Epigenetic modifications ensure local changes in the architecture of chromatin, thus controlling accessibility of the machinery of transcription, replication, or DNA repair to the chromatin. Histone PTMs are fundamental players of chromatin regulation, as they contribute to editing histone chemical properties and recruiting proteins for gene transcription and DNA repair.[98] The most relevant posttranslational changes on histone tails include acetylation; ubiquitylation; sumoylation at K (lysine) residues; methylation at K, R (arginine), or H (histidine) residues; and phosphorylation at S (serine), T (threonine), or Y (tyrosine) residues; which affect transcription, DNA replication, and DNA repair.[22] Proteins with domains that recognize and bind PTMs of histones are collectively termed epigenetic readers. Numerous interactions between specific reader protein domains and histone PTMs and their regulatory outcomes have been reported.

The Roadmap Epigenomics Consortium generated a reference catalog of several key histone modifications across >100 human cell types and tissues, and Wang et al.[99] characterized cell type-specific regulatory elements (CSREs) and their histone modification codes in the human epigenomes of five histone modifications across 127 tissues or cell types.

1.2.3.3.1 Histone Methylation-Demethylation

Histone methylation is catalyzed by histone lysine methyltransferases (HKMT) (Table 1.7) and histone demethylation by histone lysine demethylases (Table 1.8). Some histone methylations (H3K4, H3K36, H3K79) are associated with transcription activation and an open euchromatin structure, whereas other histone methylations (H3K9, H3K27, H4K20) associate with gene silencing and a close heterochromatin structure.[22] Enhancers act to regulate cell

TABLE 1.7 Histone Methyltransferases

Gene	Name	Locus	Other names	MIM number	Phenotype
ASH1L	Ash1 (absent, small, or homeotic), *Drosophila*, homolog of	1q22	KIAA1420, ASH1, MRD52, ASH1L1, huASH1, KMT2H	607999	Autoimmune diseases; beta thalassemia; brain cancer; breast cancer; developmental disorders; leukemia; lung cancer; mental retardation, autosomal dominant 52
ASH2L	ASH2, *Drosophila*, homolog of	8p11.23	ASH2L2, ASH2L1	604782	Leukemia
CARM1	Coactivator associated arginine methyltransferase 1	19p13.2	PRMT4	603934	Breast cancer; prostate cancer
DOT1L	Dot1, yeast, homolog of	19p13.3	DOT1, KIAA1814, KMT4	607375	Colorectal cancer; dilated cardiomyopathy; leukemia
DPY30	DPY30, *Caenorhabditis elegans*, homolog of	2p22.3	Cps25, HDPY-30, Saf19	612032	Gastric cancer
EED	Embryonic ectoderm development protein, mouse, homolog of	11q14.2	WAIT1, COGIS	605984	Cohen-Gibson syndrome
EHMT1	Euchromatic histone methyltransferase 1	9q34.3	EUHMTASE1, DEL9q34, GLP, KLEFS1, KMT1D	607001	Kleefstra syndrome 1
EHMT2	Euchromatic histone lysine *N*-methyltransferase 2	6p21.33	KMT1C, BAT8, C6orf30, GAT8, G9A, NG36	604599	Autism spectrum disorders (ASD); bladder cancer; lung cancer
EZH1	Enhancer of zeste, *Drosophila*, homolog of, 1	17q21.2	KIAA0388, KMT6B	601674	Leukemia; thyroid cancer
EZH2	Enhancer of zeste, *Drosophila*, homolog of, 2	7q36.1	ENX-1, EZH1, KMT6, KMT6A, MGC9169, WVS	601573	Endometrial carcinoma; Ewing tumors; Melanoma; primary myelofibrosis; prostate cancer; Weaver syndrome
KMT2A	Lysine methyltransferase 2A	11q23.3	ALL-1, CXXC7, HRX, HTRX1, MLL1, MLL1A, TRX1	159555	Leukemia, myeloid/lymphoid or mixed lineage; Wiedemann-Steiner syndrome
KMT2B	Lysine (K)-specific methyltransferase 2B	19q13.12	CXXC10, MLL4, KIAA0304, DYT28, HRX2, RX2, WBP7	606834	Dystonia 28, childhood onset; pancreatic carcinoma; spindle cell sarcoma
KMT2C	Lysine (K)-specific methyltransferase 2C	7q36.1	MLL3, KIAA1506, KLEFS2	606833	Kleefstra syndrome 2
KMT2D	Lysine (K)-specific methyltransferase 2D	12q13.12	MLL2, ALR, CAGL114, KABUK1	602113	B cell lymphoma; Kabuki syndrome 1
KMT2E	Lysine (K)-specific methyltransferase 2E	7q22.3	FLJ10078, FLJ14026, HDCMC04P, PMCB, MGC70452, MLL5	608444	Leukemia
KMT5A	Lysine methyltransferase 5A	12q24.31	SETD8, PR-Set7, SET07, SET8	607240	Breast cancer
KMT5B	Lysine methyltransferase 5B	11q13.2	SUV420H1, CGI85, MRD51	610881	Mental retardation, autosomal dominant 51
KMT5C	Lysine methyltransferase 5C	19q13.42	SUV420H2, MGC2705	613198	Pancreatic cancer
NSD1	Nuclear receptor binding SET domain protein 1	5q35.3	ARA267, STO, SOTOS1	606681	Leukemia, acute myeloid; Sotos syndrome 1
PAGR1	PAXIP1-associated glutamate-rich protein 1	16p11.2	C16orf53, PA1, GAS	612033	

TABLE 1.7 Histone Methyltransferases—cont'd

Gene	Name	Locus	Other names	MIM number	Phenotype
PRMT1	Protein arginine methyltransferase 1	19q13.33	ANM1, HCP1, HRMT1L2, IR1B4	602950	Breast cancer; head and neck cancer; Hirschsprung disease; liver cancer
PRMT2	Protein arginine methyltransferase 2	21q22.3	HRMT1L1, MGC111373	601961	Breast cancer; dyslexia; endometriosis
PRMT3	Protein arginine methyltransferase 3	11p15.1	HRMT1L3	603190	
PRMT5	Protein arginine methyltransferase 5	14q11.2	HRMT1L5, SKB1, SKB1Hs	604045	Breast cancer; gastric cancer; lung cancer
PRMT6	Protein arginine methyltransferase 6	1p13.3	ANM6, HRMT1L6, FLJ10559	608274	Colorectal cancer; prostate cancer
PRMT7	Protein arginine methyltransferase 7	16q22.1	KIAA1933, FLJ10640, SBIDDS	610087	Short stature, brachydactyly, intellectual developmental disability, and seizures
PRMT8	Protein arginine methyltransferase 8	12p13.32	HRMT1L3, HRMT1L4	610086	Colorectal cancer; Intellectual developmental disability
PRMT9	Protein arginine methyltransferase 9	4q31.23	PRMT10, FLJ46629	616125	Liver cancer; Osteosarcoma
SETD1A	SET domain-containing protein 1A	16p11.2	SET1A, SET1, KIAA0339, KMT2F	611052	Leukemia
SETD1B	SET domain-containing protein 1B	12q24.31	SET1B, KIAA1076, KMT2G	611055	Breast cancer; intellectual disability, epilepsy and autism
SETD2	SET domain-containing protein 2	3p21.31	SET2, HYPB, HBP231, KIAA1732, LLS	612778	Luscan-Lumish syndrome
SETD3	SET domain-containing protein 3	14q32.2	FLJ23027	615671	Liver cancer
SETD6	SET domain-containing protein 6	16q21	FLJ21148	616424	Bladder cancer; colorectal cancer
SETD7	SET domain-containing protein 7	4q31.1	KIAA1717, KMT7, SET7, SET7/9, Set9, SET9	606594	Breast cancer; prostate cancer
SETDB1	SET domain protein, bifurcated, 1	1q21.3	ESET, KG1T, KIAA0067, KMT1E, TDRD21	604396	Breast cancer; hepatocellular carcinoma; Huntington disease; nonsmall-cell lung cancer; small-cell lung cancer; ovarian cancer
SETDB2	SET domain protein, bifurcated, 2	13q14.2	CLLD8, CLLL8, KMT1F	607865	Leukemia
SETMAR	SET and Mariner transposase domains-containing protein	3p26.1	METNASE	609834	Glioblastoma; leukemia
SMYD2	SET and MYND domain-containing protein 2	1q32.3	HSKM-B, KMT3C, ZMYND14	610663	Breast cancer; liver cancer
SMYD3	SET and MYND domain-containing protein 3	1q44	KMT3E, ZMYND1, ZNFN3A1	608783	Breast cancer; colorectal cancer; esophageal squamous cell carcinoma; liver cancer
SUV39H1	Suppressor of variegation 3–9, *Drosophila*, homolog of, 1	Xp11.23	KMT1A, SUV39H	300254	Inflammation; melanoma
SUV39H2	Suppressor of variegation 3–9, *Drosophila*, homolog of, 2	10p13	FLJ23414, KMT1B	606503	Lung cancer
SUZ12	Suppressor of zeste 12 homolog (*Drosophila*)	17q11.2	KIAA0160, CHET9, JJAZ1	606245	Chronic myeloid leukemia; endometrial stromal sarcoma; epithelial ovarian cancer; neurofibromatosis type 1

TABLE 1.8 Histone Demethylases

Gene	Name	Locus	Other names	OMIM	Phenotype
HIF1AN	Hypoxia-inducible factor 1 subunit alpha inhibitor	10q24.31	DKFZp762F1811, FIH1, FLJ20615, FLJ22027	606615	Breast cancer; liver cancer
HR	HR, lysine demethylase and nuclear receptor corepressor	8p21.3	AU, ALUNC, APL, HSA277165, HYPT4, MUHH, MUHH1	602302	Alopecia; Atrichia with papular lesion
HSPBAP1	HSPB1-associated protein 1	3q21.1	FLJ22623, FLJ39386, PASS1	608263	Renal cancer
JARID2	Jumonji and AT-rich interaction domain containing 2	6p22.3	JMJ, HY, SMCY, KIAA0234	601594	Heart diseases; Schizophrenia
JMJD1C	Jumonji domain-containing protein 1C	10q21.3	TRIP8, KIAA1380	604503	Breast cancer; intellectual disability; leukemia; Rett syndrome
JMJD4	Jumonji domain containing 4	1q42.13	FLJ12517, MGC129896		
JMJD6	Jumonji domain containing 6 (phosphatidylserine receptor)	17q25.1	PSR, KIAA0585, PTDSR1	604914	Breast cancer; neuroglioma; preeclampsia
JMJD7	Jumonji domain containing 7	15q15.1			Head and neck cancer
JMJD8	Jumonji domain containing 8	16p13.3	C16orf20, PP14397		
KDM1A	Lysine-specific demethylase 1[a]	1p36.12	LSD1, AOF2, BHC110, KIAA0601, CPRF	609132	Cleft palate, psychomotor retardation, and distinctive facial features; Multiple myeloma; Neurodegenerative diseases
KDM2A	Lysine demethylase 2[a]	11q13.2	CXXC8, DKFZP434M1735	605657	Breast cancer; colorectal cancer
KDM1B	Lysine-specific demethylase 1B	6p22.3	LSD2, AOF1	613081	Breast cancer
KDM2B	Lysine-specific demethylase 2B	12q24.31	FBXL10, FBL10, CXXC2, JHDM1B	609078	Colorectal cancer; leukemia; prostate cancer; ovarian cancer
KDM3A	Lysine-specific demethylase 3[a]	2p11.2	JMJD1A, JHDM2A, TSGA, KIAA0742	611512	Bladder cancer; Ewing sarcoma; renal cancer
KDM3B	Lysine-specific demethylase 3B	5q31.2	C5orf7, KIAA1082, JMJD1B, NET22	609373	Myelodysplasia; myeloid leukemia
KDM4A	Lysine-specific demethylase 4[a]	1p34.2-p34.1	JMJD2A, JHDM3A, KIAA0677	609764	Squamous cell cancer
KDM4B	Lysine-specific demethylase 4B	19p13.3	JMJD2B, KIAA0876	609765	Prostate cancer; renal cancer
KDM4C	Lysine-specific demethylase 4C	9p24.1	JMJD2C, GASC1, KIAA0780	605469	Breast cancer; esophageal squamous cell cancer
KDM4D	Lysine demethylase 4D	11q21	JMJD2D, FLJ10251	609766	Hodgkin lymphoma; pancreatic cancer
KDM4E	Lysine-specific demethylase 4E	11q21	JMJD2E	616581	
KDM5A	Lysine (K)-specific demethylase 5[a]	12p13.33	JARID1A, RBP2, RBBP2	180202	Acute myeloid leukemia; acute megakaryoblastic leukemia (AMKL)
KDM5B	Lysine-specific demethylase 5B	1q32.1	JARID1B, PUT1, PLU1, RBBP2H1A	605393	Breast cancer; melanoma; prostate cancer
KDM5C	Lysine-specific demethylase 5C (Jumonji, AT-rich interactive domain 1C)	Xp11.22	JARID1C, SMCX, DXS1272E, XE169, MRXSCJ	300534	Mental retardation, X linked, syndromic, Claes-Jensen type; prostate tumors and seminomas
KDM5D	Lysine-specific demethylase 5D	Yq11.223	JARID1D, SMCY, HYA	426000	Prostate cancer; renal cancer
KDM6A	Lysine (K)-specific demethylase 6[a]	Xp11.3	UTX, KABUK2	300128	Kabuki syndrome 2

TABLE 1.8 Histone Demethylases—cont'd

Gene	Name	Locus	Other names	OMIM	Phenotype
KDM6B	Lysine-specific demethylase 6B	17p13.1	JMJD3, KIAA0346	611577	Autoimmune diseases
KDM7A	Lysine demethylase 7A	7q34	JHDM1D, KDM7, KIAA1718		Systemic lupus erythematosus
KDM8	Lysine demethylase 8	16p12.1	JMJD5	611917	Adrenal cancer; bladder cancer; breast cancer; liver cancer; thyroid cancer; uterine cancer
PHF2	PHD finger protein-2	9q22.31	CENP-35, JHDM1E, KDM7C, KIAA0662	604351	Colorectal cancer; gastric cancer; obesity; renal cancer
PHF8	PHD finger protein 8	Xp11.22	JHDM1F, ZNF422, KIAA1111, MRXSSD, KDM7B	300560	Mental retardation syndrome, X linked, Siderius type
RIOX1	Ribosomal oxygenase 1	14q24.3	FLJ21802, JMJD9, MAPJD, NO66	611919	Colorectal cancer
RIOX2	Ribosomal oxygenase 2	3q11.2	MINA, FLJ14393, JMJD10, mdig, MINA53, NO52	612049	Breast cancer; gastric cancer; glioblastoma; heart diseases; liver cancer; multiple myeloma
UTY	Ubiquitously transcribed TPR gene on Y chromosome	Yq11.221	KDM6AL, KDM6C	400009	Bladder cancer; hypogonadism

type-specific gene expression by facilitating the transcription of target genes. Active or primed enhancers are commonly marked by monomethylation of histone H3 at lysine 4 (H3K4me1) in a cell type-specific manner. Studies in mononucleosomes identified multiple H3K4me1-associated proteins, including many involved in chromatin remodeling. H3K4me1 enhances the association of the chromatin-remodeling complex BAF to enhancers and H3K4me1-marked nucleosomes are more efficiently remodeled by the BAF complex. Monomethylation is accommodated by BAF45C's H3K4-binding site. H3K4me1 has an active role at enhancers by facilitating binding of the BAF complex and other chromatin regulators.[100] Trimethylation of histone 3 at lysine 27 (H3K27me3) is a repressive mark that associates with developmental gene regulation during differentiation programs.[101] Methylation of histone H3 lysine 4 is linked to active transcription and can be removed by LSD1 or the JmjC domain-containing proteins by aminooxidation or hydroxylation, respectively. Its deamination can be catalyzed by lysyl oxidase-like 2 protein (LOXL2). By regulating H3K4me3 deamination, LOXL2 activity is linked with transcriptional control of the *CDH1* gene.[102]

Histone Lys-to-Met (K-to-M) mutations act as gain-of-function mutations to inhibit a wide range of histone methyltransferases and are thought to promote tumorigenesis. In *Arabidopsis thaliana* a transgene exogenously expressing histone 3 Lys-36 to Met mutation (K36M) acts in a dominant-negative manner to cause global reduction of H3K36 methylation. This dominant repressive activity is dosage dependent and causes strong developmental perturbations.[103]

Histone lysine methylation participates in diverse mechanisms associated with health, disease, early development, aging, and cancer. Recent studies in red-eared sliders (*Trachemys scripta elegans*), which tolerate anoxic conditions for months by reducing their overall metabolic rate by 90%, demonstrated the presence of histone lysine methyltransferases (HKMTs) and corresponding histone H3 lysine methylation in the liver of this species. H3K4me1, a histone mark associated with active transcription and two corresponding histone lysine methyltransferases that modify H3K4me1, increase in response to anoxia; H3K27me1, another transcriptionally active histone mark, decreases during anoxia; and H3K9me3, a transcriptionally repressive histone mark, and corresponding KMTs increase with anoxia. These results reported by Wijenayake et al.[104] suggest dynamic regulation of histone lysine methylation in the liver of this anoxia-tolerant species for selective upregulation of the genes necessary for anoxia survival, and repression of other genes for energy conservation.

The euchromatic G9a histone methyltransferase (G9a) (KMT1C, EHMT2) is a lysine methyltransferase (KMT) whose primary function is to dimethylate lysine 9 of histone H3 (H3K9me2). G9a-dependent H3K9me2 is associated with gene silencing and acts primarily by recruiting H3K9me2-binding proteins that prevent transcriptional activation. Gene repression via G9a-dependent H3K9me2 is critically required in embryonic stem (ES) cells for the development of cellular lineages by repressing the expression of pluripotency factors. G9a also plays an important role

in the immune system where lymphoid cells and innate lymphoid cells (ILCs) can differentiate from a naive state into one of several effector lineages that require both activating and repressive mechanisms to maintain the correct gene expression program.[105]

The dysregulation of G9a in catalyzing histone H3 methylation on lysines 9 and 27 has been linked to uncontrolled proliferation of tumor cells and silencing on cell proliferation of microvascular endothelial cells, a process necessary to sustain tumor growth through the formation of a vascular capillary network. BIX-01294 and chaetocin are effective inhibitors of G9a HMT activity in human microvascular endothelial cells (HMEC-1), inducing attenuation of HMEC-1 proliferation, nuclear localization of phosphorylated Chk1, cell cycle arrest in the G1 phase, increased gene expression of the cyclin-dependent kinase (CDK) inhibitor p21, and also of Rb1. G9a HMT plays a central role in the promotion of endothelial cell proliferation.[106]

Lysine methyltransferases (KMTs) mediate methylation marks on histone and nonhistone proteins to regulate gene expression in cycling and noncycling cells. The SUV39 subfamily of KMTs (SUV39H1, SUV39H2, G9a, GLP, SETDB1, and SETDB2) (Table 1.7) is involved in cell cycle regulation, differentiation programs, and cellular senescence.[107]

SET and MYND domain-containing proteins (Smyd) are a special class of lysine methyltransferases whose catalytic SET domain is split by an MYND domain (Table 1.7). The hallmark feature of this family is the methylation of histone H3 on lysine 4. The role of the Smyd family is dynamic, targeting unique histone residues associated with both transcriptional activation and repression. Smyd proteins also methylate several nonhistone proteins to regulate various cellular processes in development, cell growth, and differentiation and disease conditions.[108]

The H3K4 methyltransferase Set1, which is commonly linked to transcriptional activation, has been implicated in telomere silencing. Set5 is an H4 K5, K8, and K12 methyltransferase that functions with Set1 to promote repression at telomeres. Set1 and Set5 promote a Sir protein-independent mechanism of repression that may primarily rely on regulation of H4K5ac and H4K8ac at telomeric regions. Cells lacking both Set1 and Set5 have highly correlated transcriptomes to mutants in telomere maintenance pathways and display defects in telomere stability, linking their roles in silencing to protection of telomere.[109] Histone methylation at H3K4 and H3K36 is commonly associated with genes actively transcribed by RNA polymerase II (RNAPII) and is catalyzed by *Saccharomyces cerevisiae* Set1 and Set2, respectively. Set1 is strongly bound to the *SET1* mRNA, Ty1 retrotransposons, and noncoding RNAs from the ribosomal DNA (rDNA) intergenic spacers, consistent with its silencing roles. Set1 lacking RNA recognition motif 2 (RRM2) shows reduced cross-linking to RNA and reduced chromatin occupancy. RNA binding by Set1 contributes to both chromatin association and methyltransferase activity.[110] H3K36 methylation is a common epigenetic mark that is thought to be associated with the activities of the RNA polymerase 2C-terminal domain. A novel silencing mechanism regulated by Set2-dependent H3K36 methylation involving exosome-dependent RNA processing has been identified.[111]

The Suv39h1 and Suv39h2 histone lysine methyltransferases are hallmark enzymes at mammalian heterochromatin. The mouse Suv39h2 enzyme differs from Suv39h1 by containing an N-terminal basic domain that facilitates retention at mitotic chromatin and provides an additional affinity for major satellite repeat RNA. Suv39h1 and Suv39h2 exclusively associate with poly-nucleosomes. Major satellite repeat transcripts remain chromatin-associated and have a secondary structure that favors RNA-DNA hybrid formation. This is an RNA-mediated mechanism for the stable chromatin interaction of the Suv39h KMT, suggesting a function for major satellite noncoding RNA in the organization of an RNA-nucleosome scaffold.[112]

The presence of H3K9me3 and heterochromatin protein 1 (HP1) are hallmarks of heterochromatin conserved in eukaryotes. The spreading and maintenance of H3K9me3 is affected by the functional interplay between the H3K9me3-specific histone methyltransferase Suv39h1 and HP1. The three HP1 isoforms, HP1α, β, and γ, may play a redundant role in Suv39h1-dependent deposition of H3K9me3 in pericentric heterochromatin (PCH). HP1α and, to a lesser extent, HP1γ have a closer functional link to Suv39h1, compared to HP1β. HP1α and γ preferentially interact in vivo with Suv39h1, regulate its dynamics in heterochromatin, and increase Suv39h1 protein stability by inhibiting MDM2-dependent Suv39h1-K87 polyubiquitination. The interplay between Suv39h1 and HP1 isoforms appears to be relevant under genotoxic stress. Loss of HP1α and γ isoforms inhibits the upregulation of Suv39h1 and H3K9me3 that is observed under stress conditions. Suv39h1 deficiency abrogates stress-dependent upregulation of HP1α and γ and enhances HP1β levels.[113]

Genome-wide correlation studies have revealed that histone activation marks and repression marks are associated with activated and repressed gene expression, respectively. Histone H3 K79 methylation is carried out by only a single methyltransferase, disruptor of telomeric silencing-1-like (DOT1L). Studies of thyroid hormone (T3)-dependent amphibian metamorphosis in the pseudotetraploid *Xenopus laevis* and diploid *Xenopus tropicalis*, as models for postembryonic development, showed that H3K79 methylation levels are induced at T3 target genes during natural and T3-induced metamorphosis and that Dot1L is itself a T3 target gene. T3 induces Dot1L expression, and Dot1L in turn

functions as a T3 receptor (TR) coactivator to promote vertebrate development. Overexpression of Dot1L enhances gene activation by TR in the presence of T3. Endogenous Dot1L is critical for T3-induced activation of endogenous TR target genes, while transgenic *Dot1L* enhances endogenous TR function in premetamorphic tadpoles in the presence of T3.[114]

Histone methyltransferase DOT1L is implicated in various biological processes including cell proliferation, differentiation, and embryogenesis. Gene ablation of Dot1L results in embryonic lethality and cardiovascular defects including decreased vasculature. DOT1L is required for angiogenesis. Silencing of *DOT1L* in human umbilical vein endothelial cells (HUVECs) leads to decreased cell viability, migration, tube formation, and capillary sprout formation. DOT1L cooperates with transcription factor ETS-1 to stimulate the expression of VEGFR2, thereby activating the ERK1/2 and AKT signaling pathways and promoting angiogenesis.[115]

Monomethylation of histone H3 at lysine 4 (H3K4me1) and acetylation of histone H3 at lysine 27 (H3K27ac) are correlated with transcriptionally engaged enhancer elements. Loss of H3K4me1 from enhancers in Mll3/4 catalytically deficient cells causes partial reduction of H3K27ac, with minor effects on transcription from either enhancers or promoters. In contrast, loss of Mll3/4 proteins leads to strong depletion of enhancer Pol II occupancy and eRNA synthesis, concomitant with downregulation of target genes.[116]

The KMT2A/MLL1 lysine methyltransferase complex is an epigenetic regulator of selected developmental genes, in part through SET domain-catalyzed methylation of H3K4. It is essential for normal embryonic development and hematopoiesis and frequently mutated in cancer. KMT2A/MLL1 and Msk1 (RPS6KA5) coimmunoprecipitate in various cell types. *KMT2A/MLL1* and *Msk1* knockdown demonstrate that the great majority of genes whose activity changed on *KTM2A/MLL1* knockdown respond comparably to *Msk1* knockdown, as did levels of H3K4 methylation and H3S10 phosphorylation at KTM2A target genes *HoxA4* and *HoxA5*. KMT2A/MLL1 is required for the genomic targeting of Msk1. The KMT2A/MLL1 complex is associated with, and functionally dependent on, the kinase Msk1, part of the MAP kinase signaling pathway. Wiersma et al.[117] proposed that Msk1-catalyzed phosphorylation at H3 serines 10 and 28 supports H3K4 methylation by the KMT2A/MLL1 complex both by making H3 a more attractive substrate for its SET domain, and improving target gene accessibility by prevention of HP1- and Polycomb-mediated chromatin condensation.

ESET protein (SETDB1) catalyzes methylation of histone H3 at lysine 9 (H3-K9). The *ESET* gene also exhibits alternative splicing variants encoding truncated proteins capable of retaining interaction with other epigenetic enzymes. Mesenchyme-specific knockout of exon 4 completely eliminates full-length *ESET* and its truncated protein products, leading to bone defects, ectopic hypertrophy of growth plate chondrocytes, and downregulation of Indian hedgehog protein. Exon 4 deletion also results in reduced thickness of articular cartilage in E17.5 embryos, whereas deletion of exons 15–16 fails to do so. These data reported by Yang et al.[118] indicate that ESET plays a critical role in the control of chondrocyte hypertrophy and skeletal development.

SETD8/SET8/Pr-SET7/KMT5A is the only known lysine methyltransferase (KMT) that monomethylates lysine 20 of histone H4 (H4K20). Lysine residues of nonhistone proteins including proliferating cell nuclear antigen (PCNA) and p53 are also monomethylated. The methyltransferase activity of the enzyme is implicated in many essential cellular processes including DNA replication, DNA damage response, transcription modulation, and cell cycle regulation.[119]

Methylation of arginine residues is an important modulator of protein function involved in epigenetic gene regulation, DNA damage response, RNA maturation, and cellular signaling. The enzymes catalyzing this posttranslational modification are called protein arginine methyltransferases (PRMTs) (Table 1.7), of which PRMT1 is the predominant enzyme. Arginine methylation on histones is a central player in epigenetics and in gene activation and repression. Protein arginine methyltransferase (PRMT) activity, associated with PRMT1-9 enzymes, is implicated in stem cell pluripotency, cancer metastasis, and tumorigenesis. PRMT5 affects the levels of symmetric dimethylarginine (SDMA) at Arg-3 on histone H4, leading to the repression of genes that are related to disease progression in lymphoma and leukemia. PRMT7 also affects SDMA levels at the same site despite its unique monomethylating activity and the lack of any evidence for PRMT7-catalyzed histone H4 Arg-3 methylation. PRMT7-mediated monomethylation of histone H4 Arg-17 regulates PRMT5 activity at Arg-3 in the same protein.[120]

Human *PRMT1* is expressed in seven splicing isoforms, which are differentially abundant in various tissues with distinct substrate specificity and intracellular localization. A novel splicing isoform does not affect the amino-terminus of the protein like the seven known isoforms, but rather lacks exons 8 and 9 that encode the dimerization arm of the enzyme, which is essential for enzymatic activity. This isoform does not form catalytically active oligomers with the other endogenous PRMT1 isoforms. This isoform is found in a variety of cell lines, and it is preferentially increased in oncocells or after expression of the EMT-inducing transcriptional repressor Snail1. It has been proposed that this novel isoform might act as a modulator of PRMT1 activity in cancer cells by acting as a competitive inhibitor that shields substrates from access to active PRMT1 oligomers.[121]

Protein arginine methyltransferase 5 (PRMT5) plays multiple roles in cellular processes at different stages of the cell cycle in a tissue-specific manner. PRMT5 in complex with MEP50/p44/WDR77 associates with a plethora of partner proteins to symmetrically dimethylate arginine residues on target proteins in both the nucleus and the cytoplasm. PRMT5 overexpression is frequent in cancer. Recent studies illustrate the structure of the 453-kDa heterooctameric PRMT5-MEP50 complex bound to an S-adenosylmethionine analog and a substrate peptide.[122]

PRMT6, a protein arginine methyltransferase, is responsible for asymmetric dimethylation of histone H3 arginine 2 (H3R2me2a), negatively regulates DNA methylation, and its upregulation contributes to global DNA hypomethylation in cancer. PRMT6 overexpression impairs chromatin association of UHRF1, an accessory factor of DNMT1, resulting in passive DNA demethylation. Elevated H3R2me2a inhibits the interaction between UHRF1 and histone H3.

The type II arginine methyltransferase PRMT5 is responsible for the symmetric dimethylation of histone to generate H3R8me2s and H4R3me2s marks, which correlate with the repression of transcription. Loss of PRMT5 causes reduction in the levels of proteins encoded by the *MEP50*, *CCND1*, *MYC*, *HIF1a*, *MTIF*, and *CDKN1B* genes, with unchanged levels of their respective mRNAs. The genes regulated by PRMT5, at the posttranscriptional level, express mRNA containing an internal ribosome entry site (IRES). PRMT5 facilitates the translation of a subset of IRES-containing genes. The heterogeneous nuclear ribonucleoprotein, hnRNP A1, is an IRES transacting factor (ITAF) that regulates the IRES-dependent translation of Cyclin D1 and c-Myc. hnRNP A1 is methylated by PRMT5 on R218 and R225 residues, and this methylation facilitates the interaction of hnRNP A1 with IRES RNA to promote IRES-dependent translation. This is a new role for PRMT5 regulation of cellular protein levels, beyond the role of PRMT5 as a transcription and splicing regulator.[123]

CARM1 is an arginine methyltransferase that asymmetrically dimethylates protein substrates on arginine residues. CARM1 is often overexpressed in human cancers. EZH2 inhibition is effective in CARM1-expressing epithelial ovarian cancer. CARM1 promotes EZH2-mediated silencing of EZH2/BAF155 target tumor suppressor genes by methylating BAF155, which leads to the displacement of BAF155 by EZH2. Pharmacological inhibition of EZH2 represents a novel therapeutic strategy for CARM1-expressing cancers.[124]

Posttranslational modifications, such as methylation, acetylation, and phosphorylation, of histone proteins play important roles in regulating dynamic chromatin structure. The demethylases for all major lysine methylation sites have been discovered, with the exception of histone H3 lysine 79 methylation.[125] Lysine-specific demethylase 1 (LSD1), also known as KDM1A (Table 1.8), was the first identified histone demethylase. LSD1 plays a pivotal role in a wide range of biological processes, including development, cellular differentiation, embryonic pluripotency, and disease.[126] LSD1 demethylases H3K4me1/2 and H3K9me1/2 at target loci in a context-dependent manner. LSD1 regulates the balance between self-renewal and differentiation of stem cells, and is highly expressed in various cancers, playing an important role in differentiation and self-renewal of tumor cells.[127]

Two current unresolved questions in epigenetics concern the existence of histone arginine demethylases and the removal of histone tails by proteolysis as a major epigenetic modification process. Liu et al.[128] found that two orphan Jumonji C domain (JmjC)-containing proteins, JMJD5 and JMJD7 (Table 1.8), have divalent cation-dependent protease activities that preferentially cleave the tails of histones 2, 3, or 4 containing methylated arginines. JMJD5 and JMJD7 act as aminopeptidases digesting C-terminal products. JMJD5-deficient fibroblasts exhibit dramatically increased levels of methylated arginines and histones. Depletion of JMJD7 in breast cancer cells greatly decreases cell proliferation. The protease activities of JMJD5 and JMJD7 represent a mechanism for removal of histone tails bearing methylated arginine residues and define a potential mechanism of transcription regulation.[128]

Fe(II)/2-oxoglutarate-dependent dioxygenases are important enzymes in the modulation of distinct biological processes such as epigenetics, hypoxic signaling, and DNA/RNA repair. Jumonji C domain-containing histone lysine demethylases (JMJCs) and prolyl hydroxylases are potential drug targets due to their relevance to human diseases.[129]

Caenorhabditis elegans JMJD-1.2, a member of the KDM7 family, is a demethylase active toward several lysine residues on histone 3 (H3). Jmjd-1.2 is expressed in the germline where it controls the level of H3 lysine 9, lysine 23, and lysine 27 dimethylation (H3K9/K23/K27me2) both in mitotic and meiotic cells. Loss of Jmjd-1.2 is not associated with major defects in the germ cells in animals grown under normal conditions or after DNA damage induced by UV or ionizing irradiation. *Jmjd-1.2* mutants are more sensitive to replication stress, and the progeny of mutant animals exposed to hydroxyurea show increased embryonic lethality and mutational rate. Jmjd-1.2 maintains genome integrity after replication stress and regulates histone methylation in genomic stability.[130]

The E26 transformation-specific (ETS) variant 2 (ETV2) protein (ETS-related 71), a member of the ETS transcription factor family, is essential for embryonic vascular development. ETV2 plays an oncogenic role in tumorigenesis. ETV2 forms complexes with two histone demethylases: jumonji domain-containing (JMJD)2A and JMJD2D. JMJD2A is a driver of prostate cancer development. ETV2 exhibits the potential to stimulate the promoters of matrix metalloproteinases (MMPs) MMP1 and MMP7 in prostate cancer cells. JMJD2A and JMJD2D synergize with ETV2 to activate the

MMP1 promoter. *ETV2* expression is positively associated with *JMJD2A* and *JMJD2D* mRNA levels in neuroendocrine prostate tumors. ETV2, JMJD2A, and JMJD2D may jointly promote tumorigenesis.[131]

The KDM4 histone demethylases are conserved epigenetic regulators linked to development, spermatogenesis, and tumorigenesis. KDM4A/B double-tudor domains (DTDs) bind to H3K23me3, a histone modification enriched in meiotic chromatin of ciliates and nematodes. KDM4B and H3K23me3 colocalize at heterochromatin in mammalian meiotic and newly postmeiotic spermatocytes. H3K23me3 binding by KDM4B stimulates H3K36 demethylation. H3K23me3 binding by KDM4B directs localized H3K36 demethylation during meiosis and spermatogenesis.[132]

The arginine methylation status of histones dynamically changes during many cellular processes, including hematopoietic stem/progenitor cell (HSPC) development. JMJD1B, previously identified as a lysine demethylase for H3K9me2, mediates arginine demethylation of H4R3me2s and its intermediate, H4R3me1. Demethylation of H4R3me2s and H3K9me2s in promoter regions is correlated with active gene expression. Knockout of *JMJD1B* blocks demethylation of H4R3me2s and/or H3K9me2 at distinct clusters of genes and impairs the activation of genes important for HSPC differentiation and development. *JMJD1B* −/− mice show defects in hematopoiesis. JMJD1B demethylates both H4R3me2s and H3K9me2 for epigenetic programming during hematopoiesis.[131, 133]

1.2.3.3.2 Histone Acetylation

The side chain acetylation of lysine residues in histones and nonhistone proteins catalyzed by lysine acetyltransferases (KATs) (Table 1.9) represents a widespread posttranslational modification (PTM) in eukaryotic cells. Lysine acetylation plays regulatory roles in major cellular pathways inside and outside the nucleus. KAT-mediated histone acetylation has an effect on all DNA-templated epigenetic processes. Aberrant expression and activation of KATs are

TABLE 1.9 Histone Lysine Acetyltransferases

Gene	Name	Locus	Other names	OMIM	Phenotype
ACLY	ATP citrate lyase	17q21.2	ACL, ATPCL, CLATP	108728	Breast cancer
CDYL	Chromodomain Y like	6p25.1	CDYL1, DKFZP586C1622	603778	
CLOCK	Circadian locomotor output cycles kaput	4q12	bHLHe8, KAT13D, KIAA0334	601851	Attention-deficit hyperactivity disorder; diabetes; eating disorders; hypoinsulinemia; sleep disorders
CREBBP	CREB-binding protein	16p13.3	CBP, RSTS1, KAT3A, RTS	600140	Acute myeloid leukemia; B cell non-Hodgkin lymphoma; hypothalamic hamartoma with gelastic epilepsy; Rubinstein-Taybi syndrome 1
EP300	E1A-binding protein, 300kD	22q13.2	RSTS2, KAT3B, p300	602700	Acute myeloid leukemia; colorectal cancer, somatic; epithelial cancer; Rubinstein-Taybi syndrome 2
GTF3C4	General transcription factor IIIC subunit 4	9q34.13	KAT12, TFIIIC90	604892	
JADE1	Jade family PHD finger 1	4q28.2	PHF17, JADE-1	610514	Renal cancer
KANSL1	KAT8 regulatory NSL complex subunit 1	17q21.31	KIAA1267, MSL1V1, KDVS	612452	Koolen-De Vries syndrome; congenital heart defects
KANSL2	KAT8 regulatory NSL complex, subunit 2	12q13.11	NSL2, C12orf41	615488	Glioblastoma
KANSL3	Kat8 regulatory NSL complex, subunit 3	2q11.2	NSL3, KIAA1310	617742	
KAT2A	K(lysine) acetyltransferase 2A	17q21.2	GCN5, GCL2, MGC102791, PCAF-b, hGCN5, GCN5L2	602301	Abdominal aortic aneurysm; cancer; leukemia; lung cancer
KAT2B	Lysine acetyltransferase 2B	3p24.3	CAF, GCN5L, P/CAF	602303	Breast cancer; cancer; drug abuse; obesity
KAT5	K(lysine) acetyltransferase 5	11q13.1	HTATIP, TIP60, ESA1, PLIP	601409	Breast cancer; head and neck cancer; lymphoma; malignant pleural mesothelioma (MPM)
KAT6A	K(lysine) acetyltransferase 6A	8p11.21	MYST3, MOZ, ZNF220, MRD32	601408	Acute myelomonocytic leukemia; cancer; mental retardation, autosomal dominant 32

Continued

TABLE 1.9 Histone Lysine Acetyltransferases—cont'd

Gene	Name	Locus	Other names	OMIM	Phenotype
KAT6B	Lysine acetyltransferase 6B	10q22.2	MYST4, MORF, GTPTS	605880	Acute myeloid leukemia; genitopatellar syndrome; infertility; SBBYSS syndrome; uterine cancer
KAT7	K(lysine) acetyltransferase 7	17q21.33	MYST2, HBO1	609880	Androgen insensitivity syndrome (AIS); bladder cancer; breast cancer; ovary cancer; stomach/esophagus cancer; testicular cancer
KAT8	K(lysine) acetyltransferase 8	16p11.2	MYST1, MOF, FLJ14040, ZC2HC8	609912	Hepatocellular carcinoma; nonsmall-cell lung cancer
KAT14	Lysine acetyltransferase 14	20p11.23	CRP2BP, CSRP2BP, ATAC2	617501	
NCOA3	Nuclear receptor coactivator 3 (amplified in breast cancer-1)	20q13.12	ACTR, AIB1, TNRC14	601937	Breast cancer; ovarian cancer; pancreatic cancer; prostate cancer
OGA	O-GlcNAcase	10q24.32	MGEA5, MEA5, NCOAT	604039	
ORC1	Origin recognition complex subunit 1	1p32.3	ORC1L, HSORC1, PARC1	601902	Meier-Gorlin syndrome 1
PHF20	PHD finger protein 20	20q11.22-q11.23	C20orf104, dJ1121G12.1, TDRD20A	610335	Myeloproliferative neoplasms; Neuroblastoma
SIN3A	SIN3 transcription regulator family member A	15q24.2	DKFZP434K2235, KIAA0700, mSIN3A, FLJ90319, SIN3	607776	Witteveen-Kolk syndrome
SIN3B	SIN3 transcription regulator family member B	19p13.12	KIAA0700	602779	Pancreatic cancer

commonly observed in cancer.[134] Histone acetylation is catalyzed by five families of histone lysine acetyltransferases (KATs): KAT2A/GCN5, KAT2B/PCAF, KAT6-8, CREBBP/CBP, and EP300 (Table 1.9).[135] Histone acetylation is associated with transcriptional activation and open chromatin conformation. The monocytic leukemia zinc finger protein-related factor (MORF) is a transcriptional coactivator and a catalytic subunit of the lysine acetyltransferase complex implicated in cancer and developmental diseases. The double-plant homeodomain finger (DPF) of MORF binds to acetylated histone H3 and recognizes many newly identified acylation marks. The acetyltransferase MORF promotes the spreading of histone acylation.[136] K (lysine) acetyltransferase 8 (KAT8, MOF) mediates the acetylation of histone H4 at lysine 16 (H4K16ac) and is crucial for murine embryogenesis. Lysine acetyltransferases regulate various stages of normal hematopoiesis.[137] PHF20 is a core component of the lysine acetyltransferase complex MOF (male absent on the first)-NSL (nonspecific lethal) that generates the major epigenetic mark H4K16ac and is necessary for transcriptional regulation and DNA repair. There is a functional coupling between methylation readers in PHF20. The plant homeodomain (PHD) finger of PHF20 recognizes dimethylated lysine 4 of histone H3 (H3K4me2) and represents an example of a native reader that selects for this modification. Tudor2 is another reader in PHF20 with a preference for dimethylated p53. Binding of the PHD finger to H3K4me2 is required for histone acetylation, accumulation of PHF20 at target genes, and transcriptional activation. These interactions might represent a unique PHF20-mediated link between MOF histone acetyltransferase (HAT), p53, and H3K4me2, suggesting a model for rapid spreading of H4K16ac-enriched open chromatin.[138]

The development of CD1d-restricted invariant natural killer T (iNKT) cells, a population that is critical for both innate and adaptive immunity, is regulated by multiple transcription factors. The histone acetyltransferase general control nonderepressible 5 (GCN5) is essential for iNKT cell development during the maturation stage. GCN5 deficiency blocks iNKT cell development in a cell-intrinsic manner. GCN5 is a specific lysine acetyltransferase of early growth responsive gene 2 (*EGR2*), a transcription factor required for iNKT cell development. GCN5-mediated acetylation positively regulates EGR2 transcriptional activity, and both genetic and pharmacological GCN5 suppression specifically inhibits the transcription of EGR2 target genes in iNKT cells, including *Runx1*, promyelocytic leukemia zinc finger protein (*PLZF*), interleukin (*IL*)-*2Rb*, and *T-bet*.[139]

Intermediates generated in several metabolic processes may regulate transcription through covalent histone and DNA modifications. One example of this is acetyl-coenzyme A (acetyl-CoA) generated by ATP citrate lyase (ACL), which is utilized to acetylate histone H3 at *MyoD* regulatory regions, resulting in increased *MyoD* expression and improved muscle regeneration after injury.[140] Chromatin modification and cellular metabolism are tightly connected. Chromatin modifiers regulate the expression of genes involved in metabolism. The generated metabolites are utilized by chromatin modifiers to effect epigenetic modification. The corepressor SIN3 controls histone acetylation through association with the histone deacetylase RPD3. The SIN3 complex is known to regulate genes involved in a number of metabolic processes. *Drosophila* SIN3 binds to the promoter region of genes involved in methionine catabolism and this binding affects histone modification and gene expression. Reduced expression of *SIN3* leads to an increase in S-adenosylmethionine (SAM), which is the major cellular donor of methyl groups for protein modification. *Sin3A* knockdown results in increased global histone H3K4me3 levels. Decreased H3K4me3 caused by knockdown of either SAM synthetase (*Sam-S*) or the histone methyltransferase *Set1* is restored to near normal levels when SIN3 is also reduced. Knockdown of *Sin3A* directly alters the expression of methionine metabolic genes to increase SAM, which in turn leads to an increase in global H3K4me3. SIN3 is an important epigenetic regulator directly connecting methionine metabolism and histone modification.[141]

The transition from transcription initiation to elongation is a key regulatory step in gene expression, which requires RNA polymerase II (Pol II) to escape promoter proximal pausing on chromatin. Two histone marks on histone H3, lysine 4 trimethylation (H3K4me3), and lysine 9 acetylation (H3K9ac) colocalize on active gene promoters and are associated with active transcription. H3K4me3 can promote transcription initiation. H3K9ac may function downstream of transcription initiation by recruiting proteins important for the next step of transcription. Gates et al.[142] described a functional role for H3K9ac in promoting Pol II pause release by directly recruiting the superelongation complex (SEC) to chromatin. H3K9ac serves as a substrate for direct binding of the SEC, as does acetylation of histone H4 lysine 5 to a lesser extent. Lysine 9 on histone H3 is necessary for maximal Pol II pause release through SEC action, and loss of H3K9ac increases the Pol II pausing index on a subset of genes in HeLa cells. At select gene promoters H3K9ac loss or SEC depletion reduces gene expression and increases paused Pol II occupancy.[142]

The binding of histone acetyltransferase to ORC1 (HBO1) regulates DNA replication, cell proliferation, and development. HBO1 is part of a multiprotein histone acetyltransferase (HAT) complex that also contains inhibitor of growth family member (ING) 4/5, MYST/Esa1-associated factor (MEAF) 6, and the scaffolding protein Jade family PHD finger (JADE) 1/2/3, or bromodomain and PHD finger-containing protein (BRPF) 2/3 to acetylate histone H4 H4K5/8/12 or H3K14, respectively. According to studies reported by Han et al.,[143] within this four-protein complex JADE1 determines histone H4 substrate specificity of the HBO1-HAT complex. JADE1 increases the catalytic efficiency of HBO1 acetylation of an H3/H4 substrate by about fivefold through an N-terminal, 21-residue HBO1- and histone-binding domain and a nearby second histone core-binding domain. HBO1 contains an N-terminal histone-binding domain (HBD) that makes additional contacts with H3/H4 independently of JADE1 interactions with histones. The N-terminal region of JADE1 functions as a platform that brings together the catalytic HBO1 subunit with its cognate H3/H4 substrate for histone acetylation.[143]

Repressive histone modifications through generations is critical for the maintenance of cell identity. Chromodomain Y-like protein (CDYL), a chromodomain-containing transcription corepressor, is physically associated with chromatin assembly factor 1 (CAF-1) and the replicative helicase MCM complex. CDYL bridges CAF-1 and MCM, facilitating histone transfer and deposition during DNA replication. CDYL recruits histone-modifying enzymes G9a, SETDB1, and EZH2 to replication forks, leading to the addition of H3K9me2/3 and H3K27me2/3 on newly deposited histone H3. Depletion of CDYL impedes early S phase progression and sensitizes cells to DNA damage. CDYL plays an important role in the transmission/restoration of repressive histone marks, preserving the epigenetic landscape for the maintenance of cell identity.[144]

Protein-protein interactions regulate and alter histone modifications. The histone acetyltransferase p300 binds thymine DNA glycosylase (TDG). The absence of TDG in mouse embryonic fibroblasts leads to a reduction in the rate of histone acetylation. TDG interacts with the CH3 domain of p300 to allosterically promote p300 activity to specific lysines on histone H3 (K18 and K23). When TDG concentrations approach those of histones, TDG acts as a competitive inhibitor of p300 histone acetylation. Histone acetylation is fine-tuned via interaction with other proteins and participates in the regulation of DNA repair/demethylation pathways.[145]

1.2.3.3.3 Histone Deacetylation

Histone deacetylation is involved in transcriptional repression and closed chromatin structure. In mammals there are 18 HDACs (Tables 1.10 and 1.11), which are organized into four classes according to their homology to yeast. Histone deacetylation is catalyzed by these four classes of HDACs (class I, II, III, IV). Class I HDACs (HDAC1, 2, 3, and 8)

TABLE 1.10 Histone Deacetylases

Gene	Name	Locus	Other names	MIM number	Phenotype
HDAC1	Histone deacetylase-1	1p35.2-p35.1	RPD3L1, GON-10, HD1	601241	Acute promyelocytic leukemia, treatment response
HDAC2	Histone deacetylase 2	6q21	RPD3, YAF1	605164	Aggressive teratocarcinomas; B cell lymphoma; colon, gastric and endometrial tumors; cushing disease; cystic fibrosis; dilated cardiomyopathy; spinal muscular atrophy
HDAC3	Histone deacetylase 3	5q31.3	HD3, RPD3, RPD3-2	605166	Hepatic steatosis; squamous cell lung carcinomas
HDAC4	Histone deacetylase 4	2q37.3	HA6116, HD4, HDAC-4, HDAC-A, HDACA, KIAA0288	605314	Brachydactyly-mental retardation syndrome
HDAC5	Histone deacetylase 5	17q21.31	FLJ90614, KIAA0600, NY-CO-9	605315	Age-related macular degeneration (AMD); Alzheimer disease; Hepatocellular carcinoma (HCC)
HDAC6	Histone deacetylase 6	Xp11.23	CPBHM	300272	Chondrodysplasia with platyspondyly, distinctive brachydactyly, hydrocephaly, and microphthalmia
HDAC7	Histone deacetylase 7	12q13.11	HDAC7A	606542	Age-related macular degeneration (AMD)
HDAC8	Histone deacetylase 8	Xq13.1	MRXS6, CDLS5	300269	Cornelia de Lange syndrome 5
HDAC9	Histone deacetylase 9	7p21.1	MITR, HDAC7B, KIAA0744	606543	Androgenetic alopecia
HDAC10	Histone deacetylase 10	22q13.33	DKFZP761B039	608544	Cervical squamous cell carcinoma
HDAC11	Histone deacetylase 11	3p25.1		607226	Inflammation; Immune disorders

TABLE 1.11 Sirtuins

Gene	Name	Locus	Other names	MIM number	Phenotype
SIRT1	Sirtuin, *Saccharomyces cerevisiae*, homolog 1	10q21.3	SIR2L1	604479	Alzheimer disease; gastric carcinoma; hepatocellular carcinoma; obesity; Parkinson disease; prostate cancer; type 2 diabetes
SIRT2	Sirtuin, *Saccharomyces cerevisiae*, homolog 2	19q13.2	SIR2L, SIR2L2	604480	Brain tumor; gliomas; preeclampsia and fetal growth restriction
SIRT3	Sirtuin, *Saccharomyces cerevisiae*, homolog 3	11p15.5	SIR2L3	604481	Breast cancer; metabolic syndrome; type 2 diabetes
SIRT4	Sirtuin, *Saccharomyces cerevisiae*, homolog 4	12q24.23-q24.31	SIR2L4	604482	Insulinoma; type 2 diabetes
SIRT5	Sirtuin, *Saccharomyces cerevisiae*, homolog 5	6p23	SIR2	604483	Breast cancer; colorectal cancer; liver cancer; lung cancer
SIRT6	Sirtuin 6 (Sir2, *Saccharomyces cerevisiae*, homolog of, 6)	19p13.3	SIR2L6	606211	Fatty liver disease; lymphopenia; lordokyphosis; metabolic syndrome; type 2 diabetes
SIRT7	Sirtuin 7 (Sir2, *Saccharomyces cerevisiae*, homolog of, 7)	17q25.3	SIR2L7	606212	Breast cancer; leukemia; lymphomas; thyroid cancer

are nuclear proteins; HDAC1 and HDAC2 are often found in transcriptional corepressor complexes (SIN3A, NuRD, CoREST), and HDAC3 is found in other complexes (SMRT/N-CoR); class II HDACs are subdivided into class IIa (HDAC4, 5, 7, and 9), and IIb (HDAC6 and 10), which are located in the nucleus-cytoplasm interface and in the cytoplasm, respectively. Class III HDCAs belong to the sirtuin family (Table 1.11), with nuclear (SIRT1, 2, 6, 7), mitocondrial (SIRT3, 4, 5), or cytoplasmic (SIRT1, 2) localization. Class IV HDAC (HDAC11) is a nuclear protein.[22, 84, 146, 147]

Histone deacetylases deacetylate histone and nonhistone protein targets. Aberrant HDAC expression and function have been observed in several diseases. HDAC11 was initially identified as a negative regulator of the antiinflammatory cytokine IL-10. Antagonizing HDAC11 activity may have antitumor potential, whereas activating HDAC11 may be useful to treat chronic inflammation or autoimmunity.[148] Epigenetic changes in chromatin structure have been recently associated with the deregulated expression of critical genes in normal and malignant processes. HDAC11, the newest member of the HDAC family of enzymes, functions as a negative regulator of IL-10 expression in APCs. HDAC11 is a multifaceted regulator of neutrophils. HDAC11 appears to associate with the transcription machinery, possibly regulating the expression of inflammatory and migratory genes in neutrophils.[149]

SIRTUINS Sirtuins (Table 1.11) were discovered in yeast following the characterization of a yeast gene silencing modifier (Silent Information Modifier 2, SIR2) with a particular role in maintaining genomic stability. SIR2 homologs were identified in different species. This category of protein deacetylases is important in the regulation of cell cycle progression and maintenance of genomic stability. In yeast, SIR2 interacts with replication origins and protein complexes that affect both replication origin usage and gene silencing. In metazoans the largest SIR2 homolog, SIRT1, is implicated in epigenetic modifications, circadian signaling, DNA recombination, and DNA repair. Mammalian SIRT1 participates in modulating DNA replication.[150] Sirtuins (Sirt1-Sirt7) are NAD^+-dependent protein deacetylases/ADP ribosyltransferases, which play decisive roles in chromatin silencing, cell cycle regulation, cellular differentiation, cellular stress response, and metabolism. Different sirtuins control similar cellular processes, suggesting a coordinated mode of action. Sirt1 requires autodeacetylation to efficiently deacetylate targets such as p53, H3K9, and H4K16. Sirt7 restricts Sirt1 activity by preventing Sirt1 autodeacetylation causing enhanced Sirt1 activity in *Sirt7* −/− mice. Increased Sirt1 activity in *Sirt7* −/− mice blocks PPARγ and adipocyte differentiation, thereby diminishing accumulation of white fat. Reduction of Sirt1 activity restores adipogenesis in *Sirt7* −/− adipocytes. Antagonistic interactions between Sirt1 and Sirt7 are pivotal in controlling the signaling network required for maintenance of adipose tissue.[151]

SIRT1 substrates include histones and proteins related to enhancement of mitochondrial function as well as antioxidant protection. Fluctuations in intracellular NAD^+ levels regulate SIRT1 activity. SIRT1 influences the nuclear organization of protein-bound NADH. Free and bound NADH are compartmentalized inside the nucleus, and its subnuclear distribution depends on SIRT1.[152]

In the liver, SIRT1 coordinates the circadian oscillation of clock-controlled genes, including genes that encode enzymes involved in metabolic pathways. G1/S progression is affected by absence of SIRT1, as well as circadian gene expression, accompanied by lipid accumulation due to defective fatty acid beta-oxidation.[153]

2-Hydroxyglutarate (2-HG) is a hypoxic metabolite with potentially important epigenetic signaling roles. The acetylation status of the major 2-HG-generating enzymes—lactate dehydrogenase (LDH), isocitrate dehydrogenase (IDH), and malate dehydrogenase (MDH)—may govern their 2-HG-generating activity. Elevated 2-HG in hypoxia is associated with the activation of lysine deacetylases. Mice lacking mitochondrial SIRT3 exhibit hyperacetylation and elevated 2-HG.[154]

Sirt6 protects genome stability and regulates metabolic homeostasis through gene silencing. Sirt6 loss causes an accelerated aging phenotype directly linked to hyperactivation of the NFκB pathway. Sirt6 binds to the H3K9me3-specific histone methyltransferase Suv39h1 and induces monoubiquitination of conserved cysteines in the PRE-SET domain of Suv39h1. Sirt6 attenuates the NFκB pathway through IκBα upregulation via cysteine monoubiquitination and chromatin eviction of Suv39h1.[155]

Histone and DNA modifications are critical to maintaining the equilibrium between pluripotency and differentiation during early embryogenesis. A homozygous inactivating mutation in the histone deacetylase SIRT6 results in severe congenital anomalies and perinatal lethality. The amino acid change at Asp63 to a histidine results in virtually complete loss of H3K9 deacetylase and demyristoylase functions. *SIRT6 D63H* mouse embryonic stem cells (mESCs) fail to repress pluripotent gene expression, direct targets of SIRT6, and exhibit an even more severe phenotype than Sirt6-deficient ESCs when differentiated into embryoid bodies (EBs). *D63H* mutant mESCs maintain expression of pluripotent genes and fail to form functional cardiomyocyte foci. Human-induced pluripotent stem cells (iPSCs) derived from *D63H* homozygous fetuses fail to differentiate into EBs, functional cardiomyocytes, and neural progenitor cells as a result of failure to repress pluripotent genes.[156]

Pyrimidine 5′-nucleotidase (NT5C3A) is an enzyme that mediates nucleotide catabolism. *NT5C3A* gene expression is induced by type I interferons (IFNs) in multiple cell types. NT5C3A suppresses cytokine production by inhibiting the nuclear factor κB (NFκB) pathway. *NT5C3A* expression requires both an intronic IFN-stimulated response element and the IFN-stimulated transcription factor IRF1. Overexpression of *NT5C3A* suppresses IL-8 production and knockdown of *NT5C3A* enhances tumor necrosis factor (TNF)-stimulated IL-8 production. Overexpression of *NT5C3A* increases NAD^+ and activates SIRT1 and SIRT6, which are NAD^+−dependent deacetylases. NT5C3A-stimulated sirtuin activity results in deacetylation of histone H3 and the NFκB subunit RelA (p65), near the *IL-8* promoter. This antiinflammatory pathway depends on the catalytic activity of NT5C3A and functions as a negative feedback regulator of inflammatory cytokine signaling.[157]

1.2.3.3.4 Histone Acylation

Eight types of short chain Lys acylations have recently been identified on histones, including propionylation, butyrylation, 2-hydroxyisobutyrylation, succinylation, malonylation, glutarylation, crotonylation, and β-hydroxybutyrylation. These histone modifications affect gene expression and are structurally and functionally different from the widely studied histone Lys acetylation.[158] The histone acetylation-binding double PHD finger (DPF) domains of human MOZ (KAT6A) and DPF2 (BAF45d) accommodate a wide range of histone lysine acylations with the strongest preference for crotonylation. MOZ and H3K14cr colocalize in a DPF-dependent manner.[159]

1.2.3.3.5 Histone Propionylation

Histones are highly covalently modified; however, it is unclear how histone marks are coupled to cellular metabolism and how this coupling affects chromatin architecture. Kebede et al.[160] identified histone H3 Lys14 (H3K14) as a site of propionylation and butyrylation in vivo.

H3K14pr and H3K14bu are deposited by histone acetyltransferases, are preferentially enriched at promoters of active genes, and are recognized by acylation-state-specific reader proteins. Propionyl-CoA is able to stimulate transcription. Genome-wide H3 acylation profiles are redefined following changes to the metabolic state, and deletion of the metabolic enzyme propionyl-CoA carboxylase alter global histone propionylation levels. It has been proposed that histone propionylation, acetylation, and butyrylation may act in combination to promote high transcriptional output and to couple cellular metabolism with chromatin structure and function.[160]

1.2.3.3.6 N-Glycosylation and O-GlcNAcylation

N-glycosylation is a ubiquitous modification of eukaryotic secretory and membrane-bound proteins. It is estimated that over 90% of glycoproteins are N-glycosylated. The reaction is catalyzed by an eight-protein oligosaccharyltransferase complex, OST, embedded in the ER membrane. A 3.5-Å resolution cryo-EM structure of the *S. cerevisiae* OST revealed the structures of Ost1-5, Stt3, Wbp1, and Swp1. Seven phospholipids mediate many of the intersubunit interactions, and an Stt3 N-glycan mediates interactions with Wbp1 and Swp1 in the lumen. Ost3 mediates the OST-Sec61 translocon interface, funneling the acceptor peptide toward the OST catalytic site as the nascent peptide emerges from the translocon.[161]

Dynamic changes in posttranslational O-GlcNAc modification (O-GlcNAcylation) are controlled by O-linked β-*N*-acetylglucosamine (O-GlcNAc) transferase (OGT) and the glycoside hydrolase O-GlcNAcase (OGA). The nutrient sensor enzyme OGT is a modulator of chromatin remodeling. OGT acts either directly through dynamic and reversible O-GlcNAcylation of histones and chromatin effectors, or in an indirect manner through its recruitment into chromatin-bound multiprotein complexes. O-GlcNAcylation often occurs on serine (Ser) and threonine (Thr) residues of specific substrate proteins via the addition of the O-GlcNAc group by OGT.[162] There is crosstalk between OGT and the DNA dioxygenase ten-eleven translocation proteins that catalyze active DNA demethylation. The stability of OGT can also be controlled by histone lysine-specific demethylase 2 (LSD2).[163]

The dynamic modification of serine or threonine hydroxyl moieties on nuclear, mitochondrial, and cytoplasmic proteins by O-linked β-linked *N*-acetylglucosamine (O-β-GlcNAc, O-GlcNAc) represents simple carbohydrate modifications that have important repercussions in cellular physiology and disease progression. O-GlcNAc-modified proteins regulate cellular pathways such as epigenetics, gene expression, translation, protein degradation, signal transduction, mitochondrial bioenergetics, the cell cycle, and protein localization.[164]

O-GlcNAcylation is involved in fundamental cellular processes and in cancer development through various mechanisms. O-GlcNAcylation at histones or nonhistone proteins can lead to the start of subsequent biological processes. Acting as a protein/histone code, O-GlcNAcylation may provide recognition platforms or executive instructions for subsequent recruitment of proteins to carry out the specific functions. There is functional crosstalk between O-GlcNAcylation and epigenetic changes in the regulation of intracellular biological processes.[162]

Cancer cells exhibit unregulated growth, altered metabolism, enhanced metastatic potential, and altered cell surface glycans. The hexosamine biosynthetic pathway (HBP) sustains glycosylation in the endomembrane system. Elevated levels of UDP-GlcNAc drives the O-GlcNAc modification of targets in the cytoplasm, nucleus, and mitochondrion, including transcription factors, kinases, key cytoplasmic enzymes of intermediary metabolism, and electron transport chain complexes. O-GlcNAcylation alters epigenetics, transcription, signaling, proteostasis, and bioenergetics, contributing to tumorigenesis. A substantial number of cancer hallmarks are linked to dysregulation of O-GlcNAc cycling on cancer targets. Hanover et al.[165] postulated that onconutrient and oncometabolite-fueled elevation increases HBP flux and triggers O-GlcNAcylation of key regulatory enzymes in glycolysis, Kreb's cycle, pentose/phosphate pathway, and the HBP itself. The resulting rerouting of glucose metabolites leads to elevated O-GlcNAcylation of oncogenes and tumor suppressors, further escalating elevation in HBP flux creating a "vicious cycle." Downstream, elevated O-GlcNAcylation alters the DNA repair and cellular stress pathways that influence oncogenesis.[165]

1.2.3.3.7 Ubiquitination-Deubiquitination

Selective degradation of proteins in the cell occurs through ubiquitination, which consists of posttranslational deposition of ubiquitin on proteins to target them for degradation by proteases. Ubiquitination affects protein stability and promotes changes in protein function. The dynamic balance between ubiquitination and deubiquitination is essential for the development and homeostasis of organisms.[166]

Dnmt1 utilizes 2-monoubiquitylated histone H3 as a unique ubiquitin mark for its recruitment to and activation at DNA methylation sites. The crystal structure of the replication foci targeting sequence (RFTS) of Dnmt1 in complex with H3-K18Ub/23Ub reveals striking differences from the known ubiquitin recognition structures. The binding of H3-K18Ub/23Ub results in spatial rearrangement of two lobes in the RFTS, suggesting the opening of its active site. Incubation of Dnmt1 with H3-K18Ub/23Ub increases its catalytic activity in vitro.[167]

Monoubiquitylation is reversed by histone deubiquitinases. OTLD1 deubiquitylates histone 2B in *Arabidopsis*, acting as a transcriptional repressor. OTLD1 can also promote expression of a target gene, displaying a dual role. This transcriptional activation activity of OTLD1 involves occupation of the target chromatin by this enzyme, deubiquitination of monoubiquitylated H2B within the occupied regions, and formation of the euchromatic histone acetylation and methylation marks. H2B ubiquitylation acts as both a repressive and an active mark whereas OTLD1 association with and deubiquitylation of the target chromatin may represent the key juncture between two opposing effects of this enzyme on gene expression.[168]

MDM2 is an E3 ubiquitin ligase and a potent inhibitor of the p53 tumor suppressor. MDM2 tends to be elevated in many human cancers that retain wild-type p53. *MDM2 SNP309G* carriers show elevated levels of MDM2, as a result of enhanced SP1 binding to the *MDM2* promoter, and decreased p53 activity. *Mdm2SNP309G/G* mice are prone to spontaneous tumor formation. Transcriptional repressor E2F6 is a possible negative regulator of *MDM2* expression. E2F6 suppresses *Mdm2* expression in cells harboring the *SNP309G* allele but not the *SNP309T* allele.[169]

Ubiquitin C-terminal hydrolase isozyme L1 (UCHL1) is primarily expressed in neuronal cells and neuroendocrine cells. This multifunctional protein is involved in deubiquitination, ubiquitination, and ubiquitin homeostasis. UCHL1 is associated with genomic DNA in prostate cancer cells, including DU 145 cells derived from a brain metastatic site, and in HEK293T embryonic kidney cells with a neuronal lineage. UCHL1 localizes to TTAGGG repeats at telomeres and interstitial telomeric sequences, where UCHL1 interacts with TRF1 and TRF2 (RAP1) components of the shelterin complex. UCHL1, TERF2IP, and a component of the shelterin complex are bound to the nuclear scaffold. UCHL1 binds telomeres and interstitial telomeric sites.[170]

The epigenetic inheritance of DNA methylation requires UHRF1, a histone- and DNA-binding RING E3 ubiquitin ligase that recruits DNMT1 to sites of newly replicated DNA through ubiquitylation of histone H3. UHRF1 binds DNA with selectivity toward hemimethylated CpGs (HeDNA). The interaction of UHRF1 with HeDNA is required for DNA methylation, but is dispensable for chromatin interaction, which is governed by reciprocal positive cooperativity between the UHRF1 histone- and DNA-binding domains. HeDNA recognition activates UHRF1 ubiquitylation toward multiple lysines on the H3 tail adjacent to the UHRF1 histone-binding site. A DNA-protein interaction and an epigenetic modification directly regulate E3 ubiquitin ligase activity. There is an orchestrated epigenetic control mechanism involving modifications both to histones and DNA that facilitate UHRF1 chromatin targeting, H3 ubiquitylation, and DNA methylation inheritance.[171]

UHRF2 is a ubiquitin-protein ligase E3 that regulates cell cycle, genomic stability, and epigenetics. TIP60 and HDAC1 interact with UHRF2. UHRF2 regulates H3K9ac and H3K14ac differentially in normal and cancer cells. TIP60 acts downstream of UHRF2 to regulate H3K9ac and H3K14ac expression. TIP60 is stabilized in normal cells by UHRF2 ubiquitination and TIP60 is destabilized in cancer cells. Depletion or inhibition of TIP60 disrupts the

regulatory relationship between UHRF2, H3K9ac, and H3K14ac. UHRF2 mediates the posttranslational modification of histones and the initiation and progression of cancer.[172]

Histone H2B monoubiquitination is a regulator of transcription elongation. The E3-ubiquitin ligase complex of H2B: RNF20/RNF40 overexpression causes repression of the induced activity of the enhancers. H2Bub1 levels are negatively correlated with the accessibility of enhancers to transcriptional activators. The chromatin association of histone variant H2A.Z, which is evicted from enhancers for transcriptional activation, is stabilized by H2Bub1 by impairing access of the chromatin remodeler INO80. H2Bub1 acts as a gatekeeper of H2A.Z eviction and activation of inducible enhancers.[173]

Heterochromatin formation in budding yeast is regulated by the silent information regulator (SIR) complex. The SIR complex is integrated by NAD-dependent deacetylase Sir2, the scaffolding protein Sir4, and the nucleosome-binding protein Sir3. Transcriptionally active regions present a challenge to SIR complex-mediated de novo heterochromatic silencing as a result of the presence of antagonistic histone posttranslational modifications. Methylation of histone H3K4 and H3K79 is dependent on monoubiquitination of histone H2B (H2B-Ub). The SIR complex cannot erase H2B-Ub or histone methylation on its own. The deubiquitinase (DUB) Ubp10 is thought to promote heterochromatic silencing by maintaining low H2B-Ub at subtelomeres. Zukowski et al. brilliantly characterized the interactions between Ubp10 and the SIR complex machinery, demonstrating that a direct interaction between Ubp10 and the Sir2/4 subcomplex facilitates Ubp10 recruitment to chromatin via a coassembly mechanism. Sir2/4 stimulates Ubp10 DUB activity on nucleosomes, and this coupling mechanism between the silencing machinery and its DUB partner allows erasure of active PTMs and the de novo transition of a transcriptionally active DNA region to a silent chromatin state.[174] The filament structures that mirror yeast epigenetic gene silencing require Sir2, Sir3, Sir4, nucleosomes, and *O*-acetyl-ADP-ribose. Sir proteins and nucleosomes are components of these filaments. The individual localization patterns of Sir proteins on the SIR-nucleosome filament reflect those patterns on telomeres. Magnesium is present in the SIR-nucleosome filament, with a role similar to that for chromatin condensation.[175]

The adaptor protein TRAF6 has a central function in Toll-like receptor (TLR) signaling. NLRP11 inhibits TLR signaling by targeting TRAF6 for degradation. NLRP11 recruits the ubiquitin ligase RNF19A to catalyze K48-linked ubiquitination of TRAF6 at multiple sites, thereby leading to the degradation of TRAF6. Deficiency in either NLRP11 or RNF19A abrogates K48-linked ubiquitination and degradation of TRAF6, which promotes activation of NFκB and MAPK signaling and increases the production of proinflammatory cytokines. NLRP11 is a conserved negative regulator of TLR signaling and the NLRP11-RNF19A axis targets TRAF6 for degradation.[176]

USP7 (ubiquitin-specific protease 7) prevents ubiquitylation and degradation of DNA methyltransferase 1 (DNMT1) by direct binding of USP7 to the glycine-lysine (GK) repeats that join the N-terminal regulatory domain of DNMT1 to the C-terminal methyltransferase domain. The USP7-DNMT1 interaction is mediated by acetylation of lysine residues within the (GK) repeats.[177]

TBK1 is a component of the type I interferon (IFN) signaling pathway. USP38 negatively regulates type I IFN signaling by targeting the active form of TBK1 for degradation. USP38 specifically cleaves K33-linked poly-ubiquitin chains from TBK1 at Lys670, allowing subsequent K48-linked ubiquitination at the same position mediated by DTX4 and TRIP. Knockdown or knockout of *USP38* increases K33-linked ubiquitination and abrogates K48-linked ubiquitination and degradation of TBK1, thus enhancing type I IFN signaling. USP38 regulates TBK1 ubiquitination through the NLRP4 signalosome.[178]

Histone chaperone ASF1A is dysregulated in multiple tumors. ASF1A is physically associated with USP52, which is a pseudodeubiquitinase. USP52 is a ubiquitin-specific protease that promotes ASF1A deubiquitination and stabilization. USP52-promoted ASF1A stabilization facilitates chromatin assembly and favors cell cycle progression. USP52 is overexpressed in breast cancer. Impairment of USP52-promoted ASF1A stabilization results in growth arrest of breast cancer cells and sensitizes these cells to DNA damage.[179]

Decreased expression of USP44 deubiquitinase has been associated with global increases in H2Bub1 levels during mouse embryonic stem cell (mESC) differentiation. USP44 is an integral subunit of the nuclear receptor corepressor (N-CoR) complex. USP44 within N-CoR deubiquitinates H2B, and ablation of USP44 impairs the repressive activity of the N-CoR complex. USP44 recruitment reduces H2Bub1 levels at N-CoR target loci. High expression of USP44 correlates with reduced levels of H2Bub1. Depletion of either USP44 or TBL1XR1 impairs the invasiveness of breast cancer MDA-MB-231 cells in vitro and causes an increase of global H2Bub1 levels.[180]

The SAGA complex contains two enzymatic modules, which house histone acetyltransferase (HAT) and deubiquitinase (DUB) activities. USP22 is the catalytic subunit of the DUB module, but two adaptor proteins, ATXN7L3 and ENY2, are necessary for DUB activity toward histone H2Bub1 and other substrates. ATXN7L3B shares 74% identity with the N-terminal region of ATXN7L3. ATXN7L3B interacts with ENY2 but not other SAGA components. ATXN7L3B localizes in the cytoplasm and ATXN7L3B overexpression increases H2Bub1 levels, while overexpression

of ATXN7L3 decreases H2Bub1 levels. ATXN7L3B competes with ATXN7L3 to bind ENY2, and knockdown of *ATXN7L3B* leads to concomitant loss of ENY2. Unlike the ATXN7L3 DUB complex, a USP22-ATXN7L3B-ENY2 complex cannot deubiquitinate H2Bub1 efficiently. ATXN7L3B regulates H2Bub1 levels and SAGA DUB activity through competition for ENY2 binding.[181]

Tripartite motif-containing protein 24 (TRIM24) functions as an E3 ligase targeting p53 for ubiquitination, a histone "reader" that interacts with a specific signature of histone posttranslational modifications and a coregulator of nuclear receptor-regulated transcription. TRIM24 may be a liver-specific tumor suppressor and an oncogene when aberrantly overexpressed.[182]

1.2.3.3.8 SUMOylation

Histone deacetylases (HDACs) control dynamic protein acetylation by removing acetyl moieties from lysine. Histone deacetylases themselves are regulated on the posttranslational level, including modifications with small ubiquitin-like modifier (SUMO) proteins.[183] The ubiquitin-related SUMO modifier is essential in cell fate decisions. The SUMO isopeptidase SENP3 regulates chromatin assembly of the MLL1/2 histone methyltransferase complex at distinct *HOX* genes, including the osteogenic master regulator DLX3. Flightless-I homolog (FLII), a member of the gelsolin family of actin-remodeling proteins, is a regulator of SENP3. FLII is associated with SENP3 and the MLL1/2 complex. FLII determines SENP3 recruitment and MLL1/2 complex assembly on the *DLX3* gene. FLII is indispensible for H3K4 methylation and proper loading of active RNA polymerase II at this gene locus. FLII-mediated SENP3 regulation governs osteogenic differentiation of human mesenchymal stem cells.[184]

Tripartite motif-containing protein 24 (TRIM24) is a histone reader aberrantly expressed in multiple cancers. There is functional crosstalk between histone acetylation and TRIM24 SUMOylation. Binding of TRIM24 to chromatin via its tandem PHD-bromodomain, which recognizes unmethylated lysine 4 and acetylated lysine 23 of histone H3 (H3K4me0/K23ac), leads to TRIM24 SUMOylation at lysine residues 723 and 741. Inactivation of the bromodomain, either by mutation or with a small-molecule inhibitor, IACS-9571, abolishes TRIM24 SUMOylation. Inhibition of histone deacetylation increases TRIM24's interaction with chromatin and its SUMOylation. Cell adhesion is the major pathway regulated by the crosstalk between chromatin acetylation and TRIM24 SUMOylation.[182, 185]

1.2.3.3.9 Histone Phosphorylation-Dephosphorylation

Histone phosphorylation depends on protein kinases, and histone dephosphorylation is under the control of protein phosphatases. Several histone modifications can occur simultaneously leading to repression of gene silencing, with no changes in epigenetic memory.

Mitosis in metazoans is characterized by abundant phosphorylation of histone H3 and involves the recruitment of condensin complexes to chromatin. H3T3 phosphorylation decreases binding of histone readers to methylated H3K4 and is essential to displace the corresponding proteins from mitotic chromatin, suggesting a role for mitotic histone H3 phosphorylation in blocking transcriptional programs or preserving "memory" PTMs. H3 phosphorylation thus serves as an integral step in the condensation of chromosome arms.[186]

1.2.3.3.10 Histone Chaperonization

The association of histones with specific chaperone complexes is important for their folding, oligomerization, posttranslational modification, nuclear import, stability, assembly, and genomic localization. The chaperoning of soluble histones is a key determinant of histone availability and fate, which affects all chromosomal processes, including gene expression, chromosome segregation, and genome replication and repair.[187]

Incorporation of variant histone sequences, in addition to posttranslational modification of histones, serves to modulate the chromatin environment. Different histone chaperone proteins mediate the storage and chromatin deposition of variant histones. Although the two noncentromeric histone H3 variants, H3.1 and H3.3, differ by only 5 aa, replacement of histone H3.1 with H3.3 can modulate the transcription for highly expressed and developmentally required genes, lead to the formation of repressive heterochromatin, or aid in DNA and chromatin repair. The human histone cell cycle regulator (HIRA) complex composed of HIRA, ubinuclein-1, CABIN1, and transiently antisilencing function 1, forms one of the two complexes that bind and deposit H3.3/H4 into chromatin.[188] The histone chaperone chromatin assembly factor 1 (CAF-1) deposits tetrameric (H3/H4)2 histones onto newly synthesized DNA during DNA replication. Cac1 binds H3/H4 with high affinity and promotes histone tetramerization.[189] The Tousled-like kinases, TLK1 and TLK2, regulate ASF1, a histone H3/H4 chaperone, and other substrates; their activity has been implicated in transcription, DNA replication, DNA repair, RNA interference, cell cycle progression, viral latency, chromosome segregation, and mitosis.[190]

Packaging of DNA into chromatin affects all processes on DNA. Nucleosomes present a strong barrier to transcription. DNA sequence, DNA-histone interactions, and backtracking by RNA polymerase II (Pol II) contribute to formation of the barrier. After partial uncoiling of nucleosomal DNA from histone octamer by Pol II and backtracking of the enzyme, nucleosomal DNA recoils on the octamer, locking Pol II in the arrested state. Histone chaperones and transcription factors TFIIS, TFIIF and FACT facilitate transcription through chromatin using different molecular mechanisms.[191]

1.2.3.3.11 Glutathionylation

Glutathionylation is the process by which glutathione binds to proteins. Glutathione (γ-glutamyl-cysteinyl-glycine) (GSH) is an intracellular thiol molecule and a potent antioxidant that participates in the toxic metabolism phase II biotransformation of xenobiotics. Protein glutathionylation is an important posttranslational regulatory mechanism involved in the physiological function of transcriptional factors, eicosanoids, cytokines, and nitric oxide (NO).[192] Histone H3, one of the basic proteins in the nucleosomes that make up chromatin, is S-glutathionylated in mammalian cells and tissues.[193]

1.2.3.3.12 Poly ADP-Ribosylation

Poly(ADP-ribosyl)ation (PARylation) is a widespread and highly conserved posttranslational modification catalyzed by a large family of enzymes called poly(ADP-ribose) polymerases (PARPs). PARylation plays an essential role in various cardinal processes of cellular physiology and cancer. PARP-1, PARP-2, and PARP-3 are the only known DNA damage-dependent PARPs with critical roles in DNA damage response, DNA metabolism, and chromatin architecture. PARP-2 plays specific and diverse regulatory roles in cellular physiology, ranging from genomic stability and epigenetics to proliferative signaling and inflammation.[194] Poly ADP-ribose polymerases (PARPs) catalyze massive protein poly ADP-ribosylation (PARylation) within seconds after the induction of DNA single- or double-strand breaks. PARylation is mainly catalyzed by poly-ADP-ribose polymerase 1 (PARP1). PARP1 is a DNA damage sensor that catalyzes the poly (ADP-ribose) (PAR) onto a variety of target proteins, such as histones, DSB repair factors, and PARP1 itself under consumption of NAD^+.[195] PARylation occurs at or near the sites of DNA damage and promotes the recruitment of DNA repair factors via their poly ADP-ribose (PAR) binding domains. PARylation may be the critical event that mediates the first wave of the DNA damage response.[196] In response to LPS exposure, PARP1 interacts with the adenylateuridylate-rich element-binding protein embryonic lethal abnormal vision-like 1 (Elavl1)/human antigen R (HuR), resulting in its PARylation, primarily at site D226. PARP inhibition and the *D226* mutation impair HuR's PARylation, nucleocytoplasmic shuttling, and mRNA binding. Increases in mRNA level or stability of proinflammatory cytokines/chemokines are abolished by PARP1 ablation or inhibition.[197]

CCCTC-binding factor (CTCF) regulates the structure of chromatin by binding DNA strands for defining the boundary between active and heterochromatic DNA. CTCF is quickly recruited to the sites of DNA damage. Fast recruitment is mediated by the zinc finger domain and PAR. CTCF-deficient cells are hypersensitive to genotoxic stress such as ionizing radiation. CTCF participates in DNA damage response via poly(ADP-ribosylation).[198]

1.2.3.3.13 Oxidative Stress

Bioactive electrophiles generated from the oxidation of endogenous and exogenous compounds cause toxicity that is attributed to the covalent modification of cellular nucleophiles, including protein and DNA. In protein modifications the side-chains of Cys, His, Lys, and Arg residues are critical targets, resulting in the generation of undesired protein posttranslational modifications (PTMs) that can trigger cellular dysfunction. Histones are Lys- and Arg-rich proteins, providing a fertile source for adduction by both exogenous and endogenous electrophiles. The regulation of histone PTMs plays a critical role in the regulation of chromatin structure and gene expression.[199]

Oxidative stress and the resulting damage to genomic DNA affect cellular processes and cellular response to environmental exposures. The oxidation of guanine to premutagenic 8-oxo-7,8-dihydroguanine (8-oxoG) is one of the most frequent reactions of reactive oxygen species with DNA. Over 72% of the promoters are rich in GC content where 8-oxoG may serve as an epigenetic mark. When complexed with the oxidatively inactivated repair enzyme 8-oxoguanine DNA glycosylase 1, 8-oxoG contributes to the initiation of DNA repair and the assembly of the transcriptional machinery for the expression of redox-regulated genes. Alterations in the coordination of this efficient cellular process may lead to disease and accelerated aging.[200]

Alterations in mitochondrial metabolism affect cell differentiation and growth. This process is regulated by the activity of 2-oxoglutarate (2OG)-dependent dioxygenases (2OGDDs), a diverse superfamily of oxygen-consuming enzymes, through modulation of the epigenetic landscape and transcriptional responses.[201]

DNA repair protein counteracting oxidative promoter lesions may modulate gene expression. Oxidative DNA bases are primarily modified by reactive oxygen species (ROS), as 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxoG), and this can be repaired by 8-oxoguanine DNA glycosylase1 (OGG1) via the base excision repair (BER) pathway. Cellular response to oxidative challenge is accompanied by DNA damage repair. OGG1 counteracts 8-oxoG and is essential for NFκB- dependent gene expression, prior to 8-oxoG excised from DNA.[202]

ROS may become important cellular-signaling agents for cellular survival. ROS-mediated oxidation of DNA to yield 8-oxo-7,8-dihydroguanine (OG) in gene promoters is a signaling agent for gene activation. Enhanced gene expression occurs when OG is formed in guanine-rich, potential G-quadruplex-forming sequences (PQS) in promoter-coding strands, initiating base excision repair (BER) by 8-oxoguanine DNA glycosylase (OGG1), yielding an abasic site (AP). The AP enables melting of the duplex to unmask the PQS, adopting a G-quadruplex fold in which apurinic/apyrimidinic endonuclease 1 (APE1) binds, but inefficiently cleaves, the AP for activation of vascular endothelial growth factor (*VEGF*) or endonuclease III-like protein 1 (*NTHL1*) genes. The identification of the oxidatively modified DNA base OG to guide BER activity in a gene promoter and its impact on cellular phenotype indicates a potential epigenetic role for OG.[203]

Many redox-responsive gene promoters contain evolutionarily conserved guanine-rich clusters that are susceptible to oxidative modifications. 7,8-Dihydro-8-oxoguanine (8-oxoG) is one of the most abundant base lesions in promoters and is primarily repaired via the 8-oxoguanine DNA glycosylase-1 (OOG1)-initiated base excision repair pathway. The 8-oxoG lesion and the cognate repair protein OGG1 are utilized in transcriptional gene activation. TNFα-induced enrichment of both 8-oxoG and OGG1 in promoters of proinflammatory genes precedes interaction of NFκB with its DNA-binding motif. OGG1 bound to 8-oxoG upstream from the NFκB motif increases its DNA occupancy by promoting an on-rate of both homodimeric and heterodimeric forms of NFκB. OGG1 depletion decreases both NFκB binding and gene expression, whereas Nei-like glycosylase-1 and -2 have a marginal effect. The DNA repair protein OGG1 bound to its substrate is coupled to DNA occupancy of NFκB and functions in epigenetic regulation of gene expression.[204]

Peroxiredoxin I to VI (PRX I–VI) is a family of highly conserved antioxidants that has been implicated in numerous diseases. PRXs are expressed aberrantly in a variety of tumors, implying that they could play an important role in carcinogenesis. DNA methylation, histone modifications, and microRNAs modulate expression of PRXs. Histone deacetylases restore PRX to normal levels.[205]

1.2.3.4 Other Posttranslational Changes

Degranulation of mast cells causes the release of bioactive compounds from their secretory granules, including mast cell-restricted proteases such as tryptase. Tryptase is present within the nuclei of mast cells where it truncates core histones at their N-terminal ends. Tryptase truncates nucleosomal histone 3 and histone 2B (H2B) and its absence results in accumulation of the epigenetic mark, lysine 5-acetylated H2B.[206]

Posttranslational modifications provide versatility to the biological functions of highly conserved proteins. Posttranslational modifications in nonhistone proteins such as methylation, acetylation, phosphorylation, glycosylation, ubiquitination, sumoylation, and other posttranslational changes are linked to the regulation of pivotal pathways related to cellular response and stability. Dysregulation of these pathways and/or the pathogenic component of posttranslational changes lead to inflammation, cancer, and neurodegenerative disorders.[207]

1.2.3.5 Noncoding RNAs

Long noncoding (lnc) RNAs are defined as nonprotein-coding RNAs, distinct from housekeeping RNAs (tRNAs, rRNAs, and snRNAs) and independent of small RNAs with specific molecular processing machinery (micro- or piwi-RNAs).[208] Over 95% of the eukaryotic genome is transcribed into noncoding RNAs (ncRNAs) and less than 5% is translated. lncRNA-mediated epigenetic regulation depends mainly on lcnRNA interactions with proteins or genomic DNA via RNA secondary structures, and some lncRNAs rely on Watson-Crick base pairing for functional activity.[209] RNAs are classified by size into two categories. (i) Small RNAs (<200 nucleotides), which are further subdivided into (a) structural RNAs: ribosomal (rRNAs), transfer (tRNAs), small nuclear RNAs (snRNAs); (b) regulatory RNAs: microRNAs (miRNAs), small interfering RNAs (siRNAs), small nuclear RNAs (snRNAs), piwi-interacting RNAs (piRNAs), splice junction-associated RNAs. (ii) Long noncoding RNAs (lncRNAs) (>200 nucleotides), present in >8000 loci in the human genome: large intergenic noncoding RNAs (lincRNAs), natural antisense transcripts (NATs), noncoding RNA expansion repeats, promoter-associated RNAs (PARs), enhancer RNAs (eRNAs), small activating RNAs (saRNAs).[22, 208–211] Small ncRNAs (miRNAs, siRNAs, piRNAs) show mature forms of 20–30 nucleotides (nt) that associate with members of the Argonaute (AGO) superfamily of proteins, the central effectors of RNA interference (RNAi) pathways.

1.2.3.5.1 miRNAs

miRNAs and siRNAs are posttranscriptional gene silencers, guiding AGO complexes to complementary mRNAs in the cytoplasm, inducing transcript degradation and blocking translation.[210] miRNAs repress translation with RISC (RNA-induced silencing complex) and induce mRNA degradation by binding to the 3′ untranslated region (3′ UTR). Other miRNAs may enhance mRNA translation and induce gene expression by binding to the promoter of the target gene. ncRNAs are essential in the regulation of epigenetic mechanisms (silencing of transposable elements, gene expression control, X chromosome inactivation, DNA imprinting, DNA methylation, histone modifications).

Small noncoding RNAs (ncRNAs) control gene expression in a sequence-specific manner. ncRNAs are classified into different categories including small interfering RNAs (siRNAs), microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), endogenous small interfering RNAs (*endo*-siRNAs or esiRNAs), promoter associate RNAs (pRNAs), small nucleolar RNAs (snoRNAs), and sno-derived RNAs. miRNAs are important cytoplasmic regulators of gene expression, acting as posttranscriptional regulators of messenger RNA (mRNA) targets via mRNA degradation and/or translational repression. miRNAs also have specific nuclear functions such as miRNA-guided transcriptional control of gene expression. ncRNAs are a cluster of RNAs that do not encode functional proteins and whose major function is transcriptional gene silencing.[212, 213]

The evolutionary history of miRNAs in the human genome is still a mystery. Inverted duplication of target genes, random hairpin sequences, and small transposable elements constitute three main models that explain the origin of miRNA genes (*MIR*). Both interspecies and intraspecies divergence of miRNAs exhibits functional adaptation and adaptation to changing environments in evolution.[214]

RNA activation (RNAa) is the process of enhancing selective gene expression at the transcriptional level using double-stranded RNAs, targeting gene promoters. RNA molecules are usually 21 nucleotides long and are collectively called small activating RNAs (saRNAs). They are involved in gene regulation, epigenetics gain-of-function, and therapeutics. RNAa is opposite to RNA interference (RNAi), although both processes share some protein machinery. A new saRNAdb database has been developed with 2150 curated saRNA entries.[215]

Over 130 different RNA modifications have been identified. Mapping selected RNA modifications at single-nucleotide resolution has contributed to creating the epitranscriptome.[216] Epitranscriptomics refers to RNA modification-mediated regulation of gene expression. Major mRNA modifications in the transcriptome of eukaryotic cells include N6-methyladenosine, N6, 2′-*O*-dimethyladenosine, 5-methylcytidine, 5-hydroxylmethylcytidine, inosine, pseudouridine, and N1-methyladenosine.[217] RNA modifications are particularly enriched in tRNAs where they can regulate not only global protein translation, but also protein translation at the codon level. Modifications located in the vicinity of tRNA anticodons are highly conserved in eukaryotes and have been identified as potential regulators of mRNA decoding. These modifications orchestrate the speed and fidelity of translation to ensure proper protein homeostasis. Prominent modifications in the tRNA anticodon loop include queuosine, inosine, 5-methoxycarbonylmethyl-2-thiouridine, wybutosine, threonyl-carbamoyl-adenosine and 5-methylcytosine.[218]

Ribosomal RNAs (rRNAs) are effectors of messenger RNA (mRNA) decoding, peptide bond formation, and ribosome dynamics during translation. Ribose 2′-*O*-methylation (2′-O-Me) is essential for accurate and efficient protein synthesis. The intrinsic capability of ribosomes to translate mRNAs is modulated through a 2′-O-Me pattern and not by nonribosomal actors of the translational machinery.[219] Phosphoinositides are present in the plasma membrane, cytoplasmic organelles, and the nucleus. Phosphatidylinositol-4,5-bisphosphate (PIP2) is a regulator of rRNA gene transcription at the epigenetic level. PIP2 interacts with histone lysine demethylase PHF8 (PHD finger protein 8) and represses demethylation of H3K9me2. The C-terminal K/R-rich motif is the PIP2-binding site within PHF8. The PIP2-binding mutant of PHF8 increases the activity of rDNA promoter and expression of pre-rRNA genes. PIP2 contributes to the fine-tuning of rDNA transcription.[220]

Much of the newly discovered transcriptome appears to represent long noncoding RNA (lncRNA). A central topic in miRNA biology is the existence of a network of interactions with miRNA pathways. lncRNA acts as both a source and an inhibitory regulator of miRNA. At the transcriptional level, lncRNAs bridge DNA and protein by binding to chromatin and serving as a scaffold for modifying protein complexes. Such a mechanism can bridge promoters to enhancers or enhancer-like noncoding genes by regulating chromatin looping, as well as conferring specificity on histone-modifying complexes by directing them to specific loci.[221]

Traditionally, miRNAs are thought to play a negative regulatory role in the cytoplasm by binding to the 3′ UTR of target genes to degrade mRNA or inhibit translation. miRNAs are endogenous noncoding RNAs that contain approximately 22 nucleotides. miRNAs are key regulators of multiple biological processes and their dysregulation is involved in a great variety of human pathologies. The maturation of miRNAs occurs in the cytoplasm where miRNAs exert

posttranscriptional gene silencing (PTGS) via the RNA-induced silencing complex (RISC) pathway. Additionally, mature miRNAs are also present in the nucleus where miRNAs might regulate nucleus-cytoplasm transport mechanisms and participate in other active regulatory functions including PTGS, transcriptional gene silencing (TGS), and transcriptional gene activation (TGA). Liang et al.[222] reported a new type of miRNA present in the nucleus, which can activate gene expression by binding to the enhancer. This type of miRNA was named nuclear activating miRNAs (NamiRNAs). miRNAs can bind nascent RNA transcripts, gene promoter regions, or gene enhancer regions contributing to the modulation of diverse epigenetic pathways.[222] NamiRNAs activate gene expression at the transcriptional level as enhancer regulators. The regulation of enhancers mediated by NamiRNAs depends on the presence of intact enhancers and AGO2 protein. NamiRNAs promote global gene transcription through the binding and activation of their targeted enhancers. This is a novel role for miRNAs as enhancer triggers for transcriptional gene activation.[223]

The miRNA regulome represents a set of regulatory elements that modulate miRNA expression. Classification of miRNA-related genetic variability is an important issue because miRNAs interact with different genomic elements. miRNA-associated genetic variability has been presented at three levels: (i) miRNA genes and their upstream regulation, (ii) miRNA silencing machinery, and (iii) miRNA targets.[224]

ncRNAs maintain critical housekeeping functions such as transcription, RNA processing, and translation. lncRNAs regulate dosage compensation, genomic imprinting, pluripotency, cell differentiation and development, immune response, and many other homeostatic functions (translational inhibition, mRNA degradation, RNA decoys, recruitment of chromatin modifiers, regulation of protein activity, regulation of the availability of miRNAs by sponging mechanism, organization of nuclear subcompartments, and nuclear architecture).[225]

ncRNAs are crucial players in chromatin regulation. During development, long and short ncRNAs act in conjunction with each other, where long ncRNAs (lncRNAs) regulate gene expression patterns. Short ncRNAs (sRNAs) establish constitutive heterochromatin and suppress mobile elements. sRNAs also participate in lncRNA-mediated processes, including dosage compensation. ncRNAs also establish mitotically heritable epigenetic marks during development and participate in mechanisms that regulate maintenance of these epigenetic marks during the lifespan. Epigenetic traits are transmitted to the next generation via paramutations or transgenerational inheritance mediated by sRNAs.[226]

Long ncRNAs can act directly as long transcripts or can be processed into active small si/miRNAs. lncRNAs can modulate mRNA cleavage, translational repression, or the epigenetic landscape of their target genes. Some lncRNAs may play a role in the regulation of alternative splicing in response to several stimuli or during disease.[227] Noncoding RNAs act at the posttranscriptional level, modulating gene expression and leading to mRNA target cleavage and degradation and translation repression. ncRNAs are involved in the regulation of 60% of the coding genes; each ncRNA may have multiple target genes; and each gene may be regulated by several ncRNAs.[228]

Long noncoding RNAs interact with proteins, RNA, and genomic DNA. Most lncRNAs display strong nuclear localization. Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a large family of RNA-binding proteins that are important for multiple aspects of nucleic acid metabolism. The interactions of lncRNAs and hnRNPs regulate gene expression at the transcriptional and posttranscriptional level and influence glucose and lipid metabolism, immune response, DNA damage response, and other cellular functions.[229]

Production of most eukaryotic mRNAs requires splicing of introns from pre-mRNA. The splicing reaction requires definition of splice sites, which are initially recognized in either intron-spanning ("intron definition") or exon-spanning ("exon definition") pairs. In the *Drosophila* genome the modal intron length ranges from 60 to 70 nt representing a local maximum of splicing rates. Low variation in splicing rates across introns has been observed in the same gene, suggesting the presence of gene-level influences.[230] Genes can interact by small RNAs in a homology-dependent manner. Short interfering (siRNAs) can act in *trans* at the chromatin level producing stable and heritable silencing phenotypes. The silencing of endogenous genes is temperature dependent. Silencing efficiency correlates with more efficient accumulation of primary siRNAs at higher temperatures rather than higher expression of precursor RNAs.[231] Peripheral noncoding DNAs protect the genome and the central protein-coding sequences against DNA damage in the somatic genome. In the cytosol, invading exogenous nucleic acids may first be deactivated by small RNAs encoded by noncoding DNA via mechanisms similar to the prokaryotic CRISPR-Cas system. In the nucleus the radicals generated by radiation in the cytosol, radiation energy, and invading exogenous nucleic acids are absorbed, blocked, and/or reduced by peripheral heterochromatin, and damaged DNA in heterochromatin is removed and excluded from the nucleus to the cytoplasm through nuclear pore complexes. Noncoding DNAs in the genome are protective for the sperm genome through similar mechanisms to those of the somatic genome.[232]

In animal cells, mitochondria are the primary powerhouses and metabolic factories. They also contain genomes and can produce mitochondrial-specific nucleic acids and proteins. Crosstalk between mitochondria and the nucleus, mediated by encoded ncRNAs and proteins, is essential for cell homeostasis. Some lncRNAs transcribed in the nucleus

reside in mitochondria for regulating mitochondrial functions. RMRP is a component of the mitochondrial RNase MRP that regulates mitochondrial DNA replication and RNA processing. The steroid receptor RNA activator (SRA) is a key modulator of hormone signaling present in the nucleus and mitochondria. RNA-binding proteins (HuR, GRSF1, SHARP, SLIRP, PPR, and PNPASE) may play specific roles in the lncRNAs transport system. Nuclear DNA-encoded lncRNAs are implicated in mitochondria-mediated apoptosis, mitochondrial bioenergetics and biosynthesis, and glutamine metabolism. The mitochondrial genome can also encode three categories of lncRNAs, including (i) lncND5, lncND6, and lncCyt b RNA; (ii) chimeric mitochondrial DNA-encoded lncRNAs; and (iii) putative mitochondrial DNA-encoded lncRNAs; and these mitochondrial DNA-encoded lncRNAs operate in the nucleus.[233]

RNAs are candidate molecules for transfering gene-specific regulatory information from one generation to the next. Double-stranded RNAs (dsRNAs) introduced into some animals can silence genes of matching sequence and the silencing can persist in the progeny. Such persistent gene silencing is thought to result from sequence-specific interaction of the RNA within parents to generate chromatin modifications, DNA methylation, and/or secondary RNAs, which are then inherited by the progeny.[234] In *C. elegans* small RNAs can regulate genes across generations. The mysterious tendency of heritable RNA interference (RNAi) responses to terminate after three to five generations has been referred to as "the bottleneck to RNAi inheritance." The resetting of epigenetic inheritance after three to five generations is not due to passive dilution of the original RNA trigger, but instead results from an active, multigenerational, and small RNA-mediated regulatory pathway. The process that leads to the erasure of the ancestral small RNA-encoded memory is a specialized type of germline reprogramming mechanism, analogous to the processes that robustly remove parental DNA methylation and histone modifications early in the development in different organisms. Traditionally, germline reprogramming mechanisms that reset chromatin are thought to stand in the way of inheritance of memories of parental experiences. Reprogramming heritable small RNAs takes multiple generations to complete, enabling long-term inheritance of small RNA responses.[235]

miRNAs are often thought to mediate posttranscriptional epigenetic changes by mRNA degradation or translational attenuation. Several miRNAs such as miR-375, members of the miR-29 family, miR-34, and miR-200 are regulated by DNA methylation and histone modifications in various types of cancers and metabolic diseases. miRNAs such as miR-449a, miR-148, miR-101, miR-214, and miR-128 target members of the epigenetic machinery and their dysregulation leads to diverse cellular aberrations. It is becoming clear there is a connection between DNA methylation, histone modification, and miRNA function in physiological and pathological conditions.[236]

lncRNA expression and function have also been associated with many human diseases. lncRNA misregulation may result in an aberrant regulation of gene expression contributing to tumorigenesis.[237] However, many important issues still remain elusive in RNA epigenetics. Residues in exons are methylated (m6A) in nascent pre-mRNA and remain methylated in the same exonic residues in nucleoplasmic and cytoplasmic mRNA. Based on recent studies, Darnell et al.[238] argue against a commonly used "reversible dynamic methylation/demethylation" of mRNA, calling into question the concept of "RNA epigenetics" that parallels the well-established role of dynamic DNA epigenetics.

Secreted extracellular vesicles (EVs) have a function in intercellular communication as paracrine or endocrine factors, circulating in biological fluids. Exosomes are actively secreted vesicles that contain proteins, lipids, soluble factors, and nucleic acids, including miRNAs and other classes of small RNAs (sRNAs). Exosomes are linked to tumor progression and permissive premetastatic diffusion.[239]

miR-148a possesses a binding site in the 3′ UTR of DNMT1 mRNA, which can cause silencing of the DNMT1 gene. There is a physical association between DNMT1 mRNA and miR-148a. Ectopic expression of miR-148a induces programmed cell death and represses cell proliferation by targeting DNMT1. The miR-148a gene is regulated by DNA methylation and DNMT1 in prostate cancer. miR-148a is silenced by DNA methylation, and ectopic expression of miR-148a suppresses DNMT1 expression and induces apoptotic gene expression in hormone-refractory prostate cancer cells.[240]

Two large families of miRNAs (the miR-200 family and the miR-302 family) modulate pluripotency in stem cells under the regulatory effects of TGFβ.[241] SNP-miRNA-mRNA interaction networks are present in blood mononuclear cells. At least 167 trios corresponding to 56 SNPs, 20 miRNAs, and 47 target-mRNAs show SNP-miRNA-mRNA interactions. hsa-miR-222-3p, hsa-miR-181b-5p, and hsa-miR-106b-5p mediate specific correlations between SNP and mRNA in energy metabolism, cellular homeostasis, and tissue homeostasis.[242] Xist, the master regulator of X chromosome inactivation, is a classic example of how lncRNAs can exert multilayered and fine-tuned regulatory functions, by acting as a molecular scaffold for recruitment of distinct protein factors.[243]

Long noncoding RNAs are potential key regulators of the inflammatory response, particularly by modulating the transcriptional control of inflammatory genes. lncRNAs may act as an enhancer or suppressor of inflammatory transcription, function as scaffold molecules through interactions with RNA-binding proteins in chromatin-remodeling complexes, and modulate dynamic and epigenetic control of inflammatory transcription in a gene-specific and

time-dependent fashion.[244] miRNAs modulate glucocorticoid (GC) production by the adrenal glands and cells' responses to GCs. GCs influence cell proliferation, survival, and function at least in part by regulating miRNA expression. GCs are steroids with antiinflammatory and immunomodulatory activities of current use for managing chronic inflammatory and autoimmune conditions, as immunosuppressants in transplantation, and as antitumor agents in certain hematological cancers.[245]

Redox imbalance inhibits endothelial cell (EC) growth, inducing cell death and senescence, and ncRNAs participate in the oxidative stress response. In p53-silenced ECs, several p53-targets were identified among both mRNAs and lncRNAs, including MALAT1 and NEAT1. miR-192-5p is the most induced by H_2O_2 treatment, in a p53-dependent manner. Downmodulated mRNA-targets of miR-192-5p are involved in cell cycle, DNA repair, and stress response. miR-192-5p overexpression decreases EC proliferation, inducing cell death. With H_2O_2 treatment the expression of p53-dependent 5′-isoforms of MDM2 and PVT1 increase selectively. These transcriptomic alterations are also present in pathological conditions, such as ECs undergoing replicative senescence, skeletal muscles of critical limb ischemia patients, and the peripheral blood mononuclear cells of long-living individuals.[246]

Temperature influences gene expression in ectotherms, and small noncoding RNAs contribute to thermosensitive gene regulation. For instance, efficient piRNA-dependent transposon silencing is enhanced by higher temperatures. This might be important in climate-dependent transposon propagation in evolution and the putative transgenerational epigenetic effects of altered small RNA transcriptomes.[247]

lncRNA n342419 (*MANTIS*) is the most strongly regulated lncRNA. Controlled by the histone demethylase JARID1B, MANTIS is downregulated in patients with idiopathic pulmonary arterial hypertension, whereas it is upregulated in carotid arteries of *Macaca fascicularis* subjected to atherosclerosis regression diet, and in endothelial cells isolated from human glioblastoma patients. Deletion or silencing of *MANTIS* inhibits angiogenic sprouting and alignment of endothelial cells in response to shear stress. The nuclear-localized *MANTIS* lncRNA interacts with BRG1, the catalytic subunit of the switch/sucrose nonfermentable chromatin-remodeling complex, for nucleosome remodeling by keeping the ATPase function of BRG1 active. The transcription of key endothelial genes such as *SOX18, SMAD6*, and *COUP-TFII* is regulated by ensuring efficient RNA polymerase II machinery binding.[248]

The kallikrein-related peptidases (KLKs) constitute a family of 15 highly conserved serine proteases with trypsin- and chymotrypsin-like activities. Dysregulated expression and/or aberrant activation of KLKs have been associated with cancer and other pathogenic mechanisms. miRNAs are involved in the posttranscriptional regulation of KLKs and can also act as downstream effectors of KLKs.[249]

1.2.3.5.2 circRNAs

The expression patterns of endogenous circular RNA (circRNA) molecules is important during epidermal stem cell (EpSC) differentiation. High levels of circRNAs are expressed in the differentiated cells; upregulated circRNAs are derived from developmental genes such as *DLG1*. Changes in circRNA expression are independent of host gene expression, and upregulated circRNAs are prone to AGO2 binding. Upregulated circRNAs from the *HECTD1* and *ZNF91* genes show a high number of AGO2 binding sites. circZNF91 contains 24 target sites for miR-23b-3p. Upregulated circRNAs are poorly flanked by homolog-inverted Alu repeats compared to stably expressed circRNAs. Upregulated circRNAs upon differentiation are also upregulated upon *DNMT3A* or *DNMT3B* knockdown, suggesting that circRNA expression changes are unlikely regulated by epigenetic mechanisms.[250]

circRNA_0046366 antagonizes the activity of miRNA-34a via MRE-based complementation, and circRNA_0046366 upregulation abolishes the miRNA-34a-dependent inhibition of PPARα. TG-specific lipolytic genes (carnitine palmitoyltransferase 1A (*CPT1A*) and solute carrier family 27A (*SLC27A*)) are overexpressed, and circRNA_0046366-related rebalancing of lipid homeostasis leads to reduction of TG content and consequent improvement of hepatocellular steatosis.[251]

1.2.3.5.3 RNA Methylation

Modifications in mRNA constitute ancient mechanisms that regulate gene expression posttranscriptionally. N6-methyladenosine (m6A) is the most prominent mRNA modification. A large methyltransferase complex (the m6A "writer") bound by RNA-binding proteins (the m6A "readers") and removed by demethylases (the m6A "erasers") is at the base of this process. m6A mRNA modifications have been linked to regulation at multiple steps in mRNA processing. One of the main functions of m6A may be posttranscriptional fine-tuning of gene expression. In contrast to miRNA regulation, which mostly reduces gene expression, m6A might provide a fast means of posttranscriptionally maximizing gene expression. During developmental transitions, m6A might mark transcripts for degradation.[252]

The m6A methylation of RNA is being mapped at the nucleotide level. m6A modifications are tied to most aspects of the mRNA life cycle. RNA virus genomes are subject to m6A methylation with significant roles in the viral replication cycle.[253]

1.2.3.6 Other Operational Elements of the Epigenetic Machinery

1.2.3.6.1 DNA Replication Regulators

DNA replication in hyperacetylated euchromatin is activated preferentially during the early S phase. TICRR/TRESLIN is an essential protein required for the initiation of DNA replication. TICRR interacts with the acetyl-histone binding bromodomain (BRD) and extraterminal (BET) proteins BRD2 and BRD4. Abrogation of this interaction impairs TICRR binding to acetylated chromatin and disrupts normal S-phase progression. The replication licensing factor CDC6 recruits the MCM2-7 replicative helicase to the replication origin, where MCM2-7 is activated to initiate DNA replication. MCM2-7 is activated by both the CDC7-Dbf4 kinase and cyclin-dependent kinase and via interactions with CDC45 and the go-ichi-ni-san complex (GINS) to form the CDC45-MCM2-7-GINS (CMG) helicase complex. TIMELESS (TIM) is important for the subsequent coupling of CMG activity to DNA polymerases for efficient DNA synthesis. TIM interacts with MCM2-7 prior to the initiation of DNA replication. TIM depletion in various human cell lines results in the accumulation of aberrant CMG helicase complexes on chromatin. These aberrant CMG complexes interact with the DNA polymerases on human chromatin, are not phosphorylated properly by cyclin-dependent kinase/CDC7-Dbf4 kinase, and exhibit reduced DNA unwinding activity. This phenomenon is accompanied by accumulation of the p27 and p21 replication inhibitors, reduced chromatin association of CDC6 and cyclin E, and a delay in S-phase entry. TIM is required for the correct chromatin association of the CMG complex to allow efficient DNA replication.[254]

1.2.3.6.2 N6-Adenine DNA Methylation

N6-methyl-2′-deoxyadenosine (m6dA) is a well-characterized DNA modification in prokaryotes. m6dA levels decrease with increasing complexity of eukaryotic genomes. m6dA participates in gene regulation, nucleosome positioning, and early development. In higher eukaryotes, m6dA is enriched in nongenic regions compared to genic regions, preferentially in chromosome X and 13, and show high levels during embryogenesis. In contrast, decreased levels of m6dA are seen in cancer and in diabetic patients, correlating with expression of fat mass and obesity-associated FTO, which acts as m6A demethylase.[255]

N6-methyladenine is the most widespread mRNA modification. A subset of human box C/D snoRNA species have target GAC sequences that lead to formation of N6-methyladenine at a key *trans* Hoogsteen-sugar A–G base pair, half of which are methylated. The assembly of the box C/D snoRNP can be regulated by RNA methylation at its critical first stage. N6-methylation of adenine prevents the formation of *trans*-Hoogsteen-sugar A–G base pairs. Sheared A–G base pairs, but not Watson-Crick base pairs, are more susceptible to disruption by N6mA methylation and are therefore possible regulatory sites. Human signal recognition particle RNA and many related Alu retrotransposon RNA species are also methylated at the N6 of an adenine that forms a sheared base pair with guanine and mediates a key tertiary interaction.[256]

N6-methyladenosine (m6A) is a widespread posttranscriptional RNA modification that occurs in tRNAs, rRNAs, snRNAs, viral RNAs, and in mRNAs in a dynamic, reversible manner. m6A modulates cell differentiation and pluripotency, cell cycle and tumorigenesis, and several types of stress responses. m6A RNA, its associated enzymes, and DNA polymerase κ constitute an early-response system that confers cellular resistance to ultraviolet irradiation, separate from the canonical nucleotide excision repair (NER) pathway that normally repairs UV-induced DNA damage.[257] Like m6A, N1-methyladenosine (m1A) is a prevalent posttranscriptional RNA modification commonly found in tRNAs, rRNAs, and mRNAs.[258]

1.2.3.6.3 Transcription Factors

Gene transcription is regulated mainly by transcription factors (TFs). ENCODE and Roadmap Epigenomics provide global binding profiles of TFs, which can be used to identify regulatory regions. In cell type-specific and species-specific maps of regulatory regions and TF-TF interactions, Diamanti et al.[259] detected ~144,000 putative regulatory regions among human cell lines, with the majority of them being ~300 bp, and ~20,000 putative regulatory elements in the ENCODE heterochromatic domains, suggesting a large regulatory potential in regions presumed transcriptionally silent. The most significant TF interactions identified in the heterochromatic regions were CTCF and the cohesin complex. Over 90% of the regions were discovered in the 3D contacting domains, with enrichment of GWAS SNPs in the putative regulatory regions, indicating that the regulatory regions play a crucial role in genomic structural stability.

Nucleosomes participate in structural and transcription regulatory functions. Histone posttranslational modifications and nucleosome remodeling are mechanisms to remove the obstacles imposed by the chromatin structure to transcription.[260]

Eukaryotic transcription is regulated through two complexes, the general transcription factor IID (TFIID) and the coactivator Spt-Ada-Gcn5 acetyltransferase (SAGA). The SAGA/TREX-2 subunit Sus1 associates with upstream regulatory regions of many genes and heat shock drastically changes Sus1 binding. SAGA deubiquitinating enzyme Ubp8 is dispensable for RNA synthesis, and Sus1 contributes to synthesis and stability of a wide range of transcripts. SAGA/TREX-2 factor Sus1 may act as a global transcriptional regulator in yeast.[261]

Some transcription factors are involved in the site-specific determination of DNA demethylation in a binding site-directed manner. After screening 15 master TFs involved in cellular differentiation, Suzuki et al.,[262] identified eight novel binding site-directed DNA demethylation-inducing TFs (RUNX3, GATA2, CEBPB, MAFB, NR4A2, MYOD1, CEBPA, and TBX5). These TFs demethylate genomic regions that are associated with corresponding biological roles.[262]

Transcriptional regulation of protein-coding genes is a major mechanism of controlling cellular functions. The enormous amount of transcription factors potentially controlling transcription of any given gene makes it difficult to quickly identify the biologically relevant transcription factors in a particular pathway. A new member of this vast category is Hnf4a, a major transcription factor of the DnaJ heat shock protein family (Hsp40) member C22 (Dnajc22), identified by combining coexpression analyses based on self-organizing maps with sequence-based transcription factor binding prediction.[263]

The ETS family of transcription factors is a functionally heterogeneous group of gene regulators that share a structurally conserved, eponymous DNA-binding domain. DNA target specificity derives from combinatorial interactions with other proteins as well as intrinsic heterogeneity among ETS domains. Molecular hydration appears to be a relevant feature defining the intrinsic heterogeneity in DNA target selection and susceptibility to epigenetic DNA modification.[264]

The vitamin D receptor (VDR) cistrome has binding motifs of the ETS-domain transcription factor GABPA. VDR is the nuclear receptor for the most active vitamin D metabolite 1α,25-dihydroxyvitamin D3 (1,25(OH)2D3). The GABPA cistrome in THP-1 human monocytes is comprised of 3822 genomic loci, 20% of which are modulated by 1,25(OH)2D3. The GABPA cistrome overlaps with accessible chromatin and the pioneer transcription factor PU.1. Some 40% of GABPA binding sites are found at transcription start sites, nearly 100 of which are of 1,25(OH)2D3 target genes. VDR and GABPA colocalize with PU.1 on 593 genomic loci, whereas only 175 VDR sites bound GABPA in the absence of PU.1. VDR sites with GABPA colocalization may control some 450 vitamin D target genes preferentially involved in cellular and immune signaling processes and in cellular metabolism pathways.[265]

Cell identity is primarily maintained by cell type-specific gene expression programs. Serum response factor (Srf), a transcription factor that is activated by various extracellular stimuli, can repress cell type-specific genes and promote cellular reprogramming to pluripotency. Diminution of β-actin monomer quantity results in nuclear accumulation of Mkl1 and activation of Srf, which downregulates cell type-specific genes and alters the epigenetics of regulatory regions and chromatin organization. Srf overexpression may lead to pathogenic phenotypes such as ulcerative colitis or pancreas metaplasia.[266]

A series of transcriptional regulators modulate the activity of transcription factors. Two examples are MeCP2, a protein whose mutated forms are involved in Rett syndrome, and CTCF, a constitutive transcriptional insulator.[267] The multidomain CCCTC-binding factor (CTCF), containing a tandem array of 11 zinc fingers (ZFs), modulates the three-dimensional organization of chromatin. CTCF is sensitive to cytosine methylation at position 2, but insensitive at position 12 of the 15-bp core sequence.[268]

Sp1 belongs to the 26 members of the strong Sp/KLF family of transcription factors. It is a paradigm for a ubiquitously expressed transcription factor and is involved in regulating the expression of genes associated with a wide range of cellular processes in mammalian cells. Sp1 can interact with a range of proteins, including other transcription factors, members of the transcription initiation complex, and epigenetic regulators, enabling tight regulation of its target genes.[229] Transcriptional silencing is a major cause of the inactivation of tumor suppressor genes.

The *EPHB2* gene encodes a receptor tyrosine kinase that controls epithelial cell migration and allocation in intestinal crypts. EPHB2 functions as a tumor suppressor in colorectal cancer whose expression is frequently lost as tumors progress to the carcinoma stage. *EPHB2* expression depends on a transcriptional enhancer whose activity is diminished in EPHB2 nonexpressing cells. Expression of *EPHB2* and *SNAIL1*, an inducer of epithelial mesenchymal transition (EMT), is anticorrelated in colorectal cancer cell lines and tumors. In a cellular model of Snail1-induced EMT, the features of active chromatin at the *EPHB2* enhancer are diminished upon expression of murine Snail1. The transcription factors FOXA1, MYB, CDX2, and TCF7L2 are *EPHB2* enhancer factors, and Snail1 indirectly inactivates the *EPHB2* enhancer by downregulation of *FOXA1* and *MYB*. Snail1 induces the expression of lymphoid enhancer factor 1

(LEF1), which competitively displaces TCF7L2 from the *EPHB2* enhancer. In contrast to TCF7L2, LEF1 appears to repress the *EPHB2* enhancer. SNAIL1 employs a combinatorial mechanism to inactivate the *EPHB2* enhancer based on activator deprivation and competitive displacement of transcription factors.[270]

The mitochondrial pyruvate dehydrogenase (PDH) complex (PDC) acts as a central metabolic node that mediates pyruvate oxidation and fuels the tricarboxylic acid cycle to meet energy demand. E4 transcription factor 1 (E4F1) controls a set of four genes (dihydrolipoamide acetyltransferase (*Dlat*), dihydrolipoyl dehydrogenase (*Dld*), mitochondrial pyruvate carrier 1 (*Mpc1*), and solute carrier family 25 member 19 (*Slc25a19*)) involved in pyruvate oxidation and reported to be individually mutated in human metabolic syndromes. E4F1 dysfunction results in an 80% decrease of PDH activity and alterations of pyruvate metabolism. Genetic inactivation of murine *E4f1* in striated muscles results in viable animals that show low muscle PDH activity, severe endurance defects, and chronic lactic acidemia, recapitulating some clinical symptoms described in PDC-deficient patients.[271] E4F1 is an essential regulator of epidermal stem cell (ESC) maintenance. E4F1 transcriptionally regulates a metabolic program involved in pyruvate metabolism that is required to maintain skin homeostasis. E4F1 deficiency in basal keratinocytes results in deregulated expression of dihydrolipoamide acetyltransferase (*Dlat*), a gene encoding the E2 subunit of the mitochondrial pyruvate dehydrogenase (PDH) complex. *E4f1* knock-out (KO) keratinocytes exhibit impaired PDH activity and a redirection of the glycolytic flux toward lactate production.[272]

The motif ACTAYRNNNCCCR (Y=C or T, R=A or G, and N any nucleotide) (M4) has been found to be a putative *cis*-regulatory element, present 520 times in human promoter regions. Of these, 317 (61%) are conserved within promoter sequences of four related organisms: humans, mice, rats, and dogs. M4 is a transcription factor (TF) binding site for THAP11 that does often overlap with SBS (STAF binding site), a second core promoter-associated TF binding module, which associates with the TFs STAF/ZNF143 and RBP-J. Human M4-promoter genes show enhanced expression in cells of hematopoietic origin, especially in B lymphoblasts and peripheral blood B and T cells. RBP-J recruits the intracellular transcriptional mediator of activated Notch1 (ICN1). THAP11 and Ikaros interact directly, while NFKB1 (NF-kappa B p50) and HCF-1 bind indirectly to M4-promoters in living cells. M4 is a bipartite composite *cis*-element, which is recognized by THAP11 via binding to the ACTAYR sequence module, thereby promoting ternary complex formation with HCF-1. Ikaros binds to the CCCR module of the M4 motif and this interaction is crucial for recruiting NFKB1 to M4 harboring genes. The M4 motif (ACTAYRNNNCCCR) is a functional regulatory bipartite *cis*-element, which engages a THAP11/HCF-1 complex via binding to the ACTAYR module, while the CCCRRNRNRC subsequence part constitutes a binding platform for Ikaros and NFKB1.[273]

1.2.3.6.4 Transcriptional Repression

The sequence and functional contribution of transcriptional repression mechanisms at high temporal resolution have been delineated. Inducible entry of the NuRD-interacting transcriptional regulator Ikaros into mouse pre-B cell nuclei triggers immediate binding to target gene promoters. Rapid RNAP2 eviction, transcriptional shutdown, nucleosome invasion, and reduced transcriptional activator binding require chromatin remodeling by NuRD-associated Mi2beta/CHD4, independently of HDAC activity. Histone deacetylation occurs after transcriptional repression. HDAC activity contributes to stable gene silencing.[274]

1.2.3.6.5 Polycomb Group Proteins: Polycomb Repressive Complex 2 (PRC2)

Polycomb group (PcG) proteins are major determinants of gene silencing and epigenetic memory in higher eukaryotes. Hauri et al.[275] mapped the human PcG complexome and identified two human PRC2 complexes and two PR-DUB deubiquitination complexes, which contain the O-linked *N*-acetylglucosamine transferase OGT1 and several transcription factors. The human PR-DUB and PRC1 complexes bind distinct sets of target genes, suggesting differential impact on cellular processes in mammals.[275] PcG proteins epigenetically repress key developmental genes and thereby control alternative cell fates. PcG proteins act as complexes that can modify histones and these histone modifications play a role in transmitting information about the repressed state as cells divide. PcG complexes do not rely on histone modifications to recognize their target genes, but use them to stabilize interactions within large chromatin domains.[276]

The Polycomb group of transcriptional repressors and the trithorax group (trxG) of transcriptional activators are mediators of cellular differentiation. These protein families, while opposed in function, work together to coordinate appropriate cellular developmental programs that allow for both embryonic stem cell self-renewal and differentiation.[277]

Polycomb Repressive Complex 2 (PRC2) methylates lysine 27 in histone H3, a modification associated with epigenetic gene silencing. This complex plays a fundamental role in regulating cellular differentiation and development, and *PRC2* overexpression and mutations have been implicated in numerous cancers.[278]

PRC2 methylates lysine 27 of histone H3 (H3K27) through its catalytic subunit Ezh2. PRC2-mediated dimethylation and trimethylation (H3K27me2/H3K27me3) have been interchangeably associated with gene repression. Modifying the ratio of H3K27me2 and H3K27me3 is sufficient for the acquisition and repression of defined cell lineage transcriptional programs and phenotypes.[279]

Polycomb-like (PCL) proteins, such as PHF1, MTF2, and PHF19, are PRC2-associated factors that form subcomplexes with PRC2 core components, and have been proposed to modulate the enzymatic activity of PRC2 or the recruitment of PRC2 to specific genomic loci. PRC2-binding sites are enriched in CG content, which correlates with CpG islands that display a low level of DNA methylation.[280]

Epigenetic maintenance of gene repression is essential for development. Polycomb complexes are central to this memory. Like Heterochromatin Protein 1 (LHP1) binds Polycomb-deposited H3K27me3 and is required for repression of many Polycomb target genes. LHP1 binds RNA through the intrinsically disordered hinge region. Both the RNA-binding hinge region and H3K27me3 (trimethylation of histone H3 at Lys27) recognition facilitate LHP1 localization and H3K27me3 maintenance. Disruption of the RNA-binding hinge region prevents formation of subnuclear foci, structures potentially important for epigenetic repression.[281]

Target genes of the Polycomb group (PcG) are transiently activated by a stimulus and subsequently repressed. In *Drosophila*, mutually exclusive binding patterns for HSF and PRC1 at the *hsp70* locus have been detected. Pleiohomeotic (Pho), a DNA-binding PcG member, dynamically interacts with Spt5, an elongation factor. The dynamic interaction switch between Pho and Spt5 is triggered by the recruitment of HSF to chromatin. Mutation in the protein-protein interaction domain (REPO domain) of *Pho* interferes with the dynamics of its interaction with Spt5. The transcriptional kinetics of the heat shock response is negatively affected by a mutation in the REPO domain of *Pho*.[282]

PRC2 can add one to three methyl groups, and the fully methylated product, H3-K27me3, is a hallmark of Polycomb-silenced chromatin. In studies with a variant of *Drosophila melanogaster PRC2*, which is converted into a monomethyltransferase, a single substitution, F738Y, in the lysine-substrate binding pocket of the catalytic subunit, E(Z), creates an enzyme that retains robust K27 monomethylation, but dramatically reduces dimethylation and trimethylation. Overexpression of *E(Z)-F738Y* triggers desilencing of Polycomb target genes suggesting that H3-K27me1 contributes positively to gene activity. The normal genomic distribution of H3-K27me1 is enriched on actively transcribed *Drosophila* genes, with localization overlapping the active H3-K36me2/3 chromatin marks. Distinct K27 methylation states link to either repression or activation depending on the number of added methyl groups. H3-K27me1 deposition may involve alternative methyltransferases beyond PRC2, which is primarily repressive. These studies reported by Wang et al.[283] suggest distinct roles for K27me1 versus K27me3 in transcriptional control and expanded the machinery for methylating H3-K27.

Chromatin-based cell memory enables cells to maintain their identity by fixing lineage-specific transcriptional programs, ensuring accurate transmission through cell division. The PcG-based memory system maintains the silenced state of developmental and cell cycle genes. However, the function of Polycomb proteins is not limited to the imposition of rigid states of genetic programs; they also have the capacity to recognize signals, allowing plastic transcriptional changes in response to different stimuli.[284]

Under stress conditions the coactivator Multiprotein bridging factor 1 (Mbf1) translocates from the cytoplasm into the nucleus to induce stress-response genes. Mbf1 associates with *E(z)* mRNA and protects it from degradation by the exoribonuclease Pacman (Pcm), thereby ensuring Polycomb silencing. Loss of mbf1 function enhances a Polycomb phenotype in mutants, and it is accompanied by a significant reduction in *E(z)* mRNA expression. *Pcm* mutations suppress the Polycomb phenotype and restore the expression level of *E(z)* mRNA, while Pcm overexpression exhibits the Polycomb phenotype in the *mbf1* mutant. Mbf1 buffers fluctuations in Pcm activity to maintain an *E(z)* mRNA expression level sufficient for Polycomb silencing.[285]

Distinct epigenomic profiles of histone marks have been associated with gene expression. O'Geen et al.[286] studied a broad collection of genomically targeted epigenetic regulators that could write epigenetic marks associated with a repressed chromatin state (G9A, SUV39H1, Krüppel-associated box (KRAB), DNMT3A as well as the first targetable versions of Ezh2 and Friend of GATA-1 (FOG1)) and found that that so-called repressive histone modifications were not sufficient for gene repression.

The Polycomb repressor complex 2 molecule EZH2 plays a role in cell fate decisions, cell cycle regulation, senescence, cell differentiation, and cancer development and progression. High expression of EZH2 correlates with an unfavorable prognosis of neuroblastoma (NB). Knockdown of *EZH2* and EZH2 inhibitors induce NB cell differentiation. *NTRK1* (TrkA) is one of the EZH2-related suppression targets. The *NTRK1 P1* and *P2* promoter regions are regulated

by DNA methylation and EZH2-related histone modifications. The *NTRK1* transcript variants 1/2, which are regulated by EZH2-related H3K27me3 modifications at the *P1* promoter region, are strongly expressed in favorable NB. EZH2 is important in preventing the differentiation of NB cells, and EZH2-related *NTRK1* transcriptional regulation may be the key pathway for NB cell differentiation.[287] Enhancer of zeste homolog 2 (EZH2), the catalytic subunit of Polycomb repressive complex 2 (PRC2), is involved in the development and maintenance of many types of cancer. PRC2 can have both oncogenic and tumor-suppressive functions. These apparently opposing roles of PRC2 in cancer are a consequence of the molecular function of the complex in maintaining, rather than specifying, the transcriptional repression state of its several thousand target genes.[288]

Gene regulatory networks are pivotal for many biological processes. In mouse embryonic stem cells (mESCs) the transcriptional network can be divided into three functionally distinct modules: Polycomb, Core, and Myc. The Polycomb module represses developmental genes, while the Myc module is associated with proliferative functions, and its misregulation is linked to cancer development. The Polycomb repressive complex 2 (PRC2)-associated protein EPOP (Elongin BC and Polycomb Repressive Complex 2-associated protein; a.k.a. C17orf96, esPRC2p48, and E130012A19Rik) colocalizes at chromatin with members of the Myc and Polycomb module. EPOP interacts with the transcription elongation factor Elongin BC and the H2B deubiquitinase USP7 to modulate transcriptional processes in mESCs similar to MYC. EPOP is commonly upregulated in human cancer, and its loss impairs the proliferation of several human cancer cell lines. EPOP is a transcriptional modulator that impacts both Polycomb and active gene transcription in mammalian cells.[289]

The Polycomb PRC1 plays essential roles in development and disease pathogenesis. Targeting of PRC1 to chromatin is thought to be mediated by the Cbx family proteins (Cbx2/4/6/7/8) binding to histone H3 with a K27me3 modification (H3K27me3). H3K27me3 contributes to the targeting of Cbx7 and Cbx8 to chromatin, but less to Cbx2, Cbx4, and Cbx6. Genetic disruption of the complex formation of PRC1 facilitates the targeting of Cbx7 to chromatin. The CD and AT-hook-like (ATL) motif of Cbx7 constitute a functional DNA-binding unit. Cbx7 is targeted to chromatin by corecognizing of H3K27me3 and DNA. This is a novel hierarchical cooperation mechanism by which histone modifications and DNA coordinate to target chromatin regulatory complexes.[290]

Elongin BC is a binding factor at the promoters of bivalent sites. Elongin BC is associated with Polycomb Repressive Complex 2 in pluripotent stem cells. Elongin BC is recruited to chromatin by the PRC2-associated factor EPOP, a protein expressed in the inner cell mass of the mouse blastocyst. Both EPOP and Elongin BC are required to maintain low levels of expression at PRC2 genomic targets.[291]

1.2.3.6.6 BET (Bromodomain and Extraterminal Domain) Proteins

Bromodomain proteins (Table 1.12) are epigenetic readers that recognize acetylated histone tails to facilitate the transcription of target genes.[292] There are approximately 60 human bromodomains, which are divided into eight subfamilies based on structural conservation (Table 1.12). The bromodomain-containing proteins in family IV include seven members (BRPF1, BRPF2, BRPF3, BRD7, BRD9, ATAD2, and ATAD2b). The BRPF1 subunit of the MOZ histone acetyltransferase (HAT) recognizes acetylated histones H2AK5ac, H4K12ac, H3K14ac, H4K8ac, and H4K5ac. The bromodomain of BRD7 is a member of the SWI/SNF complex that preferentially recognizes acetylated histones H3K9ac, H3K14ac, H4K8ac, H4K12ac, and H4K16ac. The bromodomains of BRPF2 and BRPF3 have similar sequences, and function as part of the HBO1 HAT complex. The ATAD2 bromodomain binds to the diacetylated H4K5acK12ac mark found in newly synthesized histones following DNA replication.[293]

Bromodomains are protein modules adopting conserved helix bundle folds. Bromodomains bind to acetylated lysine residues on histone tails, facilitating reading of the histone code.

Lysine acetylation of histone proteins is a fundamental posttranslational modification that regulates chromatin structure and plays an important role in gene transcription. Acetyl-lysine modifications create docking sites for bromodomains, which are structurally conserved modules present in transcription-associated proteins (reader proteins). Bromodomain-containing reader proteins are part of multiprotein complexes that regulate transcription programs, which are often associated with profound phenotypic changes.[294]

Double bromodomain and extraterminal domain (BET) proteins are critical epigenetic readers that bind to acetylated histones in chromatin and regulate transcriptional activity and modulate changes in chromatin structure and organization. The testis-specific BET member regulates male sterility. BRDT is expressed in both spermatocytes and spermatids, and loss of the first bromodomain of BRDT leads to severe defects in spermiogenesis without overtly compromising meiosis. In contrast, complete loss of BRDT blocks the progression of spermatocytes into the first meiotic division, resulting in complete absence of postmeiotic cells. BRDT is an essential regulator of chromatin organization and reprogramming during prophase I of meiosis. Loss of BRDT function disrupts the epigenetic state of meiotic

TABLE 1.12 Bromodomains

Gene	Name	Locus	Other names	MIM number	Phenotype
ASH1L	Ash1 (absent, small, or homeotic), *Drosophila*, homolog of	1q22	KIAA1420, ASH1, MRD52, ASH1L1, huASH1, KMT2H	607999	Autoimmune diseases; beta thalassemia; brain cancer; breast cancer; developmental disorders; leukemia; lung cancer; mental retardation, autosomal dominant 52
ATAD2	ATPase family, AAA domain containing 2	8q24.13	ANCCA, CT137, DKFZp667N1320, MGC29843, MGC5254, PRO2000	611941	Breast, uterus, colon, ovary, and stomach tumors
ATAD2B	ATPase family, AAA domain-containing, member 2B	2p24.1-p23.3	KIAA1240	615347	
BAZ1A	Bromodomain adjacent to zinc finger domain, 1A	14q13.1-q13.2	ACF1, hACF1, WALp1, WCRF180	605680	Esophageal squamous cell carcinoma
BAZ1B	Bromodomain adjacent to zinc finger domain, 1B	7q11.23	WSTF, WBSCR9	605681	Williams-Beuren syndrome
BAZ2A	Bromodomain adjacent to zinc finger domain, 2A	12q13.3	KIAA0314, TIP5	605682	
BAZ2B	Bromodomain adjacent to zinc finger domain, 2B	2q24.2	WALp4	605683	Sudden cardiac death (SCD), prolonged QRS/QT intervals
BPTF	Bromodomain PHD finger transcription factor	17q24.2	FALZ, FAC1, NURF301, NEDDFL	601819	Neurodevelopmental disorder with dysmorphic facies and distal limb anomalies
BRD1	Bromodomain-containing protein 1	22q13.33	BRL, BRPF2	604589	Myocardial infarction
BRD2	Bromodomain-containing protein 2	6p21.32	RING3, FSRG1, D6S113E, KIAA9001, NAT	601540	Epilepsy, juvenile myoclonic; Leukemia; B cell lymphoma
BRD3	Bromodomain-containing protein 3	9q34.2	KIAA0043, ORFX, RING3L	601541	
BRD4	Bromodomain-containing protein 4	19p13.12	CAP, HUNK1, HUNKI, MCAP	608749	Carcinoma
BRD7	Bromodomain containing 7	16q12.1	BP75, CELTIX1		Nasopharyngeal carcinoma
BRD8	Bromodomain-containing protein 8	5q31.2	SMAP	602848	Pancreatic cancer
BRD9	Bromodomain containing 9	5p15.33	FLJ13441		Nonsmall-cell lung cancer
BRDT	Bromodomain, testis-specific	1p22.1	SPGF21, BRD6, CT9	602144	Spermatogenic failure 21
BRPF1	Bromodomain- and PHD finger-containing protein 1, 140kD	3p25.3	BR140, IDDDFP	602410	Intellectual developmental disorder with dysmorphic facies and ptosis
BRPF3	Bromodomain- and PHD finger-containing protein 3	6p21.31	KIAA1286	616856	
BRWD1	Bromodomain- and WD repeat domain-containing protein 1	21q22.2	WRD9, C21orf107, DCAF19, FLJ11315, N143	617824	Down syndrome
BRWD3	Bromodomain- and WD repeat-containing protein 3	Xq21.1	MRX93	300553	Mental retardation, X-linked 93
CECR2	CECR2, histone acetyl-lysine reader	22q11.1-q11.21	KIAA1740	607576	Anorectal, renal, and preauricular anomalies in patients with cat eye syndrome (CES)
CREBBP	CREB-binding protein	16p13.3	CBP, KAT3A, RSTS, RTS	600140	Acute lymphoblastic leukemia; acute myeloid leukemia; hypothalamic hamartoma with gelastic epilepsy; non-Hodgkin lymphoma; Rubinstein-Taybi syndrome 1

Continued

TABLE 1.12 Bromodomains—cont'd

Gene	Name	Locus	Other names	MIM number	Phenotype
EP300	E1A binding protein p300	22q13.2	KAT3B, p300, RSTS2	602700	Acute lymphoblastic leukemia; acute myeloid leukemia; acute monocytic leukemia; colorectal cancer, somatic; epithelial cancer; non-Hodgkin lymphoma; Rubinstein-Taybi syndrome 2
KAT2A	K(lysine) acetyltransferase 2A	17q21.2	GCN5L2, GCN5, PCAF-b	602301	Leukemia
KAT2B	Lysine acetyltransferase 2B	3p24.3	PCAF, GCN5, GCN5L, P/CAF	602303	Breast cancer; drug abuse
KMT2A	Lysine methyltransferase 2A	11q23.3	ALL-1, CXXC7, HRX, HTRX1, MLL, MLL1, MLL1A, TRX1	159555	Leukemia, myeloid/lymphoid or mixed lineage; Wiedemann-Steiner syndrome
PBRM1	Polybromo 1	3p21.1	BAF180, PB1	606083	Breast cancer
PHIP	Pleckstrin homology domain-interacting protein	6q14.1	DIDOD	612870	Developmental delay, intellectual disability, obesity, and dysmorphic features
SMARCA2	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 2	9p24.3	SNF2L2, BAF190, BRM, hBRM, hSNF2a, SNF2, SNF2LA, Sth1p, SWI2	600014	Gastric cancer; lung cancer; Nicolaides-Baraitser syndrome; Coffin-Siris syndrome; Schizophrenia
SMARCA4	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4	19p13.2	BRG1, RTPS2, MRD16, CSS4	603254	Coffin-Siris syndrome 4; rhabdoid tumor predisposition syndrome 2
SP100	SP100 nuclear antigen	2q37.1	LEU5, RFP2, LYSP100B	604585	HCMV infection
SP110	SP110 nuclear body protein	2q37.1	IFI41, IFI75, VODI	604457	Hepatic venoocclusive disease with immunodeficiency; *Mycobacterium tuberculosis*, susceptibility to
SP140	SP140 nuclear body protein	2q37.1	LYSP100-A, LYSP100-B	608602	Multiple sclerosis
SP140L	SP140 nuclear body protein like	2q37.1		617747	Primary biliary cirrhosis
TAF1	TAF1 RNA polymerase II, TATA box-binding protein-associated factor, 250kD	Xq13.1	TAF2A, CCG1, BA2R, DYT3, MRXS33	313650	Dystonia Parkinsonism, X linked; Mental retardation, X linked, syndromic 33
TAF1L	TATA box-binding protein-associated factor 1 like	9p21.1	TAF2A2, MGC134910, TAF(II)210	607798	Colorectal cancer; gastric cancer
TRIM24	Tripartite motif containing 24	7q33-q34	TIF1, hTIF1, RNF82, Tif1a, TIF1A	603406	Breast cancer; papillary thyroid carcinoma; myeloproliferative syndrome; hepatocellular carcinoma
TRIM28	Tripartite motif containing 28	19q13.4	KAP1, PPP1R157, RNF96, TF1B, TIF1B	601742	Colorectal cancer
TRIM33	Tripartite motif containing 33	1p13.2	TIF1G, RFG7, PTC7, FLJ11429, KIAA1113	605769	Thyroid cancer
TRIM66	Tripartite motif containing 66	11p15.4	KIAA0298, TIF1D, TIF1DELTA, C11orf29	612000	Nonsmall-cell lung cancer; osteosarcoma
ZMYND8	Zinc finger MYND-type containing 8	20q13.12	PRKCBP1, RACK7	615713	Acute erythroid leukemia
ZMYND11	Zinc finger MYND-type containing 11	10p15.3	BS69, PRKCBP1L1, BRAM1, MGC111056, RP11-486H9.1	608668	Chromosome 10p subtelomeric deletion syndrome; mental retardation, autosomal dominant 30

sex chromosome inactivation in spermatocytes, affecting the synapsis and silencing of the X and Y chromosomes. BRDT also controls global chromatin organization and histone modifications of the chromatin attached to the synaptolemal complex.[295]

BET proteins bind acetylated chromatin to facilitate access by transcriptional regulators to chromatin, as well as to assist the activity of transcription elongation complexes via CDK9/pTEFb. NFκB (Nuclear Factor-κ-light-chain-enhancer of activated B cells) signaling elicits global transcriptional changes by activating cognate promoters and through genome-wide remodeling of cognate regulatory elements called "superenhancers." BRD4 and other BET proteins are involved in NFκB-dependent promoter and superenhancer modulation. BRD4 binds nonhistone proteins and modulates their activity. BRD4 binds acetylated RELA, an NFκB coactivator, increasing its transcriptional transactivation activity and stability in the nucleus.[296]

Bromodomain adjacent to zinc finger 2B (BAZ2B) is a multidomain histone-binding protein that contains two histone reader modules, a plant homeodomain (PHD) and a bromodomain (BRD), linked by a largely disordered linker. The PHD domain is specific for the unmodified N-terminus of histone H3 and of the BRD domain for H3 acetylated at Lys14 (H3K14ac). BAZ2B PHD-BRD establishes a polyvalent interaction with H3K14ac, and the disordered interdomain linker modulates the histone-binding affinity by interacting with the PHD domain. Phosphorylation, acetylation, or poly(ADP-ribosyl)ation of the linker residues may therefore act as a cellular mechanism to transiently tune BAZ2B histone-binding affinity.[292]

ATPase family AAA domain-containing protein 2 (ATAD2), isoform A, is a bromodomain-containing protein overexpressed in many types of cancer.[297]

The heat shock response is characterized by transcriptional activation of both hsp genes and noncoding and repeated satellite III DNA sequences located at pericentric heterochromatin. Both events are under the control of heat shock factor I (HSF1). HSF1 recruits major cellular acetyltransferases, GCN5, TIP60, and p300 to pericentric heterochromatin leading to a targeted hyperacetylation of pericentric chromatin. Redistribution of histone acetylation toward the pericentric region in turn directs the recruitment of bromodomain and extraterminal (BET) proteins BRD2, BRD3, BRD4, which are required for satellite III transcription by RNAP II.[298]

BET proteins regulate the expression of inflammatory genes. Both proliferation and IgG production are reduced by BET inhibitors (JQ1) in a concentration-dependent manner, as well as immunoglobulin gene transcription.[299] The bromodomain protein BRD4 has been identified as an integral member of the oxidative stress as well as the inflammatory response, mainly due to its role in the transcriptional regulation process. BRD4 is also involved in the splicing process. There is an increase in splicing inhibition—in particular, intron retentions (IRs)—following heat treatment in BRD4-depleted cells, leading to a decrease of mRNA abundancy of affected transcripts, most likely due to premature termination codons. BRD4 interacts with heat shock factor 1 (HSF1) such that under heat stress BRD4 is recruited to nuclear stress bodies and noncoding SatIII RNA transcripts are upregulated. BRD4 is an important regulator of splicing during heat stress.[300]

1.2.3.6.7 UHRF1

UHRF1 is a mediator of inheritance of epigenetic DNA methylation patterns during cell division. Interdomain interactions influence UHRF1's chromatin-binding properties. Houliston et al.[301] characterized the dynamics of the tandem tudor domain-plant homeodomain (TTD-PHD) histone reader module, including its 20-residue interdomain linker. The apo TTD-PHD module in solution comprises a dynamic ensemble of conformers, approximately half of which are compact conformations, with the linker lying in the TTD peptide-binding groove. These compact conformations are amenable to cooperative, high-affinity histone binding. These authors also identified a compound, 4-benzylpiperidine-1-carboximidamide, which binds to the TTD groove, competes with linker binding, and promotes open TTD-PHD conformations that are less efficient at H3K9me3 binding. These studies show a mechanism by which the dynamic TTD-PHD module can be allosterically targeted with small molecules to modulate its histone reader function for therapeutic purposes.

UHRF1 targets newly replicated DNA by cooperatively binding hemimethylated DNA and H3K9me2/3. There is a direct recruitment of UHRF1 by the replication machinery via DNA ligase 1 (LIG1). A histone H3K9-like mimic within LIG1 is methylated by G9a and GLP and avidly binds UHRF1. Interaction with methylated LIG1 promotes the recruitment of UHRF1 to DNA replication sites and is required for DNA methylation maintenance.[302]

1.2.3.6.8 Plant Homeodomain (PHD) Fingers

Plant homeodomain (PHD) fingers are among the largest family of epigenetic domains, first characterized as readers of methylated H3K4.[303]

1.2.3.6.9 HDACs

The chromatin-remodeling factor HDAC4 regulates satellite cell proliferation and commitment. The proliferation and differentiation of *HDAC4* KO satellite cells are compromised, and inhibition of HDAC4 in satellite cells blocks the differentiation process. *P21* and *Sharp1* are the HDAC4 target genes.[304] HDAC9 is a novel suppressing factor involved in AGT regulation in proximal tubular cells, leading to low levels of intrarenal AGT in females.[305]

1.2.3.6.10 Chromatin-Remodeling Factors

ATRX. ATRX is a chromatin-remodeling factor found in a wide range of tandemly repeated sequences including telomeres (TTAGGG)n. *ATRX* mutations are found in tumors that maintain their telomeres via alternative lengthening of the telomere (ALT) pathway, which is suppressed by ATRX. Recruitment of ATRX to telomeric repeats depends on repeat number, orientation, and critically on repeat transcription. Loss of *ATRX* is also associated with increased R-loop formation. The presence of ATRX at telomeres may have a central role in suppressing deleterious DNA secondary structures that form at transcribed telomeric repeats.[306]

NUPR1. The protein NUPR1 is a multifunctional intrinsically disordered protein (IDP) involved in chromatin remodeling and in the development and progression of pancreatic cancer. Polycomb proteins are involved in specific transcriptional cascades and gene silencing. One of the proteins of the Polycomb complex is the Ring finger protein 1 (RING1). RING1 is related to aggressive tumor features in multiple cancer types. NUPR1 interacts with the paralog RING1B through the C-terminal region of RING1B (C-RING1B). This interaction is inhibited by trifluoperazine, a drug known to hamper binding of wild-type NUPR1 with other proteins. NUPR1 may play an active role in chromatin remodeling and carcinogenesis, together with Polycomb proteins.[307]

LSH. Lsh is a chromatin-remodeling factor that regulates DNA methylation and chromatin function in mammals. Lsh assists gene repression upon binding to the *Oct4* promoter region. Upon differentiation, association of Lsh promotes transcriptional repression of the reporter gene accompanied by an increase in repressive histone marks and a gain of DNA methylation at distal and proximal Oct4 enhancer sites.[308]

SMARCAD1. Chromatin in embryonic stem cells (ESCs) exhibits a more open chromatin configuration than in somatic cells. ATP-dependent chromatin-remodeling complexes are important regulators of ESC homeostasis. Depletion of the remodeler SMARCAD1, an ATPase of the SNF2 family, affects stem cell state. KRAB-associated protein 1 (KAP1) is the stoichiometric binding partner of SMARCAD1 in ESCs. This interaction occurs in chromatin when SMARCAD1 binds to different classes of KAP1 target genes, including zinc finger protein (ZFP) and imprinted genes. The RING B-box coiled-coil (RBCC) domain in KAP1 and the proximal coupling of ubiquitin conjugation to ER degradation (CUE) domain in SMARCAD1 mediate their direct interaction. Retention of SMARCAD1 in the nucleus depends on KAP1; and mutations in the CUE1 domain of *SMARCAD1* alter the binding to KAP1. An intact CUE1 domain is required for tethering this remodeler to the nucleus.[309]

1.2.3.6.11 Heterochromatin and Gene Silencing

Heterochromatic DNA domains regulate gene expression and maintain genome stability by silencing repetitive DNA elements and transposons. Heterochromatin assembly at DNA repeats involves the activity of small noncoding RNAs (sRNAs) associated with the RNA interference (RNAi) pathway. sRNAs, originating from long noncoding RNAs, guide Argonaute-containing effector complexes to complementary nascent RNAs to initiate histone H3 lysine 9 dimethylation and trimethylation (H3K9me2, H3K9me3) and the formation of heterochromatin. H3K9me is in turn required for the recruitment of RNAi to chromatin to promote the amplification of sRNA. H3K9me2 defines a functionally distinct heterochromatin state that is sufficient for RNAi-dependent cotranscriptional gene silencing at pericentromeric DNA repeats. Unlike H3K9me3 domains, which are transcriptionally silent, H3K9me2 domains are transcriptionally active, contain modifications associated with euchromatic transcription, and couple RNAi-mediated transcript degradation to the establishment of H3K9me domains. The two H3K9me states recruit reader proteins with different efficiencies, explaining their different downstream silencing functions. The transition from H3K9me2 to H3K9me3 is required for RNAi-independent epigenetic inheritance of H3K9me domains. These studies reported by Jih et al.[310] demonstrate that H3K9me2 and H3K9me3 define functionally distinct chromatin states and uncover a mechanism for the formation of transcriptionally permissive heterochromatin that is compatible with its broadly conserved role in sRNA-mediated genome defense.

1.2.3.6.12 MEN1

Men1 is a tumor suppressor gene that encodes the protein Menin, with effect in the control of epigenetic gene regulation. Menin interaction with the MLL complex favors transcriptional activation of target genes through

H3K4me3 marks. Menin represses gene expression via mechanisms involving the Polycomb repressing complex (PRC). Ezh2, the PRC-methyltransferase that catalyzes H3K27me3 repressive marks, and Menin have been shown to cooccupy a large number of promoters. ActivinB, a TGFβ superfamily member encoded by the *Inhbb* gene, is upregulated in insulinoma tumors caused by Men1 invalidation. Menin may participate in the epigenetic repression of *Inhbb* gene expression. Loss of Menin is associated with ActivinB-induced expression. ActivinB expression is mediated through direct modulation of H3K27me3 marks on the *Inhbb* locus in Menin-KO cell lines. Menin binds on the promoter of *Inhbb* gene where it favors the recruitment of Ezh2 via an indirect mechanism involving Akt-phosphorylation. Menin may affect the Ezh2-epigenetic repressive landscape by modulating Akt phosphorylation.[311]

1.2.3.6.13 NPC, HOXA, and Nup93

The nuclear pore complex (NPC) mediates nuclear transport of RNA and proteins into and out of the nucleus. Nucleoporins have additional functions in chromatin organization and transcription regulation. Nup93 is a scaffold nucleoporin at the nuclear pore complex that is associated with human chromosomes 5, 7, and 16 and with the promoters of the *HOXA* gene. Labade et al.[312] studied the association of Nup93 with the *HOXA* gene cluster and its consequences on *HOXA* gene expression in diploid colorectal cancer cells (DLD1). Nup93 shows a specific enrichment ~1 Kb upstream of the transcription start site of each of the *HOXA1, HOXA3*, and *HOXA5* promoters. The association of Nup93 with HOXA is assisted by its interacting partners Nup188 and Nup205. Depletion of the Nup93 subcomplex upregulates *HOXA* gene expression levels. Nup93 may repress the *HOXA* gene cluster. *Nup93* knockdown increases active histone marks (H3K9ac), decreases repressive histone marks (H3K27me3) on the *HOXA1* promoter, and increases transcription elongation marks (H3K36me3) within the *HOXA1* gene. The nucleoporin Nup93 assisted by its interactors Nup188 and Nup205 mediates the repression of *HOXA* gene expression.[312]

1.2.3.6.14 CCR4

The multisubunit CCR4 (carbon catabolite repressor 4)-NOT (Negative on TATA) complex serves as a central coordinator of all different steps of eukaryotic gene expression. The CCR4-NOT subunits CNOT1, CNOT2, and CNOT3 are individually downregulated using doxycycline-inducible shRNAs. Downregulation of any of the CNOT subunits results in elevated expression of major histocompatibility complex class II (*MHC II*) genes which are found in a gene cluster on chromosome 6. CNOT2-mediated repression of *MHC II* genes occurs also in the absence of the master regulator class II transactivator (CIITA) and does not cause detectable changes in the chromatin structure at the chromosomal MHC II locus. CNOT2 downregulation results in an increased de novo transcription of mRNAs, whereas tethering of CNOT2 to a regulatory region governing MHC II expression results in diminished transcription. CNOT proteins are a novel group of corepressors restricting class II expression.[313]

1.2.3.6.15 HP1

Chromatin proteins control gene activity in a concerted manner. Recruitment to over 1000 genomic locations revealed that HP1a is a potent repressor able to silence even highly expressing reporter genes. The local chromatin context can modulate HP1a function. In pericentromeric regions, HP1a-induced repression is enhanced twofold. In regions marked by a H3K36me3-rich chromatin signature, HP1a-dependent silencing is significantly decreased.[314]

1.2.3.6.16 TORC1

The conserved nutrient-regulated target of rapamycin complex 1 (TORC1) pathway and the histone H3N-terminus at lysine 37 (H3K37) function collaboratively to restrict specific chromatin-binding high mobility group box (HMGB) proteins to the nucleus to maintain cellular homeostasis and viability. Reducing TORC1 activity in an *H3K37* mutant causes cytoplasmic localization of the HMGB Nhp6a, organelle dysfunction, and both nontraditional apoptosis and necrosis. Under nutrient-rich conditions the *H3K37* mutation increases basal TORC1 signaling. This effect is prevented by deletion of the genes encoding HMGBs whose cytoplasmic localization increases when TORC1 activity is repressed. TORC1 and histone H3 collaborate to retain HMGBs within the nucleus to maintain cell homeostasis and promote longevity.[315]

1.2.3.6.17 WDR5

WDR5 is a highly conserved WD40 repeat-containing protein that is essential for proper regulation of multiple cellular processes. WDR5 is a core scaffolding component of histone methyltransferase complexes and is potentially involved in controlling the integrity of cell division.[316]

1.2.3.6.18 Nudt21

Cell fate transitions involve rapid gene expression changes and global chromatin remodeling. The RNA-processing factor Nudt21 controls cell fate by connecting alternative polyadenylation to chromatin signaling. Suppression of Nudt21 enhances the generation of induced pluripotent stem cells, facilitates transdifferentiation into trophoblast stem cells, and impairs differentiation of myeloid precursors and embryonic stem cells. Nudt21 directs differential polyadenylation of over 1500 transcripts in cells acquiring pluripotency. These proteins are strongly enriched for chromatin regulators, and their suppression neutralizes the effect of Nudt21 during reprogramming.[317]

1.2.3.6.19 Trithorax-Group Proteins

Trithorax-group proteins (TrxGs) play essential regulatory roles in chromatin modification for activation of transcription. In *Arabidopsis*, TrxGs function in the dehydration and abscisic acid (ABA)-mediated modulation of downstream gene expression. Two evolutionarily conserved *A. thaliana* TrxGs, ATX4 and ATX5, play essential roles in the drought stress response. ATX4 and ATX5 regulate the expression of genes involved in dehydration stress.[318]

1.2.3.6.20 SUUR Protein

In eukaryotes, heterochromatin replicates late in the S phase of the cell cycle and contains specific covalent modifications of histones. *SUUR* mutation found in *Drosophila* makes heterochromatin replicate earlier than in the wild type and reduces the level of repressive histone modifications. SUUR protein associates with moving replication forks through interactions with PCNA. *SUUR*-sensitive chromosomal regions do not contain Polycomb and require SUUR function to sustain the H3K27me3 level. SUUR protein contributes to heterochromatin maintenance during chromosome replication.[319]

1.2.3.6.21 Kap123

Kap123 is a major karyopherin protein of budding yeast that recognizes the nuclear localization signals (NLSs) of cytoplasmic histones H3 and H4 and translocates them into the nucleus during DNA replication. Cytoplasmic histone H4 diacetylation weakens the Kap123-H4-NLS interaction, thereby facilitating histone Kap123-H3-dependent H3-H4/Asf1 complex nuclear translocation.[320]

1.2.3.6.22 SET Complex

Chromatin-modifying complexes are targeted to the appropriate gene promoters in vertebrates. The SET1 complex is targeted to actively transcribed gene promoters through CFP1, which engages in a form of multivalent chromatin reading that involves recognition of nonmethylated DNA and histone H3 lysine 4 trimethylation (H3K4me3). CFP1 defines SET1 complex occupancy on chromatin, and its multivalent interactions are required for the SET1 complex to place H3K4me3. According to studies reported by Brown et al.,[321] in the absence of CFP1, gene expression is perturbed, suggesting that normal targeting and functioning of the SET1 complex are central to creating an appropriately functioning vertebrate promoter-associated epigenome.

1.2.3.6.23 Histone Code Reader Spin1

The histone code reader Spin1 is associated with tumorigenesis, cancer growth, and physiological functions. In *Spin1M5* mice with ablation of *Spin1* in myoblast precursors using the Myf5-Cre deleter strain, *Spin1M5* mice die after birth with severe sarcomere disorganization and necrosis. Surviving *Spin1M5* mice are growth-retarded and show defects in several muscles as a result of aberrant fetal myogenesis and deregulated skeletal muscle (SkM) functional networks. Deregulation of helix-loop-helix transcription factor networks appears to be responsible for developmental defects in *Spin1M5* fetuses. Aberrant expression of titin-associated proteins, abnormal glycogen metabolism, and neuromuscular junction defects contribute to SkM pathology in *Spin1M5* mice.[322]

1.2.3.6.24 Proteasome

The proteasome displays proteolytic and nonproteolytic functions that are essential in the regulation of cell activity. The 19S proteasome mediates heterochromatin spreading of centromeric heterochromatin in a nonproteolytic manner. The 19S proteasome is involved in regulating subtelomere silencing and facultative heterochromatin formation in fission yeast, and through a distinct pathway regulates subtelomere silencing and facultative heterochromatin formation through the Paf1 complex subunit Leo1, indicating that the proteasome is involved in global regulation of facultative and constitutive heterochromatin.[323]

Nonproteolytic functions of the proteasome are involved in transcriptional regulation, mRNA export, and ubiquitin-dependent histone modification. Seo et al.[323, 324] identified the mutant allele *rpt4-1* that disrupts a nonproteolytic function of the proteasome. The proteasome is involved in the regulation of heterochromatin spreading to prevent its uncontrolled invasion into neighboring euchromatin regions. The phenotype of the nonproteolytic *rpt4-1* mutant resembles that of epe1Δ cells, which lack the Epe1 protein that counteracts heterochromatin spreading. Both mutants exhibit variegated gene-silencing phenotypes across yeast colonies, spreading of heterochromatin, bypassing the requirement for RNAi in heterochromatin formation at the outer repeat region (otr), and upregulation of RNA polymerase II. Mst2 is another factor that antagonizes heterochromatin spreading, showing a redundant function with Rpt4. The 19S proteasome may be involved in modulating the activities of Epe1 and Mst2.[324]

Cellular models of memory formation have focused on the need for protein synthesis. Protein degradation mediated by the ubiquitin-proteasome system (UPS) also appears to be relevant for this process. Nonproteolytic ubiquitin-proteasome signaling is involved in histone modifications and DNA methylation, suggesting that ubiquitin and the proteasome can regulate chromatin remodeling independent of protein degradation. Both ubiquitin signaling and the proteasome can act independently to regulate epigenetic-mediated transcriptional processes necessary for learning-dependent synaptic plasticity.[325]

1.2.3.6.25 Lipid Mediators

Phospholipids, sphingolipids, and cholesterol are integral components of cell organelles and the nucleus. Nuclear lipid composition is distinct from that of the cytoplasm and plasma membrane. Nuclear sphingolipids, sphingoid bases (sphingosine, ceramide, sphingosine-1-phosphate (S1P)), and sphingolipid signaling are involved in physiological and pathological conditions. S1P is generated in the nucleus by phosphorylation of SphK2 and modulates HDAC activity to regulate cell cycle and proinflammatory gene expression.[326]

Lipid-derived acetyl-CoA is a major source of carbon for histone acetylation. Up to 90% of acetylation on histone lysines can be derived from fatty acid carbon, even in the presence of excess glucose. By repressing both glucose and glutamine metabolism, fatty acid oxidation reprograms cellular metabolism, leading to increased lipid-derived acetyl-CoA. Gene expression profiling of octanoate-treated hepatocytes shows a pattern of upregulated lipid metabolic genes, demonstrating a specific transcriptional response to lipid.[327]

1.2.3.6.26 Transposable Elements

Transposable elements (TEs) comprise nearly half the human genome and play an essential role in the maintenance of genomic stability, chromosomal architecture, and transcriptional regulation. TEs are repetitive sequences consisting of RNA transposons, DNA transposons, and endogenous retroviruses that can invade the human genome making a substantial contribution to human evolution and genomic diversity. TEs are therefore firmly regulated from early embryonic development and during the entire course of human life by epigenetic mechanisms—in particular, DNA methylation and histone modifications.

The genome is enrolled in the generation of different epigenomic landscapes that define each cell type. These epigenomic profiles can be deregulated under disease conditions. About 80% of human DNA is biochemically active and approximately 10%–15% displays signals of purifying selection. TEs make up at least 50% of the human genome and can be actively transcribed. TEs can also act as regulatory elements either for their own purposes or to be coopted for the benefit of their host. TEs contribute to the functional genome, and coopted TEs can be differentiated from noisy genomic elements.[328] The deregulation of TEs has been reported in some developmental diseases, as well as for different types of human cancers.[329]

TEs were initially conceived as genomic parasites with the ability to mobilize and replicate themselves in a genome. Mammalian genomes are dominated by thousands of TEs that impact on mammalian evolution. Most genomes are dominated by LINE and SINE retrotransposons, more limited LTR retrotransposons, and minimal DNA transposon accumulation. The mammalian genome contains at least one family of actively accumulating retrotransposons. Horizontal transfer of TEs among lineages is rare. TE exaptation events are relatively frequent and, despite beneficial aspects of TE content and activity, the majority of TE insertions are neutral or deleterious. The genome has evolved several defense mechanisms that act at the epigenetic, transcriptional, and posttranscriptional levels to limit the deleterious effects of TE proliferation.[330] TEs have beneficial roles in the evolution of diverse biological processes and as sources of selectable phenotypic variation.[331]

The first genome-wide quantification of TEs in *Drosophila melanogaster* and *Drosophila simulans* revealed that the spread of repressive epigenetic marks (histone H3K9me2) to nearby DNA occurs in over 50% of euchromatic TEs, extending up to 20 kb. The lower TE content correlates with the stronger epigenetic effects of TEs and higher levels of host genetic factors known to promote epigenetic silencing.[332]

Human endogenous retroviruses (HERVs) and other long terminal repeat (LTR)-type retrotransposons (HERV/LTRs) have regulatory elements that possibly influence the transcription of host genes. A total of 794,972 HERV-TFBSs have been identified. HERV/LTR-shared regulatory element (HSRE), defined as a TF-binding motif in HERV-TFBSs, shares fractions of a HERV/LTR type. A total of 2201 HSREs, comprising specific associations of 354 HERV/LTRs and 84 TFs, have been identified by Ito et al.[333] HERV/LTRs can be grouped according to TF binding patterns; HERV/LTR groups bind to pluripotent TFs (SOX2, POU5F1, NANOG), embryonic endoderm/mesendoderm TFs (GATA4/6, SOX17, FOXA1/2), hematopoietic TFs (SPI1 (PU1), GATA1/2, TAL1), and CTCF. Regulatory elements of HERV/LTRs tend to locate nearby and/or interact three-dimensionally with the genes involved in immune responses, indicating that the regulatory elements play an important role in controlling the immune regulatory network. Subgroup-specific TF binding within LTR7, LTR5B, and LTR5_Hs indicates that gain or loss of regulatory elements might occur during genomic invasions of HERV/LTRs.[333]

Repetitive genomic regions include tandem sequence repeats and interspersed repeats, such as endogenous retroviruses and LINE-1 elements. Repressive heterochromatin domains silence the expression of these sequences through mechanisms that remain poorly understood. The retinoblastoma protein (pRB) utilizes a cell-cycle-independent interaction with E2F1 to recruit Enhancer of zeste homolog 2 (EZH2) to diverse repeat sequences. These include simple repeats, satellites, LINEs, and endogenous retroviruses as well as transposon fragments. A mutant mouse strain carrying an F832A mutation in *Rb1* is defective for recruitment to repetitive sequences. Loss of pRB-EZH2 complexes from repeats disperses H3K27me3 from these genomic locations and permits repeat expression. Consistent with maintenance of H3K27me3 at the *Hox* clusters, these mice are developmentally normal.[334]

Variation in the presence or absence of transposable elements (TEs) is a major source of genetic variation between individuals. Stuart et al.[335] identified 23,095 TE presence/absence variants between 216 *Arabidopsis* accessions. Most TE variants were rare and associated with local extremes of gene expression and DNA methylation levels within the population. Of the common alleles identified, two-thirds were not in linkage disequilibrium with nearby SNPs, implicating these variants as a source of novel genetic diversity. Many common TE variants were associated with significantly altered expression of nearby genes, and a major fraction of interaccession DNA methylation differences were associated with nearby TE insertions.

The hallmark of retrogenes in the genome is the presence of DCCGTAGCCATTTTGGCTCAAG, a spliced leader (DinoSL) constitutively *trans*-spliced to the 5′ end of all nucleus-encoded mRNAs. Although retrogenes have often lost part of the 22-nt DinoSL, the putative promoter motif from the DinoSL, TTT(T/G), is consistently retained in the upstream region of these genes, providing an explanation for the high survival rate of retrogenes in dinoflagellates.[336] DNA methylation drives origination, survival, evolution, and expression of retrogenes.[337] Studies in *Sus scrofa* identified a total of 964 retrocopies as well as new retrocopies for the synthesis of glycans and lipids corresponding to phenotypic traits in pigs. Retrogene DNA methylation negatively correlates with evolutionary time and regulates retrogene tissue-specific expression patterns. Retrogenes are consistently hypermethylated and hypomethylation of parental genes shows higher susceptibility to retroposition.[337]

Repetitive DNA, represented by transposons and satellite DNA, constitutes a large portion of eukaryotic genomes, being the major component of constitutive heterochromatin. This genomic component regulates several nuclear functions including chromatin state and the proper functioning of centromeres and telomeres. In *Drosophila* the 1.688 satellite is one of the most abundant repetitive sequences, with the longest array being located in the pericentromeric region of the X chromosome. Short arrays of 1.688 repeats are widespread within the euchromatic part of the X chromosome, and these arrays assist in recognition of the X chromosome by the dosage compensation male-specific lethal complex. A short array of 1.688 satellite repeats is essential for recruitment of the protein POF to a site on the X chromosome (PoX2) and to various transgenic constructs. The 1.688 array promotes POF targeting to the roX1-proximal PoX1 site in trans. Binding of POF to the 1.688-related satellite-enriched sequences is conserved in evolution. Kim et al.[338] postulate that the 1.688 satellite functions in an ancient dosage compensation system involving POF targeting at the X chromosome.

Sleeping Beauty transposon (SB) has become an increasingly important genetic tool for generating mutations in vertebrate cells. It was widely thought that SB exclusively integrates into TA dinucleotides. However, recent studies indicate that TA dnts are not exclusive integrating sites for SBs. Guo et al.[339] identified 28,000 SB insertions in non-TA sites. The consensus sequence of these non-TA sites shows an asymmetric pattern distinct from the symmetric pattern of the canonical TA sites. Perfect similarity between the downstream flanking sequence and SB transposon ends indicates there may be interaction between the transposon DNA binding domain of transposase and the target DNA. The SB integrations at non-TA sites might be guided by the interaction between the transposon DNA binding domain of SB transposase and the target DNA.[339]

tRNA-derived small RNAs participate in genome protection against retrotransposons. tRNAs are involved in the replication cycle of retroviruses, pararetroviruses, and retrotransposons as primers of their reverse transcription. tRNA-derived small RNAs, as functional small RNAs or as mere tRNA degradation products, have emerged as important players in the regulation of genic transcription.[340]

Long Interspersed Nuclear Element-1 (*LINE-1*), the most ubiquitous repetitive element in mammalian genomes, plays an important role in the pathogenesis of disease and in the response to exposure to environmental stressors. Ionizing radiation is a genotoxic stressor that induces alterations in *LINE-1* DNA methylation.[341]

Intracisternal A particle (*IAP*) is one of the most transpositionally active retrotransposons in the mouse genome, with a great expression viariability among cell types as a result of differences in the methylation status of the 5′ long terminal repeat (LTR), where transcription starts. There is subfamily- and locus-specific hypomethylation of *IAP* LTRs. Binding of TFs might be involved in protection from DNA methylation, whereas the *IAP* internal sequence might enhance methylation.[342]

In mouse prospermatogonia, PIWI-interacting small RNAs (piRNAs) combat retrotransposon activity to maintain genomic integrity. The piRNA system destroys retrotransposon-derived RNAs and guides de novo DNA methylation at some retrotransposon promoters. Inoue et al.[343] performed studies of DNA methylation and *polyA*$^+$ RNAs (transcriptome) in developing male germ cells from *Pld6/Mitopld* and *Dnmt3l* knockout mice defective in piRNA biogenesis and de novo DNA methylation, respectively, and found that *Dnmt3l* mutation reduced DNA methylation levels at most retrotransposons. In *Pld6* mutant germ cells, although only a few retrotransposons exhibited reduced DNA methylation, many showed increased expression at the RNA level. The increase in retrotransposon expression was larger in *Pld6* mutants than in *Dnmt3l* mutants, suggesting that RNA degradation by the piRNA system plays a more important role than does DNA methylation in prospermatogonia, but DNA methylation has a long-term effect. Hypomethylation caused by the *Pld6* or *Dnmt3l* mutation results in increased retrotransposon expression in meiotic spermatocytes. Posttranscriptional silencing plays an important role in the early stage of germ cell development, and transcriptional silencing becomes important in later stages. Retrotransposon silencing is important for the maintenance of genomic and transcriptomic integrity.[343]

Piwi proteins and piRNAs protect eukaryotic germlines against the spread of transposons. During development in the ciliate *Paramecium*, two Piwi-dependent sRNA classes are involved in the elimination of transposons and transposon-derived DNA: scan RNAs (scnRNAs), associated with Ptiwi01 and Ptiwi09, and iesRNAs, whose binding partners are Ptiwi10 and Ptiwi11. scnRNAs derive from the maternal genome and initiate DNA elimination during development, whereas iesRNAs continue DNA targeting until the removal process is complete. Furrer et al.[344] showed that scnRNAs and iesRNAs are processed by distinct Dicer-like proteins and bind Piwi proteins in a mutually exclusive manner, suggesting separate biogenesis pathways. The *PTIWI10* gene is transcribed from the developing nucleus and its transcription depends on prior DNA excision, suggesting a mechanism of gene expression control triggered by the removal of short DNA segments interrupting the gene.

Alu elements belong to the short interspersed nuclear element (*SINE*) family of repetitive elements, and with over 1 million insertions they make up more than 10% of the human genome. Alu elements can be mutagenic to the host as they can act as splice acceptors, inhibit the translation of mRNAs, and cause genomic instability. Alu elements are the main targets of the RNA-editing enzyme ADAR, and the formation of Alu exons is suppressed by the nuclear ribonucleoprotein HNRNPC. DHX9, a nuclear RNA helicase, binds specifically to inverted-repeat Alu elements that are transcribed as parts of genes. Loss of DHX9 leads to an increase in the number of circular-RNA-producing genes and the amount of circular RNAs, translational repression of reporters containing inverted-repeat Alu elements, and transcriptional rewiring of susceptible loci. The interferon-inducible isoform of ADAR (p150), but not the constitutively expressed ADAR isoform (p110), is an RNA-independent interaction partner. Codepletion of ADAR and DHX9 increases double-stranded RNA accumulation defects, leading to increased circular RNA production, revealing a functional link between these two enzymes. Based on these studies, Aktaş et al.[345] proposed that DHX9 acts as a nuclear RNA resolvase that neutralizes the immediate threat posed by transposon insertions and allows these elements to evolve as tools for the posttranscriptional regulation of gene expression.

LTR retrotransposons are repetitive DNA elements comprising approximately 10% of the human genome. They are silenced by hypermethylation of cytosines in CpG dinucleotides and are considered parasitic DNA serving no useful function for the host genome. However, hypermethylated *LTRs* contain enhancer and promoter sequences and can promote tissue-specific transcription of *cis*-linked genes. The *ERV-9 LTR* retrotransposon is located at the 5′ border of the transcriptionally active β-globin gene locus in human erythroid progenitor and erythroleukemia K562 cells. The *ERV-9 LTR*, containing 65 CpGs in 1.7 kb DNA, is hypermethylated (>90% CpGs) and displays transcriptional enhancer activity. The hypermethylated *LTR* enhancer spanning recurrent CCAATCG and GATA motifs associate with key transcription factors (TFs) NF-Y and GATA-1 and -2, respectively. Hypermethylation reduces the binding

affinities of the enhancer motifs to the key TFs to assemble the *LTR-Pol II* transcription complex that activates the transcription of *cis*-linked genes at reduced efficiency.[346]

Ten-eleven translocation (TET) enzymes oxidize DNA methylation as part of an active demethylation pathway. There is a complex relationship between ten-eleven translocation (TET) proteins and retrotransposons in mouse embryonic stem cells (ESCs), implicating TETs as enhancers in the exaptation and function of retroelement sequences. TET1 and TET2 bind multiple TE classes that harbor a variety of epigenetic signatures indicative of different functional roles. TETs cobind with pluripotency factors to enhancer-like TEs that interact with highly expressed genes in ESCs whose expression is partly maintained by TET2-mediated DNA demethylation. TETs and 5-hydroxymethylcytosine (5hmC) are also strongly enriched at the 5′ UTR of full-length, evolutionarily young *LINE-1* elements, a pattern that is conserved in human ESCs. TETs drive *LINE-1* demethylation, but *LINE-1s* are kept repressed through additional TET-dependent activities. The SIN3A corepressive complex binds to *LINE-1s*, ensuring their repression in a TET1-dependent manner. Active demethylation of retrotransposons does not correlate with their increased expression in ESCs, calling into question long-held assumptions regarding the importance of DNA demethylation for retrotransposon expression, and revealing novel epigenetic players in retrotransposon control.[347, 348]

Aberrant expression of coding genes or long noncoding RNAs (lncRNAs) with oncogenic properties can be caused by translocations, gene amplifications, point mutations, or other less characterized mechanisms. One such mechanism is the inappropriate usage of normally dormant, tissue-restricted, or cryptic enhancers or promoters that serve to drive oncogenic gene expression. Dispersed across the human genome, endogenous retroviruses (ERVs) provide an enormous reservoir of autonomous gene regulatory modules, some of which have been coopted by the host during evolution to play important roles in the normal regulation of genes and gene networks.[349] Human endogenous retroviruses (HERVs) constitute 8% of the human genome and contribute substantially to the transcriptome. HERVs generate RNAs that modulate host gene expression.

Broecker et al.[350] characterized the HERV-K (*HML-10*) endogenous retrovirus family which invaded the ancestral genome of Old World monkeys about 35 million years ago and is enriched within introns of human genes when compared to other HERV families. Long terminal repeats (LTRs) of *HML-10* exhibit variable promoter activity in human cancer cell lines. One identified *HML-10* LTR-primed RNA was in opposite orientation to the proapoptotic Death-associated protein 3 (DAP3). Inactivation of *HML-10* LTR-primed transcripts induces DAP3 expression levels, which leads to apoptosis. *HML-10* may have been evolutionarily coopted for gene regulation more than other HERV families. *HML-10* RNA suppresses DAP3-mediated apoptosis, and its upregulation in various tumors may contribute to evasion of apoptosis in malignant cells.[350]

A common aberration in cancer is the activation of germline-specific proteins. The DNA-binding proteins among them could generate novel chromatin states, not found in normal cells. The germline-specific transcription factor BORIS/CTCFL, a paralog of chromatin architecture protein CTCF, is often erroneously activated in cancers and rewires the epigenome for the germline-like transcription program. Another common feature of malignancies is the changed expression and epigenetic states of genomic repeats, which could alter the transcription of neighboring genes and cause somatic mutations upon transposition. BORIS serves as a repressor of SVA expression, alongside DNA and histone methylation, with the exception of promoter capture by SVA. The global germline-specific transcriptional regulator BORIS directly binds to and regulates *SVA* repeats, which are essentially movable CpG islands, via clusters of BORIS binding sites. BORIS regulates and represses the newest class of transposable elements that are actively transposed in the human genome when activated.[351]

Hippocampal retrotransposon (RT) elements are regulated by acute stress via the accumulation of the repressive H3K9me3 mark at RT loci. Dysregulation of RT expression is predicted to result in functional deficits in affected brain areas. Transposons may have a variety of adaptive functions.[352]

TEs are active in mammalian oocytes and early embryos, and this activity, albeit counterintuitive because TEs can lead to genomic instability in somatic cells, correlates with successful development. TEs bridge genetic and epigenetic landscapes because TEs are genetic elements whose silencing and derepression are regulated by epigenetic mechanisms that are sensitive to environmental factors. Transposition events can change the size, content, and function of mammalian genomes.[353]

Tumor suppressor protein 53 (p53) plays a central role in the control of genome stability, acting primarily through the transcriptional activation of stress-response genes. However, many p53 binding sites are located at genomic locations with no obvious regulatory link to known stress-response genes. Lieberman[354] discovered p53 binding sites within retrotransposon-derived elements in human and mouse subtelomeres. These retrotransposon-derived p53 binding sites protect chromosome ends through transcription activation of telomere repeat RNA, as well as through the direct modification of local chromatin structure in response to DNA damage. A class of p53 binding sites, including

the retrotransposon-derived p53 sites found in subtelomeres, provide a primary function in genome stability by mounting a direct and local protective chromatin response to DNA damage.

1.2.3.6.27 Intragenic Enhancers

Enhancers are *cis*-regulatory genetic elements crucial for controlling temporal and cell type-specific patterns of gene expression. Active enhancers generate bidirectional noncoding RNA transcripts called enhancer RNAs (eRNAs). eRNAs are important for stimulating gene expression. There is a direct interaction between RNAs and the transcriptional coactivator Creb-binding protein (CBP). RNA binding could stimulate the core histone acetyltransferase activity of the enzyme, observable in cells as a link between eRNA production, CBP-dependent histone acetylation, and expression of genes regulated by specific enhancers. According to Bose and Berger,[355] by modulating the activity of chromatin-modifying enzymes, enhancers might directly impact transcription by altering the chromatin environment.

CBP/p300 are transcription coactivators whose binding is a signature of enhancers, *cis*-regulatory elements that control patterns of gene expression in multicellular organisms. Active enhancers produce bidirectional enhancer RNAs (eRNAs) and display CBP/p300-dependent histone acetylation. CBP binds directly to RNAs, and RNAs bound to CBP include a large number of eRNAs. An RNA binding region in the HAT domain of CBP, a regulatory motif unique to CBP/p300, allows RNA to stimulate CBP's HAT activity. At enhancers, where CBP interacts with eRNAs, stimulation manifests in RNA-dependent changes in the histone acetylation mediated by CBP, such as H3K27ac, and by corresponding changes in gene expression. By interacting directly with CBP, eRNAs contribute to the unique chromatin structure at active enhancers, which in turn is required for regulation of target genes.[356]

Eukaryotic gene transcription is regulated at many steps, including RNA polymerase II (Pol II) recruitment, transcription initiation, promoter-proximal Pol II pause release, and transcription termination. The enhancers that activate gene transcription undergo Pol II-mediated transcription. Transcription at intragenic enhancers interferes with and attenuates host gene transcription during productive elongation. The extent of attenuation correlates positively with nascent eRNA expression. Intragenic enhancers not only enhance transcription of one or more genes from a distance but also fine-tune transcription of their host gene through transcription interference, facilitating differential utilization of the same regulatory element for disparate functions.[357]

1.2.3.6.28 Genomic Regulatory Regions

The identification of genomic regulatory regions is an important issue to understand the roles of genomic variants in evolution, domestication, and animal production. A computational method to predict regulatory DNA sequences (promoters, enhancers, and transcription factor binding sites) in mammals has been developed.[358]

1.3 SINGULAR EPIGENETIC PHENOMENA

1.3.1 Aneuploidy

Aneuploidies are copy number variants that affect entire chromosomes. They are seen commonly in cancer, embryonic stem cells, human embryos, and in various trisomic diseases. Mosaic aneuploidy occurs when aneuploidies affect only a subset of cells. A cell that harbors an aneuploidy exhibits disrupted gene expression patterns that can alter its behavior.[359]

1.3.2 X Chromosome Inactivation

The nuclear long noncoding RNA *Xist* ensures X chromosome inactivation (XCI) in female placental mammals. X chromosome inactivation is a dosage compensation process that was adopted by female mammals to balance gene dosage between XX females and XY males. XCI starts with the upregulation of noncoding RNA *Xist*, after which most X-linked genes are silenced and acquire a repressive chromatin state.[360]

After the pioneering studies of Mary Lyon in 1961, it has been stated that in early development one of the major differences between XX female and XY male embryos is the conserved process of X chromosome inactivation, which compensates gene expression of the two female X chromosomes to match the dosage of the single X chromosome of males. Recent advances illustrate the control of X chromosome dosage compensation in early human embryonic development.[27, 361]

Xist is indispensable for X chromosome inactivation. Ectopic *Xist* expression faithfully recapitulates endogenous X chromosome inactivation from any location on the X chromosome, whereas long-range silencing of autosomal genes

is less efficient. Long interspersed elements facilitate inactivation of genes located far away from the *Xist* transcription locus, and genes escaping X chromosome inactivation show enrichment of CTCF on X chromosomal but not autosomal loci.[362]

Recruitment of the Polycomb repressive complexes PRC1 and PRC2 by *Xist* RNA is an important paradigm for chromatin regulation by long noncoding RNAs. The noncanonical Polycomb group RING finger 3/5 (PCGF3/5)-PRC1 complex initiates recruitment of both PRC1 and PRC2 in response to *Xist* RNA expression. PCGF3/5-PRC1-mediated ubiquitylation of histone H2A signals recruitment of other noncanonical PRC1 complexes and of PRC2, the latter leading to deposition of histone H3 lysine 27 methylation chromosome wide. *Pcgf3/5* gene knockout results in female-specific embryo lethality and abrogates *Xist*-mediated gene repression, highlighting a key role for Polycomb in *Xist*-dependent chromosome silencing.[363]

Vallot et al.[364] identified human-specific mechanisms regulating X chromosome activity in early embryonic development. There is coactivation and accumulation of the lncRNA *XACT* and *XIST* on active X chromosomes in both early human preimplantation embryos and naive human embryonic stem cells. *XIST* RNA adopts an unusual, highly dispersed organization, which may explain why it does not trigger X chromosome inactivation at this stage. *XACT* influences *XIST* accumulation in *cis* and it is very likely an antagonistic activity of *XIST* and *XACT* in controlling X chromosome activity in early human embryos.[364]

Xist is an lncRNA with capacity to trigger chromosome-wide gene silencing, the formation of facultative heterochromatin, and an unusual 3D conformation of the inactive X chromosome.[365]

Rett syndrome (RS) is a neurological disorder affecting mostly girls with heterozygous mutations in the gene encoding the methyl-CpG-binding protein MeCP2 on the X chromosome. Restoration of *MeCP2* expression in a mouse model reverses neurologic deficits in adult animals. In RS, reactivation of the wild-type copy of *MeCP2* on the inactive X chromosome (Xi) presents a therapeutic opportunity. To identify genes involved in *MeCP2* silencing, Sripathy et al.[366] screened a library of 60,000 shRNAs using a cell line with an *MeCP2* reporter on the Xi and found 30 genes clustered in seven functional groups. Over 50% of encoded proteins have known enzymatic activity, and six were members of the bone morphogenetic protein (BMP)/TGFβ pathway. shRNAs directed against each of these six genes downregulated X-inactive specific transcript (*XIST*), a key player in X chromosome inactivation that encodes an RNA that coats the silent X chromosome. Rnf12, an X-encoded ubiquitin ligase important for initiation of X chromosome inactivation and *XIST* transcription in ES cells, is active for the maintenance of the inactive state through regulation of BMP/TGFβ signaling.[366]

To equalize gene expression between the sexes and balance X and autosomal expression, sequential steps are required in *C. elegans*. Initially, there is an upregulation of the X chromosome in both sexes to balance the X to autosomal expression in males, creating X overexpression in hermaphrodites. Finally, to restore the balance, hermaphrodites downregulate gene expression twofold on both X chromosomes. The H4K16 histone acetyltransferase MYS-1/Tip60 mediates decondensation of male X chromosomes, and MYS-1 contributes only slightly to upregulation of gene expression on the X chromosome. The level of chromosome decondensation does not correlate with the degree of gene expression change. The X chromosome is more sensitive to MYS-1-mediated decondensation than the autosomes. H4K16ac levels weakly correlate with gene expression levels on both the X and the autosomes, but highly expressed genes on the X chromosome do not contain exceptionally high levels of H4K16ac. H4K16ac and chromosome decondensation influence regulation of the male X chromosome.[367]

Sex-biased gene expression is abundant in many species, although its extent may vary greatly among tissues or developmental stages. In species with genetic sex determination, sex chromosome-specific processes, such as dosage compensation, also may influence sex-biased gene expression. Sex-biased genes, especially those with male-biased expression, often show elevated rates of both protein sequence and gene expression divergence between species, which could have a number of causes, including sexual selection, sexual antagonism, and relaxed selective constraint.[368]

1.3.3 Meiotic Silencing

Some filamentous fungi, such as *Neurospora crassa*, show meiotic silencing by unpaired DNA (MSUD). MSUD scans homologous chromosomes for unpaired DNA during meiosis. When unpaired DNA is identified, MSUD silences all RNA from the unpaired DNA along with any RNA transcribed from homologous sequences at other locations in the genome. Unpaired DNA segments occupy 1.3 kb, and DNA sequences with a small level of polymorphism (6%) can be considered unpaired by MSUD. At least nine proteins are required for meiotic silencing; three proteins are homologs of the canonical RNA interference (RNAi) proteins Dicer, Argonaute, and RNA-dependent RNA polymerase. Most MSUD proteins dock outside the nuclear envelope during early stages of meiosis. Only two proteins are intranuclear where they participate in the unpaired DNA detection process.[369]

The degradation of small RNAs is associated with small RNA 3′ truncation and 3′ uridylation by the action of exonucleases and nucleotidyl transferases. Argonaute (AGO) proteins associated with small RNAs are essential for the activity and stability of small RNAs. *AGO1* is the miRNA effector in *Arabidopsis*, and its closest homolog *AGO10* maintains stem cell homeostasis in meristems by sequestration of miR165/6, a conserved miRNA acting through *AGO1*. Small RNA degrading nucleases (SDNs) initiate miRNA degradation by acting on *AGO1*-bound miRNAs to cause their 3′ truncation, and the truncated species are uridylated and degraded. *AGO10* reduces miR165/6 accumulation by enhancing its degradation by SDN1 and SDN2. AGO10 promotes the degradation of miR165/6, which is contrary to the stabilizing effect of AGO1.[370]

1.3.4 RNA Splicing

Alternative splicing represents an important level of the regulation of gene function in eukaryotic organisms and has an impact on the regulation of cell division and cell death, differentiation of tissues in the embryo and the adult organism, as well as in cellular response to diverse environmental factors. Determinants of alternative splicing include RNA-protein interactions, epigenetic regulation via chromatin remodeling, coupling of transcription-to-alternative splicing, effects of secondary structures in pre-RNA, and the function of RNA quality control systems. The final structure of RNA is predetermined by a complex interplay between *cis*- and *trans*-acting factors.[371]

Alternative splicing of the antiapoptotic and proliferation-associated survivin (*BIRC5*) gene generates six isoforms, which regulate key aspects of cancer initiation and progression. One isoform, survivin *DEx3*, exhibits an exclusion of exon 3 that generates a unique carboxyl terminus with specific antiapoptotic functions, highly expressed in advanced stages of breast and cervical tumors. The first 22 bp of exon 3 contain *cis*-acting elements that enhance the exclusion of exon 3 to generate the survivin *DEx3* mRNA isoform. Sam68 is a possible *trans*-acting factor that binds to this region and regulates exon 3 splicing.[372]

1.3.5 Copy Number Variation (CNV)

Copy number variation (CNV) is rife in eukaryotic genomes and has been implicated in many human disorders. CNV promotes both tumorigenesis and chemotherapy resistance. CNVs are random mutations that arise through replication defects. Transcription can interfere with replication fork progression and stability, leading to increased mutation rates at highly transcribed loci. Hull et al.[373] investigated whether inducible promoters can stimulate CNV to yield reproducible, environment-specific genetic changes. CNV of the copper resistance gene *CUP1* is stimulated by environmental copper. CNV stimulation accelerates the formation of novel alleles conferring enhanced copper resistance, such that copper exposure actively drives adaptation to copper-rich environments. CNV is regulated by both promoter activity and acetylation of histone H3 lysine 56 (H3K56ac). H3K56ac is required for *CUP1* CNV and efficient copper adaptation.[373]

1.3.6 The Epigenetic Clock

The mammalian molecular clock comprises a complex network of transcriptional programs that integrates environmental signals with physiological pathways in a tissue-specific manner.[374] A central biological clock maintains the daily rhythm in accordance with the external environment in mammals. The circadian rhythm is maintained by epigenetic regulation of the circadian pathway.[375] The epigenetic clock is interpreted as diverse estimates of biological age derived from DNA methylation patterns that are associated with mortality, physical and cognitive function, frailty, and physical activity.[376]

The circadian clock regulates the daily rhythms of several physiological and behavioral processes. Disruptions in clock genes have been associated with obesity and related comorbidities. DNA methylation patterns of nine CpG sites at six circadian rhythm pathway genes are strongly correlated with BMI. These CpGs encompassed cg09578018 (*RORA*), cg20406576 (*PRKAG2*), cg10059324 (*PER3*), cg01180628 (*BHLHE40*), cg23871860 (*FBXL3*), cg16964728 (*RORA*), cg14129040 (*CREB1*), cg07012178 (*PRKAG2*), and cg24061580 (*PRKAG2*). Methylation signatures at cg09578018 (*RORA*), cg24061580 (*PRKAG2*), cg01180628 (*BHLHE40*), and cg10059324 (*PER3*) also correlated with insulin resistance and mean arterial blood pressure. Methylation at cg09578018 (*RORA*) and cg01180628 (*BHLHE40*) correlates with total energy and carbohydrate intake.[377]

Genome-wide differences in DNA methylation occur in shift workers.[378] Rotating night work may be associated with methylation of the promoter regions within tumor suppressor and DNA repair genes (*BRCA1* and *BRCA2*).

Current night shift work or night work history is not associated with methylation status of the promoter sites within the *BRCA1* and *BRCA2* genes. There are weak associations between smoking and the methylation status of *BRCA1* for current smoking.[379]

1.3.7 Epigenetic Reprogramming Memory

Computational procedures to find DNA methylation somatic memory sites (SMSs) at single CpGs, integrated with genomics, epigenomics, transcriptomics, and imprinting information, indicates that reprogramming memory persists at late passage through low methylated regions. In contrast, hypermethylated SMSs persist at evolutionary conserved sites overlapping active transcription loci in dynamic chromatin regions. According to Luu et al.[380] the epigenetic memory molecular origin is the expression of source cell transcription factors protecting hypomethylated SMSs in euchromatin from de novo methylation, keeping source cell lineage-specific loci in induced pluripotent stem (iPS) cells incompletely silenced. SMSs cause differential expression between iPS cells and embryonic stem cells through two mechanisms: (i) "epigenetic/expression memory rule" and (ii) "imprinting control."[380]

1.3.8 Transgenerational Epigenetic Inheritance

It is argued that epigenetic signatures acquired through experience may be passed to offspring, constituting what has been called transgenerational epigenetics. Transgenerational inheritance can be defined as heritable changes to the state of DNA that may be passed on to subsequent generations without alterations to the underlying DNA sequence. Transmission of an epigenetic blueprint may predispose offspring to specific epigenetic patterning following a transgenerational inheritance modality.[381, 382]

Early-life programmed changes may be transmitted to successive generations. Prenatal restricted diet can induce changes in the expression of major genes involved in DNA methylation and histone modifications (*Dnmt1, Dnmt3a, Dnmt3b, Mecp2, Hdac1,* and *Sin3a*) in the liver across generations.[383]

Tremblay et al.[384] studied familial resemblances in DNA methylation levels in blood leukocytes on 485,577 CpG sites and computed maximal heritability, genetic heritability, and common environmental effect for all probes (12.7%, 8.2%, and 4.5%, respectively). Higher maximal heritability was observed in the major histocompatibility complex region on chromosome 6. Familial resemblances in DNA methylation levels are mainly attributable to genetic factors when considering the average across the genome, but common environmental effects play an important role when considering statistically significant probes.[384]

Small RNAs are increasingly emerging as transgenerational carriers of epigenetic information in *C. elegans* and in other organisms. Several factors are required for the inheritance of small RNAs and for heritable RNAi in worms, which typically persist for a finite number of generations.[385]

Maternal effects may in some cases be adaptive. miRNAs are regulators of gene expression that have been shown to play roles in intergenerational information transfer. In crustaceans, miRNAs are differentially expressed in mothers of different ages or nutritional status. The maternal generation exhibits differential expression of miRNAs, as do their eggs; however, this is reduced in adult daughters and lost in great-granddaughters. According to these results, reported by Hearn et al.,[386] miRNAs are a component of maternal provisioning, but do not appear to be the cause of transgenerational responses under particular experimental conditions.

Maternal obesity is associated with an increased risk for metabolic disease and obesity in offspring. Under maternal metabolic conditions the placenta is responsible in part for fetal programming during pregnancy as a result of changes in the transcriptome of placenta progenitor cells of the trophectoderm and ectoplacental cone in preimplantation and early postimplantation periods with later effects on placenta development and function.[387] Recent studies illustrate the DNA methylation-based epigenetic inheritance (intergenerational and transgenerational) of metabolic diseases through the male germline.[388]

Many epigenetic traits are linked to self-perpetuating changes in the individual or collective activity of proteins. Some of these proteins are prions with capacity to adopt one conformation that self-templates over long biological timescales, allowing them to serve as protein-based epigenetic elements that are readily broadcast through mitosis and meiosis. Under some circumstances self-templating can ignite a pathological process, and under other circumstances they permit access to multiple activity states from the same polypeptide and transmission of that information across generations. Protein-based epigenetic inheritance allows genetically identical cells to express a great variety of adaptive phenotypes.[389]

Chromatin is assembled by histone chaperones such as chromatin assembly factor CAF-1. In some species, such as *A. thaliana*, the vitality of *CAF-1* mutants decreases over several generations. Epigenetic rather than genetic mechanisms underlie the progressive developmental phenotype aggravation in *Arabidopsis CAF-1* mutants, and preferred maternal transmission reveals more efficient reprogramming of epigenetic information in the male than in the female germline.[390]

The mechanisms of the epigenetic inheritance of a repressive chromatin state suggest that (i) epigenetic information is inherited in a relatively stable but imprecise fashion; (ii) multiple *cis* and *trans* factors are involved in the maintenance of epigenetic information during mitosis; and (iii) the maintenance of a repressive epigenetic state requires both recruitment and self-reinforcement mechanisms. According to Wang et al.[283] these mechanisms crosstalk with each other and form interconnected feedback loops to shape a stable epigenetic system while maintaining certain degrees of flexibility.

1.3.9 DNA Repair Pathways and Genomic Instability

Damaged DNA is repaired by specialized repair factors that are recruited to the damage site. DNA damage is accompanied by posttranslational modifications of DNA repair factors and the chromatin environment surrounding the lesion. Mono- and polyubiquitylation events are an integral part of DNA damage signaling. Ubiquitylation events occur during nucleotide excision repair (NER), the major pathway to remove bulky helix lesions. The global genomic (GG-NER) and the transcription-coupled (TC-NER) branches of NER are subject to ubiquitylation and deubiquitylation processes that drive DNA repair in the NER.[391] One of the major cellular DNA repair pathways is nucleotide excision repair (NER). It is the primary pathway for repair of various DNA lesions caused by exposure to ultraviolet (UV) light, such as cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts. NER preferentially occurs in specific nuclear areas, such as the nucleolus. H2A ubiquitylation via the UV-RING1B complex localizes chromatin close to the nucleolus. H2A-ubiquitin binding protein ZRF1 resides in the nucleolus where it anchors ubiquitylated chromatin along with XPC.[392]

The high level of compaction and the abundance of repeated sequences in heterochromatin pose multiple challenges for the maintenance of genome stability.[393] The integrity of the genome is maintained by specific DNA repair pathways. The chromatin configuration surrounding the DNA damaged site undergoes dramatic remodeling to facilitate access of DNA repair factors and subsequent removal of the DNA lesion. Cellular DNA repair pathways overcome the chromatin barrier, the chromatin environment is rearranged to facilitate efficient DNA repair, several proteins mediate this reorganization process, and the altered chromatin landscape is involved in the regulation of DNA damage responses.[394]

The maintenance of eukaryotic genome stability is ensured by the interplay of transcriptional as well as posttranscriptional mechanisms that control recombination of repeat regions and the expression and mobility of transposable elements. Mutations in two (cytosine-5) RNA methyltransferases, *Dnmt2* and *NSun2*, impact the accumulation of mobile element-derived sequences and DNA repeat integrity in *Drosophila*.[395]

Global DNA hypomethylation promoting genomic instability leads to cancer and deterioration of human health with age. There is an inverse correlation between *Alu* element methylation and endogenous DNA damage in white blood cells. Cells transfected with *Alu* siRNA exhibit high *Alu* methylation levels, increased proliferation, reduced endogenous DNA damage, and improved resistance to DNA-damaging agents.[396]

The main pathway removing DNA lesions induced by exposure to UV light is the nucleotide excision repair (NER) pathway. The DNA damage response at chromatin is accompanied by the recruitment of DNA repair factors to the lesion site and the deposition of specific histone marks. The methyltransferase MMSET catalyzes the dimethylation of histone H4 at lysine 20 (H4K20me2) at the lesion site. The deposition of H4K20me2 at DNA damage sites elicits the recruitment of the NER factor XPA providing evidence for an H4K20me2-dependent DNA repair factor recruitment mechanism during lesion recognition in the global-genomic branch of NER.[397] The endoribonuclease DICER facilitates chromatin decondensation during lesion recognition in the global-genomic branch of NER and mediates recruitment of the methyltransferase MMSET to the DNA damage site. MMSET is required for efficient NER and catalyzes the dimethylation of histone H4 at lysine 20 (H4K20me2). H4K20me2 at DNA damage sites facilitates recruitment of the NER factor XPA.[391, 392]

Members of the SWI/SNF and INO80 families and PARP1 participate in nucleotide excision repair. The endonuclease DICER is implicated in chromatin decondensation during NER. In response to UV irradiation, DICER is recruited to chromatin in a ZRF1-mediated manner. The H2A-ubiquitin binding protein ZRF1 and DICER together impact on chromatin conformation via PARP1. DICER-mediated chromatin decondensation is independent of its catalytic activity.[392, 398]

The DNA damage checkpoint is activated in response to DNA double-strand breaks (DSBs). Chromatin assembly mediated by the histone chaperone Asf1 triggers inactivation of the DNA damage checkpoint in yeast after DSB repair. Chromatin assembly factor 1 (CAF-1) also contributes to chromatin reassembly after DSB repair. The damage sensors Ddc1 and Ddc2 are present after DSB repair in *asf1* mutants. The genes encoding the E3 ubiquitin ligase complex Rtt101Mms1 are epistatic to ASF1 for survival following induction of a DSB, and Rtt101Mms1 are required for checkpoint recovery after DSB repair but not for chromatin assembly. The Mms22 substrate adaptor, degraded by Rtt101Mms1, is required for DSB repair per se. Deletion of *MMS22* blocks loading of Rad51 at the DSB, while deletion of *ASF1* or *RTT101* leads to persistent Rad51 loading. Based on these results Diao et al.[399] proposed that checkpoint recovery is promoted by Rtt101Mms1-mediated ubiquitylation of Mms22 to halt Mms22-dependent loading of Rad51 onto double-stranded DNA after DSB repair, in concert with the chromatin assembly-mediated displacement of Rad51 and checkpoint sensors from the site of repair.

DNA damage repair (DDR) pathways modulate cancer risk, progression, and therapeutic response. Studies on DDR deficiency across 33 cancer types revealed mutations with accompanying loss of heterozygosity in over one-third of DDR genes, including *TP53* and *BRCA1/2*. Other prevalent alterations include epigenetic silencing of the direct repair genes *EXO5*, *MGMT*, and *ALKBH3* in approximately 20% of samples. Homologous recombination deficiency (HRD) is present in many cancer types with worse outcomes.[400]

Trimethylation of lysine 36 in histone H3 (H3K36me3) and acetylation of lysine 16 in histone H4 (H4K16ac) have important roles in transcriptional regulation and DNA damage response signaling. Li and Wang[401] discovered a new pathway through which H3K36me3 stimulates H4K16ac upon DNA double-strand break (DSB) induction in human cells. The levels of H3K36me3 and H4K16ac in cells after exposure to various DSB-inducing agents, including neocarzinostatin, γ rays, and etoposide, are elevated. DSB-induced H4K16 acetylation is abolished in cells upon depletion of the histone methyltransferase gene SET-domain containing 2 (*SETD2*) and the ensuing loss of H3K36me3. The H3K36me3-mediated increase in H4K16ac necessitates lens epithelium-derived growth factor p75 splicing variant (LEDGF), which is a reader protein of H3K36me3, and the KAT5 (TIP60) histone acetyltransferase. The chromatin-bound LEDGF, through its interaction with KAT5, promotes chromatin localization of KAT5, stimulating H4K16 acetylation.[401]

Homologous recombination (HR) is a DNA double-strand break (DSB) repair pathway that protects the genome from chromosomal instability. RAD51 mediator proteins are critical for efficient HR in mammalian cells. *RAD51D*-deficient cells have a reduced capacity for HR-mediated gene conversion both spontaneously and in response to I-*Sce*I-induced DSBs. *RAD51D*-deficiency shifts DSB repair toward highly deleterious single-strand annealing (SSA) and end-joining processes that lead to the loss of large chromosomal segments surrounding site-specific DSBs at an exceptionally high frequency. Deletions in the proximity of the break are due to a nonhomologous end-joining pathway, while larger deletions are processed via an SSA pathway. In addition to leading to chromosomal abnormalities, *RAD51D* deficiency results in a high frequency of deletions. The *RAD51* paralog is involved in maintaining genomic stability and its deficiency may predispose cells to tumorigenesis.[402]

Loss of expression of the fragile site-encoded Wwox protein was found to contribute to radiation and cisplatin resistance of cells, responses that could be associated with cancer recurrence and poor outcome. *WWOX* gene deletions occur in a variety of human cancer types, and reduced Wwox protein expression can be detected during cancer development. *Wwox* loss is followed by mild chromosome instability in genomes of mouse embryo fibroblast cells from *Wwox* knockout mice. Human and mouse cells deficient for *Wwox* also exhibit significantly enhanced survival of ionizing radiation and bleomycin treatment, agents that induce double-strand breaks (DSBs). *Wwox*-deficient cells exhibit shorter tumor latencies than *Wwox*-expressing cells. *Wwox*-deficient cells exhibit enhanced homology directed repair (HDR) and decreased nonhomologous end-joining (NHEJ) repair, suggesting that *Wwox* contributes to DNA DSB repair pathway choice. Upon silencing of *Rad51*, a protein critical for HDR, *Wwox*-deficient cells are resensitized to radiation. Schrock et al.[403] proposed a genome caretaker function for *WWOX*, in which the Brca1-Wwox interaction supports NHEJ as the dominant DSB repair pathway in *Wwox*-sufficient cells.

DNA double-strand breaks (DSBs) are highly cytotoxic DNA lesions, whose accurate repair by nonhomologous end-joining (NHEJ) or homologous recombination (HR) is crucial for genome integrity and is strongly influenced by the local chromatin environment. SCAI (suppressor of cancer cell invasion) is a 53BP1-interacting chromatin-associated protein that promotes the functionality of several DSB repair pathways in mammalian cells. SCAI undergoes prominent enrichment at DSB sites through dual mechanisms involving 53BP1-dependent recruitment to DSB-surrounding chromatin and 53BP1-independent accumulation at resected DSBs. Cells lacking *SCAI* display reduced DSB repair capacity, hypersensitivity to DSB-inflicting agents, and genome instability. SCAI is a mediator

of 53BP1-dependent repair of heterochromatin-associated DSBs, facilitating ATM kinase signaling at DSBs in repressive chromatin environments. *SCAI* deficiency in mice leads to germ cell loss and subfertility associated with impaired retention of DMC1 recombinase on meiotic chromosomes. SCAI is an important component of both NHEJ- and HR-mediated pathways that potentiates DSB repair efficiency in specific chromatin contexts.[404]

γH2AX is a central player in the DDR (DNA damage response), with specificity for double-strand breaks (DSBs). Upon generation of DSBs, γ-phosphorylation extends along megabase-long domains in chromatin on both sides of the damage. This is a biological amplification mechanism where one DSB induces the γ-phosphorylation of thousands of H2AX molecules along megabase-long domains of chromatin that are adjusted to the sites of DSBs. γ-Phosphorylation is an early event in the DSB damage response, induced in all phases of the cell cycle, and participates in both DSB repair pathways, homologous recombination, and nonhomologous end-joining. γH2AX functions as a guardian of the genome by preventing misrepaired DSB that increase the mutation load of the cells and may further lead to genome instability and carcinogenesis.[405]

DNA repair pathways enable cancer cells to survive DNA damage induced after genotoxic therapies. Tyrosine kinase receptors (TKRs) have been reported as regulators of the DNA repair machinery. TIE2 is a TKR overexpressed in human gliomas at levels that correlate with the degree of increasing malignancy. After ionizing radiation, TIE2 translocates to the nucleus, conferring cells with an enhanced nonhomologous end-joining mechanism of DNA repair that results in a radioresistant phenotype. Nuclear TIE2 binds to key components of DNA repair and phosphorylates H4 at tyrosine 51, which in turn is recognized by the protooncogene ABL1, indicating a role for nuclear TIE2 as a sensor for genotoxic stress by action as a histone modifier. H4Y51 constitutes the first tyrosine phosphorylation of core histones recognized by ABL1, defining this histone modification as a direct signal to couple genotoxic stress with the DNA repair machinery.[406]

BRCA1 mutations strongly predispose individuals to breast and ovarian cancer. Homozygous deletion of exon 2 of the mouse *Brca1* gene normally causes embryonic lethality. Exon 2-deleted alleles of *Brca1* are expressed as a mutant isoform that lacks the N-terminal RING domain. This "RING-less" BRCA1 protein is stable and efficiently recruited to the sites of DNA damage. Robust RAD51 foci form in cells expressing RING-less BRCA1 in response to DNA damage, but cells display substantial genomic instability. Genomic instability can be rescued by the deletion of *Trp53bp1*, which encodes the DNA damage response factor 53BP1, and mice expressing RING-less BRCA1 do not show increased susceptibility to tumors in the absence of 53BP1. Genomic instability in cells expressing RING-less BRCA1 correlates with the loss of BARD1 and a defect in restart of replication forks after hydroxyurea treatment, suggesting a role of BRCA1-BARD1 in genomic integrity that is independent of RAD51 loading.[407]

O-linked *N*-acetylglucosamine linkage (O-GlcNAcylation) to serine or threonine residues regulates numerous biological processes, including DNA damage response. O-GlcNAcylation is induced by DNA damage response. O-GlcNAc transferase (OGT), the solo enzyme for O-GlcNAcylation, relocates to the sites of DNA damage and induces the O-GlcNAcylation of histone H2AX and mediator of DNA damage checkpoint 1 (MDC1). The O-GlcNAcylation negatively regulates DNA double-strand break-induced phosphorylation of H2AX and MDC1 by restraining the expansion of these phosphorylation events from the sites of DNA damage.[408]

Russo et al.[409] characterized the changes in chromatin structure, DNA methylation, and transcription during and after homologous DNA repair (HR). HR modifies the DNA methylation pattern of the repaired segment. HR also alters local histone H3 methylation as well as chromatin structure by inducing DNA chromatin loops connecting the 5′ and 3′ ends of the repaired gene. During a two-week period after repair, transcription-associated demethylation promoted by base excision repair enzymes further modifies methylation of the repaired DNA. The repaired genes display stable but diverse methylation profiles that govern the levels of expression in each clone. DNA methylation and chromatin remodeling induced by HR may be a source of permanent variation of gene expression in somatic cells.[409]

Chromosome segregation during mitosis is monitored by the mitotic checkpoint and is dependent on DNA methylation. ZBTB4 is a mammalian epigenetic regulator with high affinity for methylated CpGs that localizes at pericentromeric heterochromatin and is frequently downregulated in cancer. Decreased ZBTB4 expression correlates with high genome instability across many frequent human cancers. ZBTB4 depletion is sufficient to increase the prevalence of micronuclei and binucleated cells in parallel with aberrant mitotic checkpoint gene expression, a weakened mitotic checkpoint, and an increased frequency of lagging chromosomes during mitosis. *Zbtb4* −/− mice are smaller than their wild-type littermates. Primary cells isolated from *Zbtb4* −/− mice exhibit diminished mitotic checkpoint activity, increased mitotic defects, aneuploid cells marked by a specific transcriptional signature, and increased genomic instability. *Zbtb4* −/− mice are also susceptible to 7,12-dimethylbenz(a)anthracene/12-O-tetradecanoylphorbol-13-acetate (DMBA/TPA)-induced skin carcinogenesis.[410]

1.3.10 Antibody Maturation

Epigenetic modifications contribute to antibody maturation during somatic hypermutation (SHM) and class switch recombination (CSR). Histone modifications alter the chromatin landscape and help recruit activation-induced cytidine deaminase (AID) to the immunoglobulin (Ig) locus. AID is a potent DNA mutator that catalyzes cytosine-to-uracil deamination on single-stranded DNA to create U-G mismatches. Alternate chromatin modifications, in concert with ncRNAs and potentially DNA methylation, regulate AID recruitment and stabilize DNA repair factors that lead to enhanced antibody antigen binding affinity (SHM) or antibody isotype switching (CSR).[411]

1.3.11 Sexual Dimorphism

Most diseases exhibit some degree of sexual dimorphism. Studies in isogenic mice of both sexes revealed that DNA methylation patterns varied significantly from tissue to tissue and between the sexes, with thousands of sexually dimorphic loci identified. Gender is underwritten in the epigenome in a tissue-specific and potentially sex hormone-independent manner. Gender-specific epigenetic states are likely to have important implications for understanding sexually dimorphic phenotypes in health and disease.[412] Epigenetics may mechanistically explain the effects that endocrine-disrupting chemicals (EDCs) can exert on sexual dimorphism.[413]

Genetic and environmental factors are determinant in the mechanisms underlying female sexual dysfunction (FSD). A differentially methylated CpG pattern was found in monozygotic twin pairs discordant for sexual functioning. Two differentially methylated CpG positions (cg09580409 and cg14734994) were found for overall sexual functioning, mapping to *MGC45800* and the threonine synthase-like 2 gene (*THNSL2*), respectively. Potential candidates for sexual desire (CUB and zona pellucida-like domains 1, *CUZD1*) and satisfaction (solute carrier family 6 member 19, *SLC6A19*) were also identified.[414]

Methylation has been implicated in gender determination in plants. The sex-determining region (SDR) of balsam poplar (*Populus balsamifera*) encompasses 13 genes with differentiated X and Y copies. The only SDR gene to show a marked pattern of gender-specific methylation is *PbRR9*, a member of the two-component response regulator (type A) gene family, involved in cytokinin signaling. It is an ortholog of *Arabidopsis* genes *ARR16* and *ARR17*. The strongest patterns of differential methylation are found in the putative promoter and the first intron. The fourth intron is strongly methylated in both sexes and the fifth intron is unmethylated in both sexes. It has been proposed that PbRR9 has a direct, epigenetically mediated, role in poplar sex determination.[415]

1.3.12 Heritable RNAi

Germline nuclear RNAi is a transgenerational gene-silencing pathway that leads to H3K9 trimethylation (H3K9me3) and transcriptional silencing at the target genes. H3K9me3 induced by either exogenous double-stranded RNA (dsRNA) or endogenous siRNA (*endo*-siRNA) is highly specific to the target loci and transgenerationally heritable. siRNA-mediated H3K9me3 requires combined activities of three H3K9 histone methyltransferases (MET-2, SET-25, SET-32). Mutant *C. elegans* for these enzymes exhibit reductions in H3K9me3 throughout the genome. However, loss of H3K9me3 at native nuclear RNAi targets has no effect on the transcriptional silencing state. Exogenous dsRNA-induced transcriptional silencing and heritable RNAi at *oma-1*, a nuclear RNAi reporter gene, are resistant to the loss of H3K9me3. siRNA-mediated transcriptional silencing in *C. elegans* can be maintained by an H3K9me3-independent mechanism.[416]

Silencing is dependent on germline nuclear RNAi factors and posttranscriptional mechanisms. Heritable germline silencing directs somatic epigenetic silencing. Somatic silencing does not require somatic nuclear RNAi, but instead requires both maternal germline nuclear RNAi and chromatin-modifying activity.[417]

1.3.13 Genomic Imprinting

Imprinting is an epigenetic phenomenon in which genes are expressed selectively from either the maternal or paternal alleles. Inheritance of DNA methylation states from gametes determines genomic imprinting, and repressive chromatin in oocytes can also confer imprinting.[418] Epigenetic marks reset in the germline to enable differentiation of sperm and eggs and at fertilization to create the totipotent zygote that then begins growth and differentiation into a new human. A small group of genes can evade the second zygotic wave of epigenetic reprogramming retaining an epigenetic imprint of the parent from whom they were inherited. Imprinted genes are in general expressed from one

parental allele only. Some imprinted genes are critical regulators of growth and development, and disruption of their monoallelic expression may cause congenital imprinting disorders that affect growth, development, behavior, and metabolism.[419]

Human inbreeding in cases of parents biologically related affects embryo development, with smaller organs, and consequently causes infants with lower body weight at birth. Fetal growth is regulated by imprinted genes that are in conflict, promoting growth when derived from the father and suppressing growth when derived from the mother. Undersized organs are less likely to develop malignant conversion.[420]

DNA methylation controls the expression of imprinted genes, which are expressed monoallelically and in a parent-of-origin-specific manner. Parental allele-specific DNA methylation at imprinting control regions (ICRs) is necessary for appropriate imprinting. Deregulation of DNA methylation of imprinted loci is associated with specific disorders. DNA methylation patterns are modified during mammalian development. Most genomic regions, with the exception of ICRs, are demethylated after fertilization, and subsequently undergo genome-wide de novo DNA methylation. After primordial germ cell specialization in the embryo a new wave of demethylation is activated, with ICR demethylation occurring late in the process. Finally, ICRs reacquire DNA methylation imprints in developing germ cells.[421]

A hallmark of imprinted genes in mammals is the occurrence of parent-of-origin-dependent asymmetry of DNA cytosine methylation (5mC) of alleles at CpG islands (CGIs) in their promoter regions. This 5mCpG asymmetry between parental alleles creates allele-specific imprinted differentially methylated regions (iDMRs). iDMRs are often coupled to the transcriptional repression of the methylated allele and the activation of the unmethylated allele in a tissue-specific, developmental stage-specific, and/or isoform-specific fashion.[422] Studies with the maternal origin of the 5mCpG imprints of one gametic (PARD6G-AS1) and one secondary (GCSAML) iDMR revealed that PARD6G-AS1 and GCSAML are expressed biallelically in multiple tissues. Tissue-specific monoallelic expression of *ZNF124* and *OR2L13* was found to be located 363 kb upstream and 419 kb downstream, respectively, of the GCSAML iDMR, suggesting that the GCSAML iDMR regulates the tissue-specific, monoallelic expression of *ZNF124* but not of *OR2L13*. Maternal 5mCpG imprints at PARD6G-AS1 and GCSAML iDMRs are decoupled from parent-of-origin transcriptional expression effects in multiple tissues.[422]

Uniparental disomy of certain chromosomes is associated with imprinting disorders. The extreme form of uniparental disomy affecting the whole genome is usually not compatible with life, with the exception of very rare cases of patients with mosaic genome-wide uniparental disomy. Bens et al.[423] reported the case of a fetus with intrauterine growth retardation, malformations, and mosaicism for one cell line with genome-wide maternal uniparental disomy and a second diploid cell line of biparental inheritance with trisomy X due to paternal isodisomy X. DNA methylation changes were observed in all imprinted loci.[423]

Intergenic/intronic hypomethylated regions (iHMRs) may be noncanonical enhancers for imprinted genes. *Peg3*- and *H19*-iHMRs show context-dependent promoter and enhancer activity. Deletion of *Peg3*-iHMR results in allele- and sex-specific misregulation of several imprinted genes within the domain. Some iHMRs may function as domain-wide regulators for the associated imprinted domains.[424] Imprinted domains have been identified as targets for aberrant DNA methylation during carcinogenesis. Epigenetic instability at key *cis*-regulatory elements within imprinted domains can concomitantly activate protooncogenes and turn off tumor suppressor genes. Imprinted domains remain stable in benign processes, but are highly susceptible to epigenetic alterations in infiltrative lesions. Imprinted genes are not involved in the initiation of carcinogenesis or tumor growth. Imprinted domains are targeted for DNA hypermethylation when benign tumor cells evolve into malignant stages.[425]

Differential DNA methylation is a critical factor in the regulation of imprinted genes. The differentially methylated state of the imprinting control region is inherited via the gametes at fertilization, and is stably maintained in somatic cells throughout development, influencing the expression of genes across the imprinting cluster. The examination of CpG dyad methylation at differentially methylated regions associated with the murine Dlk1/Gtl2 imprinting cluster on both complementary strands shows homomethylation at greater than 90% of the methylated CpG dyads at the IG-DMR, which serves as the imprinting control element. In contrast, homomethylation is only present at 67%–78% of the methylated CpG dyads at the secondary differentially methylated regions. According to Guntrum et al.[426] this high degree of hemimethylation could explain the variability in DNA methylation patterns at secondary differentially methylated regions associated with imprinted loci. 5-Hydroxymethylation at secondary differentially methylated regions may result in hemimethylation and methylation variability as a result of passive and/or active demethylation mechanisms.

Alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV) is a rare lethal lung developmental disorder caused by heterozygous point mutations or genomic deletions involving *FOXF1* or its 60-kb tissue-specific enhancer region mapping 270 kb upstream of *FOXF1* and involving fetal lung-expressed long noncoding RNA genes and CpG-enriched sites. The *FOXF1* locus at 16q24.1 may be a subject of genomic imprinting. Szafranski et al.[427]

Identified a novel de novo 104-kb genomic deletion upstream of *FOXF1* in a patient with histopathologically verified full phenotype of ACDMPV. Narrowing the *FOXF1* enhancer region the authors identified its critical 15-kb core interval, essential for lung development. This interval harbors binding sites for lung-expressed transcription factors, including GATA3, ESR1, and YY1, and is flanked by lncRNA genes and CpG islands. Sequencing of one of these CpG islands on the nondeleted allele showed that it is predominantly methylated on the maternal chromosome 16.

1.4 EVOLUTION

Epigenetics is a potential contributing factor to evolution.[428] Evolutionary simulations based on the cooperative model reproduced the process of genetic assimilation, as defined by Conrad H. Waddington. Genetic assimilation is a process in which epigenetically induced phenotypic changes are incrementally and statistically replaced with multiple minor genetic mutations through natural selection. According to Nishikawa and Kinjo[429] epigenetic and genetic changes may be considered mutually independent but equivalent in terms of their effects on phenotypic changes, rejecting the common hypothesis that epigenetically induced phenotypic changes depend on genetic mutations. Cytosine methylation at the C5 position, generating 5-methylcytosine (5mC), is a DNA modification found in many eukaryotic organisms, including fungi, plants, invertebrates, and vertebrates, albeit its levels vary greatly in different organisms. In mammals, cytosine methylation occurs predominantly in the context of CpG dinucleotides, with the majority (60%–80%) of CpG sites in their genomes being methylated. DNA methylation plays crucial roles in the regulation of chromatin structure and gene expression and is essential for mammalian development.[430] Uncovering the mechanisms of epigenome evolution is an essential step toward understanding the evolution of different cellular phenotypes. DNA methylation is a conserved epigenetic mechanism in mammalian development. Studies of comparative epigenomics comparing the tissue-specific DNA methylation patterns of rats against those of mice and humans across three shared tissue types confirmed that tissue-specific differentially methylated regions are strongly associated with tissue-specific regulatory elements. Comparisons between species revealed that at a minimum 11%–37% of tissue-specific DNA methylation patterns are conserved. Conserved DNA methylation is accompanied by conservation of other epigenetic marks including histone modifications. Although a significant amount of locus-specific methylation is epigenetically conserved, the majority of tissue-specific DNA methylation is not conserved across species and tissues.[431]

Evolutionary analysis in metazoa, dictyosteliida, and algae, including multiple previously unreported vertebrate clades and versions from urochordates, nematodes, echinoderms, arthropods, lophotrochozoans, cnidarians, and porifera, suggests a fundamental division of AADs early in metazoan evolution into secreted deaminases (SNADs) and classical AADs, followed by diversification into several clades driven by rapid-sequence evolution, gene loss, lineage-specific expansions, and lateral transfer to various algae. Biological conflicts of AADs with viruses and genomic retroelements are drivers of rapid AAD evolution, suggesting a widespread presence of mutagenesis-based immune defense systems.[432]

Ultraconserved noncoding elements (UCNEs) are impervious to accumulating mutations and represent <1 Mb of vertebrate genomes. Vertebrate genomes exhibit about 4000 UCNEs of 200 nucleotides in length, with over 95% sequence identity between humans and chickens. Colwell et al.[433] studied UCNEs with high CpG density in 56 species and found half to be intermediately methylated and the remaining near 0% or 100%. In humans most UCNEs show greater variation than the *LINE1* transposon. In vertebrates global methylation is found to be inversely correlated with hydroxymethylation. The DNA methylation of UCNEs is flexible, conserved between related species, and relaxed from the underlying sequence selection pressure, while remaining heritable through speciation.

Epigenetic mechanisms are a key component of dosage compensation on sex chromosomes and a source of phenotypic variation influencing plasticity and adaptive evolutionary processes.[434] Changes in gene expression resulting from epigenetic and/or genetic changes play an important role in the evolutionary divergence of phenotypes. Genome-wide DNA methylation profiles (methylomes) of humans and chimpanzees, which have a 1.2% DNA sequence divergence, revealed species-specific differentially methylated regions (S-DMRs), ranging from several hundred base pairs (bp) to several kilo base pairs (kb). These differences are frequently associated with sequence changes in transcription factor-binding sites and insertions of *Alu* and *SVA* retrotransposons. Human and chimpanzee S-DMRs arose more frequently owing to methylation loss rather than gain. The sperm methylomes contain more hypomethylated domains (HMDs), ranging from 20 to 500 kb, than somatic methylomes. Sperm HMDs changed rapidly during primate evolution. Hundreds of sperm HMDs were specific to humans, whereas most somatic HMDs were highly conserved between humans and chimpanzees. Human-specific sperm HMDs frequently occur in regions exhibiting copy number variations.[435]

In many lineages of complex genomes in the vegetal kingdom, polyploidy is followed by "biased fractionation," the unequal loss of genes from ancestral progenitor genomes. Biased fractionation results from changes in the epigenetic landscape near genes, probably mediated by transposable elements. These epigenetic changes result in unequal gene expression between duplicates, establishing differential fitness that leads to biased gene loss with respect to ancestral genomes. Wendel et al.[436] proposed a unifying conceptual framework and a set of testable hypotheses based on this model, relating genome size, the proximity of transposable elements to genes, epigenetic reprogramming, chromatin accessibility, and gene expression.

1.5 POPULATION EPIGENETICS

Novel epigenetic technologies allow the epigenetic characterization of different species and populations. The applicability of population epigenetics may help to optimize health epidemiology and medical epidemiology, especially in those human disorders in which epigenetic aberrations participate in pathogenic events.

Populations are often divided categorically into distinct racial/ethnic groups based on social rather than biological constructs. Genetic ancestry is an alternative to this categorization. Galanter et al.[437] typed over 450,000 CpG sites in whole blood of 573 individuals of diverse Hispanic origin and found that both self-identified ethnicity and genetically determined ancestry were each significantly associated with methylation levels at 916 and 194 CpGs, respectively, and that shared genomic ancestry accounted for a median of 75.7% of the variance in methylation associated with ethnicity.

Genome-wide association studies (GWASs) have identified loci for erythrocyte traits in primarily European ancestry populations. A GWAS metaanalysis of six erythrocyte traits in 71,638 individuals from European, East Asian, and African ancestries identified seven loci for erythrocyte traits including a locus (*RBPMS/GTF2E2*) associated with mean corpuscular hemoglobin and mean corpuscular volume. RBPMS is a regulator of erythropoiesis.[438]

1.6 FERTILITY AND GESTATION

Both female and male germline development follow distinct paths of epigenetic events, and both oocyte and sperm possess their own unique epigenomes. Fertilizing male and female germ cells deliver not only their haploid genomes but also their epigenomes, which contain the code for preimplantation and postimplantation reprogramming and embryonal development. Sperm RNAs delivered upon fertilization provide initial contacts with the oocyte, directly confronting the maternal with the paternal contribution as a prelude to genome consolidation. Following syngamy, early embryo development may in part be modulated by paternal RNAs that can include epididymal passengers. This provides a direct path to relay an experience and then initialize a paternal response to the environment to the oocyte and beyond. Their epigenetic impact is likely felt prior to embryonic genome activation when the population of sperm-delivered transcripts markedly changes.[439]

Epigenetic changes influence multiple facets of female/male fertility and gestation. The timing of the first cell divisions may predict the developmental potential of an embryo. Embryos with different speeds of development present distinct patterns of gene expression, mainly related to energy and lipid metabolism. Genome-wide DNA methylation analysis identified 11,584 differently methylated regions (DMRs) (7976 hypermethylated regions in fast and 3608 hypermethylated regions in slow embryos). Fast embryos present more regions classified as hypermethylated distributed throughout the genome as introns, exons, promoters, and repeat elements, while in slow embryos hypermethylated regions are more present in CpG islands. According to studies reported by Ispada et al.[440] the kinetics of the first cleavages influences the DNA methylation and expression profiles of genes related to metabolism and differentiation pathways and may affect embryo viability.

In some species high frequencies of male-deleterious alleles are attributable to Y chromosomal distorter-suppressor pair activity, and these alleles are suppressed through epigenetic modification. Suppression of male-deleterious alleles results in negative frequency-dependent selection of the Y distorter and suppressor, a prerequisite for a stable polymorphism of the Y distorter-suppressor pair. The Y distorter seems to be responsible for positive selection of male-deleterious alleles, and the Y suppressor for positive selection of these alleles. Male-deleterious alleles are associated with susceptibility to diseases and deleterious environmental stressors.[441]

In spermatozoa the DNA methylation profile, DNA-associated proteins, protamine 1:protamine 2 ratio, nucleosome distribution pattern, histone modifications, and other factors make up a unique epigenetic landscape. Developmental programming of the embryo is not only controlled by genetic information but also dictated by epigenetic information contained in spermatozoa and oocyte. Paternal and maternal lifestyle, including physical activity, nutrition, and

exposure to hazardous substances, can alter the epigenome and can affect the health of their children.[442, 443] Gametogenesis represents one of the most active cellular differentiation pathways in females and males. Genomically, meiosis ensures that diploid germ cells become haploid gametes and, epigenetically, extensive changes are required to turn on and shut off gene expression in a precise manner.[444] Chromatin remodeling during spermatogenesis culminates in the exchange of nucleosomes for transition proteins and protamines as an important part of spermatid development to give rise to healthy sperm. Histone H4 hyperacetylation is considered a key event of histone removal during the nucleoprotein transition to a protamine-based sperm chromatin structure. Transition protein 1 (*TP1*), protamine 1, H2A histone family member Z (*H2AFZ*), and testis-specific histone H2B variant (*TH2B*) expression are involved in spermatid development. H4K16 acetylation, which is dependent on DNA damage signaling, may be more important for nucleosome replacement in spermiogenesis.[445]

Preconceptional exposure to certain lifestyle and environmental factors, such as diet, physical activity, and smoking, affects the phenotype of the next generation by remodeling the epigenetic blueprint of spermatozoa. Like somatic cells the epigenome of spermatozoa has proven to be dynamically reactive to a wide variety of environmental and lifestyle stressors.[443]

In fertile males the sperm methylome is highly homogeneous and hypomethylated. Genes with hypomethylated promoters are ontologically associated with biological functions related to spermatogenesis and embryogenesis. Sex chromosomes are the most hypomethylated chromosomes. Over 90 genes are resistant to demethylation, being strong candidates for transgenerational inheritance.[446] Methylenetetrahydrofolate reductase (*MTHFR*) gene promoter hypermethylation in spermatozoa is associated with idiopathic male infertility. The percentage of *MTHFR* promoter methylation in infertile men with normozoospermia (11%) is significantly higher than that in the healthy control (4.3%) group.[447] Epigenetic aberrations affect male fertility. Studies of sperm DNA methylation showed at least three CpG sites (cg23081194, cg25750688, and cg04807108) with clear differences in methylation levels between oligospermic males and controls.[448] Estrogen receptors (ESR1 and ESR2) play important roles in various processes during spermatogenesis that are crucial for male fertility.[449] Homozygous mutations in autosomal candidate genes are identified in 63% of infertile men presenting with multiple morphological anomalies of the sperm flagella.[450]

Splicing can be epigenetically regulated and involved in cellular differentiation in somatic cells and in spermatogenesis. MORF-related gene on chromosome 15 (*MRG15*) is a multifunctional chromatin organizer that binds to methylated histone H3 lysine 36 (H3K36) in introns of transcriptionally active genes and that participates in the regulation of histone acetylation, homology-directed DNA repair, and alternative splicing in somatic cells. Conditional KO (cKO) males lacking *MRG15* in the germline are sterile secondary to spermatogenic arrest at the round spermatid stage. In round spermatids MRG15 colocalizes with splicing factors PTBP1 and PTBP2 at H3K36me3 sites between the exons and single intron of transition nuclear protein 2 (*Tnp2*). *MRG15* is essential for pre-mRNA splicing during spermatogenesis.[451]

Zygotic genome activation denotes the initiation of gene expression after fertilization, as a part of the complex oocyte-to-embryo transition (OET) in which the oocyte is fertilized and transformed into a zygote that gives rise to an embryo that will develop into a newborn. The OET reflects the reprogramming of germ cell gene expression into the new developmental program of the zygote, and this reprogramming occurs at the transcriptional and posttranscriptional level with full involvement of the epigenetic machinery.[452]

Global DNA demethylation is a hallmark of embryonic epigenetic reprogramming. Embryos engage noncanonical DNA methylation maintenance mechanisms to ensure inheritance of exceptional epigenetic germline features to the soma. The Y-linked gene *Rbmy1a1* is highly methylated in mature sperm and resists DNA demethylation post fertilization. Aberrant hypomethylation of the *Rbmy1a1* promoter results in its ectopic activation, causing male-specific periimplantation lethality. *Rbmy1a1* is a novel target of the TRIM28 complex, which is required to protect its repressive epigenetic state during embryonic epigenetic reprogramming.[453]

Germ cell development involves major reprogramming of the epigenome to prime the zygote for totipotency. Histone 3 lysine 4 (H3K4) methylation is a universal epigenetic mark mediated in mammals by six H3K4 methyltransferases related to fly Trithorax, including two yeast Set1 orthologs (Setd1a and Setd1b). Setd1a plays no role in oogenesis, and Setd1b deficiency causes female sterility in mice. Oocyte-specific *Gdf9-iCre* conditional knockout (*Setd1bGdf9* cKO) ovaries develop through all stages; however, follicular loss accumulates with age and unfertilized metaphase II (MII) oocytes exhibit irregularities of the zona pellucida and meiotic spindle. Most *Setd1bGdf9* cKO zygotes remain in the pronuclear stage and display polyspermy in the perivitelline space. Setd1b promotes expression of the major oocyte transcription factors including Obox1, 2, 5, 7, Meis2, and Sall4. Setd1b also promotes expression of negative regulators of oocyte development with multiple Zfp-KRAB factors implicated. *Setd1b* serves as a maternal effect gene through regulation of the oocyte gene expression program.[454]

Paternally expressed gene 3 (*Peg3*) encodes a DNA-binding protein with 12 C2H2 zinc finger motifs. A set of 16 *PEG3* genomic targets has been identified, the majority of which overlap with the promoter regions of genes with oocyte expression. These potential downstream genes are upregulated in MEF cells lacking PEG3 protein, suggesting a potential repressor role for PEG3. The imprinting control region (ICR) of H19 is a genomic target. PEG3 binds to a specific sequence motif located between the third and fourth CTCF binding sites of the H19-ICR. PEG3 also binds to the active maternal allele of the H19-ICR. The expression levels of H19 are upregulated in MEF cells lacking PEG3, and this upregulation is mainly derived from the maternal allele. PEG3 may function as a transcriptional repressor for the maternal allele of H19.[455]

During embryogenesis, DNA methylation together with other epigenetic factors plays an essential role in selecting and maintaining cell identity. DNA methylation seems to be essential for the pluripotency stages of embryonic development. Targeted deposition and removal of DNA methylation by DNMTs and TET proteins, respectively, appears to be required for vertebrate gastrulation.[456] Epigenetic modifications affect key chromatin regulation, including transcription and DNA repair, which are critical for normal embryo development. In porcine embryos around the period of embryonic genome activation (EGA), Brahma-related gene 1 (*BRG1*) and Lysine demethylase 1A (*KDM1A*), which can alter the methylation status of lysine 4 in histone 3 (H3K4), localize to the nucleus at day 3–4 of development. The mRNA abundance of *BRG1*, *KDM1A*, as well as other lysine demethylases (*KDM1B*, *KDM5A*, *KDM5B*, and *KDM5C*), are elevated in late-compared to early-cleaving embryos near the EGA period, although these differences disappeared at the blastocyst stage. The abundance of H3K4 monomethylation (H3K4me) and dimethylation (H3K4me2) during the EGA period is reduced in late-cleaving and less developmentally competent embryos, whereas BRG1, KDM1A, and H3K4me2 abundance is greater in embryos with more than eight cells at day 3–4 of development compared to those with fewer than four cells. Altered epigenetic modifications of H3K4 around the EGA period may affect the developmental capacity of porcine embryos to reach the blastocyst stage.[457]

Extensive chromatin remodeling after fertilization is thought to take place to allow a new developmental program to start. This includes dynamic changes in histone methylation and, in particular, the remodeling of constitutive heterochromatic marks such as histone H4 Lys20 trimethylation (H4K20me3). The essential function of H4K20me1 in preimplantation mouse embryos is well established. Suv4-20h1/h2 are mostly absent in mouse embryos before implantation, underscoring the rapid decrease of H4K20me3 from the two-cell stage onward. Ectopic expression of *Suv4-20h2* leads to sustained levels of H4K20me3, developmental arrest, and defects in S-phase progression. The developmental phenotype can be partially overcome by inhibiting the ATR pathway, suggesting that the main function for the remodeling of H4K20me3 after fertilization is to allow the timely and coordinated progression of replication.[458] Global epigenetic reprogramming is considered to be essential during embryo development to establish totipotency. Genome-wide DNA demethylation is asymmetric between the paternal and the maternal genome. The paternal genome undergoes ten-eleven translocation (TET)-mediated active DNA demethylation, which is completed before the end of the first cell cycle. Since TET enzymes oxidize 5-methylcytosine to 5-hydroxymethylcytosine, the latter is postulated to be an intermediate stage toward DNA demethylation. The maternal genome is protected from active demethylation and undergoes replication-dependent DNA demethylation. However, several species do not show the asymmetric DNA demethylation process. According to studies reported by Heras et al.,[459] 5-methylcytosine and 5-hydroxymethylcytosine both are explicitly present throughout pronuclear development, with similar intensity levels in both parental genomes, in equine zygotes produced by ICSI. However, the localization patterns of 5-methylcytosine and 5-hydroxymethylcytosine are different, with 5-hydroxymethylcytosine homogeneously distributed in the DNA, while 5-methylcytosine tended to be clustered in certain regions. 5-Methylcytosine levels are increased in the maternal genome from PN1 to PN2, while no differences are found in PN3 and PN4. No differences are observed in the paternal genome. Horses do not seem to follow the classic murine model of asymmetric demethylation as no evidence of global DNA demethylation of the paternal pronucleus during the first cell cycle was demonstrated.

Stress-induced epigenetic changes in the germline can be inherited and can have a profound impact on offspring development. *Drosophila* oocytes transmit the repressive histone mark H3K27me3 to their offspring. Maternal contribution of the histone methyltransferase Enhancer of zeste, the enzymatic component of Polycomb repressive complex 2, is required for active propagation of H3K27me3 during early embryogenesis. H3K27me3 in the early embryo prevents aberrant accumulation of the active histone mark H3K27ac at regulatory regions and precocious activation of lineage-specific genes at zygotic genome activation. Disruption of the germline-inherited Polycomb epigenetic memory causes embryonic lethality that cannot be rescued by late zygotic reestablishment of H3K27me3. According to the studies reported by Zenk et al.,[460] maternally inherited H3K27me3, propagated in the early embryo, regulates the activation of enhancers and lineage-specific genes during development.

Mammalian embryos undergo dramatic epigenetic remodeling that can have a profound impact on both gene transcription and overall embryo developmental competence. Members of the SWI/SNF (Switch/Sucrose nonfermentable) family of chromatin-remodeling complexes reposition nucleosomes and alter transcription factor accessibility. These large, multiprotein complexes possess an SNF2-type ATPase (SMARCA4, SMARCA2) as their core catalytic subunit and are directed to specific loci by associated subunits. ARID1A, one of the SWI/SNF complex subunits, can affect histone methylation in somatic cells. ARID1A transcript levels are reduced in four-cell porcine embryos as compared to germinal vesicle-stage oocytes, suggesting that ARID1A would be required for porcine cleavage-stage development.[461]

The gene coding for histone H3 lysine 9 methyltransferase G9A is conditionally deleted in neural crest cells with Wnt1-Cre. Mutants display incomplete ossification. Twist1 and Twist2 regulatory regions contain the repressive H3K9me2 marks catalyzed by G9A, which are removed when the G9A inhibitor BIX-01294 is added.[462]

Recent studies also indicate that paternal experience can influence offspring development via germline inheritance, but mothers can serve as a modulating factor in determining the impact of paternal influences on offspring development.[463]

Phenotypic and epigenetic similarities in monozygotic twins are largely due to their genetic identity. Genome-scale studies of DNA methylation in monozygotic and dizygotic twins revealed genomic regions at which the epigenetic similarity of monozygotic twins is substantially greater than can be explained by their genetic identity. This "epigenetic supersimilarity" may result from locus-specific establishment of epigenotype prior to embryo cleavage during twinning. Epigenetically supersimilar loci exhibit systemic interindividual epigenetic variation and plasticity in the periconceptional environment and are enriched in subtelomeric regions. Blood DNA methylation at these loci years before diagnosis is associated with risk for developing several types of cancer.[464]

Epigenetic modifications in the C-terminal domain of histones coordinate important events during early development including embryo genome activation (EGA) and cell differentiation. The mRNA expression profile of the main lysine demethylases (KDMs) acting on lysine 4 (H3K4), 9 (H3K9), and 27 (H3K27) of the histone H3 was determined at pre-, during and post- EGA stages of bovine and porcine embryos produced by in vitro fertilization (IVF) and somatic cell nuclear transfer (SCNT). In IVF embryos, mRNA expression was highest around the EGA period: D3 for porcine (*KDM2B*, *KDM5B*, *KDM5C*, *KDM4B*, *KDM4C*, *KDM6A*, *KDM6B*, and *KDM7A*); and D4 for bovine (*KDM1A*, *KDM5A*, *KDM5B*, *KDM5C*, *KDM3A*, *KDM4A*, *KDM4C*, and *KDM7A*). The mRNA profile of *KDM1A*, *KDM2B*, *KDM3A*, *KDM3B*, *KDM6A*, and *KDM6B* differed between porcine and bovine IVF embryos. Other epigenetic differences observed between SCNT and IVF indicate the existence of species-specific changes during cell reprogramming in embryos.[465]

A successful pregnancy needs to initiate immune biases toward T helper 2 (Th2) responses. Studies on changes in DNA methylation of Th1, Th2, Th17, and regulatory T cell pathway genes before and during pregnancy indicate that 27.7% of Th1-related CpGs change during the first half of pregnancy and 36.1% in the second half. The Th2 pathway CpGs change in a similar proportion. These methylation changes suggest involvement of both Th1 and Th2 pathway CpGs in the immune bias during pregnancy.[466]

Histone variant H3.3 is encoded by two distinct genes, *H3F3A* and *H3F3B*, that are closely associated with actively transcribed genes. H3.3 replacement is continuous and essential for maintaining the correct chromatin structure during mouse oogenesis. Upon fertilization, H3.3 is incorporated in parental chromatin, and is required for blastocyst formation in mice. The H3.3 exchange process is facilitated by the chaperone HIRA, particularly during zygote development. H3.3 is required for bovine early embryonic development. *H3F3A* mRNA abundance is stable, whereas *H3F3B* and *HIRA* mRNA are relatively dynamic during early embryonic development. *H3F3B* mRNA quantity is also considerably higher than *H3F3A*. Knockdown of H3.3 decreases the expression of the pluripotency marker NANOG and trophectoderm marker CTGF (connective tissue growth factor) in bovine blastocysts. Histone H3 lysine 36 dimethylation and linker histone H1 abundance is reduced in H3.3-deficient embryos, which was similar to effects following knockdown of *CHD1* (chromodomain helicase DNA-binding protein 1). H3.3 is required for correct epigenetic modifications and H1 deposition.[467]

Osteogenic lineage commitment and progression is controlled by multiple signaling pathways (WNT, BMP, FGF) that converge on bone-related transcription factors. Access of osteogenic transcription factors to chromatin is controlled by epigenetic regulators that generate posttranslational modifications of histones. Cells with increased osteogenic potential have higher levels of the H4K20 methyltransferase Suv420h2 compared to other methyltransferases (Suv39h1, Suv39h2, Suv420h1, Ezh1, Ezh2). Six epigenetic regulators are transiently expressed at different stages of osteoblast differentiation, with maximal mRNA levels of *Suv39h1* and *Suv39h2* preceding maximal expression of *Suv420h1* and *Suv420h2* and developmental stages that reflect, respectively, early and later collagen matrix deposition. Loss-of-function analysis of *Suv420h2* by siRNA depletion shows loss of H4K20 methylation and

decreased expression of bone biomarkers. Suv420h2 is required for matrix mineralization during osteoblast differentiation. Suv420h2 controls the H4K20 methylome of osteoblasts and is critical for normal progression of osteoblastogenesis.[468]

The group III chromodomain-helicase-DNA-binding (CHD) family of ATP-dependent chromatin-remodeling enzymes (CHD6, CHD7, CHD8, CHD9) regulate transcription and are involved in development and disease (i.e., CHARGE syndrome, autism spectrum disorders). CHD6 and CHD7 bind with high affinity to short linker DNA, whereas CHD8 requires longer DNA for binding. CHD8 slides nucleosomes into positions with more flanking linker DNA than CHD7. CHD6 disrupts nucleosomes in a distinct nonsliding manner.[469]

Derepression of chromatin-mediated transcriptional repression of paternal and maternal genomes is one of the first major steps in zygotic gene expression after fertilization. The histone variant H3.3 participates in remodeling the paternal and maternal genomes for activation along the process of fertilization and embryogenesis. H3.3-mediated paternal chromatin remodeling is essential for the development of preimplantation embryos and the activation of the paternal genome during embryogenesis.[470] It has been shown that sperm-derived H3.3 protein (sH3.3) is removed from the sperm genome after fertilization and extruded from the zygotes via the second polar bodies (PB II) during embryogenesis. Maternal H3.3 (mH3.3) protein is incorporated into the paternal genome immediately after fertilization and remains in the paternal genome until the morula stage. Alterations in maternal H3.3 may affect embryonic development, and mH3.3 depletion in oocytes impairs both activation of the Oct4 pluripotency marker gene and global de novo transcription from the paternal genome in early embryonic development.[470]

Dynamic generation and erasure of the repressive histone modification trimethyl histone H3 lysine 27 (H3K27me3) in decidual stromal cells dictate both elements of pregnancy success in mice. In early gestation, H3K27me3-induced transcriptional silencing of select gene targets ensured uterine quiescence by preventing the decidua from expressing parturition-inducing hormone receptors, manifesting type 1 immunity and, most unexpectedly, generating myofibroblasts and associated wound-healing responses. In late gestation, genome-wide H3K27 demethylation allowed for target gene upregulation, decidual activation, and labor entry.[471]

The histone code is an established epigenetic regulator of early embryonic development. The lysine residue K9 of histone H3 (H3K9) is a prime target of SIRT1, a member of the NAD^+-dependent histone deacetylase family of enzymes targeting both histone and nonhistone substrates. Recent studies on the effect of SIRT1 activity on H3K9 methylation and acetylation in zygotes and the significance of H3K9 modifications for early embryonic development revealed that SIRT1 activators (resveratrol, BML-278) increase H3K9 methylation and suppress H3K9 acetylation in both the paternal and maternal pronucleus. In contrast, SIRT1 inhibitors (nicotinamide, sirtinol) suppress methylation and increase acetylation of pronuclear H3K9. At early embryonic development the positive effect of selective SIRT1 activation on the blastocyst formation rate correlates with the signal intensity of ooplasmic ubiquitin ligase MDM2, a known substrate of SIRT1 and limiting factor of epigenome remodeling. SIRT1 modulates the zygotic histone code through direct deacetylation and via nonhistone targets resulting in increased H3K9me3.[472]

The *CDKN1C* gene encodes the p57Kip2 protein, the third member of the CIP/Kip family (p27Kip1 and p21Cip1). p57Kip2 inhibits cyclin/cyclin-dependent kinase complexes and modulates cell division cycle progression. p57Kip2 has been classically associated with correct embryogenesis, since *CDKN1C*-ablated mice are not vital. CDKN1C alterations cause three human hereditary syndromes, characterized by altered growth rate. *CDKN1C* maps on 11p15.5 and shows regional imprinting with transcription of the maternal allele. *CDKN1C* transcription is regulated by DNA methylation, specific histone methylation/acetylation, lncRNAs, and miRNAs.[473]

Crosstalk between growth factors (GFs) and steroid hormones is a recurrent phenomenon in embryogenesis. The crosstalk between the EGF and glucocorticoids (GCs) involves transcription factors like p53 and NFκB, along with reduced pausing and traveling of RNA polymerase II (RNAPII) at both promoters and bodies of GF-inducible genes. GCs inhibit positive feedback loops activated by GFs and stimulate reciprocal inhibitory loops. According to data reported by Enuka et al.,[474] no alterations in DNA methylation accompany the transcriptional events instigated by either stimulus; however, forced demethylation of regulatory regions broadened the repertoire of GF-inducible genes. Enhancers and promoters are poised for activation by GFs and GCs. GFs enhance binding of the GC receptor to DNA and promote productive RNAPII elongation. Unmethylated genomic regions that encode feedback regulatory modules and differentially recruit RNAPII and acetylases/deacetylases underlie the crosstalk between GFs and a steroid hormone.[474]

Placental insufficiency, high-altitude pregnancies, maternal obesity/diabetes, maternal undernutrition, and stress can result in a poor setting for growth of the developing fetus. An adverse intrauterine environment can influence the expression of miRNAs and these changes may impact heart development. Potential consequences of altered miRNA expression in the fetal heart include hypoxia-inducible factor activation, dysregulation of angiogenesis, mitochondrial abnormalities, and altered glucose and fatty acid transport/metabolism.[475]

Maternal undernutrition or overnutrition during pregnancy alters organ structure, impairs prenatal and neonatal growth and development, and reduces feed efficiency for lean tissue gains. These adverse effects may be carried over to the next generation or beyond. Like maternal malnutrition, undernutrition during the neonatal period also reduces growth performance and feed efficiency.[476] An inappropriate nutritional environment during early development with alterations in maternal nutrition, including both under- and overnutrition, increase the risk for a range of cardiometabolic and neurobehavioral disorders in adult offspring characterized by both adipokine resistance and obesity. Alterations in the epigenome and other underlying mechanisms (altered gut-brain axis) may contribute to lasting cardiometabolic dysfunction in offspring.[477]

Prepregnancy maternal obesity is associated with adverse offspring outcomes at birth and later in life. The Pregnancy and Childhood Epigenetics (PACE) Consortium metaanalyzed the association between prepregnancy maternal BMI and methylation at over 450,000 sites in newborn blood DNA, across 19 cohorts (9340 mother-newborn pairs), and found that in newborns maternal BMI was associated with small methylation variation at 9044 sites throughout the genome.[478]

Expression of 27 miRNAs was positively associated with prepregnancy body mass index. Some of these differentially expressed miRNAs are associated with adipogenesis (i.e., let-7d*, miR-103-2*, -130b, -146b-5-p, -29c, and -26b) and other physiological and pathological pathways (injury, reproductive system disease, connective tissue disorders, cancer, cellular development, growth, and proliferation).[479]

A suboptimal intrauterine environment can perturb the metabolic programming of the growing fetus, thereby increasing the risk for developing obesity in later life. Maternal adiposity, smoking, blood glucose, and plasma unsaturated fatty acid levels are associated with birth weight. Polygenic defects are also associated with birth weight and child adiposity, indicating an overlap between the genetic pathways influencing metabolic health in early and later life. Neonatal methylation markers from seven gene loci (*ANK3, CDKN2B, CACNA1G, IGDCC4, P4HA3, ZNF423,* and *MIRLET7BHG*) are significantly associated with birth weight.[480]

Paternal obesity impairs hormones, metabolism, and sperm function, which can be transmitted to offspring. Paternal obesity results in insulin resistance/type 2 diabetes and increased levels of cortisol in umbilical cord blood, which increases the risk factors for cardiovascular disease. Epigenetics is the primary mechanism for the transmission of phenotypes from the father to offspring.[481]

Maternal diet can modify the epigenome of the offspring, producing different phenotypes and altered disease susceptibilities.[480, 482] Differential DNA methylation defects of H19/IGF2 are associated with congenital growth disorders characterized by opposite clinical pictures.[483] Maternal dietary methyl donor (methionine, folate, and choline) and cofactor (zinc and vitamins B2, B6, and B12) intake in one-carbon metabolism and DNA methylation impacts on fetal growth and lifelong health outcomes.[484] Maternal obesity induces fetal liver epigenetic changes resulting in dysregulation of key metabolic pathways that impact lipid metabolism.[485] Grandpaternal chronic high-fat diet (HFD) transgenerationally impairs glucose metabolism in subsequent generations altering the transcriptome and lipidome in skeletal muscle.[486]

Factors predisposing for type 2 diabetes including an adverse intrauterine environment, increasing age, overweight, physical inactivity, a family history of the disease, and an unhealthy diet affect the DNA methylation pattern in target tissues for insulin resistance in humans.[487]

Pregnancy anxiety is associated with differential DNA methylation patterns in newborns.[488] Intrauterine growth restriction (IUGR) leads to increased preference for palatable foods at different ages, and altered striatal dopamine signaling associates with a preference for palatable foods. The multiloci genetic score reflecting dopamine-signaling capacity is differentially associated with spontaneous palatable food intake in children depending on the fetal growth status.[489]

Another important issue is the long-term epigenetic effects of gestational drug treatments. Studies on maternal treatment with α1-adrenergic antagonism (Prazosin) during pregnancy indicate that maternal α1-adrenergic blockade may cause dwarfism, hyperthermia, and insulin resistance in male offspring, accompanied by reduced IGF-1 serum concentrations as a result of reduced hepatic growth hormone receptor (GHR) expression, and increased CpG DNA methylation at the transcriptional start site of the alternative *GHR* promoter.[490]

Long-term cigarette smoking during pregnancy affects the risk for childhood cardiovascular morbidity of the offspring.[491]

Fetal intolerance of labor is a common indication for delivery by Cesarean section. DNA methylation patterns at CpG sites across the genome interrogated for associations with fetal intolerance of labor identified four CpG sites in the *SLC9B1* gene, a Na^+/H^+ exchanger. DNA methylation and gene expression were negatively associated when examined longitudinally during pregnancy. Fetal intolerance of labor may be accurately predicted from maternal blood samples obtained between 24 and 32 weeks' gestation by assessing the DNA methylation patterns of *SLC9B1*.[492]

Mutation of highly conserved residues in transcription factors may affect protein-protein or protein-DNA interactions, leading to gene network dysregulation and human disease. Human mutations in *GATA4*, a cardiogenic transcription factor, cause cardiac septal defects and cardiomyopathy. iPS-derived cardiomyocytes from subjects with a heterozygous *GATA4-G296S* missense mutation show impaired contractility, calcium handling, and metabolic activity. In human cardiomyocytes, GATA4 broadly cooccupied cardiac enhancers with TBX5, another transcription factor that causes septal defects when mutated. The *GATA4-G296S* mutation disrupted TBX5 recruitment, particularly to cardiac superenhancers, concomitant with dysregulation of genes related to phenotypic abnormalities, including cardiac septation. The *GATA4-G296S* mutation can lead to failure of GATA4 and TBX5-mediated repression at noncardiac genes and enhanced open chromatin states at endothelial/endocardial promoters.[493]

Late gestation has been associated with increased prevalence of obstructive sleep apnea (OSA). Late gestation intermittent hypoxia may induce long-term effects on somatic growth, energy homeostasis, and metabolic function in offspring. Fetal perturbations by OSA during pregnancy cause long-term metabolic dysfunction in adult male offspring. This process may involve 1520 DMRs associated with 693 genes related to metabolic regulation and inflammation.[494]

1.6.1 In Vitro Fertilization and Assisted Reproduction Technology

The rate of in vitro fertilization is growing worldwide, especially in developed countries. Although the general somatic health status and cognitive development do not differ from spontaneously fertilized children, in vitro fertilization treatments are associated with a slightly elevated risk for preterm delivery, low birth weight, and structural abnormalities. The in vitro fertilization process affects the embryonic epigenome, which organizes itself during early embryonic development. These changes may influence the phenotype and health profile of the child during development and in adult life.[495]

DNA methylation can be considered a component of epigenetic memory with a critical role during embryo development, and dramatic reprogramming after fertilization. Assisted reproductive technologies (ART) might impair DNA methylation reprogramming, and ART-associated defects might affect the health conditions of ART-derived children.[496]

A study of the effects of high progesterone levels on the day of hCG administration in IVF cycles on epigenetic modifications of the endometrium in the periimplantation period revealed that (i) in luminal epithelium the expression of H3K9me2 in women with high progesterone levels is higher than in women with normal progesterone levels; (ii) in glandular epithelium the expression of 5-mC, H3K9me2, and H3K9ac is also higher in cases with hyperprogesteronemia; (iii) in stroma the expression of H3K27me3 is higher in women with high progesterone levels. The altered epigenetic modification status in the endometrium may disrupt the endometrial receptivity on the day of hCG administration in hyperprogesteronemic women.[497]

The association of in vitro fertilization (IVF) and DNA methylation has been studied predominantly at regulatory regions of imprinted genes and at just thousands of the ~28 million CpG sites in the human genome. Castillo-Fernandez et al.[498] studied the links between IVF and DNA methylation patterns in whole cord blood cells and cord blood mononuclear cells from newborn twins and found one significant whole blood DNA methylation change linked to conception via IVF, which was located ~3 kb upstream of *TNP1*, a gene previously linked to male infertility. The 46 most strongly associated signals included a second region in *C9orf3*, a gene also previously linked to infertility. Individual-specific environmental factors might be the main overall contributors to methylation variability.

Individual semen parameters differentially affect the offspring birth weight and body mass index (BMI) in an assisted reproductive technology (ART) patient compared to fertile controls. The offspring of men with impaired sperm parameters have lower birth weight compared to fertile control offspring. In subfertile subjects there is a tendency to increasing birth weight across levels of total motile count and total sperm count compared to azoospermic subjects.[499]

ART and subfertility appear to be linked to lower birth weight outcomes, setting infants up for poor long-term health. Prenatal growth is in part regulated via epigenetically controlled imprinted genes in the placenta. Differences in DNA methylation between ART and control infants have been found. A total of 45 genes were identified as having significantly different expression between subfertile infants and controls, whereas no significant differences were identified between the IVF and control groups. The expression of *IGF2, NAPIL5, PAX8-AS1,* and *TUBGCP5* was significantly downregulated in the IVF compared to the subfertile group. Methylation levels in *GRB10, NDN, CD44, MKRN3, WRB, DHCR24,* and *CYR61* correlated with expression. According to Litzky et al.[500] epigenetic differences in placentas resulting from in vitro fertilization (IVF) pregnancies may be related to underlying subfertility in parents using IVF rather than the IVF procedure itself.

Approximately 50% of the cases of infertility are linked to male factor infertility. Differential DNA methylation patterns are seen in spermatozoa from males who are suffering from a reduction in fecundity. Differences are relevant in the methylation levels of CpGs of the *PRICKLE2* (CpG1, CpG2) and CpGs in *ALS2CR12* gene-related amplicons (CpG1, CpG2), as well as in CpGs of the *ALDH3B2* gene (CpG2, CpG6, CpG9, CpG10, CpG11, CpG12, CpG13) and *PTGIR* gene-related amplicons (CpG4, CpG6, CpG8, CpG9, CpG11, CpG15, CpG19, CpG23, CpG26).[448]

Krieg et al.[501] assessed expression of the histone demethylases KDM4A and KDM4B in granulosa collected from women undergoing oocyte retrieval and found that KDM4A and KDM4B were localized in oocytes, granulosa cells, and theca and luteal cells in ovaries from reproductive-aged women. Histone demethylases *KDM4A* and *KDM4B* mRNA are differentially expressed in cumulus and mural granulosa. Expression of both *KDM4A* and *KDM4B* mRNA was lower in cumulus granulosa and mural granulosa from pregnant compared to nonpregnant patients. Altered expression of histone demethylases may impact epigenetic changes in granulosa cells associated with pregnancy.[501]

1.7 PLACENTA

The placenta is a fundamental organ for the mother and the embryo-fetal entity during pregnancy. Human placental epigenetic signatures are associated with psychosocial stress, maternal psychopathology, maternal toxicology, and exposure to environmental pollutants.[502] The placenta is also recognized as the "third brain" linking the developing fetal brain and the maternal brain. Epigenetic regulation of placenta affect infant neurodevelopment.[503]

DNA methylation is globally reprogrammed after fertilization, and as a result the parental genomes have similar DNA-methylation profiles after implantation except at germline differentially methylated regions (gDMRs). Human blastocysts might contain thousands of transient maternally methylated gDMRs (transient mDMRs), whose maternal methylation is lost in embryonic tissues after implantation. Genome-wide allelic DNA methylation analyses of purified trophoblast cells from human placentas revealed that over one-fourth of transient-in-embryo mDMRs maintain their maternally biased DNA methylation. Some placenta-specific mDMRs are associated with expression of imprinted genes (*TIGAR, SLC4A7, PROSER2-AS1,* and *KLHDC10*), and three imprinted gene clusters have been identified. These data reported by Hamada et al.[504] highlight the incomplete erasure of germline DNA methylation in the human placenta and potential consequences for pathogenesis of developmental disorders with imprinting effects.

The Tousled-like kinases (TLKs) are fundamental proteins in the placenta. TLK1 is dispensable for murine viability, and TLK2 loss leads to late embryonic lethality because of placental failure. TLK2 is required for normal trophoblast differentiation, and the phosphorylation of ASF1 is reduced in placentas lacking TLK2. TLK2 is essential for placental function during mammalian development, and both TLK1 and TLK2 have largely redundant roles in genome maintenance.[190]

Intrauterine growth restriction (IUGR) is caused by dysregulation of placental metabolism. Paternally inherited IUGR mutations in the fetus influence maternal physiology via the placenta. A 3′ deletion in the noncoding MER1 repeat containing imprinted transcript 1 (*MIMT1*) gene causes IUGR and late abortion in Ayrshire cattle with variable levels of severity. Transcriptome analysis shows fewer differentially expressed genes in maternal than fetal MIMT1-Del/WT placentome. *AST1*, within the PEG3 domain, is the only gene consistently reduced in IUGR in both fetal and maternal samples. Several genes show an imprinting pattern associated with IUGR; only secernin 3 (*SCRN3*) and paternally expressed 3 (*PEG3*) are differentially imprinted in both placentome components. Loss of strictly monoallelic, allele-specific expression of *PEG3* in the maternal MIMT1Del/WT placenta was associated with incomplete penetrance of *MIMT1Del*. Dysregulation of the PEG3 domain is involved in IUGR, and maternal placental tissues may affect penetrance of the paternally inherited IUGR mutation.[505]

TERRA ncRNA is a negative regulator of telomerase. Human placenta tissue and trophoblasts show longer telomere lengths compared to gestational age-matched somatic cells. However, telomerase (hTERT) expression and activity in the placenta is low, suggesting a role for alternate lengthening of telomeres (ALT). While ALT is observed in 10%–15% of human cancers and in some mouse stem cells, ALT has never been reported in noncancerous human tissues. Human placenta tissue and purified first-trimester trophoblasts show low subtelomeric (*TERRA*) DNA methylation compared to matched CBMCs and other somatic cells. Placental *TERRA* methylation is lower than ALT cancer cell lines. Low *TERRA* methylation is associated with higher expression of *TERRA* RNA in the placenta compared to matched CBMCs.[506]

Placental transfer of amino acids via amino acid transporters is essential for fetal growth. Expression of specific amino acid transporters is inversely associated with DNA methylation. Amino acid transporters expressed in terms of the placenta show low levels of promoter DNA methylation. These transporters tend to be more highly methylated

at gene promoter regions. The *SLC1A2, SLC1A3, SLC1A4, SLC7A5, SLC7A11,* and *SLC7A10* transporter genes exhibit changes in enhancer DNA methylation across gestation.[507]

The fibroblast growth factor receptor 2 (*FGFR2*) gene encodes a protein of the fibroblast growth factor receptor family. *FGFR2* gene expression is associated with regulation of the implantation process of the placenta, which plays a vital role in fetal growth. DNA methylation of the *FGFR2* gene in the placenta is associated with full-term low birth weight. Placental surface area mediates the association between DNA methylation of *FGFR2* in the placenta and full-term low birth weight in a sex-specific manner.[508]

5,10-Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in one-carbon metabolism facilitating the availability of methyl groups for methylation reactions. Two SNPs in the *MTHFR* gene (677C > T; 1298A > C) result in a thermolabile enzyme with reduced function. These variants have been associated with pregnancy complications including miscarriage, neural tube defects, and preeclampsia, potentially due to altered capacity for DNA methylation. However, large-scale, genome-wide disruption in DNA methylation does not occur in placentas with high-risk *MTHFR* 677TT or 1298CC genotypes.[509]

Circadian pathway methylation of the human placenta is an important molecular target for healthy development. The study of placental methylation of CpG sites within the promoter regions of the *CLOCK, BMAL1, NPAS2, CRY1-2,* and *PER1-3* genes in newborns revealed that air pollution, an environmental risk factor leading to a proinflammatory state of the mother and fetus, is associated with the methylation pattern of genes in the circadian pathway. Observed alterations in the placental *CLOCK* epigenetic signature might form a relevant molecular mechanism through which fine-particle air pollution exposure might affect placental processes and fetal development.[375]

Mammalian reproductive performance declines rapidly with advanced maternal age as a result of the exponential increase in chromosome segregation errors in the oocyte with age. However, pregnancy complications and birth defects, frequent in older mothers, occur in the absence of karyotypic abnormalities. Abnormal embryonic development in aged females is associated with severe placentation defects, which result from major deficits in the decidualization response of the uterine stroma.[510]

Fetal development largely depends on thyroid hormone availability and proper placental function with an important role played by placental mitochondria. Cord blood FT3 and FT4 are inversely associated with placental mtDNA methylation at the MT-RNR1 and D-loop regions, whereas a positive association is observed for both hormones with placental mtDNA content.[511]

Early pregnancy loss (EPL) (early miscarriage) is the unintentional expulsion of an embryo or fetus prior to the 12th week of gestation, which occurs with a frequency of about 15% during pregnancy. miRNAs are the primary epigenetic regulators in placental development and function. Four miRNAs (hsamiRNA (miR) 125a3p, has-miR-3663-3p, hsa-miR-423-5p, hsa-miR-575) are upregulated in EPL placentas. Target genes in EPL pathogenesis are associated with cell migration, proliferation, implantation, adhesion, angiogenesis, and differentiation.[512]

An epigenome-wide association study (EWAS) of DNA methylation in the placenta in relation to maternal tobacco smoking during pregnancy showed that 50 CpGs were differentially methylated in the placenta between smokers and nonsmokers during pregnancy. Differential methylation was relevant at top-ranking loci: cg27402634 located between *LINC00086* and *LEKR1*, cg20340720 (*WBP1L*) and cg25585967 and cg12294026 (*TRIO*). Differential methylation at cg27402634 explained up to 36% of the lower birth weight in the offspring of smokers. Decreases in methylation levels at cg27402634 lead to decreases in birth weight.[513]

Transplacental in utero exposure to particulate matter is associated with an increased overall placental mutation rate, which occurs in concert with epigenetic alterations in key DNA repair and tumor suppressor genes (*APEX1, OGG1, PARP1, ERCC1, ERCC4, p53,* and *DAPK1*). Exposure to air pollution can induce changes to fetal and neonatal DNA repair capacity.[514]

Psychosocial stress contributes to placental oxidative stress. Mitochondria are vulnerable to oxidative stress, which can lead to changes in mitochondrial DNA copy number (mtDNAcn). Higher lifetime stress and depressive symptoms account for most of the effect on mtDNAcn. Increased maternal psychosocial stress correlates with reduced placental mtDNAcn.[515]

1.8 LACTATION

DNA methylation seems to be an essential regulatory element during lactation, modulating the activity of milk-related genes. The highest CG levels (>70%) occur in the 3′ UTR region, followed by the gene body region (>60%). In dairy goat mammary glands, methylation levels of 95 and 54 genes in the lactation period are upregulated or downregulated, respectively, relative to the dry period.[516]

1.9 DEVELOPMENT

Epigenetics plays a fundamental role in development. The early environment has a major impact on the developing embryo, fetus, and infant. Parental adversity before conception and during pregnancy has profound effects on development and behavior. These effects are species-, sex-, and age-specific and depend on the timing and duration of exposure to environmental pressure. The impact of these early exposures can extend across multiple generations, via both the maternal and paternal lineage.[517]

Most developmental genes are devoid of DNA methylation at promoters even when they are repressed. These hypomethylated regions are large and extend beyond proximal promoters, forming DNA methylation valleys (DMVs) or DNA methylation canyons. DMVs are hypomethylated in development and are highly conserved across vertebrates. DMVs are hotspots of regulatory regions for key developmental genes and show low levels of deamination mutation rates. A subset of DMVs is dynamically methylated and enriched for Polycomb-deposited H3K27me3 when the associated genes are silenced, showing elevated DNA methylation upon gene activation. Polycomb-bound DMVs form insulated and self-interacting chromatin domains. Polycomb may regulate hypomethylation of DMVs through ten-eleven translocation (TET) proteins.[518]

Methylation of histone 3 lysine 4 (H3K4) is largely associated with promoters and enhancers of actively transcribed genes and is finely regulated during development by the action of histone methyltransferases and demethylases. H3K4me3 demethylases of the KDM5 family are implicated in development. The H3K4 demethylase RBR-2, the unique member of the KDM5 family in *C. elegans*, acts cell-autonomously and in a catalytic-dependent manner to control vulva precursor cell fate acquisition, by promoting the LIN-12/Notch pathway. RBR-2 reduces the H3K4me3 level at transcription start sites (TSSs) and in regions upstream of TSSs, and acts both as a transcription repressor and activator. RBR-2 controls the epigenetic signature of the lin-11 vulva-specific enhancer and lin-11 expression, providing evidence that RBR-2 can positively regulate transcription and cell fate acquisition by controlling enhancer activity.[519]

Intrauterine growth restriction (IUGR) may cause postnatal body developmental and metabolic diseases as a result of their impaired growth and development in the mammalian embryo/fetus during pregnancy. IUGR may lead to abnormally regulated DNA methylation in the intestine, causing intestinal dysfunctions. Some of the genes affected by epigenetic abnormalities include *AIFM1, MTMR1, TWIST2, BCAP31, IRAK1,* and *AIFM1*, with influence in cell apoptosis, cell differentiation, and immunity.[520]

Precise control of gene expression during development is orchestrated by transcription factors and coregulators including chromatin modifiers. How particular chromatin-modifying enzymes affect specific developmental processes is not well defined. GCN5, a histone acetyltransferase essential for embryonic development, is required for proper expression of multiple genes encoding components of the fibroblast growth factor (FGF) signaling pathway in early embryoid bodies (EBs). *Gcn5* −/− EBs display deficient activation of ERK and p38, mislocalization of cytoskeletal components, and compromised capacity to differentiate toward mesodermal lineage. At least 7 genes are putative targets of GCN5 during early differentiation, four of which are cMYC targets.[521]

Neonates have dampened expression of proinflammatory cytokines and difficulty clearing pathogens. This makes them uniquely susceptible to infections. Studies on the role of histone modifications in neonatal immune function revealed increased activation of H3K4me3 on proinflammatory *IL1B, IL6, IL12B,* and *TNF* cytokine promoters with no change in repressive H3K27me3, suggesting that these promoters in preterm neonates are less open and accessible to transcription factors than in term neonates. As development progresses from neonate to adult, monocytes lose the poised enhancer mark H3K4me1 and gain the activating mark H3K4me3, without a change in additional histone modifications. This decreased H3K4me3 abundance at immunologically important neonatal monocyte gene promoters, including *CCR2, CD300C, ILF2, IL1B,* and *TNF*, is associated with reduced gene expression.[522] Exposure to heat in early life leads to either resilience or vulnerability to heat stress later in life. Epigenetic alterations in genes belonging to the cell proteostasis pathways are attributed to long-term responses to heat stress. Hypermethylation of the *HSP70* promoter in high-temperature-conditioned chicks is accompanied by a reduction in both POU Class 2 Homeobox 1 (POU2F1) binding and recruitment of the nucleosome remodeling deacetylase (NuRD) chromatin-remodeling complex. As a result histone H3 acetylation levels at the *HSP70* promoter are higher in harsh-temperature-conditioned chicks. The methylation level of a distal part of the *HSP70* promoter and POU2F1 recruitment may reflect heat stress-related epigenetic memory and might be useful in differentiating between individuals that are resilient or vulnerable to stress.[523]

Lineage-specific genes regulate biological programs during odontogenesis. The activity of these genes is modified by H3K4me3 and H3K27me3 marks at promoter regions in progenitors. These bivalent domains regulate activation or repression during differentiation. Wnt5a promotes odontogenic differentiation in dental mesenchyme. H3K4me3

methylases (mixed lineage leukemia (MLL) complex) and H3K27me3 demethylases (JMJD3 and UTX) are dynamically expressed between the early and late bell stage of human tooth germs during odontogenic induction. The *WNT5A* gene is marked by bivalent domains containing repressive marks (H3K27me3) and active marks (H3K4me3) on promoters. The bivalent domains resolve during induced differentiation, with removal of the H3K27me3 mark in a JMJD3-dependent manner. *JMJD3* knockdown suppresses odontogenic differentiation. *JMJD3* depletion represses WNT5A activation by increasing H3K27me3, and JMJD3 interacts with ASH2L, a component of the MLL complex, to form a coactivator complex, modulating H3K4me3 on *WNT5A* promoters.[524]

Milaniak et al.[525] studied the relationship between DNA methylation at birth and resilience to prenatal environmental stressors (conduct, hyperactivity, emotional problems, and global symptomatology) in middle childhood, focusing on DNA methylation in the vicinity of the oxytocin receptor (*OXTR*) gene. *OXTR* DNA methylation was predictive of resilience in the conduct problems domain in middle childhood. Children who were resilient to conduct problems were also broadly resilient across multiple domains.

Maltreatment is linked to methylation of the glucocorticoid receptor gene, nuclear receptor subfamily 3, group C, member 1 (*NR3C1*), which participates in the regulation of the corticotropinergic system (hypothalamus-pituitary-adrenal axis). Maltreated children show higher baseline levels of *NR3C1* methylation, a decreasing methylation profile over time, and lower levels of methylation at follow-up.[526]

Development and homeostasis of the epidermis are governed by a complex network of sequence-specific transcription factors and epigenetic modifiers cooperatively regulating the subtle balance of progenitor cell self-renewal and terminal differentiation. Histone H2A deubiquitinase 2A-DUB/Mysm1 plays an important role in suppression of p53-mediated inhibitory programs during epidermal development.[527]

In adipose tissue white fat stores energy in lipids, while brown fat is responsible for nonshivering thermogenesis through UCP1-mediated energy dissipation. Lysine-Specific Demethylase 1 (LSD1) inhibitors repress brown adipocyte differentiation. RNAi-mediated *Lsd1* knockdown causes a similar effect, which can be rescued by expression of wild-type but not catalytic-inactive LSD1. LSD1 promotes brown adipogenesis by demethylating H3K4 on promoter regions of Wnt signaling components and repressing the Wnt pathway. Deletion of *Lsd1* in mice leads to inhibition of brown adipogenesis.[528]

Epigenetic age is an indicator of biological aging, capturing the impact of environmental and behavioral influences across time on cellular function. Deviance between epigenetic age and chronological age (AgeAccel) is a predictor of health. Pubertal timing has similarly been associated with cancer risk and mortality rate among females. A five-year increase in average adolescent AgeAccel was associated with a significant decrease in time to menarche adjusting for birth weight, maternal prepregnancy body mass index, maternal height, maternal education, B2 height, fat percentage, and cell composition. AgeAccel displayed a stronger inverse association with pubertal tempo.[529]

Newborn neurons undergo migration to their final destinations during neocortical development. Reelin-induced tyrosine phosphorylation of disabled 1 (Dab1) is a critical mechanism controlling cortical neuron migration. Deleted in colorectal carcinoma (DCC) interacts with Dab1 via its P3 domain. Netrin 1 is a DCC ligand that induces Dab1 phosphorylation at Y220 and Y232. Knockdown of *DCC* or truncation of its P3 domain delays neuronal migration and impairs the multipolar-to-bipolar transition of migrating neurons. Migration delay and morphological transition defects are rescued by the expression of a phosphomimetic Dab1 or a constitutively active form of Fyn protooncogene (Fyn), a member of the Src family of tyrosine kinases that effectively induces Dab1 phosphorylation. DCC-Dab1 interaction ensures proper neuronal migration during neocortical development.[530]

Fetal exposure to high levels of glucocorticoids (GCs) affects the physiologic stress response and behaviors. Prenatal exposure to GCs alters expression of genes in the prefrontal cortex and hypothalamic paraventricular nucleus (PVN) and induces reprogramming of large transgenerational changes in PVN gene expression, with potential repercussion on type II diabetes, thermoregulation, and collagen formation gene networks.[531]

The evolutionarily conserved Hedgehog (Hh) signaling pathway is involved in development and tissue homeostasis. Aberrant activation of this signaling pathway occurs in a wide range of human diseases. miRNAs participate in the regulation of this pathway.[532]

Differentiation of B lymphocytes into isotope-specific plasma cells represents a hallmark event in adaptive immunity. During B cell maturation, expression of the class II transactivator (*CIITA*) gene is downregulated. Hypermethylated in cancer 1 (*HIC1*) is upregulated in differentiating B lymphocytes paralleling *CIITA* repression. Overexpression of *HIC1* directly represses endogenous *CIITA* transcription in B cells. HIC1 bound to the proximal *CIITA* type III promoter (−545/−113), and mutation of a conserved HIC1 site within this region abrogates *CIITA trans*-repression. Depletion of *HIC1* with small interfering RNA (siRNA) restores *CIITA* expression in differentiating B cells. HIC1 preferentially interacts with and recruits DNMT1 and DNMT3b to the *CIITA* promoter to synergistically repress

CIITA transcription. Silencing of *DNMT1/DNMT3b* or inhibition of DNMT activity with 5-aza-dC attenuates *CIITA* *trans*-repression. HIC1 is a novel factor involved in B cell differentiation, acting as an epigenetic repressor of *CIITA* transcription.[533]

Age-related differences in DNA methylation have been identified during the first years of life, represented by 14,150 consistent age-differential methylation sites (a-DMSs) in genes involved in cell signaling, and enriched in H3K27ac, which can predict developmental state. Maternal smoking tends to decrease methylation levels at the identified a-DMSs. a-DMS-associated genes during early development are linked to different disease conditions.[534]

1.10 MATURATION, AGING, AND LONGEVITY

Maturation and aging, in terms of healthy or pathological conditions, are probably the consequence of genomic, epigenomic, and environmental factors impacting previous life stages. Advanced paternal age (>40 years) is associated with accumulated damage to sperm DNA and mitotic and meiotic quality control mechanisms (mismatch repair) during spermatogenesis. This causes numerical and structural abnormalities in sperm chromosomes, increased sperm DNA fragmentation, and single gene mutations. Abnormalities in offspring have also been described, including miscarriage and fetal loss, increase in genetic disorders and congenital anomalies. Epigenetic alterations in several genetic pathways are also currently seen.[535]

Tissue maturation is a paradigmatic example of genomic-epigenomic-xenobiotic interactions. For instance, respiratory function is an important predictor of morbidity and mortality. Studies on possible associations between genome-wide DNA methylation levels and lung function in monozygotic twins showed several differentially methylated CpG sites associated with forced expiratory volume the first second (FEV1) and forced vital capacity (FVC). Some probes identified for level of FVC were located in the *GLIPR1L2* gene, which is involved in innate immunity and tumor suppressor/prooncogenic mechanisms; and changes in DNA methylation levels in the *TRIM27* gene, potentially involved in CD4 T cell regulation and cancer development, were associated with FEV1, as well as other pathways such as the TGFBR.[536] Epigenetic factors are involved in the beneficial effects of exercise on bone health, preservation of bone mass and strength, and prevention of osteoporosis.[537]

Mammalian species exhibit a wide range of lifespans. Aging-associated differentially methylated positions (aDMPs) have been identified in humans and mice. These are CpG sites at which DNA methylation dynamics shows significant correlations with age. Lowe et al.[538] hypothesized that aDMPs are pan-mammalian and are a dynamic molecular readout of lifespan variation among different mammalian species. A large-scale integrated analysis of aDMPs in six different mammals reveals a strong negative relationship between rate of change of methylation levels at aDMPs and lifespan.

Recent studies on transgenerational determinants of longevity postulate that the chance of reaching a high age is transmitted from parents to children in a modest, but robust way. Longevity inheritance is paralleled by the inheritance of individual resilience. Individual resilience might develop in the first part of life as a response to adversity and early experience, giving rise to a transgenerational pathway distinct from social class trajectories. New theories of longevity inheritance should integrate new concepts on genomic susceptibility, frailty and resilience, epigenetic events, and social epidemiology.[539]

The histone deacetylase Sirtuin 1 (SIRT1) is linked to p53 activity. SIRT1 deacetylates p53 in a NAD^+-dependent manner to inhibit transcription activity of p53. The SIRT1/p53 pathway is involved in cellular aging, cancer, and reprogramming. The regulatory pathway SIRT1-p53-LDHA-Myc and the miR-34a-Let7 regulatory network conform to a positive feedback loop that controls cell cycle, metabolism, proliferation, differentiation, and epigenetic modifications. *SIRT1* expression is reduced as a protective mechanism against oncogenesis and for maintenance of tissue homeostasis. *SIRT1* activation in aged cells responds to DNA damage to protect cells from p53-dependent apoptosis or senescence.[540]

Cellular senescence is a physiological barrier against tumor and represents an option for therapeutic intervention. A critical intracellular stimulus causing senescence is the DNA damage response, while the senescence-associated heterochromatin in cancer limits the strength of the DNA damage response to endogenous genotoxic stress or DNA-damaging agents. It has been hypothesized that pharmacologically disrupting methylation potential, defined as the ratio of cellular S-adenosylmethionine to S-adenosylhomocysteine, might affect the chromatin structures in cancer cells enhancing their sensitivity to DNA damage response signaling. 3-Deazaneplanocin A, a chemical inhibitor of S-adenosylhomocysteine hydrolase, induces cellular senescence in hepatoma cells. Therapy-induced senescence by 3-deazaneplanocin A is mediated through the p53/p21 pathway and triggered by enhanced ataxia telangiectasia-mutated activation related to chromatin changes.[541]

Epigenetic remodeling is one of the major features of the aging process. DNA methylation of *ELOVL2* and *FHL2* CpG islands correlates with age in whole blood. Studies on age-associated hypermethylation of *ELOVL2* and *FHL2* showed that *ELOVL2* methylation is different in primary dermal fibroblast cultures from donors of different ages. Most tissues show *ELOVL2* and *FHL2* hypermethylation with age. *ELOVL2* hypermethylation is not found in tissues that have a very low replication rate. *ELOVL2* hypermethylation is associated with in vitro cell replication rather than with senescence. Methylation of the two loci is not associated with longevity/mortality, but *ELOVL2* methylation is associated with cytomegalovirus status in nonagenarians, which could be informative of a higher number of replication events in a fraction of whole blood cells. *ELOVL2* methylation is a marker of cell divisions occurring during human aging.[542]

Advancing age progressively impacts on risk and severity of chronic disease. It also modifies the epigenome, with changes in DNA methylation, as a result of both random drift and variation within specific functional loci. The study of peripheral blood genome-wide DNA methylomes identified 71 age-associated differentially methylated regions within the linkage disequilibrium blocks of SNPs from the NIH genome-wide association study catalog. These age-associated differentially methylated regions also show marked enrichment for enhancers and poised promoters across multiple cell types.[543]

1.10.1 Telomeres

Telomeric sequences are located at the very ends or terminal regions of chromosomes. Some vertebrate species show blocks of (TTAGGG)n repeats present in nonterminal regions of chromosomes, such as interstitial telomeric sequences (ITSs), interstitial telomeric repeats, or interstitial telomeric bands, which include those intrachromosomal telomeric-like repeats located near (pericentromeric ITSs) or within the centromere (centromeric ITSs) and those telomeric repeats located between the centromere and the telomere (truly interstitial telomeric sequences) of eukaryotic chromosomes. ITSs can be classified into four types: (i) short ITSs, (ii) subtelomeric ITSs, (iii) fusion ITSs, and (iv) heterochromatic ITSs. ITSs play a significant role in genome instability and evolution.[544]

The telomere is a key component of the hallmarks of aging and age-related disorders. Telomere homeostasis is linked to cellular metabolism and mitochondrial function, which declines during cellular senescence and normative physiological aging. Epigenetics and cellular ions play important roles in the regulation of telomere structure, integrity, and function.[545]

Telomere length (TL) declines with age in most human tissues, and a shorter TL is thought to accelerate senescence. In contrast, older men have sperm with longer TL, and older paternal age at conception (PAC) predicts longer TL in offspring. This PAC effect might be a unique form of transgenerational genetic plasticity that modifies somatic maintenance in response to cues of recent ancestral experience. Eisenberg et al.[546] studied the PAC effect in chimpanzees (*Pan troglodytes*). The PAC effect on TL is thought to be driven by continual production of sperm. As chimpanzees have both greater sperm production and greater sperm mutation rates with PAC than humans, the PAC effect on TL should be more pronounced in chimpanzees. TL with PAC in chimpanzees is increased with a slope six times that in humans. No associations between TL and grandpaternal ages were found in humans or chimpanzees. Sperm production rates across species might be a determinant of the PAC effect on offspring TL, and sperm production rates within species might influence the TL passed on to offspring.[546]

Telomere length is essential for embryonic stem cell (ESC) self-renewal and pluripotency. Zscan4 is required for telomere extension, facilitating telomere elongation by inducing global DNA demethylation through downregulation of *Uhrf1* and *Dnmt1*. Zscan4 recruits Uhrf1 and Dnmt1 and promotes their degradation, which depends on the E3 ubiquitin ligase activity of Uhrf1. Blocking DNA demethylation prevents telomere elongation associated with *Zscan4* expression.[547]

Limitless self-renewal is one of the hallmarks of cancer and is attained by telomere maintenance, essentially through telomerase (hTERT) activation. Transcriptional regulation of hTERT is believed to play a major role in telomerase activation in human cancers. Cancer cells have acquired the ability to overcome their fate of senescence via telomere length maintenance mechanisms, mainly by telomerase activation. *hTERT* expression is upregulated in tumors via multiple genetic and epigenetic mechanisms including *hTERT* amplifications, *hTERT* structural variants, *hTERT* promoter mutations, and epigenetic modifications through hTERT promoter methylation. Genetic (*hTERT* promoter mutations) and epigenetic (*hTERT* promoter methylation and miRNAs) events were shown to have clinical implications in cancers that depend on hTERT activation.[548]

Eukaryotic cells undergo continuous telomere shortening as a consequence of multiple rounds of replications. During tumorigenesis, cells have to acquire telomere DNA maintenance mechanisms (TMMs) to counteract telomere shortening, to preserve telomeres from DNA damage repair systems, and to avoid telomere-mediated senescence and/or apoptosis. Telomere maintenance is an essential step in cancer progression. Cancer cells maintain their telomeres by enhancing telomerase or by activation of the pathway for alternative lengthening of telomeres.[549]

1.11 EPIGENETIC MENDELIAN DISORDERS

Epigenetic Mendelian disorders (EMDs) are a group of multiple congenital anomaly and intellectual disability syndromes resulting from mutations in genes encoding components of the epigenetic machinery (Table 1.13).[550, 551] Within this category, genetic mutations may affect writers, erasers, or readers of epigenetic marks, and chromatin remodelers as well. Many EMDs fall within the category of neurodevelopmental and imprinting disorders, and some of them may manifest in adults. EMDs involving the DNA methylation machinery have been described for writers and readers of DNA methylation: (i) Rett syndrome, an X-linked disorder affecting mostly females and resulting from loss-of-function mutations in a reader of CpG methylation (*MeCP2*) (methyl-CpG-binding protein); (ii) 2q23.1 microdeletion/microduplication syndrome, an autosomal dominant syndrome with deletion/duplication in the *MBD5* locus, encoding a methyl-CpG-binding protein; (iii) immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome, caused by homozygous or compound heterozygous hypomorphic mutations in the *DNMT3B* gene; (iv) hereditary sensory and autonomic neuropathy with dementia and hearing loss (HSAN1E) (mutations in *DNMT1* exon 20); (v) autosomal dominant cerebellar ataxia, deafness, and narcolepsy (ADCADN) (mutations in *DNMT1* exon 21). EMDs of the histone machinery have been described for writers, erasers, readers, and chromatin remodelers, including: (i) Kabuki syndrome, an autosomal dominant trait with mutations in mixed lineage leukemia 2 (*MLL2*) (a histone H3K4 methyltransferase) or lysine-specific demethylase 6A (*KDM6A*) genes; (ii) Rubinstein-Taybi syndrome (RTS), an autosomal dominant syndrome caused by haploinsufficiency of histone acetyltransferase enzyme genes (*CREBBP* and *EP300*); (iii) genitopatellar syndrome (GPS) and Say-Barber-Biesecker-Young-Simpson (SBBYS) syndrome (mutations in the histone acetyltransferase *KAT6B*); (iv) Widerman-Steiner syndrome (WSS) (mutations in the *MLL* gene, histone methyltransferase *H3K4*); (v) Kleefstra syndrome (KLFS) (mutations in *EHMT1*, histone methyltransferase *H3K9*); (vi) Weaver syndrome (WS) (mutations in *EZH2*, histone methyltransferase *H3K27*); (vii) Sotos syndrome (SS) (mutations in *NSD1*, histone methyltransferase *H3K36* and *H4K20*); (viii) brachydactyly-mental retardation (BDMR) syndrome (haploinsufficiency of the histone deacetylase gene, *HDAC4*); (ix) Cornelia de Lange syndrome 5 (CDLS5) (X-linked) and Wilson-Turner syndrome (WTS) (X-linked) (mutations in histone deacetylase *HDAC8*); (x) Claes-Jensen syndrome (CJS) (X-linked) (mutations in *KDMSC*, histone demethylase *H3K4*); (xi) Kabuki syndrome (X-linked) (mutations in *KDM6A*, histone demethylase *H3K27*); (xii) Siderius X-linked mental retardation syndrome (MRXSSD) (mutations in *PHF8*, plant homeodomain finger protein); (xiii) Börjeson-Forssman-Lehmann syndrome (BFLS) (X-linked recessive trait, missense mutations in the *PHF6* gene, plant homeodomain finger protein); and (xiv) X-linked mental retardation and macrocephaly (mutations in *BRWD3*, bromodomain-containing protein). EMDs of chromatin remodelers include the following: (i) alpha-thalassemia/mental retardation X-linked (ATRAX) syndrome (mutations in *ATRAX*, SWI/SNF ATP-dependent chromatin remodeler); (ii) four variants of Coffin-Siris syndrome: mental retardation autosomal dominant 14 (MRD14) (mutations in *ARID1A*), mental retardation autosomal dominant 12 (MRD12) (mutations in *ARID1B*), mental retardation autosomal dominant 16 (MRD16) (mutations in *SMARCA4*), and mental retardation autosomal dominant 15 (MRD15) (mutations in *SMARCB1*); (iii) rhabdoid tumor predisposition syndrome 2 (mutations in *SMARCA4*); (iv) Schwannomatosis (mutations in *SMARCB1*); (v) rhabdoid tumor predisposition syndrome 1 (mutations in *SMARCB1*); (vi) Nicolaides-Baraitser syndrome (mutations in *SMARCA2*); (vii) floating harbor syndrome (mutations in *SRCAP*, INO80/SWR1 ATP-dependent chromatin remodeler); (viii) CHARGE syndrome (mutations in *CHD7*, CHD ATP-dependent chromatin remodeler); and (ix) mental retardation autosomal dominant 21 (MRC21) (mutations in *CTCF*, chromatin-organizing zinc finger protein)[550, 551] (Table 1.13).

1.12 PHARMACOEPIGENETICS NETWORK

Pharmacoepigenomics deals with the influence that epigenetic alterations (DNA methylation, histone modifications, chromatin remodeling, noncoding RNA dysregulation) exert on drug efficacy and safety, and on the effects that

TABLE 1.13 Epigenetic Mendelian Disorders

Disease	Defective gene	Locus	Gene size (kb)	Protein	Protein size (aa)	Molecular weight (kDa)	Defective epigenetic function	Consequence	Other related diseases
Hereditary sensory and autonomic neuropathy with dementia and hearing loss (HSAN1E)	*DNMT1 DNA (cytosine-5-)-methyltransferase 1*	19p13.2	97.94	DNMT1 DNA (cytosine-5)-methyltransferase 1	1616	183.16	DNA methylation (Writer)	Impaired targeting of DNMT1 to heterochromatin. Aberrant hypermethylation at some gene promoters	Lymphosarcoma; Loeys–Dietz syndrome; Narcolepsy; Cerebellar ataxia; Hereditary sensory neuropathy; Mutagen sensitivity; Myelodysplastic syndrome; Breast cancer
Autosomal-dominant cerebellar ataxia, deafness, and narcolepsy (ADCADN)									
Immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome	*DNMT3B DNA (cytosine-5-)-methyltransferase 3 beta*	20q11.2	46.97	DNM3B DNA (cytosine-5)-methyltransferase 3B	853	95.75	DNA methylation (Writer)	Aberrant hypomethylation of particular DNA sequences	Malignant glioma; leukemia; carcinoma; colorectal cancer; lung cancer
Rett syndrome	*MECP2 Methyl-CpG-binding protein 2*	Xq28	76.18	MECP2 Methyl-CpG-binding protein 2	486	52.44	Binding to methylated DNA (Reader)	Impaired binding of MECEP2 or MBD5 to methylated target sequences leading to aberrant changes on gene expression	Intellectual disability; autistic disorder; angelman syndrome; microcephaly; gait apraxia
2q23.1 microdeletion/ microduplication syndrome	*MBD5 Methyl-CpG-binding domain protein 5*	2q23.1	497.22	MBD5 Methyl-CpG-binding domain protein 5	1494	159.89	Binding to methylated DNA (Reader)		Lagophthalmos; exposure keratitis, keratopathy
Rubinstein–Taybi syndrome (RTS)	*CREBBP CREB-binding protein*	16p13.3	155.67	CBP CREB-binding protein	2442	265.35	Histone acetylation (Writer)	Histone methylation (by methyltransferases) or acetylation (by acetylases) promotes an open chromatin conformation and the activation of critical gene promoters. Defective histone methyltransferases or acetylases lead to the downregulation of those genes and promote the onset of the disease	Acute myeloid leukemia, with t (8;16)(p11;p13) translocation; neonatal leukemia, floating-harbor syndrome, alpha-thalassemia/mental retardation syndrome, melanoma of soft parts, acute monocytic leukemia, monocytic leukemia, myeloid leukemia
	EP300 E1A-binding protein p300	22q13.2	88.29	EP300 Histone acetyltransferase p300	2414	264.16	Histone acetylation (Writer)		Acute monocytic leukemia; colorectal cancer; monocytic leukemia; hypoxia; breast cancer
Genitopatellar syndrome (GPS)	*KAT6B K(lysine) acetyltransferase 6B*	10q22.2	207.69	KAT6B Histone acetyltransferase KAT6B	2073	231.37	Histone acetylation (Writer)		Blepharophimosis-intellectual disability syndrome; KAT6B-related disorders; Ohdo syndrome; monocytic leukemia; noonan syndrome 1; leukemia

Continued

TABLE 1.13 Epigenetic Mendelian Disorders—cont'd

Disease	Defective gene	Locus	Gene size (kb)	Protein	Protein size (aa)	Molecular weight (kDa)	Defective epigenetic function	Consequence	Other related diseases
Say–Barber-Biesecker-Young-Simpson (SBBYS) syndrome									
Wiedemann–Steiner syndrome (WSS)	*KMT2A (MLL) Lysine (K)-specific methyltransferase 2A*	11q23	90.34	KMT2A Histone lysine *N*-methyltransferase 2A	3969	431.76	Histone methylation (Writer)		Acute biphenotypic leukemia; acute myeloid leukemia, with t (9;11)(p22;q23); bilineal acute leukemia; acute myeloid leukemia, with 11q23 abnormalities; precursor B cell acute lymphoblastic leukemia; chronic neutrophilic leukemia; acute leukemia; congenital mesoblastic nephroma; intravascular large B cell lymphoma; mesoblastic nephroma; acute myelomonocytic leukemia; primary mediastinal large B cell lymphoma; leukemia; myelodysplastic syndrome; lymphoblastic leukemia
Kleefstra syndrome (KLFS)	*EHMT1 Euchromatic histone lysine N-methyltransferase 1*	9q34.3	251.02	EHMT1 Histone lysine *N*-methyltransferase EHMT1	1298	141.46	Histone methylation (Writer)		
Weaver syndrome (WS)	*EZH2 Enhancer of zeste 2 Polycomb repressive complex 2 subunit*	7q35-q36	76.98	EZH2 Histone lysine *N*-methyltransferase EZH2	746	85.36	Histone methylation (Writer)		EZH2-related overgrowth; periodic fever; aphthous stomatitis; pharyngitis and adenitis; marshall syndrome; wrinkly skin syndrome; Marshall–Smith syndrome; salivary gland adenoid cystic carcinoma; chronic myelomonocytic leukemia; prostate cancer
Sotos syndrome (SS)	*NSD1 Nuclear receptor-binding SET domain protein 1*	5q35	167.19	NSD1 Histone lysine *N*-methyltransferase, H3 lysine-36 and H4 lysine-20 specific	2696	296.65	Histone methylation (Writer)		Weaver syndrome 1; 5q35 microduplication syndrome; beckwith-wiedemann syndrome, due to nsd1 mutation; lipidemia; Beckwith-Wiedemann syndrome; abdominal wall defect; alpha thalassemia/mental retardation syndrome; macroglossia leukemia; acute myeloid leukemia

Kabuki syndrome (KS)	*KMT2D (MLL2) Lysine (K)-specific methyltransferase 2D*	12q13.12	41.17	KMT2D Histone lysine *N*-methyltransferase 2D	5537	593.38	Histone methylation (Writer)	Defective MLL2 or KDM6A interferes with the upregulation of critical target genes	Spinocerebellar ataxia 2
	KDM6A Lysine (K)-specific demethylase 6A	Xp11.2	239.6	KDM6A Lysine-specific demethylase 6A	1401	154.17	Histone demethylation (Eraser)		
Claes-Jensen syndrome (CJS)	*KDM5C Lysine (K)-specific demethylase 5C*	Xp11.22-p11.21	34.1	KDM5C Lysine-specific demethylase 5C	1560	175.72	Histone demethylation (Eraser)	Demethylases or deacetylases are associated with condensed chromatin and downregulation at target loci. Defects on those enzymes promote the overexpression of specific genes that favor pathological conditions	Mental retardation, X linked, syndromic; syndromic X-linked intellectual disability, due to JARID1C mutation
Brachydactyly-mental retardation (BDMR) syndrome	*HDAC4 Histone deacetylase 4*	2q37.3	353.48	HDAC4 Histone deacetylase 4	1084	119.04	Histone deacetylation (Eraser)		2q37 Microdeletion syndrome; Albright hereditary osteodystrophy; Neuronal intranuclear inclusion disease
Cornelia de Lange syndrome 5 (CDLS5) Wilson-Turner syndrome (WTS)	*HDAC8 Histone deacetylase 8*	Xq13	243.58	HDAC8 Histone deacetylase 8	377	41,75	Histone deacetylation (Eraser)		Obesity
Siderius X-linked mental retardation syndrome (MRXSSD)	*PHF8 PHD finger protein 8*	Xp11.22	112.28	PHF8 Histone lysine demethylase PHF8	1060	117.86	Histone deacetylation (Eraser)		
Börjeson-Forssman*Lehmann syndrome (BFLS)	*PHF6 PHD finger protein 6*	Xq26.3	55.54	PHF6 PHD finger protein 6	365	41.29	Transcriptional regulation (Reader)	Mutations on this gene are associated with impaired cell growth and differentiation	Hypogonadism; obesity
X-linked mental retardation and macrocephaly	*BRWD3 Bromodomain and WD repeat domain containing 3*	Xq21.1	140.24	BRWD3 Bromodomain and WD repeat-containing protein 3	1802	203.59	Chromatin remodeling (Reader)	Possible defects on transcriptional regulation	Chronic lymphocytic leukemia
Alpha thalassemia/ mental retardation X-linked (ATRX) syndrome	*ATRX Alpha thalassemia/ mental retardation syndrome X-linked*	Xq21.1	281.39	ATRX Transcriptional regulator ATRX	2492	282.58	Chromatin remodeling (Remodeler)	Mutations of these proteins are associated with altered gene expression or DNA methylation mediated by a defective chromatin conformation around specific target genes	Mental retardation-hypotonic facies syndrome; myelodysplasia syndrome; Intellectual disability syndrome; mental retardation, Smith-Fineman-Myers type; spastic diplegia
Mental retardation autosomal-dominant 14 (MRD 14; syndrome)	*ARID1A AT-rich interactive domain 1A (SWI-like)*	1p35.3	86.08	ARI1A AT-rich interactive domain-containing protein 1A	2285	242.04	Chromatin remodeling (Remodeler)		Coffin-Siris syndrome; ARID1A-related coffin-Siris syndrome; ovarian clear cell carcinoma; adenofibroma

Continued

TABLE 1.13 Epigenetic Mendelian Disorders—cont'd

Disease	Defective gene	Locus	Gene size (kb)	Protein	Protein size (aa)	Molecular weight (kDa)	Defective epigenetic function	Consequence	Other related diseases
Mental retardation autosomal-dominant 12 (MRD 12; syndrome)	*ARID1B AT-rich interactive domain 1B (SWI1-like)*	6q25.1	432.93	ARI1B AT-rich interactive domain-containing protein 1B	2236	236.12	Chromatin remodeling (Remodeler)		Chromosome 6q25 microdeletion syndrome; ARID1B-related coffin-Siris syndrome; Nicolaides-Baraitser syndrome; Coffin-Siris syndrome; Spinocerebellar ataxia 2; Ladd syndrome; acrocephalosyndactylia
Mental retardation autosomal-dominant 16 (MRD 16; syndrome) Rhabdoid tumor predisposition syndrome 2	*SMARCA4 SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4*	19p13.2	104.47	SMCA4 Transcription activator BRG1	1647	184.64	Chromatin remodeling (Remodeler)		Ovarian small-cell carcinoma; SMARCA4-related Coffin-Siris syndrome; Coffin-Siris syndrome; Tumor predisposition syndrome; Peutz-Jeghers syndrome; Oculopharyngeal muscular dystrophy; Bacteremia; Choroid plexus carcinoma; primary-pigmented nodular adrenocortical disease; Stargardt disease
Mental retardation autosomal-dominant 15 (MRD 15; syndrome) Schwannomatosis Rhabdoid tumor predisposition syndrome 1	*SMARCB1 SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily b, member 1*	22q11.23; 22q11	47.55	SNF5 SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1	385	44.14	Chromatin remodeling (Remodeler)		SMARCB1-related Coffin-Siris syndrome; Coffin-Siris syndrome; epithelioid sarcoma; Rhabdoid cancer; rhabdoid meningioma; chromosome 22q deletion, monosomy 22; Familial multiple meningioma; choroid plexus carcinoma; Tumor predisposition syndrome; choroid plexus papilloma; retroperitoneal leiomyosarcoma; pleomorphic xanthoastrocytoma; epithelioid malignant peripheral nerve sheath tumor; malignant peripheral nerve sheath tumor; neurilemmoma; mesoblastic nephroma; congenital mesoblastic nephroma; mesenchymal chondrosarcoma; leiomyosarcoma; chondrosarcoma; HIV-1

Nicolaides-Baraitser syndrome	*SMARCA2 SWI/SN- related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 2*	9p22.3	178.4	SMCA2 Probable global transcription activator SNF2L2	1590	181.27	Chromatin remodeling (Remodeler)		Schizophrenia
Floating harbor syndrome	*SRCAP Snf2-related CREBBP activator protein*	16p11.2	46.98	SRCAP Helicase SRCAP	3230	343.55	Chromatin remodeling (Remodeler)		Eosinophilic angiocentric fibrosis; Rubinstein-Taybi syndrome
CHARGE syndrome	*CHD7 Chromodomain helicase DNA-binding protein 7*	8q12.2	189.26	CHD7 Chromodomain helicase DNA-binding protein 7	2997	335.92	Chromatin remodelling (Remodeler)		Scoliosis; hypogonadotropic hypogonadism 5, with or without anosmia; Kallmann syndrome 5; CHD7-related isolated gonadotropin-releasing hormone deficiency; normosmic congenital hypogonadotropic hypogonadism; omenn syndrome; esophageal atresia; tracheoesophageal fistula; choanal atresia
Mental retardation autosomal-dominant 21 (MRD 21)	*CTCF CCCTC-binding factor (zinc finger protein)*	16q21-q22.3	76.77	CTCF Transcriptional repressor CTCF	727	82.78	Chromatin organizing (Insulator)	Defective insulator genes lead to a lack in prevention for inappropriate interactions between adjacent chromatin domains	Intellectual disability-feeding difficulties-developmental delay-microcephaly syndrome; mesoblastic nephroma; Beckwith-Wiedemann syndrome; Cornelia de Lange syndrome; Silver-Russell syndrome

drugs may have on the epigenetic machinery. Genes involved in pharmacogenomics (pathogenic, mechanistic, metabolic, transporter, pleiotropic genes) are also affected by epigenetic modifications conditioning the therapeutic outcome.

Pharmacogenomics accounts for 30%–90% variability in pharmacokinetics and pharmacodynamics; however, pharmacogenetics alone does not predict all phenotypic variations in drug response. Individual differences in drug response are associated with genetic and epigenetic variability and disease determinants.[10, 13, 14, 552, 553] The genes involved in the pharmacogenomic response to drugs fall into five major categories: (i) genes associated with disease pathogenesis; (ii) genes associated with the mechanism of action of drugs (enzymes, receptors, transmitters, messengers); (iii) genes associated with drug metabolism: (a) phase I reaction enzymes: alcohol dehydrogenases (ADH1-7), aldehyde dehydrogenases (ALDH1-9), aldo-keto reductases (AKR1A-D), amine oxidases (MAOA, MAOB, SMOX), carbonyl reductases (CBR1-4), cytidine deaminase (CDA), cytochrome P450 family (CYP1-51, POR, TBXAS1), cytochrome *b*5 reductase (CYB5R3), dihydropyrimidine dehydrogenase (DPYD), esterases (AADAC, CEL, CES1, CES1P1, CES2, CES3, CES5A, ESD, GZMA, GZMB, PON1, PON2, PON3, UCHL1, UCHL3), epoxidases (EPHX1-2), flavin-containing monooxygenases (FMO1-6), glutathione reductase/peroxidases (GPX1-7, GSR), short chain dehydrogenases/reductases (DHRS1-13, DHRSX, HSD11B1, HSD17B10, HSD17B11, HSD17B14), superoxide dismutases (SOD1-2), and xanthine dehydrogenase (XDH); and (b): phase II reaction enzymes: amino acid transferases (AGXT, BAAT, CCBL1), dehydrogenases (NQO1-2, XDH), esterases (CES1-5), glucuronosyl transferases (UGT1-8), glutathione transferases (GSTA1-5, GSTK1, GSTM1-5, GSTO1-2, GSTP1, GSTT1-2, GSTZ1, GSTCD, MGST1-3, PTGES), methyl transferases (AS3MT, ASMT, COMT, GNMT, GAMT, HNMT, INMT, NNMT, PNMT, TPMT), *N*-acetyl transferases (ACSL1-4, ACSM1, ACSM2B, ACSM3, AANAT, GLYAT, NAA20, NAT1-2, SAT1), thioltransferase (GLRX), and sulfotransferases (CHST2-13, GAL3ST1, SULT1A1-3, SULT1B1, SULT1C1-4, SULT1E1, SULT2A1, SULT2B1, SULT4A1, SULT6B1, CHST1); (iv) genes associated with drug transporters (ABC genes), especially ABCB1 (ATP-binding cassette, subfamily B, member 1; P-glycoprotein-1, P-gp1; Multidrug Resistance 1, MDR1), ABCC1, ABCG2 (White1), genes of the solute carrier superfamily (SLC) and solute carrier organic (SLCO) transporter family, responsible for the transport of multiple endogenous and exogenous compounds, including folate (SLC19A1), urea (SLC14A1, SLC14A2), monoamines (SLC29A4, SLC22A3), aminoacids (SLC1A5, SLC3A1, SLC7A3, SLC7A9, SLC38A1, SLC38A4, SLC38A5, SLC38A7, SLC43A2, SLC45A1), nucleotides (SLC29A2, SLC29A3), fatty acids (SLC27A1-6), neurotransmitters (SLC6A2 (noradrenaline transporter), SLC6A3 (dopamine transporter), SLC6A4 (serotonin transporter, SERT), SLC6A5, SLC6A6, SLC6A9, SLC6A11, SLC6A12, SLC6A14, SLC6A15, SLC6A16, SLC6A17, SLC6A18, SLC6A19), glutamate (SLC1A6, SLC1A7), and others); and (v) pleiotropic genes involved in multifaceted cascades and metabolic reactions.[10, 13–15, 552–555]

Epigenetic regulation is responsible for the tissue-specific expression of genes involved in pharmacogenetic processes, and epigenetics plays a key role in the development of drug resistance. Epigenetic changes affect cytochrome P450 enzyme expression, major transporter function, and nuclear receptor interactions compromising drug efficiency and safety.

1.13 CONCLUSIONS

Our present knowledge on epigenetics is still limited; however, epigenetics has been evolving at a very fast rate over the past few years, creating great expectations in biology and medicine. It is very likely that many of our present concepts and interpretations on the pathogenesis, molecular diagnosis, and therapeutics of current human disorders will change in the near future with the advent of novel *epi*genetic data. Epigenetics adds complexity, diversity, and evolutionary clues to the central dogma of biology by which gene expression entails the flow of genetic information from DNA to RNA and proteins. Epigenetic states help to shape differential utilization of genetic information and to preserve long-lived memory of past signals.[556]

The development of new techniques and procedures for epigenome editing and for simplification and interpretation of results is necessary for a rapid translation of epigenetic knowledge into the clinic and for drug development. The completion of genome, epigenome, and transcriptome mapping demands precision biomolecular tools for DNA manipulation, chromatin structure reconstruction, and reshaping of gene expression patterns. Some molecular platforms for epigenetic editing have been developed, such as zinc finger proteins (ZFs), transcription activator-like effectors (TALEs), Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), and CRISPR-associated (Cas) proteins.[557] Massive DNA sequencing has generated a large body of genomic, transcriptomic, and epigenomic information that has provided clues for establishing three-dimensional (3D) genomic landscapes in various cells and tissues.[558]

Epigenetics challenges the conventional understanding of the gene-environment interaction and intergenerational inheritance, and probably might contribute to change some modern political ideologies and/or philosophical beliefs (atomistic individualism).[559] Experiences of racial discrimination have been associated with poor health outcomes. Significant epigenetic associations between disease-associated genes and perceived discrimination as measured by the Major Life Discrimination (MLD) Scale have been reported. Consequently, future health disparities research should include epigenetics in high-risk populations to elucidate functional consequences induced by the psychosocial environment.[560]

Advances in the field of epigenetics will contribute to applying this knowledge in environmental health risk assessment.[561] Pathogens pose serious threats to human health, agricultural investment, and biodiversity conservation through the emergence of zoonoses, spillover to domestic livestock, and epizootic outbreaks. Understanding the epigenetics of wildlife disease will enable more accurate risk assessment, reconstruction of transmission pathways, discernment of optimal intervention strategies, and development of more effective and ecologically sound treatments to minimize damage to the host population and the environment.[562]

Despite significant technological advances in epigenetic profiling, there is still a need for a systematic understanding of how epigenetics shapes cellular circuitry and disease pathogenesis. The development of accurate computational approaches for analyzing complex epigenetic profiles is essential for disentangling the mechanisms underlying cellular development and the intricate interaction networks determining and sensing chromatin modifications and DNA methylation to control gene expression. Computational epigenetics is a very necessary aid for the implementation of epigenetic procedures in the clinical setting and in pharmacoepigenetics.[563]

The physicians of the 21st century have to adapt their mentality and aptitude to understand new concepts, new interpretations of disease pathology, new biomarkers for an early (or presymptomatic) diagnosis, and new strategies for a personalized medicine to efficiently serve their patients and preserve the health conditions of the population.[564, 565]

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CHAPTER

2

Pharmacoepigenetics: Basic Principles for Personalized Medicine

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2.1 INTRODUCTION: PERSONALIZED OR PRECISION MEDICINE?

Personalized medicine is defined by therapy decisions tailored to individual patients to achieve the highest possible therapeutic effect and minimize side effects.[1] The idea is not new since even in the 5th century BCE Hippocrates commanded "give different ones [drugs] to different patients, for the sweet ones do not benefit everyone, nor do the astringent ones, nor are all the patients able to drink the same thing."[2] He also said, "It's far more important to know what person the disease has than what disease the person has." Moreover, Hippocrates believed that disease was a product of environmental forces, diet, and lifestyle habits and that treatment should focus on a patient's care (prevention and prognosis prediction). Accordingly, personalized medicine emphasizes the prevention, prediction, diagnosis, and treatment of a disease as it pertains to the individual patient.[3]

The term "precision medicine," although used in scientific literature for as long as "personalized medicine," has become more prevalent since the year 2015, when the Precision Medicine Initiative was announced by President Barack Obama, which was based on the report published by the US National Research Council in 2011.[4, 5] According to this report, "precision medicine" is the tailoring of medical treatment to the individual characteristics of each patient. However, in contrast to "personalized medicine" it does not literally mean the creation of drugs or medical devices that are unique to a patent, but rather the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease, and/or disease prognosis, or in their response to a specific treatment.

Thus, the precision medicine approach utilizes individuals and defines (sub)population-based cohorts that have a common network of disease (or health) taxonomy. In addition, it requires an integrated molecular and clinical profile of both the individual as well as the subpopulation-based cohort. The precision medicine approach has attracted huge attention and is in the ascendancy compared with personalized medicine because it is more reliable.

In this chapter we will highlight the role of pharmacoepigenetics in personalized therapy focusing on predictive therapeutic biomarkers and therapeutic targets. Thus we will refer equally to personalized and precision medicine.

Several reasons contribute to the diverse responses of patients or subsets of patients to pharmacological treatment. Drug efficacy and safety depends on its interaction with on-target and off-target molecules, but how the drug is processed in the body to access the pharmacological target depends on its absorption, distribution, metabolism, and excretion (ADME). The most important part of this system is the drug-metabolizing enzymes and transporters (DMETs). Variations in drug ADME, common among a defined population of patients, influence the efficacy of treatment and/or its toxicity. In this regard (i) inhibition or activation of the protein functions of DMETs leads to remarkable variations in the pharmacokinetics of a drug, influencing its efficiency and/or toxicity; (ii) changes in transcriptional gene expression of DMETs, controlled by xenobiotic receptors and transcription factors, are one of the major causes of variations in drug metabolism and toxicity; (iii) genetic variations of ADME genes often lead to significant changes in DMET expression and ultimately enzyme/protein activities; and (iv) epigenetics, noncoding RNAs, and gut microbiota may modulate ADME gene expression and cause variations in drug metabolism and toxicity.[6]

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The therapeutic response to anticancer drugs/DNA-damaging agents depends to a great extent on the activity of DNA repair systems and/or enzymes and proteins involved in apoptosis induction and signal transduction. All these systems are the subject of epigenetic modification, which influences their expression and should be considered in personalized or precision medicine.

The need for the personalized medicine approach is clearly visible when we realize that the top 10 highest grossing drugs in the United States help only between 1 in 25 and 1 in 4 of the people who take them.[7] It is estimated that up to 40% of the medicines that individuals take every day are not effective.[8, 9] Moreover, the results of some classical clinical trials are biased. For instance, often only white Western participants are enrolled, leading to the creation of therapeutic strategies harmful to certain other ethnic groups.[7] It is estimated that phase II clinical trial success rates are as low as 18%, whereas the failure rate is around 50% in phase III, with 60% of drugs failing as a result of the lack of efficacy and 21% failing as a result of safety concerns.[8] Therefore precision medicine today attracts much attention when designing clinical trials, which account for variability between patients much more effectively. So-called "basket trials," "umbrella trials," or "adaptive trials" try to answer these needs. Numerous initiatives, such as the US Precision Medicine Initiative Project or Ubiquitous Pharmacogenomic Project are intended to fill the missing gap between theoretical drug efficacy/safety and its influence on a particular patient in real life.[10] An important element of this strategy is pharmacoepigenetics.

2.2 PHARMACOGENOMICS: THE CORE DISCIPLINE OF PERSONALIZED MEDICINE

Pharmacogenetics and pharmacogenomics (PGx) are the core disciplines of personalized medicine and have provided some important information that is now applied to individual therapy. Pharmacogenomics is defined as the genome-wide analysis of genetic determinants of DMETs, drug receptors, and targets that influence therapeutic efficacy and safety and drug-related phenotypes. The term pharmacogenomics is often used interchangeably with pharmacogenetics, which refers to the analysis of specific single nucleotide polymorphisms (SNPs) in distinct genes with known functions that are plausibly connected to drug response.[11] Several pharmacogenomic biomarkers have been approved for clinical use by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA). About 15% of medical products approved by EMA and 138 medicines approved by FDA contain pharmacogenomic labels.[6] These biomarkers include germline or somatic gene variants (polymorphisms, mutations), functional deficiencies with a genetic etiology, gene expression differences, and chromosomal abnormalities. Selected proteins that are used for treatment selection are also included. The most important pharmacogenomic biomarkers are related to genes encoding HLA molecules, enzymes, transporters, drug targets, and specific markers and mutations in the somatic genome. The analysis of a panel of PGx markers instead of providing evidence for individual drug-gene pairs is considered even more relevant.

Nowadays, PGx analyses are routinely performed during drug development.[12, 13] There are also numerous tests available to analyze an individual's genetic makeup and help predict drug response to guide optimal drug and dose selection. To date, several randomized controlled trials have provided gold standard evidence of the clinical utility of single drug-gene PGx tests to guide dosing for warfarin, acenocoumarol, phencopromon, and thiopurines, and guide the drug selection of abacavir.[10] Moreover, several prospective cohort studies have been performed indicating the clinical utility of single drug-gene PGx tests to guide the drug selection of carbamazepine and allopurinol.[10] In addition, cancer is one of the therapeutic areas where PGx is already being applied in the clinical setting. Cetuximab, trastuzumab, imatinib, and vemurafenib are just a few examples of anticancer drugs that have PGx labels.

Predicting an individual's drug-metabolizing phenotype can be evaluated using cytochrome P450 (CYP450) genotype analysis. The genetic polymorphism in the genes encoding CYP members was first reported for *CYP2D6*. Although constituting only 2%–4% of the total amount of CYPs in the liver, *CYP2D6* actively metabolizes approximately 20%–25% of all drugs.[14, 15] The extensive presence of polymorphism in the *CYP2D6* gene significantly affects phenotypic drug responses, and up to a 10-fold difference in the required dose was observed to achieve the same plasma concentration in different individuals.[14]

In the last decade genome-wide association studies (GWAS) allowed the analysis of hundreds of thousands of genetic markers instead of groups of candidate genes. However, it is now clear that GWAS will not provide all the answers for any given drug response phenotype and explain all the phenotypic variations in this respect.[16] Apparently, the number of rare variants is much higher than previously thought. This was investigated by a study covering 231 genes involved in drug metabolism and transport; it was found that each individual carries over 18,000 variants and the actual number of SNPs in the CYP family of genes was more than twice those considered in common pharmacogenomics databases. Among the P450 genes, *CYP1A2*, *CYP2A6*, *CYP2C19*, and *CYP3A4* have a high contribution

of rare alleles to genetic variation.[6] Overall, one can estimate that rare variations in ADME genes, not determined by common pharmacogenomics tests available, account for 30%–40% of all genetically linked variability in drug response in a specific individual.[17]

Moreover, phenotype complexity/variations result not only from genotype information, but can be modified by environmental factors and specific cell-type gene expression. Thus, even taking genetic polymorphisms into consideration and adjusting the drug dosage regimen to body weight, gender, or age of an individual, frequently it cannot be explained why some patients respond to therapy and others do not. Novel biomarkers beyond pharmacogenetics are needed to predict individual responses to given drugs and to develop new treatment strategies.

2.3 PHARMACOEPIGENETICS: AN ADDITIONAL LAYER OF REGULATORY AND ENVIRONMENTAL EXPOSURE INFORMATION AFFECTING INDIVIDUAL DRUG RESPONSE

The broad complexity of a living organism, which includes spatial and temporal variations in biological function or phenotypic expression within the same individual, results to a large extent from epigenetic control of gene expression. Epigenetics, which provides layers of regulatory mechanisms and environmental exposure information on top of each individual's unique genome, enables better design of the personal drug regimen and better evaluation of disease susceptibility and cure (Fig. 2.1).[18, 19]

It is now evident that epigenetic changes in the expression of phase I and II DMEs are important contributors to individual drug response. The majority of epigenetic alterations regulating DME gene expression concern DNA methylation changes in CpG islands. An increasing number of reports on differential expression of DMEs point out the alterations in histone modifications, which in most cases are accompanied by changes in DNA methylation.[20] In addition to these mechanisms the expression of CYP genes and phase II enzymes was found to be directly regulated by miRNAs or indirectly regulated by nuclear receptors through the binding of miRNAs.[21] The new players in this game are long noncoding RNAs (lncRNAs). They are involved in the epigenetic control of coding genes through the upregulation or downregulation of mRNAs, methylation, and transcription of specific gene polymorphisms.[22] Their aberrant levels are likely to cause disorders associated with protein dysregulations.[23] However, their association with the majority of diseases and DMEs has still not been elucidated and requires further study. Thus the expression of DME genes is controlled by a plethora of epigenetic modifications, including DNA methylation, histone modifications, miRNAs, and lncRNAs, all of which may significantly alter drug effects.

In fact, CYP bioactivity is characterized by high intersubject variability. This phenomenon had largely been attributed to gene polymorphisms until pharmacoepigenetic studies revealed other mechanisms regulating their expression. It is now established that of 57 putatively functional genes in the P450 family around 20 are reported to be under epigenetic control.[24] A systematic analysis of DME genes (55 CYPs and 62 phase II enzymes) in different tissues and cell lines

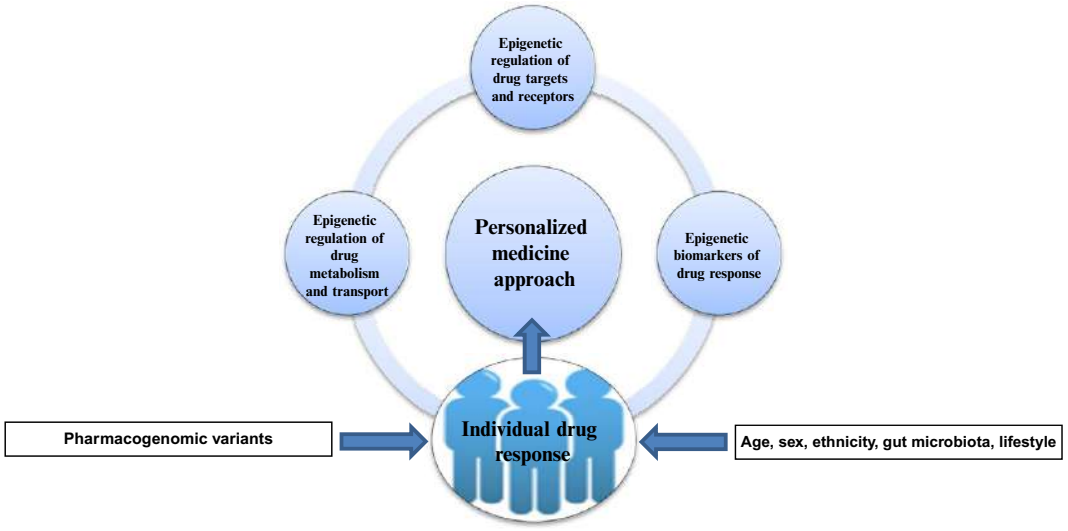

FIG. 2.1 Factors determining individual drug response that may contribute to the personalized medicine approach.

revealed that their expression differs markedly between tissue groups. Some CYPs showed variable DNA methylation statuses (in particular, CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4). Most interestingly, there was an inverse correlation between DNA methylation of CpG sites and mRNA expression, explaining almost 30% of the variability in gene expression. Interestingly, it was shown that DNA methylation silences the promoter of cytochrome P450 1B1 (CYP1B1), preventing gene induction through dioxin. This halogenated aromatic hydrocarbon is a well-known inducer of the battery of CYP1 enzymes controlled by the aromatic hydrocarbon receptor. Treatment with the DNA methyltransferase inhibitor 5-aza-deoxycytidine could restore inducibility.[17, 25] Since CYP1B1 plays an important role in estrogen metabolism and subsequently breast cancer carcinogenesis and its induction occurs via ERα silencing of its gene expression, this CYP isoform may interfere with selective estrogen receptor modulator activity.[26]

Critical transcriptional control of CYP expression by several nuclear receptors is also well established. Most nuclear receptors (e.g., PXR, CAR, VDR, and PPARα) are regulated by epigenetic mechanisms.[17, 20] Thus the modification of nuclear receptors may equally contribute to individual variations in DME expression. Based on bioinformatics analysis it was suggested that all the major, clinically important CYP450 genes are targeted by miRNAs.[27] Subsequently, in vitro functional studies have validated these predictions for some of the *P450* genes or nuclear receptors. For example, it was shown that miR-148a targets the PXR and consequently suppresses *CYP3A4* expression.[28]

Among the genes encoding phase II enzymes, *GSTP1* methylation was found in many cancers, particularly hormone-dependent ones.[29] GSTP1 was proposed as a biomarker to predict the response of breast cancer cells to treatment with doxorubicin and DNA methyltransferase inhibitors.[30]

In addition to DMEs, genetic and epigenetic variations of drug transporters such as ABC and SLC may alter drug disposition and response. Transporters mediate the translocation of many drugs and endogenous compounds across the cell membrane and consequently influence the ADME processes of drugs as well as their pharmacokinetic and dynamic properties. Pharmacoepigenetic studies provide increasing evidence supporting the significant role of epigenetic modifications in the regulation of expression of efflux and uptake transporter genes.

It is important to note that epigenetic modifications and miRNA expression patterns may differ between different tissues. It is well established that DMETs, in particular, are expressed at various interfaces. Most data on epigenetic regulation of ADME come from studies of the human liver. The epigenetic alterations of ADME in extrahepatic tissues are poorly described, although they certainly may affect the target tissue response.

In addition to the enzymes and proteins involved in ADME regulation, aberrant DNA methylation has been demonstrated in enzymes involved in DNA repair processes, which has an impact on anticancer therapy. The most prominent example of gene promoter hypermethylation resulting in transcriptional repression and drug resistance is displayed in the DNA repair gene *MGMT*.[1] MGMT is involved in the mismatch DNA repair system and protects cells against transition mutations by removing alkyl groups from the guanine base. This process protects cells from genetic modifications caused by chemical carcinogens, but its activity is a negative predictor for treatment with alkylating therapeutics, whose mechanism of action is counteracted by this enzyme. Thus gene silencing by promoter hypermethylation is a good prognostic marker for patients treated with such drugs as temozolomide. MGMT is basically useful in glioma therapy stratification, but can potentially be applied to various other tumor types.[31]

Hypermethylation of the mismatch repair gene *MLH1* has been associated with tumor resistance to platinum-based therapies. *MLH1* hypermethylation was found here to lead to resistance to cisplatin in ovary cancer cell lines.[32] Moreover, the resistant phenotype could be reverted using a demethylating agent resulting in reexpression of the repair gene and increasing the sensitivity of cells. However, administration of a demethylating agent has a global effect on DNA methylation and ultimately gene expression.

It is noteworthy that reactivation and drug sensitivity could be increased by combining demethylating agents with histone acetyltransferases, suggesting a conjoined function of multiple layers of epigenetic regulation with clinical value regarding treatment toxicity and efficiency.[33]

The *BRCA1* gene involved in homologous recombination DNA repair is often hypermethylated in breast and ovarian cancer and might be considered a potential marker of sensitivity to cisplatin in this cancer type. Epigenetic inactivation of *BRCA1* is also related to sensitivity to PARP inhibitors targeting DNA base excision repair. Other DNA repair hypermethylated genes such as *WRN*, *ERCC1*, and *ERCC5* are also directly associated with drug resistance.[34]

To sum up, hypermethylation of DNA repair enzymes represents an epigenetic event that has high translational potential for application in the clinical setting and might contribute to personalized/precision medicine.

Programmed cell death/apoptosis and signal-transducing pathways, usually aberrant in cancer cells, are common targets of anticancer drugs and may also affect personal/individual responses through epigenetic mechanisms. It has been found here that *TP53*, one of the key elements of apoptosis induction-associated genes *TP73* and *APAF1*, are frequently hypermethylated in cancer cells, making them suitable targets for drug sensitivity screening using such medications as cisplatin and adriamycin.[35, 36]

The *CDK10* gene encodes cyclin-dependent kinase 10, which is responsible for inducing cell division in the G2/M phase of the cell cycle. Its reduced expression, as a result of methylation of the CpG island of the *CDK10* promoter, has been strongly associated with survival and resistance to antiestrogen treatment in some ERα-positive breast cancer patients.[1, 37] Repression of *CDK10* may lead to activation of MAPK-driven mitogenic signaling. Moreover, *CDK10* transcription is regulated by miRNA, and miR-210 appears to be a good prognostic marker for patients undergoing tamoxifen treatment.[38]

On the other hand, ERα-positive patients treated with tamoxifen can be stratified in low- and high-risk resistance groups using promoter hypermethylation of *PITX2* (paired-like homeodomain transcription factor 2) as a predictive epigenetic biomarker.[39] Finally, it has been found that *EGFR* mutation and *CHFR* hypermethylation are mutually exclusive.[40] Non-small cell lung carcinoma patients with unmethylated *CHFR* survived longer when receiving second-line treatment with EGFR inhibitors (gefinitib or erlotinib).

2.4 PHARMACOEPIGENETICS IN PERSONALIZED/PRECISION MEDICINE

2.4.1 Epigenetic Biomarkers of Drug Response

2.4.1.1 DNA Methylation Markers

DNA methylation represents an epigenetic biomarker with the highest translational potential in the personalized medicine approach. The abnormal distribution of DNA methylation is one of the hallmarks of many cancers, as methylation changes occur early during carcinogenesis. DNA methylation is stable in fixed samples over time and there are reliable detection technologies, which recently have been enforced by high-throughput profiling techniques such as whole genome bisulfite sequencing, and CpG methylation-specific array technology.[1] Moreover, next generation sequencing (NGS) opens up an avenue for routine testing of DNA methylation biomarker panels. DNA methylation profiling can be performed not only in fresh-frozen material, but also in formalin-fixed or poorly conserved material.[41, 42] Almost any biological tissue sample or body fluid can be used for DNA methylation analysis. Furthermore, in the case of cancer cells, circulating, cell-free DNA (cfDNA) isolated from blood can be applied.[43, 44] Circulating cfDNA is extracted from plasma or serum and is derived from dying tumor cells that release their DNA into the bloodstream. Such a "liquid biopsy" is fast, minimally invasive, and can be repeated over time for disease monitoring and predicting drug response. Even though available in very small concentrations, cfDNA isolated from plasma/serum is suitable for the detection of aberrant methylation and reflects the methylation profile found in tumor tissue. In addition to blood (plasma/serum) there are other easy-to-access tissues or body fluids, such as saliva, urine, stools, or bronchial aspirates, that can be used for DNA methylation marker detection. At birth the placenta, umbilical cord, and fetal membranes are also suitable tissues for the analysis of DNA methylation.[45]

Numerous methods for DNA methylation detection are available, including qPCR-based methods (such as MethyLight, SMART-MSP, and HeavyMethyl), pyrosequencing, MS-HRM, and MS-MLPA, just to list a few. Unfortunately, currently there is no agreement about the methodology that could be used as the gold standard for DNA methylation analysis. This definitely slows down the clinical implementation of published data. Discussion of the pros and cons of different methods is beyond the scope of this chapter, and we refer the interested reader elsewhere.[46–48] However, it is important to note that in any assay used in personalized/precision medicine the test should have the highest possible sensitivity and specificity (both ideally 100%, but such an ideal scenario is rarely achieved). Several recent studies assessing the clinical utility of different methodologies for DNA methylation detection favor quantitative approaches such as bisulfite pyrosequencing or qPCR-based methods over qualitative assays. However, in quantitative approaches cutoff values need to be determined for methylation ranges related to clinical information such as prognosis. Methylation cutoff values are not universal for a particular gene and strongly depend on the method used for DNA methylation analysis. Even if the same methodology is used, cutoff value determination is required for each assay, as these values also depend on the region in which the gene investigated is found, PCR primers, and PCR conditions used as well as minimal tumor content required.[47]

The robustness of DNA methylation marks makes DNA methylation analysis very attractive in the clinical setting and as a tool in personalized/precision medicine, including pharmacotherapy. Several markers have already been implemented in clinics and are available for cancer screening/detection. For instance, Cologuard was approved by the FDA in 2014 and since then it has been used for the analysis of stool DNA samples collected as part of colorectal cancer (CRC)-screening protocols. This test analyzes *NDRG4* and *BMP3* methylation together with mutated *KRAS* and involves immunochemical assay for hemoglobin. Epi proColon (analyzing the methylation of *SEPT9*) also has FDA approval and is intended for the analysis of blood samples also taken for CRC screening. Similar to Epi proColon there

are other tests, ColoVantage and RealTime mS9 CRC assay, that detect *SEPT9* gene methylation in cfDNA and are used for early detection of CRC.[46]

While the number of approved diagnostic markers, particularly for cancer diagnostics and prediction, is increasing, epigenetic predictive/therapeutic biomarkers are scant.

MGMT methylation was one of the first DNA methylation biomarkers to be identified, clinically validated, and reviewed.[49] GBM patients participating in clinical trials can take advantage of MDxHealth's MGMT assay, which determines the methylation status of the *MGMT* gene in tumor tissue, and can be used as a treatment predictive assay. This test is used as a laboratory-developed test or as an investigational use-only tool in the assessment of patients who are likely to respond to alkylating agents. This patented methylated gene test is also attractive for new brain cancer drug developers since they can more easily target their new drugs to patients who usually do not respond to the traditional alkylating agent drug regime.

Methylation of ABC drug transporters and genes encoding the proteins involved in apoptosis and signal transduction might potentially be useful biomarkers to predict the development of drug resistance, but so far there are no tests available that use this approach.

In general, despite the massive amount of scientific data that have been reviewed,[50] well-established/approved epigenetic biomarkers and predictive biomarkers, particularly, that have been translated into the clinical setting, are scarce. A recent systematic review of publications on this subject[51] revealed that of >14,000 scientific publications describing DNA methylation-based biomarkers and their clinical associations in cancer only 14 have been translated into commercially available clinical tests. Of these 14 biomarker assays, only nine—*GSTP1*, *APC*, *RASSF1*, *NDRG4*, *BMP3*, two *SEPT9*, *SHOX2*, and *MGMT*—have been included in one or more clinical guidelines.[51] Thus the rate of translation from laboratory bench to clinic is only 0.8% and has not changed in the last 5 years.[52, 53]

Therefore, to improve the speed of transition of laboratory results to clinical validated tests used in the personalized/precision medicine approach, a good strategy and validation of tests for DNA methylation biomarkers should be applied. It needs to be emphasized that in addition to such factors as methodological problems/objections (biased patient selection, wrong study design, data analysis, lack of validation) and financial investment needed to develop the tests that refer to any specific biomarker, in the case of DNA methylation its genomic location might be the significant contributor to this low rate of translational success. Traditionally, DNA methylation biomarker studies have been concentrated on the effects of hypermethylation of promoter CpG islands in specific genes. However, even within a single promoter region not all CpG islands are functionally equivalent because transcriptional silencing is often controlled by methylation of one or more small parts of a promoter, rather than by the entire promoter region.[54–56] Thus identifying the precise location of clinically relevant CpG islands has to be considered an important step in the development of a DNA methylation-based biomarker. It has been found here that the detailed analysis of *GSTP1* showed that methylation of the 5′ region of the promoter was significantly more specific in distinguishing HCC from nonmalignant liver diseases or liver tissue with no established pathological alterations other than methylation of the 3′ end of the promoter (97.1% vs 60%; $P < .001$).[57] Another example of this is the previously mentioned promoter methylation of *MGMT*. Although the predictive power of this marker is well established, detailed evaluation of the correlation between a specific methylated region or regions and *MGMT* expression has provided conflicting results.[58–60] The region encompassing CpGs 75–78 is the best studied. The region is covered by the commercially available PredictMDx test, which has been consistently shown to be associated with favorable patient prognosis.[60] However, the debate on the exact region of the promoter at which methylation provides the highest level of clinical value is still not over.

Moreover, preprocessing analyzed samples is an extremely critical issue in DNA methylation testing. Freshly deep-frozen specimens that have not undergone any fixation procedure represent the best starting material for DNA methylation analysis.[46] Isolation of cfDNA for methylation assay is far more challenging. Thus a variety of precautions, such as using specific anticoagulators, deep-frozen plasma instead of serum, and short time of processing, are recommended.[46]

Since most of the methods for DNA methylation analysis require bisulfite-treated DNA as the starting material, it is crucial that bisulfite conversion is performed efficiently; otherwise it can lead to false-positive results. The use of commercially available bisulfite conversion kits can help to improve DNA recovery and guarantees maintenance of a proper bisulfite conversion rate.[47] Ideally, DNA methylation detection methods chosen for the personalized/precision medicine approach should be relatively cheap, easy to use, automatable, and capable of processing many samples in parallel to minimize the cost of future tests. Methods suitable for quantitative DNA methylation detection are critical since only small differences in methylation values determine a diseased or disease-free state. Therefore, qPCR-based assays will remain the first choice of investigators for validation studies.

Moreover, as nicely presented in the review of Noehammer et al.[46] sample-size calculations for genome-wide methylation-screening studies that, irrespective of the platform technology used, reveal sample numbers of

approximately 30 per group still lead to false discovery rates of 20%. As far as sample size in validation studies is concerned, it seems that they are highly dependent on assay performance and standardization. Testing different sources of samples should be avoided since they appear not to be useful for direct comparison.

To conclude, DNA methylation pharmacoepigenetic biomarkers have great potential to contribute to personalized/precision medicine. As described above, DNA methylation biomarkers with diagnostic/prognostic power are already being used in clinical trials or in clinical applications in oncology. However, much more needs to be done to elaborate pharmacoepigenetic biomarkers that are suitable to monitor pharmacotherapy.

2.4.1.2 MicroRNA Markers

Besides DNA methylation markers, there are other epigenetic markers that can be used in the personalized medicine approach. It has been found that miRNA markers show great potential, especially in clinical oncology, as novel diagnostic and/or prognostic biomarkers.[61] The expression of several *CYP* genes and nuclear receptors are modified by specific miRNAs. They are characterized by temporal and spatial specificity, sensitivity, and stability in both paraffin sections and body fluids. Digital PCR, miRNA microarrays, and high-throughput deep-sequencing techniques are believed to improve their translation to clinics.

miRNA-based diagnostics have finally entered clinics. For instance, RosettaGX Reveal is a qPCR-based thyroid microRNA classifier that helps stratify thyroid cancers in a better way. This assay distinguishes benign from malignant thyroid nodules using a single fine needle aspirate cytology smear, and does not require fresh tissue or special collection and shipment conditions. It is therefore a valuable tool for the preoperative classification of thyroid samples with indeterminate cytology.[62] Another test used for the same purpose is ThyraMIR, a miRNA gene expression classifier, based on evaluating the expression of 10 miRNAs.[63] A second test sold by the same company (Interpace Diagnostics) is ThyGenX, which is a highly specific oncogene (mutational) panel that assesses the most common genetic alterations across eight genes associated with papillary carcinoma and follicular carcinoma. In fact, ThyraMIR can identify malignancy in nodules that are negative for ThyGenX, thereby improving overall sensitivity and the ability to detect malignancy. Therefore these two tests are recommended to be used together to achieve highly predictive results.

Nevertheless, to date there are no validated miRNA-based biomarkers used as therapeutic and disease outcome predictors (pharmacoepigenetic biomarkers). miRNA-based tests are challenging because, in contrast to DNA or RNA-based tests, miRNA assays produce results that are difficult to interpret. Most miRNAs are expressed widely in a noncell-specific manner, and they do not differ drastically in level between cases and controls.[64] In regard to sampling and analyzing miRNA, it has to be mentioned that miRNAs have remarkable extracellular stability; however, their levels are very sensitive to preprocessing and postprocessing factors. Therefore clinical labs should ensure strict standardization in collecting and processing the sample.[65, 66] Fortunately, as far as circulating miRNAs are concerned, the results show a limited overall impact of blood-sampling conditions on circulating miRNA patterns.[67] Nevertheless, the abundance of single miRNAs can be significantly altered by different blood-sampling protocols.[67] Moreover, it must be emphasized that circulating miRNA patterns differ between plasma and serum preparations.[65] Furthermore, age and gender could influence the pattern of circulating miRNAs. Unambiguous annotation and differentiation from other noncoding RNAs can be equally challenging. Additionally, more consistent and reliable miRNA signatures or miRNome in both FFPET and circulation urgently need to be established by having an adequately large sample size of cohort studies on multiple, independent populations.[68]

To sum up, despite the increasing number of potential miRNA biomarkers reported in the literature the transition of miRNA-based biomarkers from the bench to the bedside is still relatively slow, similar to the case of DNA methylation markers. None of the proposed issues addresses how pharmacotherapy should be guided.

2.4.2 Epigenetic Drugs

It is now evident that not only can epigenetic status influence drug response, but it can also be modulated by drugs. Epigenetic therapy, defined as the use of drugs to treat or prevent epigenetic defects associated with disease, may represent a step forward in the treatment of cancer and other diseases in which epigenetic regulation plays a role.[20, 69] In cancer cells the general decrease in the methylated cytosine level (genomic hypomethylation) is accompanied by local CpG hypermethylation. Both hypo- and hypermethylation may promote cancer development and thus are "natural" targets of drugs interfering with the epigenetic machinery. Besides cancer, epigenetics is thought to play a major role in the pathogenesis of many other multifactorial diseases such as schizophrenia and bipolar disorder, depression, cardiac hypertrophy and heart failure, and several neurological diseases.[69] To date, the most studied epidrugs have been DNA methyltransferase inhibitors (DNMTi), histone acetyltransferase inhibitors (HATi/KATi), histone methyltransferase inhibitors (HMTi/KMTi), histone *N*-methyl lysine demethylase inhibitors (HDMi/KDMi), histone deacetylase

inhibitors (HDACi), and bromodomain inhibitors.[18] Today two classes of epigenetic drugs have been approved by the FDA for clinical use in the United States: DNMTi and HDACi. Representatives of the first class, azacitidine (5-azacytidine or Vidaza) and its deoxyderivative decitabine (5-aza-2′-deoxycytidine), have been indicated to treat chronic myelomonocytic leukemia and myelodysplastic syndrome.[18, 70] Both drugs cause broad hypomethylation that leads to cellular dysregulation, which affects rapidly dividing cells, in particular. Basically, these drugs were designed to induce genes that have been silenced in cancer,[71] but they may also activate the expression of oncogenes and prometastatic genes[72] since their effect is not highly locus specific.

The potential application of DNMTi to other diseases includes multiple sclerosis,[73] HIV,[74] pain,[75] and memory deficit.[76] For the therapy of AIDS patients a combination of antiretroviral drugs and epidrugs has been suggested in which latent HIV-1 genomes are reactivated. These epidrugs include DNMTi, HDACi, histone methyltransferase inhibitors (HMTi), and histone demethylase inhibitors.[74]

Interestingly, inhibition of DNMT activity has also been demonstrated for certain drugs currently on the market, such as the cardiovascular drugs hydralazine, procainamide, and procaine. Moreover, it has been shown that some approved drugs may act through previously uncharacterized epigenetic processes.[20] However, the side effects of these compounds are serious concerns. On the other hand, epidrugs may be used to reduce the toxicity of other drugs. It has been proposed here that combining the potential anticancer drug dichloroacetate (DCA), which targets cancer metabolism, with an inhibitor of SLC5A8 transporter methylation would offer a means to reduce the doses of DCA to avoid detrimental effects, but without compromising antitumor activity.[77]

A vast array of both natural and synthetic chemical compounds functioning as HDACi have been discovered. Three HDACi, vorinostat (suberoylanilide hydroxamic acid), romidepsin (depsipeptide), and belinostat (hydroxamic acid derivative) have been approved for treatment of cutaneous/peripheral T cell lymphoma.[78] Currently, all three drugs are being further evaluated for other diseases, as well as other hematological malignancies and solid tumors, either as a single agent or in combination with other drugs.[78] Panobinostat, another hydroxamic acid analog, is the latest HDACi. It was approved by the FDA in 2015 for the treatment of multiple myeloma in combination with bortezomib and dexamethasone.[79] Although most HDACi are approved for cancer-type indications, some studies suggest their potential roles in schizophrenia[80] and type 2 diabetes.[81]

Similar to the case to DNMTi, HDACi drugs lack locus specificity and can have serious side effects.[79] Thus, it is recommended to use them when other treatments have failed, or as combination therapies.[18]

In addition to these two approved classes of epidrugs, HMTi, KMTs, bromodomain inhibitors, and HATi are under investigation and development. Pinometostat, an inhibitor of histone methyltransferase DOT1L, has here been proposed for the treatment of MLL-fusion leukemia.[82] Tazemetostat has been proposed to treat multiple types of hematological malignancies and genetically defined solid tumors.[83] Moreover, the histone lysine methyltransferase inhibitor (KMTi) BIX-012294 has shown not only anticancer activity, but also unexpected antiparasitic activity as a result of its action on the KMT of parasites.[84]

Bromodomain proteins are readers that recognize acetylated lysine and transduce the gene activation signal.[85] OTX-015 and CPI-0610 are bromodomain protein inhibitors that are being used in phase I trials for cancer. HATi are currently used in preclinical studies and some have been shown to be effective. For example, PU139, which inhibits several HAT subfamilies, has been shown to block neuroblastoma xenograft growth in mice.[86]

miRNAs are also considered treatment targets. The feasibility of miRNA-based therapeutics in the treatment of cancer and cognitive disorders has been discussed.[87] It is believed that recent progress in the development of effective strategies to block miRNAs will ultimately lead to anti-miRNA drugs that will be used in clinics.[88]

Finally, it is worth stressing that paramount among the priorities for future epidrug development is improving target specificity.

2.5 EPIGENETIC TOOLS IN PERSONALIZED CELL THERAPY

Different cell types have been suggested as candidates for use in regenerative medicine. Embryonic pluripotent stem cells can give rise to all cells of the body and possess unlimited self-renewal potential. However, obtaining them from blastocyst raises ethical concerns. Moreover, they are unstable, difficult to control, and have a risk for neoplastic transformation. Adult stem cells are safe but have limited proliferation and differentiation abilities and usually are not within easy access. In recent years induced pluripotent stem cells (iPSCs) have become a new promising tool in regenerative medicine.[89] Somatic cell reprogramming involves erasing somatic memories and obtaining a pluripotent state similar to that of embryonic stem cells. iPSCs generated by ectopic expression of key transcriptional factors exhibit similar characteristics to ESCs that have a remarkable developmental plasticity and capacity for

indefinite self-renewal, offering significant prospects for disease modeling and potential clinical therapy/application. Moreover, the forced expression of a set of transcription factors can not only achieve somatic cell reprogramming, but also fulfill conversions among different types of cells or trans-differentiation. Several studies have shown that transcriptional factors require the assistance of different epigenetic modifiers at different stages during somatic cell reprograming.[90–93] These include histone posttranslational modifying enzymes and DNA methylation. Comparison of the somatic state with the pluripotent state reveals that somatic cells show a dense chromatin state (heterochromatin), while most stem cells exhibit an open state more amenable to accommodating quick changes in transcriptome (euchromatin). The reprograming process is very inefficient at overcoming such a barrier[94] and this is the reason the efficiency of iPSC induction remains low. To overcome the limits related to low efficiency and the introduction of exogenous transcription factors, small molecules with an epigenetic mode of action have been used to modulate the epigenetic state and increase reprogramming efficiency by inhibiting and activating, in a reversible way, specific signaling pathways.[95–98] In this regard application of the HDACi, valproic acid, not only improves reprograming efficiency by >100-fold but also enables efficient induction of human and murine iPSCs, without introducing the myelocytomatosis oncogene (c-Myc).[95]

ESCs are enriched in DNMTs, which suggests their important role in supporting the pluripotency of ESCs. Indeed, addition of the DNMTi 5-azacytidine during reprogramming greatly improves, by >30-fold, iPSC induction efficiency indicating that DNA methylation may serve as a barrier to somatic reprograming.

To sum up, the development of iPSC reprogramming techniques that include epigenetic modifiers provides a reliable platform for stem cell research and regenerative medicine studies. It is now clear that epigenetic modification and remodeling play key roles during the reprogramming process. Besides widely described epigenetic modifications the recently discovered N^6-methyladenosine, a conserved epitranscriptomic modification of eukaryotic mRNAs, has been shown to have a positive effect on reprogramming to pluripotency.[99] Thus, the crosstalk between RNA methylation and DNA methylation during reprogramming requires further studies. Deeper knowledge of epigenetic modification in the induction of iPSC and elaborating protocols, as a result of applying small molecules/epidrugs in this process, will pave the way to personal regenerative medicine/therapy.

2.6 CONCLUSIONS AND PERSPECTIVES

Growth in the new era of pharmacoepigenetic biomarkers and drug targets will certainly be important for optimization of a true precision medicine, based on genetically assisted drug therapy of many diseases (in particular, for cancer). One area that should significantly contribute to this goal is the use of cfDNA released to the bloodstream from dead cells. Diseased tissue DNA is the major source of cfDNA, so it provides more direct information on diseased tissue methylation levels than surrogate easily accessible cell types such as lymphocytes. This is important when considering the cell-specific nature of methylation patterns. In addition, circulating miRNAs could most likely be used as predictive biomarkers for disease progression and chemotherapy response.

So far, only a small number of epigenetic biomarkers of drug response have been reported, and one (*MGMT* assay) is commercially available, but none are yet approved for general clinical use. NGS will certainly enable the discovery of new epigenetic biomarkers, while adaptations of the CRISP/dCas9 system of epigenome editing will improve the development of drugs targeting specific genetic loci. Moreover, epigenetic modifiers may replace transcription factors/transgens for the generation of iPSCs. This makes epigenetic conversion a very promising tool for regenerative medicine.

Ultimately, both pharmacogenomics and epigenomic biomarkers, which contribute differently to interindividual variations in drug response, should be taken into consideration in the development of personalized/precision therapy.

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CHAPTER

3

Epigenetic Mechanisms in the Regulation of Drug Metabolism and Transport

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3.1 INTRODUCTION

Clinically, interindividual differences in drug metabolism result in the development of erratic therapeutic interventions and adverse drug reactions, which have been reported in 30%–90% of patients subjected to pharmacological treatment.[1, 2] Drug effectiveness, required dosage, and toxicity depend on drug pharmacodynamics and pharmacokinetics. However, it is necessary to know individual pharmacogenetic profiles for adequate personalized treatment. There are a number of well-characterized polymorphisms associated with interindividual pharmacogenomic profiles. These polymorphisms allow the prediction of many phenotypic variations in drug response.[3–8] Pharmacogenomics provides multiple benefits for clinical trials and even for chronic treatment including: (i) identification of candidate patients with ideal genomic profiles for a particular drug; (ii) regulation of drug dosage according to pharmacogenomic profiles; (iii) enhancement of drug efficiency; (iv) reduction of drug interactions and adverse reactions; (v) reduction of costs derived from inappropriate drug selection. Pharmacogenomics thus allows personalized treatments according to the requirements of each individual. Pharmacogenomics provides information about polymorphisms affecting the pathogenic, mechanistic, metabolic, transporter, and pleiotropic genes affecting drug pharmacokinetics and pharmacodynamics. Nevertheless, interindividual gene polymorphisms only explain 20%–30% of this variability in drug response and toxicity. The remainder is caused by epigenetic factors (i.e., physiological and environmental factors interacting with genes, such as age, diet, exposure to toxicants or pollutants, and coadministered drugs, among others[6, 7, 9–12]). These factors can modulate gene regulatory networks and entail epigenetic changes that amplify and preserve alterations in gene expression patterns and molecular phenotypes.[13, 14] Importantly, the effects of these epigenetic modifications persist throughout life and are inherited through at least three or four generations.[15, 16] Hence the integration of an epigenetic signature that reinforces gene expression patterns opens uppromising perspectives for the identification of novel biomarkers for predictive diagnosis, or for patients who do not respond to treatment. In addition, the epigenetic hallmarks on genes involved in drug metabolism, transport, and detoxification provide important insights into the mechanisms of drug resistance mediated by complex treatments, such as chemotherapeutic interventions. Thus the analysis of drug efficiency according to the interplay between interindividual genetic (pharmacogenetics) and epigenetic (pharmacoepigenetics) profiles refines the stratification of patients within a personalized medicine framework.

Importantly, the reversibility and potential restoration of epigenetic aberrations, unlike genetic mutations, have positioned epigenetic-based therapy as a promising tool to treat complex disorders, such as those involving neurodegeneration or cancer. In addition, the acquisition of drug resistance is tightly regulated by epigenetic modifications, such as DNA methylation, histone posttranslational modification, and noncoding RNAs, that affect the genes associated with drug metabolism and transport.[8, 17] Acquired drug resistance is the major cause of chemotherapeutic failure. Importantly, identifying the epigenetic hallmarks of drug resistance along with potential restoring of such epigenetic aberrations allows the development of epidrugs that sensitize cancer cells to chemotherapeutic interventions, improving patient prognosis.[8, 17]

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3.2 EPIGENETIC MECHANISMS

The epigenetic machinery has prompted great interest among the scientific community as it is positioned among the major regulatory elements controlling metabolic pathways at the molecular level. In this regard mechanisms such as memory and learning, elderly associated cognitive impairment, or behavior disorder are to some extent epigenetically regulated.[18–20] Alterations to this epigenetic control by endogenous (hormonal changes, synaptic alterations, response to medication) or exogenous factors (diet habits, physical exercise, stress, environment modifications) lead to abnormal gene expression, which results in being pathogenic despite the genetic code remaining intact.

Epigenetic mechanisms regulate gene expression at both the transcriptional and the posttranscriptional level. The DNA methylation status, histone modifications, and chromatin structure control gene expression, whereas interference RNAs modulate gene expression posttranscriptionally[21] (Table 3.1).

3.2.1 DNA Methylation

DNA methyltransferases (DNMTs) execute methylation by adding methyl groups to cytosine molecules located in the gene promoter or within the gene. The roles of DNMTs in mammals include the addition of methyl groups to unmethylated cytosines (DNMT3a, DNMT3b) and maintenance of the methylated status (DNMT1).[22, 23] Methylation normally occurs at CpG islands defined as regions where the CG content is greater than 60%. The level of gene

TABLE 3.1 Epigenetic Mechanisms and Their Implications in Biological Processes

Epigenetic targets	Effectors	Activity	Biological implications
DNA methylation	DNMTs	DNA methylation	Repressed transcription
	DNDMs	DNA demethylation	Activated transcription
Chromatin structure	ATP-CRCs	Chromatin remodeling to allow accessibility of TFs	Activated/repressed transcription
	Coactivators	Accumulation of transcription activators	Activated transcription
	Corepressors	Accumulation of transcription repressors	Repressed transcription
Histone modifications	HATs	Histone acetylation	Activated transcription; DNA repair; DNA replication; chromosome condensation
	HDACs/ SIRTs	Histone deacetylation	Repressed transcription
	HMTs	Histone methylation	Activated/repressed transcription; DNA repair
	HDMTs	Histone demethylation	Activated/repressed transcription
	Protein kinases	Histone phosphorylation	Activated transcription; DNA repair; chromosome condensation
	Others	Histone ubiquitylation	Activated transcription; DNA repair
		Histone sumoylation	Repressed transcription
		ADP ribosylation	Transcription regulation under DNA repair
Noncoding RNAs	lncRNAs	Protein and genomic DNA binding	Translational regulation; posttranslational regulation
	miRNAs	Posttranslational gene silencers	Repressed translation
	siRNAs	Posttranslational gene silencers	Repressed translation
	piRNAs	Transposon silencers in the germline	Repressed translation

ATP-CRCs, ATP-dependent chromatin-remodeling complexes; *DNDMs*, DNA demethylases; *DNMTs*, DNA methyltransferases; *HATs*, histone lysine acetyltransferases; *HDACs/SIRTs*, histone deacetylases/sirtuins; *HDMTs*, histone lysine demethylases; *HMTs*, histone lysine methyltransferases; *lncRNAs*, long noncoding RNAs; *miRNAs*, micro-RNAs; *piRNAs*, Piwi-interacting RNAs; *siRNAs*, small interference RNAs; *TFs*, transcription factors.

expression inversely correlates with the methylation level of the gene promoter. The level of methylation of a given gene promoter can be predicted by its CpG island content, since approximately 70% of CpG islands within the human genome are methylated. Gene promoters with a rich CpG content tend to be hypermethylated by DNMT activity. Promoter hypermethylation limits the access of transcription factors or promotes the binding of transcription repressors, which leads to reduced gene expression[14–26] (Table 3.1). However, CpG methylation within the gene, rather than at the promoter level, may activate transcription.[27]

Unlike DNMTs, DNA demethylases are another kind of enzyme that instead promote the removal of methyl groups from CpGs. In this case hypomethylated gene promoters are more accessible to transcription factors that promote gene expression. Hypomethylation involves several types of enzymes including: (i) the ten-eleven translocation (TET) family mediating the conversion of 5-methyl-cytosine (5mC) to 5-hydroxymethyl-cytosine (5hmC); (ii) the AID/APOBEC family acting as mediators of 5mC or 5hmC deamination; and (iii) the BER (base excision repair) glycosylase family involved in DNA repair.[28]

3.2.2 Chromatin Remodeling

Chromatin structure and stability determine the accessibility of gene promoters to the transcription machinery, and therefore constitute an essential regulator of gene expression and transposable elements and maintain genome integrity. Chromatin conformation depends on the affinity of DNA and histones, controlled by ATP-dependent chromatin regulator complexes (ATP-CRCs) and posttranslational histone modifications (HMs) (Table 3.1).

ATP-CRCs use ATP hydrolysis to move, destabilize, eject, or restructure nucleosomes thus allowing transcription factors to access DNA. The effects of ATP-CRCs on gene expression depend on the recruitment of coactivators or corepressors in accessible promoters. The main CRCs correspond to (i) the SWI/SNF (switching defective/sucrose nonfermenting) family; (ii) the ISWI (imitation SWI) family; (iii) the CHD (chromodomain, helicase, DNA binding) family; and (iv) the INO (inositol-requiring 80 family).[29, 30]

Posttranslational modifications of histones alter their electrostatic affinity with DNA, leading to different degrees of chromatin condensation that modify the accessibility of gene promoters to the transcription machinery.[29, 30] The most relevant histone modifications affecting chromatin structure include acetylation/deacetylation, methylation, phosphorylation, ubiquitylation, and sumoylation.

Histone acetylation promotes gene transcription and participates in DNA repair, DNA replication, and chromosome condensation.[30–36] The addition of acetyl groups by histone lysine acetyltransferases (HATs or KATs) decreases the electrostatic DNA-histone interaction, leading to an open chromatin conformation and therefore promoting gene expression. The main HATs involve Gcn5-related *N*-acetyltransferases (GNATs), which include GCN5, p300/cAmp response element-binding protein (CBP)-associated factor (PCAF), KAT6-8, CREB-binding protein/CBP (CREBBP/CBP), and EP300.[10, 30, 37, 38] Alternately, histone deacetylases (HDACs) mediate histone deacetylation, which involves the removal of acetyl residues. *Histone deacetylation*, promoted by HDACs or by deficient HAT activity, results in a condensed chromatin conformation and repressed gene expression. There are four classes of HDACs described in mammals: (i) class I HDACs (HDAC1, 2, 3, and 8) are nuclear proteins, HDAC1 and HDAC2 are often found in transcriptional corepressor complexes (*SIN3A*, *NuRD*, *CoREST*), and HDAC3 is found in other complexes (SMRT/N-CoR); (ii) class II HDACs are subdivided into classes IIa (HDAC4, 5, 7, and 9) and IIb (HDAC6 and 10) that which are located in the nucleus-cytoplasm interface and the cytoplasm, respectively; (iii) class III HDACs belong to the sirtuin (SIRT) family, with nuclear (SIRT1, 2, 6, 7), mitochondrial (SIRT3, 4, 5), or cytoplasmic (SIRT1, 2) localization; and (iv) class IV HDACs (made up of a single member HDAC11), a nuclear protein.[10, 30, 37, 38]

Histone methylation is a process that is mediated by histone methylases (HMTs). Unlike the unspecific lysine residue acetylation mediated by HATs, each HMT usually targets a single lysine in the histones that may promote activation or repression of transcription. In this regard histone methylations at H3K4, H3K36, and H3K79 are associated with activation of gene expression, whereas methylations at H3K9, H3K27, and H4K20 correspond to gene silencing.[30, 37, 39–41] Independent of their role in gene expression, histone methylations at H3K79 and H4K20 are also involved in DNA repair molecular signaling.[30, 39, 42, 43]

The effects of the other histone posttranslational modifications remain less clear or controversial, although some studies report that *histone phosphorylation* plays an important role in activating gene expression,[30, 44–46] DNA repair,[30, 44, 47] and chromosome condensation[30, 44, 48, 49]; that *histone ubiquitylation* plays an important role in activating[30, 44, 50] and repressing[30, 44, 51] transcription and is associated with DNA repair[30, 44, 50, 52]; and that *histone sumoylation* plays an important role in transcriptional repression.[30, 44, 53]

3.2.3 Noncoding RNAs

Only 5% of the eukaryotic genome transcribes into mRNA, which is crucial for protein synthesis and cell function. The remaining 95% turns into noncoding RNAs (ncRNAs)[54, 55] (Table 3.1). Of these ncRNAs, long noncoding RNAs (lncRNAs) and regulatory RNAs are the most important posttranslational gene regulators.[56–58]

lncRNAs (>200 nucleotides) are present at over 8000 loci in the human genome, and regulate gene expression by interacting with proteins or RNA secondary structures, by genomic imprinting, by silencing genes in somatic cells involved in brain development, or by interacting with membraneless subnuclear bodies that participate in nuclear organization.

Regulatory RNAs (<200 nucleotides) associate with members of the Argonaute (AGO) superfamily of proteins, which are the central effectors of RNA interference pathways. Micro-RNAs (miRNAs) and silencing RNAs (siRNAs) are posttranscriptional gene silencers, guiding AGO complexes to complementary mRNAs in the cytoplasm, inducing transcript degradation and thus blocking translation. Specific small RNAs (piRNAs), associated with the PIWI clade of AGO, are essential for fertility by silencing transposons in the germline.

3.3 EPIGENETIC REGULATION OF GENES ASSOCIATED WITH DRUG METABOLISM AND TRANSPORT (PHARMACOEPIGENETICS)

Pharmacogenomics accounts for 30%–90% of the variability in pharmacokinetics and pharmacodynamics.[1, 8] Individual differences in drug response are associated with the genetic and epigenetic variability of genes involved in drug metabolism and transport as well as those related to detoxification mechanisms. The pharmacogenetic response to drugs can be classified into five different gene categories: (i) Pathogenic genes involved in disease development or potential risk. Not all individuals carrying the same disease present the same affected pathogenic genes. (ii) Genes associated with the mechanism of action of drugs (enzymes, receptors, messengers, etc.). (iii) Genes associated with drug metabolism. This category includes genes related to phase I enzymes such as alcohol dehydrogenases (*ADHs*), monoamine oxidases (*MAOs*), and cytochrome p450 family genes (*CYPs*), and phase II enzymes such as UDP glucuronosyltransferases (*UGTs*), gluthatione *S*-transferase family genes (*GSTs*), *N*-acetyltransferases (*NATs*), and sulfonotransferases (*SULTs*). (iv) Genes associated with drug transporters (phase III) such as ATP-binding cassette family members (*ABCs*), solute carrier superfamily members (*SLCs*), and solute carrier organic transporter family members (*SLCOs*). (v) Pleiotropic genes involved in multiple pathways and metabolic reactions.[3–8]

The epigenetic machinery modulates all these genes, all of which are involved in pharmacogenetic processes. Individual epigenomic profiles provide information about the efficiency of drug transport and metabolism. Therefore, epigenetics plays a crucial role in drug efficiency and safety, and is highly involved in drug resistance.[6, 7, 10, 11, 17] Pharmacoepigenomics deals with the influence of epigenetic alterations of genes involved in the pharmacogenomic network responsible for the pharmacokinetics and pharmacodynamics of drugs. At the same time pharmacoepigenomics is also related to the effects that drugs may exert on epigenetic pathways. Consequently, drug development and personalized treatments should entail exhaustive pharmacoepigenetic analysis.

Pioneering pharmacoepigenetic studies have illustrated how genes encoding drug-metabolizing enzymes, drug transporters, and nuclear receptors during healthy development and in pathologic conditions are epigenetically regulated. These studies also illustrate the effects of drugs and toxicants on the epigenetic modulation of those genes.[9, 17, 59–63] An important part of these studies relies on the epigenetic pathways associated with acquired drug resistance, especially on chemotherapy and on complex treatments (Fig. 3.1).

3.3.1 Epigenetic Modifications of Genes Encoding Phase I Drug-Metabolizing Enzymes

3.3.1.1 Epigenetic Regulation During Sexual Differentiation

Sexual differences are not only attributable to genetic factors and hormones. Cultured cells from males or females display different gene expression patterns and sensitivity to toxins. This distinct behavior is also evidenced in embryos before sexual differentiation and persist in isolated primary cells. Cultured cells from Swiss-Webster (CWF) mice show sexual differentially methylated patterns for X-ist and for *CYP1a1*, *CYP2elm*, and *CYP7b1*. *Dnmt31* is differentially expressed but not differentially methylated, whereas *Gapdh* is neither differentially methylated nor expressed. The expression levels of *CYP* genes are 2- to 355-fold higher in females, while *Dnmt31* displays a 12- to 32-fold increase in expression in males compared with females. These different expression patterns are the result of sex-dimorphic DNA methylation patterns in the promoters of these genes. External stimuli, such as stress or estradiol, alter both

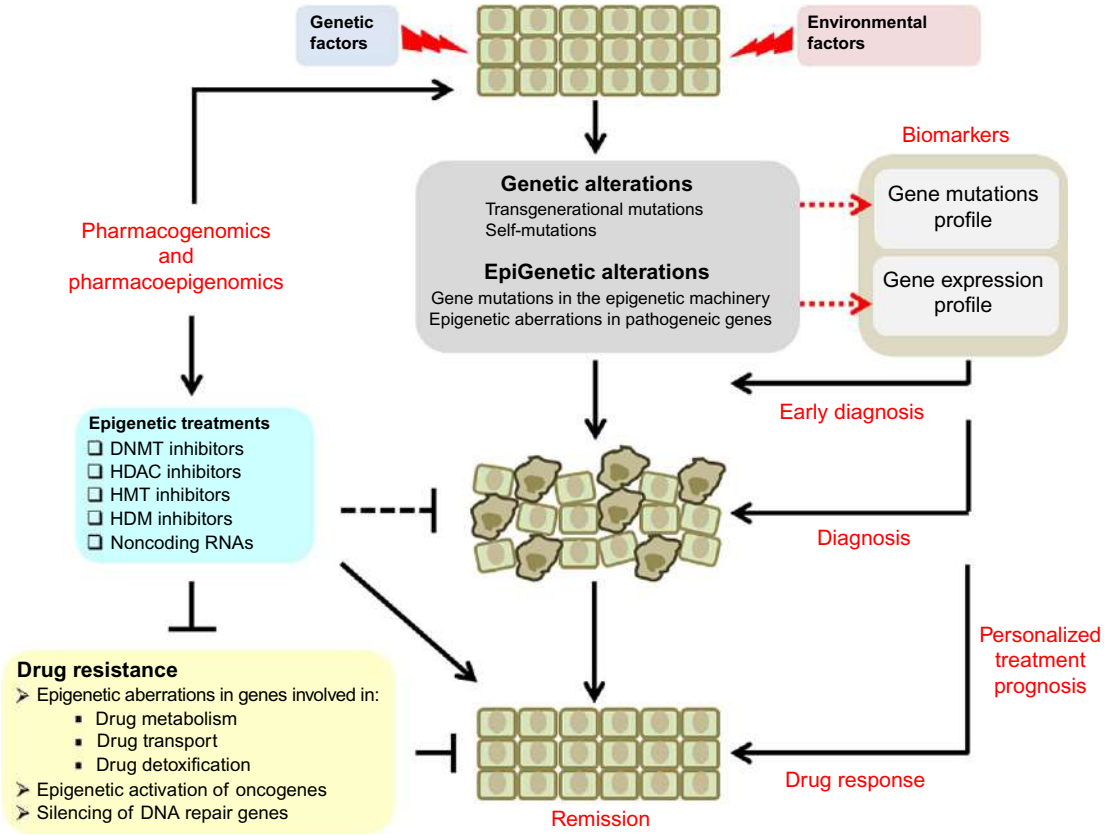

FIG. 3.1 Potential epigenetic interventions for efficient and personalized treatments. Complex diseases are highly influenced by genetic and epigenetic factors. Inherited and de novo gene mutations or polymorphisms generated during embryonic development explain most interindividual genetic variations. In addition, environmental factors such as age, diet, exposure to toxicants or pollutants, and coadministrated drugs lead to dynamic alterations in gene methylation, chromatin structure, or noncoding RNA synthesis that modulate the gene expression profile. It is important to note that epigenetic targets constitute key initiating events in complex disorders that may provide an early diagnostic of presymptomatic stages of complex diseases. Identification of the genetic and epigenetic biomarkers of diseases allows patients to be stratified to personalize treatment and prognostic applications to be made to provide information about the response of an individual patient to the chosen therapy. Clinically, interindividual differences in drug metabolism result in the development of erratic therapeutic interventions and adverse drug reactions. Drug effectiveness, required dosage, and toxicity depend on interindividual polymorphisms on genes associated with drug metabolism and transport (pharmacogenetics). However, interindividual gene polymorphisms only explain 20%–30% of variability in drug response and toxicity. Hence a complete and accurate treatment profile needs to include an extensive analysis of the epigenetic modifications of these genes (pharmacoepigenomics). The versatility and reversibility of epigenetic changes make epigenetic-based drugs (epidrugs) potential candidate treatments for complex diseases such as cancer, neurodegenerative disorders, and diabetes. These treatments can exert their effects as single agents or as part of a combinatorial therapy and could be used to prevent disease onset or reverse epigenetic alterations during disease progression. Furthermore, epidrugs can prevent or reverse epigenetically acquired drug resistance, which allows cancer cells to resist chemotherapy, either by lowering intracellular drug levels or by escaping drug-induced apoptosis. *DNMT*, DNA methyltransferase; *HDAC*, histone deacetylase; *HDM*, histone demethylase; *HMT*, histone methyltransferase.

methylation and gene expression. Different methylation patterns partially explain sex-based differences in the expression of CYP family members and X-ist, which potentially lead to inborn differences between males and females and their different responses to chronic and acute changes.[64]

Sexual dimorphism in the expression of CYP enzymes is much more evident in mice and rats (up to 500-fold male and female differences) and to a much smaller degree in humans. Nevertheless, the slight differences observed in humans are determinant of the sex dependence of hepatic drug and steroid metabolism. Sex-dependent differential expression of CYPs is mediated by dimorphic methylation or DNMT expression, as well as by hormonal pathways that alter the expression of hepatic enzymes. Growth hormone (GH) exerts sex-dependent effects on the liver in many species by means of many hepatic genes, most notably genes coding for CYP enzymes. GH activates intracellular signaling via sex-dependent temporal patterns of pituitary release, leading to the sexually dimorphic transcription of CYPs and other liver-expressed genes. The sex-dependent activities of GH include the modulation of transcription factor STAT5b (signal transducer and activator of transcription 5b), hepatocyte nuclear factors 3, 4alpha, and 6, as well as differential DNA methylation and chromatin structure.[65]

Lipopolysaccharide (LPS) inhibits *CYP19A1* expression and 17-estradiol (E2) production in granulosa cells (GCs). This is one of the major causes of infertility underlying postpartum uterine infections. GCs exposed to LPS transiently increased the expression of proinflammatory cytokine genes (*IL-1β*, *TNF-α*, *IL-6*), followed by the inhibition of *CYP19A1* expression and E2 production. The transient increase in proinflammatory cytokines was associated with HDACs. The HDAC inhibitor trichostatin A (TSA) can attenuate LPS-induced proinflammatory cytokine gene expression and prevent LPS-mediated downregulation of *CYP19A1* expression and E2 in GCs.[66]

3.3.1.2 *Epigenetic Regulation During Development in Healthy and Cancer Cells*

Understanding the epigenetic regulation of *CYP* genes during organogenesis and tissue development provides crucial information about the aberrant modulation pathways leading to the onset of complex pathologies, such as cancer.

Park et al.[67] studied the epigenetic regulation of *CYP* genes (*CYP1A1, CYP1A2, CYP1B1, CYP2D6, CYP2E1*) in human pluripotent stem cell-derived hepatocytes and in primary hepatocytes. The transcript levels of major *CYP* genes were much lower in human embryonic stem cell-derived hepatocytes (hESC-Hep) than in human primary hepatocytes (hPH). The hypermethylation of CpG islands of *CYPs* in hESC-Hep correlated with the lower expression levels of these genes in hESC-Hep, whereas the enhanced expression of *CYPs* in hPH was consistent with hypomethylation of CpG sites and permissive histone modifications. The inhibition of DNA methyltransferases (DNMTs) during hepatic maturation induced demethylation of the CpG sites of *CYP1A1* and *CYP1A2*, leading to upregulation of their transcription. Combinatorial inhibition of DNMTs and histone deacetylases (HDACs) enhanced the transcript levels of *CYP1A1*, *CYP1A2*, *CYP1B1*, and *CYP2D6*. These data suggest that epigenetic regulatory factors, such as DNMTs and HDACs, modulate the limited expression of *CYP* genes in hESC-Hep.

Aberrant epigenetic regulation of dioxin-inducible *CYP1s, CYP1A1*, and *CYP1B1* genes is associated with carcinogenesis in extrahepatic tissues. Normally, the carcinogenesis of hormone-responsive tissues correlates with abnormal *CYP1B1* expression levels. Abnormal expression of these CYPs also correlates with cancers unrelated to hormone response. Analysis of the methylation status of CpG islands within the 5′ flanking region of *CYP1B1* in 7 colorectal cancer cell lines and 40 primary colorectal cancers showed significantly higher methylation levels in 2 colorectal carcinoma cell lines (SW48 and Caco-2) and 5% of cancers than in corresponding normal tissues. Cell treatment with the DNMT inhibitor 5-aza-2′-deoxycytidine revealed a significant increase in *CYP1B1* expression levels in SW48 and Caco-2 cells, along with decreased methylation levels. Only HT29 cells showed a clear increase in *CYP1A1* mRNA, although there were no apparent differences in methylation status among these cell lines. None of these cell lines showed a significant change in the mRNA levels of aryl hydrocarbon receptor (AhR) and AhR nuclear translocator (ARNT), which directly activate *CYP1* transcription. CpG methylation of the *CYP1B1* promoter region epigenetically regulates *CYP1B1* expression during the development of some colorectal cancers, and cancers with aberrant *CYP1B1* expression might well show an altered response to procarcinogen metabolism and chemotherapy.[68]

The aryl hydrocarbon receptor (AhR) regulates many enzymes involved in xenobiotic metabolism, including CYP1A1. A combination of AhR and its high-affinity ligand, 3,3′,4,4′,5-penta chlorobiphenyl (PCB 126), can enhance the expression of *CYP1A1*. Vorrink et al.[69] studied the epigenetic determinants of *CYP1A1* induction in carcinoma cell lines. Although HepG2 hepatocarcinoma cells and HeLa cervical carcinoma cells did not display significant differences in the methylation levels of CpG islands of *CYP1A1* gene promoter, the levels of mRNA expression were highly different. *CYP1A1* gene expression was enhanced in HepG2 cells and repressed in the HeLa cell line following PCB 126 exposure. Treatment with the HDAC inhibitor trichostatin A (TSA) promoted *CYP1A1* gene expression in HeLa cells. This suggests that differences in *CYP1A1* expression between the two cell lines may be relayed to the chromatin architecture of the *CYP1A1* promoter and thus establish a role for epigenetic regulation in cell-specific *CYP1A1* expression.

Gene polymorphisms may also influence the effects methylation has on gene expression levels. According to Naselli et al.[70] different polymorphisms modulated the effects of methylation at the 5′ flanking region of *CYP2E1* on the expression of this gene in both tumor and nonneoplastic liver cell lines. In this case reduced DNA methylation differentially influenced CYP2E1 enzyme expression in the Rsa/Pst haplotype. Cells with the VNTR A4/A4 genotype displayed a reduced (20%–30%) inhibition of expression compared with A2/A2 genotypes. Cells with the A2/A3 genotype showed increased expression (25%). Therefore, the A2 and A3 *CYP2E1* alleles may play a more important role in expression of the enzyme than other epigenetic factors, since they are binding sites for trans-acting proteins.

3.3.1.3 *Toxicoepigenetics Affecting Genes of Phase I Drug-Metabolizing Enzymes*

The metabolism of xenobiotics and toxicants mediated by phase I drug-metabolizing enzymes is also epigenetically regulated. Exposure to toxicants and pollutants may alter the expression of those enzymes, thus contributing to toxicity and disease. Genome-wide RNA-seq of tongue samples of combined 4-nitroquinoline-1-oxide (4-NQO) oral carcinogenesis and Meadows-Cook alcohol mouse models revealed significant modifications in transcripts involved in

oxidative stress and alcohol metabolism, such as *Aldh2*, *Aldh1a3*, *Adh1*, *Adh7*, and *Cyp2a5*, when mice were treated with 4-NQO followed by ethanol (4-NQO/EtOH). Furthermore, the oral cavities of these mice showed specific histone acetylation and methylation biomarkers (H3K27ac, H3K9/14ac, H3K27me3, and H3K9me3). *Aldh2* mRNA levels were 10-fold reduced, which was shown to be associated with increased H3K27me3 marks at the gene promoter level in 4NQO/EtOH samples.[71]

Other environmental xenobiotics and endocrine disrupters, which interfere with the normal development of male and female reproductive systems, play important roles at different levels of epigenetic control. Vinclozolin (VZ) and methoxychlor (MXC) promote epigenetic transgenerational effects.[72] Polychlorinated biphenyls (PCBs), the most widespread environmental endocrine disrupters, affect histone posttranslational modifications in a dimorphic manner, possibly as a result of the altered gene expression of enzymes involved in histone modification, such as demethylase Jarid1b, an enzyme also involved in regulating the interaction between androgens and their receptor.[73]

Phthalates are the largest group of environmental pollutants. They are considered toxic to the endocrine system. According to previous studies using mouse models the effect of in utero exposure of di(2-ethylhexyl)phthalate (DEHP) on Leydig cell steroidogenesis in F1 male offspring demonstrated a coordinated, dose-dependent disruption of genes involved in steroidogenesis.[74] Exposure to the pollutant led to significantly reduced expression of *Cyp* genes (*Cyp11a1* and *Cyp19a1*), of genes involved in the acute regulation of steroid hormone synthesis (StAR), and of genes encoding the Hydroxy-Delta-5-Steroid Dehydrogenase family (*HSD3* and *HSD27*) involved in the production of steroid hormones. Animal models exposed to 10 and 100 mg DEHP showed significantly decreased expression of transcription factors, such as steroidogenic factor-1 (SF-1) and specific protein-1 (Sp-1), which correlated with promoter hypermethylation of these genes. Enhanced expression of the methyltransferases Dnmt3a, Dnmt3b, and Dnmt1, but not Dnmt3l, promoted this hypermethylation. These data suggest that Dnmt3a/b and Dnmt1 are methyltransferases specific to testicular Leydig cells.

Epigenetic mechanisms may also influence the cytotoxicity of certain toxins, such as polycyclic aromatic hydrocarbons (PAHs). CYP1A1 and CYP1B1 metabolize PAHs to diol epoxides, which can covalently bind to DNA and drive carcinogenesis.[75] PAHs can induce their own metabolism by binding to the nuclear receptor AHR, which causes transcriptional activation of its target genes *CYP1A1* and *CYP1B1*.[75] Interestingly, treatment with the AHR ligand TCDD in HepG2 cells recruited AHR and the transcriptional coactivators EP300 and KAT2B to the *CYP1B1* enhancer, but did not associate RNA polymerase II or TATA-binding factor with the methylated promoter.[76] However, treatment with the demethylating agent azacitidine resulted in successful polymerase binding and *CYP1B1* expression, demonstrating that the lack of *CYP1B1* activation is, at least in part, ascribable to DNA methylation in these cells.[76]

Carcinogenic compounds (such as polycyclic aromatic hydrocarbons) or volatile organic compounds are sequentially metabolized by phase I and II enzymes. First, CYP1A1 catalyzes the conversion of these compounds into harmful hydrophilic DNA adducts; then, GSTT1 enables excretion via conjugation into polar electrophiles. There is evidence that smoking affects the fetal growth of offspring. Interestingly, smoking has been shown to upregulate CYP1A1 expression. Motivated by this fact, Suter et al.[77] hypothesized that alterations of CpG islands in the promoter of the placental *CYP1A1* gene may further affect fetal growth. The authors analyzed multiple CYP expression levels among smoker and nonsmoker gravidae and found a significant increase in *CYP1A1* gene expression among smokers compared with controls. This higher expression in smokers correlated with significant hypomethylation at CpG sites immediately proximal to the 5′-xenobiotic response element transcription factor-binding element. Importantly, *CYP1A1* upregulation uniquely correlated with placental gene expression, indicating that in utero tobacco exposure significantly increases placental CYP1A1 expression associated with differential methylation of a critical xenobiotic response element.

Human exposure to volatile compounds like toluene affects *CYP2E1* expression. CYP2E1 is a pleiotropic phase I drug-metabolizing enzyme responsible for biotransformation of those compounds. Jiménez-Garza et al.[78] analyzed blood from tannery workers exposed to toluene and found significant correlations between airborne levels of toluene and *CYP2E1* promoter methylation, as well as IL6 promoter methylation levels. *CYP2E1* promoter methylation levels were higher in toluene-exposed smokers than nonsmokers. Significant correlations were also observed between *CYP2E1* promoter methylation and *GSTP1* and *SOD1* promoter methylation levels.

3.3.2 Epigenetic Modifications of Genes Encoding Phase II Drug-Metabolizing Enzymes

The tissue-specific expression of genes encoding drug-metabolizing enzymes is often regulated by the methylation status of CpG promoters. Hypermethylation of CpG-rich promoter regions may inhibit the binding of transcription and nuclear factors to these regions, which inhibits or drastically represses gene expression. This is the case for the

gene encoding human UDP-glucuronosyltransferase (UGT) 1A10. The *UGT1A10* gene is exclusively expressed in the intestine, contributing to presystemic first-pass metabolism. Constitutive transcription of this gene is promoted by intestine-specific transcription factor CDX2 (caudal-type homeobox), hepatocyte nuclear factor 1 (HNF1), and specific protein-1 SP-1. Although HNF1 and SP-1 are highly expressed in liver, the UGT1A1 enzyme is only present in the intestine (not in liver). Oda et al.[79] demonstrated that the tissue-specific presence of UGT1A1 relied on the methylation status of gene promoter CpGs. The CpG-rich region around the *UGT1A10* promoter has been shown to be hypermethylated (89%) in hepatocytes and hypomethylated (11%) in the epithelium of the small intestine. Accordingly, the *UGT1A10* promoter displayed low methylation levels (19%) in colon-derived LS180 cells, which promoted *UGT1A10* gene expression in this tissue. However, hypermethylation of the CpG promoter region correlated with inhibition of gene expression in liver-derived HuH-7 cells. Indeed, forced methylation of the *UGT1A10* promoter by SssI methylase abrogated transactivity even with overexpressed Cdx2 and HNF1. Only treatment of HuH-7 cells with the DNA methylation inhibitor 5-aza-21-deoxycitidine (5-Aza-dC) enhanced *UGT1A10* expression in these liver-derived cells on HNF1 and Cdx2 overexpression. According to these results, DNA hypermethylation could well interfere with the binding of HNF1 and Cdx2, resulting in defective expression of *UGT1A10* in human liver. Therefore, epigenetic regulation could well determine the tissue-specific expression of the *UGT1A10* gene.[79]

3.3.3 Epigenetic Modifications of Genes Encoding Drug Transporters

A few reports describe epigenetic changes in genes involved in drug transport, especially those of the ATP-binding cassette family members (ABCs). In utero or childhood exposure to chemicals, such as polycyclic aromatic hydrocarbons, or pollutants including bisphenol A and cigarette smoke, promote these epigenetic modifications on ABC genes.[80–84] The exposure of mouse models to high concentrations of bisphenol A during gestation (50 mg/kg intraperitoneal injections) induced epigenetic aberrations in the promoters of 197 genes, including the multidrug-resistant transporters *ABCC4* and *ABCC6*. Strikingly, epigenetic aberrations mediated by pollutant exposure persisted transgenerationally in animal models. The effects of exposure to bisphenol A were detectable in the third generation of exposed pups.[82] Similarly, exposure of rodents to high concentrations (100 mg/(kg day)) of the common pesticide vinclozolin led to increased prevalence of, for example, prostate and kidney disease as well as hypercholesterolemia, up to the F4 generation.[83, 84]

3.3.4 Epigenetic Modifications Involving Nuclear Receptors and Transcription Factors

Although most studies involve animal models, mounting evidence indicates that most clinically important *CYP* genes, including *CYP2C9*, *CYP2C19*, and *CYP3A4*, are subject to epigenetic regulatory mechanisms in nuclear receptors and transcription factors that affect the expression of these genes.[85–95] However, other clinically relevant genes, such as *CYP2D6*, are controlled by genetic factors instead.

Epigenetic modulation in nuclear receptors, such as constitutive androstane receptor (CAR), primarily regulates the hepatic expression of several *CYP* genes. CAR protein, encoded by the nuclear receptor 1I3 gene (*NR1I3*), plays a key role in the detoxification of endobiotic and xenobiotic substances. Transient CAR-signaling activation in neonatal mice led to chromatin remodeling within the CAR target genes *Cyp2B10* and *Cyp2C37*, which promoted transcriptional activation of these genes. Interestingly, this activation persisted throughout adulthood in these animals.[87] Repeated drug exposure reinforced the epigenetic alterations mediated by CAR activation, which were highly reproducible between individuals.[88, 89] Hepatic expression of the *CYP2C19* gene is regulated by CpG promoter methylation and by epigenetic modifications in the *NR1I3* gene. Indeed, aberrant hypermethylation of *NR1I3*, which correlates with reduced CAR expression, results in reduced transcription of *CYP2C19*.[85] Such a reduction in CYP2C19 expression correlates with the hypermethylation of CpG promoters, which may entail decreased transcription factor recruitment.[86]

Epigenetic regulation of another member of the nuclear receptor superfamily involved in drug detoxification, the Pregnane X receptor (PXR), also modulates *CYP* gene expression and activity. PXR is the principal transcription factor involved in activating the *CYP3A4* gene. The oxidative capacity of CYP3A4 may shift as much as 30-fold among individuals.[92] This large variability cannot just be explained by common genetic variants.[93] Hence, different studies describe epigenetic pathways involving *CYP3A4* transcript activation via PXR.[94, 95] PXR binding to the regulatory region of *CYP3A4* is facilitated by concomitant recruitment of the protein arginine histone *N*-methyltransferase (PRMT1).[94] This histone modification step is a prerequisite for *CYP3A4* induction since interfering RNA-mediated knockdown of PRMT1 leads to a 20-fold reduction in *CYP3A4* expression.[94] *CYP3A4* is not present at the fetal stage, and therefore *CYP3A7* plays a similar role at that time. Epigenetic mechanisms involving DNA methylation control the switch from *CYP3A7* to *CYP3A4* expression during postnatal development and control *CYP3A4* expression in adult

liver.[95] Epigenetic regulation of PXR/CYP3A4 pathways is not restricted to the liver, it also plays important roles in intestinal first-pass metabolism and in colon cancer cells. The methylation of *PXR* promoter regulates *CYP3A4* gene expression and, according to Habano et al.,[91] might explain the interindividual variability of drug responses in colon cancer cells. Habano et al.[91] classified six colon cancer cell lines into two groups according to *PXR/CYP3A4* gene expression levels. They found hypermethylation at the CpG-rich sequence of the PXR promoter in Caco-2, HT29, HCT116, and SW48 cell lines, which correlated with downregulation of both *PXR* and *CYP3A4* genes. The methyltransferase inhibitor 5-aza-2′-deoxycytidine reversed methylation levels in these cell lines, which resulted in the activation of PXR and CYP3A4 gene transcription. In addition, promoter hypomethylation led to activation of *PXR/CYP3A4* gene transcription in LS180 and LoVo cells. Colorectal cancer tissues displayed a lower level of PXR promoter methylation than that of adjacent normal mucosa, suggesting the upregulation of PXR/CYP3A4 mRNAs during carcinogenesis.[91] HNF4α (hepatic nuclear factor 4-α) is a transcription factor that regulates the activity of several genes, including *CYP2C9*, in liver cells. Englert et al.[90] demonstrated that histone modifications modulate chromatin structure within regulatory binding sites of HNF4α, affecting CYP2C9 expression and activity in HepG2 cells. In this case transcriptional activation is mediated by Mediator complex component MED25 recruitment at the *CYP2C9* promoter via HNF4α, which prevents association with the polycomb repressive complex 2 (PRC2), thereby generating a permissive chromatin structure that promotes gene expression.[90]

3.4 EPIGENETICS OF DRUG RESISTANCE

Epigenetic modifications are associated with acquired drug resistance, a common cause of chemotherapeutic failure leading to poor patient prognosis. Cells can become resistant through several other pathways including decreased drug uptake, decreased bioactivation, increased detoxification, or drug target mutation, although the most frequent path of drug resistance relies on increased drug efflux. The inhibition of drug efflux transporters, such as multidrug resistance-associated protein 1 (MRP1), encoded by *ABCC1*, seems to be one of the most promising strategies to overcome drug resistance, although clinical trials have turned out to be rather disappointing.[96] Thus alternate strategies, involving the mechanisms underlying increased transporter gene expression, have been considered. As a result it has become increasingly evident that epigenetic heterogeneity among tumor cells combined with selective pressure by chemotherapeutic drugs facilitates the emergence of acquired drug resistance[97] (Fig. 3.1).

The acquisition of drug resistance is tightly regulated by posttranscriptional regulators, such as RNA-binding proteins (RBPs) and miRNAs, which change the stability and translation of mRNA-encoding factors involved in cell survival, proliferation, epithelial-mesenchymal transition, and drug metabolism.[98] Alterations mediated by epigenetic mechanisms are important factors in cancer progression and in the response to treatment of different types of cancer.

3.4.1 Drug Resistance Mediated by DNA Methylation in Transporter Genes

The epigenetic state of drug transporter genes is an important determinant of drug response. Therapy-induced overexpression of ATP-binding cassette (ABC) transporters *ABCB1*, *ABCC1*, and *ABCG2* is associated with increased risk for treatment failure and poor prognosis.[99–103] However, downregulation of ATPase protein associated with ABC transporters, PAAT,[104] promotes mitochondrial damage and cell death. PAAT contains a nucleotide-binding domain (NBD)-like domain and a signal for intramitochondrial sorting. PAAT has intrinsic ATPase activity and localizes in both the cytoplasm and the mitochondria. PAAT interacts with mitochondrial inner-membrane ABC proteins, ABCB7, ABCB8, and ABCB10, but not with ABCB1, ABCB6, or ABCG2, and regulates the transport of ferric nutrients and heme biosynthesis. PAAT is a novel ATPase and a trans-regulator of mitochondrial ABC transporters that plays an important role in the maintenance of mitochondrial homeostasis and cell survival. Its deficiency promotes cell death, reduces mitochondrial potential, and sensitizes mitochondria to oxidative stress-induced DNA damage.

P-glycoprotein (P-gp), an *ABCB1* gene product, plays an important role in xenobiotic distribution and bioavailability by extruding multiple endogenous and exogenous substrates from the cell. This glycoprotein plays a crucial role in protecting the fetus and fetal brain from maternally administered drugs and other xenobiotics.[105] The *ABCB1* promoter region contains several binding sites, CpG islands, and GC boxes that are highly involved in epigenetic control of the gene. Leucine-Rich Pentatricopeptide Repeat Containing (LRPPRC) is a potential regulator of *ABCB1* transcription via an invMED1 binding site in *ABCB1*. This invMED1 binding site overlaps with the GC-100 box. LRPPRC binds prominently to *ABCB1* promoter in Lucena cells, an imatinib mesylate (IM)-resistant cell line. LRPPRC knockdown positively regulates *ABCB1* transcription. The methylation levels of the GC-100 box of the *ABCB1* promoter differ

significantly between K562 and Lucena cells, as well as in chronic myeloid leukemia (CML) patients who have a different response to IM. Chromatin immunoprecipitation and Pgp expression after DNA demethylation treatment indicate that the methylation status of the *ABCB1* GC-100 box affects LRPPRC binding. LRPPRC is a transcription factor related to *ABCB1* expression and highlights the importance of epigenetic regulation in CML resistance.[106]

Induced expression of the ABCB1 drug transporter often occurs in tumors in response to chemotherapy. *ABCB1* promoter hypomethylation leads to gene downregulation, which is associated with acquisition of resistance to chemotherapy drugs, such as epirubicin or paclitaxel. Treatment of control MCF-7 cells with demethylating and/or acetylating agents allowed the detection of specific CpG sites within the promoter that may play a predominant role in transcriptional activation through promoter hypomethylation. Allele-specific reductions in *ABCB1* promoter methylation regulate promoter usage within paclitaxel-resistant cells. Changes in *ABCB1* promoter methylation, ABCB1 promoter usage, and *ABCB1* transcript expression can be temporally and causally correlated with the acquisition of drug resistance in breast tumor cells.[107]

ABC transporter family efflux pumps are subject to miRNA-mediated gene regulation. ABC transporters are embedded in a concerted and miRNA-guided network of concurrently regulated proteins that mediate altered drug transport and cell survival under changing environmental conditions. miR-27a, miR-137, miR-145, miR-200c, miR-298, miR-331-5p, miR-451, and miR-1253 are associated with reduced *ABCB1* expression, and miR-27a, miR-138, miR-296, and miR-451 are associated with increased *ABCB1* expression.[108]

Epigenetic modifications in solute carrier family genes (SLCs) are also involved in acquired resistance to drugs. Epigenetic nephritic silencing of *SLC22A2*, which encodes organic cation transporter 2 (OCT2), contributes to oxaliplatin resistance in renal cell carcinoma as a result of lower *OCT2* expression and concomitant reduced platinum uptake.[109] Mechanistically, DNA methylation blocked transcription factor binding to the *SLC22A2* promoter, which could be reversed by the DNA methylation inhibitor decitabine, thereby resensitizing cancer cells to oxaliplatin in vitro and in xenografts.[109] *SLC22A3* encodes for another organic cation transporter (OCT3) that mediates the uptake of endogenous amines and basic drugs in several tissues. Polymorphic variants in the proximal promoter region of *OCT3* modify the methylation rate, and consequently the level of gene expression. Haplotypes containing common variants g.-81G > delGA (rs60515630) (minor allele frequency of 11.5% in African Americans) and g.-2G > A (rs555754) (minor allele frequency >30% in all ethnic groups) show significant increases in luciferase reporter activities and exhibit stronger transcription factor-binding affinity than haplotypes containing major alleles. OCT3 mRNA expression levels are higher in Asian and Caucasian livers from subjects homozygous for g.-2A/A than in those homozygous for the g.-2G/G allele. The methylation level of the *OCT3* promoter has been found to be higher in over 60% of prostate tumor samples. Indeed, high hypermethylation leads to severe *OCT3* downregulation in aggressive prostate cancers.[110]

Synaptically released L-glutamate, the most important excitatory neurotransmitter in the central nervous system (CNS), is removed from extracellular space by fast and efficient transport mediated by several transporters (EAAT1/GLAST and EAAT2/GLT1). There is one CpG island in the *SLC1A2* (*EAAT2/GLT1*) gene and none in *SLC1A3* (*EAAT1/GLAST*). Furthermore, there are targets for specific miRNA binding in the *SLC1A2* (*EAAT2/GLT1*) gene.[111]

Resistance to chemotherapy may arise as a result of promoter methylation/downregulation of the expression of transporters required for drug uptake. In specific cases decitabine can reverse resistance in vitro by inducing changes in the expression of the endocytosis regulator RhoA, the folate carriers FOLR1 (folate receptor 1) and RFC1 (replication factor C subunit 1), and the glucose transporter GLUT4 (solute carrier family 2 (facilitated glucose transporter), member 4).[112]

3.4.2 Drug Resistance Mediated by Other Genes

A plethora of other genes have been implicated in acquired drug resistance including spermidine/spermine *N*(1)-acetyltransferase (*SAT1*), S100 calcium-binding protein P (*S100P*), and the DNA mismatch repair genes *O*-6-methylguanine-DNA methyltransferase (*MGMT*) and MutL homolog 1 (*MLH1*).[113–115] Longitudinal studies comparing primary and secondary tumors have provided important insights into the mechanisms underlying acquired drug tolerance in vivo. Downregulation of dual specificity phosphatase 4 (*DUSP4*), a negative regulator of extracellular signal-regulated kinase (ERK) signaling, has been identified as a mechanism underlying resistance to neoadjuvant chemotherapy in breast cancer.[116] There is evidence that the epigenetic silencing of *DUSP4*, likely mediated by gene promoter methylation, is the common mechanism underlying cancer development.[117] Similarly, promoter methylation of *MLH1* exclusively occurred in resistant cells in secondary epithelial ovarian cancer tumors subsequent to platinum-based chemotherapy, providing the potential mechanism underlying acquired treatment resistance.[118]

3.4.3 Drug Resistance Mediated by Chromatin Remodeling

Chromatin structure at the level of promoter CpG islands regulates the accessibility of transcription factors and subsequently modulates gene expression. Posttranslational modifications of histones modify the chromatin structure affecting genes involved in acquired drug resistance. Hence the belief that epidrugs, which revert histone modifications affecting the chromatin structure around multidrug-resistant genes, may well constitute a promising strategy to solve resistance to chemotherapy.

Downregulation of the multidrug resistance gene *MDR1* is a common hallmark of prostate carcinoma. Gene promoter hypermethylation and histone posttranslational modifications are the main factors leading to reduced *MDR1* transcription. These epigenetic alterations correlate with the use of chemotherapeutic agents to activate MDR1 transcription.[119] Treatment with the histone deacetylase inhibitor trichostatin A alone or combined with the DNA methylase inhibitor 5-aza-2′-deoxycytidine increased the active marks of histone acetylation (H3Ac, H3K9Ac, and H4Ac) and histone methylation (H3K4me2 and H3K4me3) at the MDR1 promoter.[120]

In seminal preclinical studies using human lung adenocarcinoma cells with *EGFR* mutations, cotreatment with the HDAC inhibitor trichostatin A prevented and even reversed resistance to the tyrosine kinase inhibitors gefitinib and erlotinib.[121] Similarly, trichostatin A treatment downregulated *ABCB1* in etoposide-resistant small-cell lung carcinoma cells, reducing multidrug resistance.[122] Furthermore, cotreatment with the γ-secretase inhibitor (GSI) dibenzazepine and JQ1, a small-molecule inhibitor that blocks the binding of BRD4 fusion oncogenes to acetylated histones, resulted in growth arrest and apoptosis in GSI-resistant primary human leukemias.[123] Alterations in chromatin acetylation and DNA double-strand breaks (DSBs) in oral lichen planus (OLP) led to different responses to therapy. Patients with high levels of acetyl-histone H3 at lys9 (H3K9ac), which is associated with nuclear decondensation and enhanced transcription, failed to respond to therapy or experienced disease recurrence shortly after therapy. At the same time, patients who responded poorly to therapy had increased accumulation of DNA DSB, indicating genomic instability.[124]

Several studies demonstrate that treatment with antidepressants and related drugs involve histone modifications and DNA methylation affecting the clinical response of psychiatric patients.[125–128] Studies indicate that histone deacetylation is important for long-term changes related to stress and antidepressant treatment.[125] The classic antidepressant imipramine and the antagonist of the *N*-methyl-D-aspartate (NMDA) receptor ketamine decreased HDAC activity in selected brain regions (nucleus accumbens) of maternally deprived adult rats.[126] Transcriptional differences in IL11 after antidepressant treatment correspond to clinical response in patients with major depressive disorder. Potential predictors of antidepressant response are the SNP rs1126757 and DNA methylation at a CpG unit predictor in IL11.[127] Compound 60 (Cpd-60) is a slow-binding, benzamide-based inhibitor of the class I histone deacetylase (HDAC) family members HDAC1 and HDAC2. Cpd-60 treatment was associated with attenuated locomotor activity following acute amphetamine challenge. Selective inhibition of HDAC1 and HDAC2 in the brain may be a potential epigenetic-based target for developing novel treatments for mood disorders.[128]

3.4.4 Drug Resistance Mediated by miRNAs

In addition to changes in DNA methylation or histone modifications the deregulated miRNA expression patterns of tumor cells have been also identified as interfering with drug response.[129] Therefore, miRNAs are also involved in the mechanisms underlying chemoresistance.

Let-7 miRNA may be involved in the chemosensitivity of cancer cell lines in vitro. Indeed, reduced expression of this miRNA has been associated with resistance to epirubicin in primary breast tumors.[130] However, upregulation of let-7a expression sensitizes tumor breast cells resistant to epirubicin and promotes the apoptosis of these cells. Thus, let-7a may well hold promise as a therapeutic target to modulate epirubicin-based chemotherapy resistance.[130]

Different bladder cancer cell lines display differential resistance to chemotherapy. miRNA expression modulates this chemoresistance. The bladder cancer cell line 5637 is sensitive to five different chemotherapeutic agents, whereas H-bc cells show resistance to these treatments. High expression of the inhibitor of growth 5 gene (*ING5*) confers sensitivity to chemotherapy on the 5637 cell line, whereas siRNA-mediated inhibition of *ING5* increases chemoresistance and inhibits the DNA damage response pathway in these cells.[131] Downregulation of *ING5* gene expression mediates the drug resistance of H-bc cells. It is important to note that forced expression of EGFP-ING5 decreased the chemoresistance of and activated the DNA damage response pathway in H-bc cells.[131] miR-193a-3p inhibits *ING5* gene expression, which means this miRNA plays a key role in activating BCa chemoresistance.[131]

Lung cancer cells show both inherent and acquired resistance to chemotherapy. El-Awady et al.[132] used an isogenic pair of lung adenocarcinoma cell lines (A549-non-resistant and A549DOX11-doxorubicin-resistant) to study the role of epigenetics and miRNA in the resistance/response of nonsmall-cell lung cancer (NSCLC) cells to doxorubicin.

A549DOX11 displayed lower levels of DNMT (promoting DNA hypomethylation), HDACs 1, 2, 3 and 4, and acetylated H2B and H3 histones than A549 cells. In addition, miRNAs were dysregulated in A549DOX11 cells compared with A549 cells; 4 of the 14 dysregulated miRNAs (has-mir-1973, 494, 4286 and 29b-3p) showed a 2.99- to 4.44-fold increase in their expression. This was associated with reduced apoptosis and higher resistance of A549DOX11cells to doxorubicin and etoposide.[132] Sequential treatment with the epigenetic modifiers trichostatin A or 5-aza-2′-deoxycytidine followed by doxorubicin resulted in enhanced sensitivity of both cell lines to doxorubicin, enhanced doxorubicin-induced DNA damage in both cell lines, and dysregulation of some miRNAs in A549 cells.[132] In conclusion, A549DOX11 cells that are resistant to DNA-damaging drugs have an epigenetic profile and miRNA expression different from those of sensitive cells. Hence epigenetic modifiers may promote DNA damage and reverse the resistance of certain NSCLC cells to DNA-damaging agents.

Cisplatin treatment promotes upregulation of the mitochondrial fission protein FIS1 in tongue squamous cell carcinoma (TSCC) cells. Specific binding of miR-483-5p to the *FIS1* gene may inhibit mitochondrial fission and cisplatin sensitivity in vitro and in vivo.[133] This means a novel mitochondrial fission pathway composed of miR-483-5p and *FIS1* regulates cisplatin sensitivity, which indicates that the modulation of miR-483-5p and *FIS1* levels may well constitute a new approach to increasing cisplatin sensitivity.

3.5 CONCLUSIONS AND FUTURE DIRECTIONS

Epigenetics is a relatively novel area of research that is currently attracting a high level of interest for three main reasons: (i) the identification of epigenetic targets as key initiating events in complex disorders that could not be explained just by genetic factors; (ii) these epigenetic targets may be potential markers for an early diagnosis or prognosis of the disease; (iii) the reversibility and potential restoring of epigenetic aberrations, unlike genetic mutations, has positioned epigenetic-based therapy as a promising tool to treat these complex disorders. Epigenetic regulation is highly complex, and a number of studies continually unveil new elements in the epigenetic landscape. In addition to the identification of novel biomarkers for analyses of disease progression and treatment response, the improved analysis of epigenetic pathways has led to the development of novel epigenetic treatments for complex and multigenic diseases, such as cancer and diabetes, and neurodegenerative disorders. These epigenetic treatments (epidrugs) include DNA methyltransferase inhibitors (nucleoside analogs, small molecules, bioproducts, antisense oligonucleotides, miRNAs), histone deacetylase inhibitors (short chain fatty acids, hydroxamic acids, cyclic peptides, benzamides, ketones, sirtuin inhibitors, sirtuin activators), histone acetyltransferase modulators, histone methyltransferase inhibitors, histone demethylase inhibitors, and noncoding RNAs (ncRNAs). Some epigenetic drugs have been approved for the treatment of different modalities of cancer.

The genes involved in the pharmacogenomic process are pathogenic, mechanistic, metabolic, transporter, and pleiotropic. They are susceptible to epigenetic modifications that lead to altered expression of proteins and enzymes with consequent effects on therapeutic outcomes. Pharmacoepigenomics deals with the influence that epigenetic alterations exert on genes involved in the pharmacogenomic network that are responsible for the pharmacokinetics and pharmacodynamics of drugs (efficacy and safety), as well as the effects that drugs have on the epigenetic machinery. Epigenetically acquired drug resistance limits the clinical efficacy of chemotherapy, with negative consequences for patient outcomes. The versatility of the epigenetic machinery allows the manipulation of epigenetic aberrations leading to drug resistance. In this regard and in efforts to overcome drug tolerance, multiple studies have demonstrated that cancer cells can be resensitized to chemotherapy by reversing epigenetic signatures. Consequently, routine procedures for proper evaluation of efficacy and safety issues in drug development and clinical trials should incorporate pharmacoepigenetic studies. However, the long-term effects of treatment with epidrugs using targets without any particular cell specificity are unknown. Thus, despite substantial progress, the research areas of pharmacoepigenetics and toxicoepigenetics are still in their infancy, and more research and the development of methods are needed to establish a more comprehensive picture of the role epigenetic modifications play in human health and the treatment of disease.

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CHAPTER

4

MicroRNA-Dependent Gene Regulation of the Human Cytochrome P450☆

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4.1 INTRODUCTION

Individual drug responses and adverse drug reactions are affected to a large extent by variations in the expression and activity of drug-metabolizing enzymes and transporters (DMETs). About 80% of phase I drug metabolism is performed by the cytochrome P450 (CYP) family, which also catalyzes metabolic transformations of other xenobiotics and many endogenous chemicals.[1] While genetic polymorphisms for CYP genes have a major influence on therapeutic outcomes for many diseases,[2, 3] epigenetic regulatory mechanisms, such as DNA methylation and microRNA (miRNA) modulation, also play a prominent role in the regulation of CYP expression; these epigenetic regulatory mechanisms also contribute to interindividual variability in responses to drug therapies and toxicities.[4, 5] In this chapter we focus on the epigenetic regulation of CYP gene expression by miRNAs.

4.2 MOLECULAR MECHANISMS FOR THE REGULATION OF CYP EXPRESSION BY miRNAs

miRNAs are a family of evolutionarily conserved, small noncoding RNA molecules found in both plants and animals; they regulate the expression of genes involved in many cellular processes, including cell proliferation, differentiation, and cell death.[6, 7] Currently, 35,828 miRNAs from 223 species have been collected in the miRbase database (Release 21, http://www.mirbase.org/), an online miRNA repository. One miRNA may regulate the expression of 100 or more different mRNA targets.[8] On the other hand, a single mRNA transcript may be targeted by more than one miRNA.

Recognition of a target mRNA transcript by the RNA-induced silencing complex (RISC) relies on partial base pairing between the seed sequence of the bound miRNA (first 2–8 nucleotides from the 5′ end) and a miRNA response element (MRE) present within the target mRNA transcript, typically within the 3′ untranslated region (UTR). The formation of a stable complex between a miRNA-containing RISC and a mRNA transcript typically causes a reduction in the expression of the target gene either by facilitating the degradation of the mRNA transcript or by suppressing the efficiency of protein translation.

As the role of miRNAs in gene expression is increasingly explored, deciphering the mechanisms for miRNA-dependent modulation of DMET expression has become critical to understanding interindividual variability in drug

☆ The contents of this chapter do not necessarily reflect the views and policies of the US Food and Drug Administration, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

sensitivity, efficacy, and toxicity. A recent analysis of the www.microrna.org database predicted that as many as 56 CYP enzymes may be regulated by miRNAs.[9] A number of studies have demonstrated a fundamental role for miRNAs as epigenetic regulators in modulating CYP gene expression. Pioneering work on the regulation of CYP expression by miRNAs was done by Tsuchiya et al.[10] who reported that miR-27b negatively modulated the expression of CYP1B1, an important enzyme for the metabolism of estradiol and procarcinogens. These investigators discovered a putative miR-27-MRE in the 3′ UTR of the CYP1B1 mRNA transcript and then used luciferase reporter constructs containing this domain to demonstrate miR-27-dependent control of CYP1B1 gene expression in MCF-7 human breast cancer cells. The introduction of excess exogenous miR-27 resulted in decreased luciferase activity, whereas transfection with antisense 2′-*O*-methyl oligoribonucleotides (AsO), which acted as an inhibitor of miR-27, caused an increase in luciferase activity. Furthermore, reduced CYP1B1 protein levels and enzymatic activity caused by miR-27 were also restored by exogenous miR-27 AsO. This research strategy provided a useful template for subsequent studies performed by this group and others to evaluate the functional significance of putative miRNA/CYP mRNA interactions in live cells. Table 4.1 lists additional CYP enzymes and their corresponding miRNA regulators reported between 2015 and 2017 that have been experimentally validated (CYP/miRNA regulatory interactions from earlier publications can be found in previous reviews[9, 37–40]).

miRNAs may also affect the expression of CYP genes at the transcriptional level by directly targeting nuclear receptors (NRs), such as the pregnane X receptor (PXR), constitutive androstane receptor (CAR), or vitamin D receptor. These NRs serve as important transcriptional regulators of CYP genes. For instance, the *CYP3A4* gene, which encodes the most abundant CYP in adult human liver, is regulated by multiple NRs, including PXR, FXR, and CAR.[41, 42] An early study by Takagi et al.[43] reported that CYP3A4 expression was inhibited at both the mRNA and protein levels upon PXR downregulation by miR-148. In addition, miR-34a and miR-30c-1-3p were later found to downregulate CYP3A4 by suppressing retinoid X receptor α (RXRα) and PXR, respectively.[28, 44] Indirect and direct regulation by miRNAs could occur with the same CYP gene; for example, CYP3A4 mRNA is a direct target of multiple miRNAs, including miR-27a/b,[29, 45] miR-627,[27] miR-122, and miR-378a-5p.[25] In some cases miRNAs affect the transcription of CYPs by interfering with the assembly of transcription machinery on the promoter regions of CYP genes. For example, miR-552 can bind to the promoter region of CYP2E1 via its nonseed region, preventing SMARCE1 and RNA polymerase II from assembling on the promoter, which then blocks transcription. Mutations in the nonseed region of miR-522 eliminate the inhibitory effect of miR-522 on CYP2E1 transcription, but these miR-522 mutants are still capable of suppressing CYP2E1 expression posttranscriptionally.[22]

4.3 METHODS FOR INVESTIGATING THE REGULATION OF CYP EXPRESSION BY miRNAs

Various in silico, in vitro, and in vivo approaches have been used to investigate the direct form of miRNA-mediated CYP gene regulation (Fig. 4.1). Predictions of putative miRNA/mRNA interactions can be generated computationally and preliminary screening can be performed using powerful bioinformatics tools. Refinements of predicted miRNA-CYP mRNA regulatory interactions can be made using additional algorithms and databases to reduce the number of false predictions. Bioinformatics tools can be applied to select putative regulatory interactions for CYP gene expression based on negative correlations between the expression of a miRNA and its predicted CYP mRNA target and on evidence that both the miRNA species and the mRNA transcript are expressed adequately within the biological system of interest, typically human liver. Experimental methods may be used to characterize and elucidate miRNA/mRNA interactions at the biochemical, biophysical, and cell biological levels and determine their functional significance.

4.3.1 In Silico Predictions of miRNA-CYP Regulatory Interactions

In the Big Data Era enormous amounts of information, such as DNA/RNA/protein sequences and expression profiles, are available in digital formats. Many programs, either downloadable or online, have been developed to perform data-intensive analyses beginning with data mining through structure prediction. Most of these software tools and databases are curated and are available to the public for little or no cost. Not only is it cost-effective to utilize these computer-based tools, the rapid, versatile, and accurate processing ability of computer technologies spares researchers from performing tedious, time-consuming activities manually.

Many programs and databases provide tools to predict potential target genes for a specific miRNA and to predict multiple miRNAs that may regulate a gene of interest. Some widely used and well-accepted programs for miRNA

TABLE 4.1 Experimentally Validated miRNA Regulation of CYPs[a]

Gene ID	Regulatory miRNA	Modulation	Mediator	References
CYP1A2	miR-132-5p	Direct		11
CYP1B1	miR-187-5p	Direct		12
CYP2A6	miR-126	Direct		13
CYP2B6	miR-25-3p	Direct		14
CYP2C19	miR-29a-3p	Direct		15
CYP2C9	miR-130b	Direct		16
	miR-128-3p	Direct		17
CYP2D6	miR-370-3p	Direct		18
	miR-142-3p	Indirect	SHP	19
	miR-101 miR-128-2	Direct		20
CYP2E1	miR-214-3p	Direct		21
	miR-552	Direct		22
	miR-570	Direct		23
CYP3A4	miR-449a	Indirect	HNF4A	24
	miR-224-5p	Direct		24
	miR-122	Direct		25
	miR-628-3p miR-641	Direct		26
	miR-627	Direct		27
	miR-30c-1-3p	Indirect	PXR	28
	miR-27a	Direct		29
CYP7B1	miR-17	Direct		30
CYP11A1	miR-320a	Indirect	RUNX2	31
CYP19A1	miR-320a	Indirect	RUNX2	31
	miR-107	Indirect	NR5a1	32
	miR-764-3p	Indirect	SF-1	33
CYP24	miR-125b	Direct		34
CYP27B1	miR-550a	Indirect	TNF-a	35
	miR-195	Direct		36

[a] *From studies published between January 2015 and December 2017.*

analysis are miRanda, TargetScan, DIANA-microT, PicTar, miRTar, and MiRTarget2. Each prediction tool employs different parameters and algorithms for predicting miRNA/mRNA interactions and emphasizes different advantageous features (Table 4.2). Most tools will perform seed match analysis based on the base pairing between the seed sequence of miRNAs and MREs within target mRNAs. A perfect seed match occurs when there is no gap in alignment of the matching sequences with Watson-Crick base pairing. However, effective miRNA-mRNA binding does not rely on sequence complementarity only, but also on the accessibility of miRNA binding sites. An energy cost may be associated with changes in mRNA secondary structure required to expose a miRNA target site for efficient hybridization with a miRNA-bound RISC. A useful tool to evaluate RNA accessibility and miRNA-mRNA binding strength computationally is RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/), which calculates the minimum free energy (MFE) of miRNA-mRNA hybridization.[61] It has been found empirically that miRNA-target duplexes with a predicted MFE lower than -20 kcal mol^{-1} are more likely to be correlated with efficient gene regulation in cells.[17]

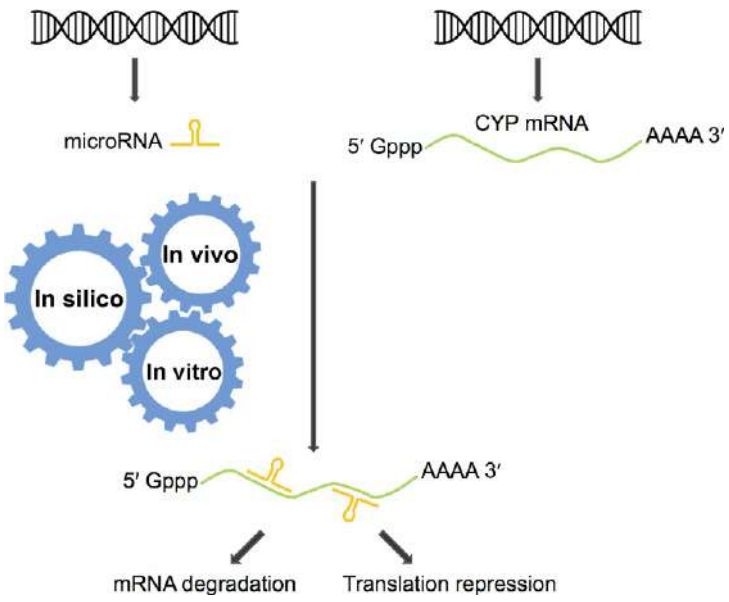

FIG. 4.1 miRNA regulation of gene expression. Genes encoding miRNAs and their targets (e.g., CYPs and NRs) are transcribed to generate mature miRNAs and target mRNAs. In silico, in vitro and in vivo approaches can be combined to identify regulatory interactions between miRNAs and mRNA targets. Mature miRNAs bind to miRNA response elements (MREs) within the 5′ UTR, coding sequence, and 3′ UTR of target mRNAs to induce the degradation of targeted mRNA transcripts or the repression of their translation to form proteins.

TABLE 4.2 Comparison of In Silico Analysis Tools for miRNA Prediction

Tool name	Websites	Features	Target region	Species	Current version and last update	References
miRBase	http://www.mirbase.org	Online searchable repository with miRNA names and annotations; links to other prediction websites	N/A	223 species	Release 21, 2014	46–48
MiRanda	http://www.microrna.org	Target site prediction; miRNA expression profile	3′ UTR	Humans, mice, rats, flies, worms	August 2010 release, 2010	49
TargetScan	http://www.targetscan.org/vert_71	Target site prediction with site conservation readout	3′ UTR	Humans, mice, fishes, flies, worms	Release 7.1, 2016	50–52
DIANA-microT	http://www.microrna.gr/webServer	Target site prediction; miRNAs and KEGG pathway search	3′ UTR, CDS[a]	Humans, mice, flies, worms	v5.0, 2013	53–56
MirTarget2	http://www.mirdb.org/	Target site prediction; miRNA functional annotation	3′ UTR, CDS, 5′ UTR	Humans, mice, dogs, rats, chickens	v5.0, 2014	57
PicTar	http://www.pictar.org	Target site prediction; multiple databases used	3′ UTR	Vertebrates, flies, nematodes	2007	58–60
RNAhybrid	https://bibiserv2.cebitec.uni-bielefeld.de/rnahybrid	Target site prediction; minimum free energy (MFE) calculation for miRNA/mRNA hybridization	3′ UTR, CDS, 5′ UTR	Humans, flies, worms	2006	61, 62
miRTar	http://mirtar.mbc.nctu.edu.tw/human	Target sit prediction; miRNA function and KEGG pathway enrichment analysis	3′ UTR, CDS, 5′ UTR	Humans	2011	63

[a] CDS, *coding sequence.*

Another important factor that some prediction tools, such as TargetScan and miRanda, include in their algorithms is the extent of conservation of base pairing regions between different species. High conservation of an MRE in an mRNA target across multiple species may indicate that the function of this putative miRNA-mRNA interaction has been selected evolutionarily. Thus, conservation status could reflect, at least partly, the reliability of predicted regulatory interactions.

It is not uncommon for discrepancies to exist between the predictions obtained using the alternative databases and algorithms employed by different software tools. Although hundreds or even thousands of potential mRNA targets could be predicted for each individual miRNA using computation-based methods alone,[64] studies have shown that 24%–70% of miRNA-mRNA interactions predicted computationally are likely to be false positives.[65, 66] Comparing results generated by multiple computational tools increases confidence in putative target identification obtained using a consensus of methods.

4.3.2 Techniques for Characterizing miRNA-CYP mRNA Regulatory Interactions In Vitro

Establishing the feasibility of predicted miRNA/CYP mRNA regulatory interactions depends first on evidence that both species are adequately expressed within relevant cells. It may be necessary to investigate the levels of multiple miRNAs or CYPs experimentally if existing databases do not provide critical information to address a specific research goal. For example, it may be necessary to obtain additional data from patients receiving a specific drug or from individuals who are representative of a specific genotype, gender, or disease state, a particular ethnic group, or certain stage of life, such as infancy, childhood, adulthood, or senectitude. Expression profiling can be performed to ascertain the levels of miRNAs and mRNAs in a given biological context. Three commonly used techniques for expression profiling are quantitative PCR (qPCR), microarray analysis, and high-throughput next generation sequencing (NGS) methods, such as RNA seq; there are specific performance advantages and drawbacks associated with each of these alternatives.[67] Choosing the optimal expression profiling technique depends largely on the goals and constraints of the study. qPCR is known for its specificity, lower costs, and facile adaptability to study newly discovered sequences; it is more appropriate for focused investigations in which the scope of the study is restricted to a selected few miRNAs and mRNA targets of specific importance. Microarray analysis is a high-throughput technique that is capable of profiling hundreds of genes in one run with as low as 100 ng of each RNA sample.[67, 68] Both qPCR and microarray analyses are hybridization based and can only measure the levels of known target sequences. RNA seq provides the best choice for novel gene discovery[69]; it can also provide reliable data on gene expression and distinguish miRNA isoforms. However, RNA seq is the most expensive and time-consuming among the three techniques and it requires expertise in bioinformatics for data analysis.

Biochemical, biophysical, and cell biological experiments are used to investigate miRNA/mRNA interactions in vitro. The RNA electrophoretic mobility shift assay (RNA EMSA) is a rapid and sensitive biophysical technique used to study the binding between miRNAs and their putative targets, which may be either mRNA sequences or DNA fragments from the promoter regions of CYP genes.[22, 70] miRNAs and target probes are labeled with fluorophores or radioactive markers. Altered electrophoretic migration of the labeled oligonucleotides occurs if they form stable intermolecular complexes with the components of the hybridization reaction mixture. Yu et al.[17] performed RNA EMSA using IRDye800-labeled CYP2C9 mRNA and cy5.5-labeled miR-128-3p and miR143-3p oligonucleotides in their study of CYP2C9 regulation by miRNAs. Although both miR-128-3p and miR143-3p were predicted to interact with CYP2C9 MREs, a gel shift was observed only with the combination of miR-128-3p and the CYP2C9 probes. Incubation of miR-143-3p with CYP2C9 probes or loss of miR-128-3p recognition sequence in the CYP2C9 probes did not result in an apparent electrophoretic gel shift, indicating that miR-128-3p, but not miR-143-3p, binds to CYP2C9 mRNA in an effective, sequence-dependent manner. In addition, purified proteins or cell lysates may be added to RNA-EMSA reaction mixtures to determine if additional factors are required to form larger ribonucleoprotein complexes.

It is critical to select an appropriate cellular context for testing the functional significance of a putative miRNA-CYP mRNA regulatory interaction. If an inappropriate cell type is used for experiments, the inadequate expression of an essential element in the regulatory pathway could lead to the false conclusion that a predicted interaction is nonfunctional. Alternatively, overexpression of alternative mRNA targets for a given miRNA may overwhelm the tested interaction in some cell systems. Thus it is important to select cells for mechanistic studies that are based on their biological relevance; that is, the use of human hepatocytes or HepaRG human hepatocyte-like cells is often preferable to nonhuman hepatocytes or immortalized hepatoma cell lines for mechanistic studies of miRNA/CYP regulatory interactions.

Reporter gene assays can be performed to test the ability of a given miRNA species to suppress CYP expression by using a reporter gene construct in which a putative MRE obtained from a CYP mRNA transcript has been introduced.

For example, HEK293T cells transfected with a luciferase reporter construct containing the CYP2B6 3′ UTR showed decreased luciferase activity upon overexpression of miR-25-3p mimics, but not by miR-504-5p mimics or a nonspecific control; miR-504-5p-dependent reduction in luciferase activity was restored when the miR-25-3p binding site in the 3′ UTR was mutated.[71] miRNA mimics and inhibitors are often used to examine the gain or loss of function in miRNA regulation of CYP expression and enzymatic activity. Zeng et al.[18] showed that transfection of multiple human liver cell lines with miR-370-3p mimics led to significant decreases of both endogenous and dexamethasone-induced CYP2D6 expression at the mRNA and protein levels, while transfection with a miR-370-3p inhibitor had the opposite effect. Additionally, DNA or RNA pull-down assays in combination with mass spectrometry can be used for mechanistic studies. One can use probes specific to a miRNA-binding sequence on the DNA or RNA of CYPs to pull down proteins that bind to the sequence; these proteins may have regulatory effects for the miRNA modulation of CYP expression and can be eluted and identified by mass spectrometry.[22]

4.3.3 Strategies for Characterizing miRNA-CYP mRNA Regulatory Interactions In Vivo

Most of the studies validating miRNA-CYP interactions and the significance of their effects are based on in vitro techniques; it is necessary to verify the interactions in living organisms. Gene expression databases (e.g., GEO Profiles, CellMiner, and TCGA) are available for examining correlations between the expression of miRNA and mRNA within the biological context of interest, such as the human liver. A number of widely available software tools, including Excel, SigmaPlot, and GraphPad Prism, can perform correlation analyses. Because most miRNA/mRNA interactions result in the repression of target gene expression, negative correlations observed between the levels of miRNAs in the human liver and their putative CYP mRNA targets enhance confidence in the biological relevance of predicted miRNA-mediated regulatory mechanisms. Conversely, evidence for inadequate expression of a given miRNA within a target tissue of interest decreases confidence that a theoretical miRNA-dependent regulatory interaction is feasible in that tissue. This type of analysis can be of great value when large amounts of data concerning the abundances of miRNAs and mRNAs have been generated via unbiased methods, such as NGS. Additionally, using rodent models to investigate the effects of miRNA-CYP interactions on pharmacokinetics in vivo can be useful but has its challenges, as discussed in a review by Nakano and Nakajima.[72] For example, exogenously administering miR-34a increased its hepatic level in mice by 80-fold but had little to no effect on the metabolism of CYP substrates.[73] Extrapolating results from rodents to humans is difficult as a result of poor conservation in the 3′ UTR across species; this could potentially be overcome by using humanized mice for in vivo studies.[72]

4.4 CLINICAL IMPLICATIONS

It is well documented that the altered expression of miRNAs is associated with a broad range of human diseases, including cancer, cardiovascular diseases, inflammatory diseases, and neurodevelopmental diseases. The Human microRNA Disease Database (HMDD) (http://www.cuilab.cn/hmdd) is a useful resource to search for associations between miRNAs and diseases. HMDD v2.0, the most recent version, was released in 2013 and included 572 miRNA genes associated with 378 diseases from 3511 published studies. Meanwhile, an increasing amount of evidence is emerging to justify the potential use of miRNAs as indicators of drug efficacy and toxicity.

4.4.1 miRNAs as Biomarkers for CYP-Dependent Drug Efficacy

Therapeutic drugs are known to influence the expression of DMETs, NRs, and miRNAs in cell culture and animal and human tissues in ways that influence drug efficacy.[37] In addition, some miRNAs target DMETs for gene silencing directly or indirectly and thus affect the metabolism and disposition of the drug itself while also potentially affecting the metabolism of other coadministered drugs; these miRNA-dependent effects may then contribute to drug resistance or drug-drug interactions (Fig. 4.2).

Interest is growing in exploring miRNAs as potential biomarkers for drug efficacy with the expectation that their use may enhance precision medicine. Circulating miRNAs are found within membrane-bound exosomes secreted normally by virtually all types of eukaryotic cells. An important feature of exosomal circulating miRNAs is their remarkable stability, attributed to protection from nucleases conferred by the exosomal membrane. Exosomal circulating miRNAs can be detected in many body fluids, including blood, urine, saliva, and cerebrospinal fluid.[74] The study of cell-free exosomal miRNAs has attracted wide attention as it permits the noninvasive or minimally invasive collection of clinical samples. Circulating hepatic miRNAs that regulate CYP expression can be explored as biomarkers in

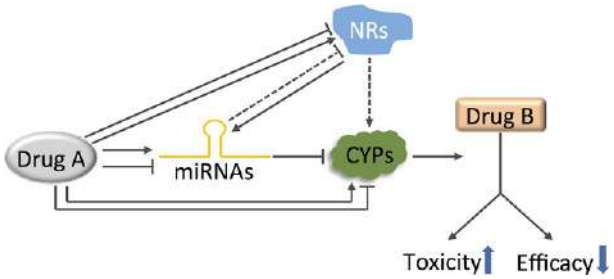

FIG. 4.2 miRNA involvement in drug-drug interactions. One drug (e.g., Drug A) may upregulate or downregulate the expression of NRs, CYPs, and miRNAs that modulate CYPs directly (*solid lines*) or indirectly (*dashed lines*) via NRs. As transcription factors, NRs may regulate the expression of miRNAs. Altered CYP expression affects the metabolism of other coadministered drugs (e.g., Drug B), potentially causing toxicity and/or reduced therapeutic effects.

drug efficacy assessment as appropriate levels of CYP expression are critical to drug efficacy, especially for prodrugs that require CYP-dependent activation to form their metabolites.[75] For example, cyclophosphamide is an anticancer drug that is activated via hydroxylation by several CYP enzymes, among which CYP2B6 plays a major role.[76] Theoretically, overexpression of miRNAs that can repress CYP2B6 (e.g., miRNA-25-3p) could inhibit the metabolic transformation of cyclophosphamide and thus decrease drug efficacy during its anticancer treatment.

4.4.2 miRNAs as Biomarkers for CYP-Dependent Drug Safety

The liver is the main organ responsible for the metabolism and detoxification of xenobiotics. Drug toxicity and adverse drug events are a major concern during pharmaceutical development, and drug-induced toxicity can lead to the withdrawal of marketed drugs.[77] Drugs can cause toxicity in multiple organs, including the liver, heart, and kidney. The detection of drug-induced toxicity is important for drug safety assessment. Injured organs release circulating miRNAs into body fluids (e.g., blood and urine) that can be collected for analysis. Due to their presence in body fluids and detectability at early stages of toxicity, circulating miRNAs can be exploited as important biomarkers for drug safety assessment.

Drug-induced hepatotoxicity is a major cause for the rejection of new drugs during development and for the withdrawal of drugs from the market.[78] The clinical biomarkers most often used for assessing hepatotoxicity currently include the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and bilirubin in the sera of patients.[79] Although the use of these classic serum biomarkers is a long-standing and well-accepted clinical practice, limitations exist with respect to the sensitivity and specificity of these biomarkers for early detection of liver injury. For example, the level of ALT and AST can also be affected by injuries to organs other than the liver, and increased serum levels of ALT and AST do not always agree with histopathological observations of hepatic injury.[79] The stability and tissue-specificity of circulating miRNAs make them good candidates as novel biomarkers for drug-induced liver injury. For instance, miR-122 is an abundant miRNA in the liver and is associated with downregulation of CYP1A2 and CYP3A4 upon acetaminophen treatment in HepaRG cells.[25] Serum levels of miR-122 are elevated in response to acetaminophen overdose in both animal models and in patients, indicating its potential use for early detection of acute liver injury in drug safety evaluation.[80, 81]

Altered expression and enzymatic activity of CYPs may lead to drug-induced toxicity as a result of drug-drug interactions, depending on the nature of parent drugs, metabolites, and catalytic reactions. For example, flutamide is a prescription drug used mainly to treat advanced prostate cancer in men and polycystic ovary syndrome in women. Nausea and vomiting are common side effects of flutamide and can be potentially treated by lansoprazole, a proton pump inhibitor. Lansoprazole induces the expression of CYP1A2 and this induction may enhance the toxicity of flutamide in HepG2 cells triggered by the CYP1A2-dependent metabolism of flutamide.[11] Importantly, cytotoxicity from coadministering flutamide and lansoprazole could be attenuated in these cells by overexpression of miR-132-5p to repress CYP1A2. These observations provide a theoretical basis for potential adverse reactions triggered by drugs that decrease miR-132-5p expression when taken in combination with flutamide and lansoprazole; however, clinical evidence has yet to be discovered.

4.4.3 miRNA-Based Therapies Involving CYP Expression

As strategic regulators of gene expression that have been associated with various diseases, miRNAs are now being explored as therapeutic targets. It is believed that existing antisense technology can be adapted easily to

miRNA-based therapy. Presumably, miRNA-based therapeutic agents will be smaller and simpler in design than other types of nucleic acid-based therapies used in the past, such as antisense oligonucleotides, DNA vaccines, or gene therapy vectors. Also, miRNAs are normal constituents in healthy cells and should be less likely to cause adverse effects and toxicity. Depending on the type of alterations associated with signature miRNAs, three types of miRNA manipulation strategies can be utilized: small molecules developed to induce or repress the expression of specific miRNAs, miRNA antagonism, and miRNA replacement.[82] High-throughput reporter gene assays containing known MREs or the 3′ UTR of CYP transcripts can be used to screen compound libraries and identify those affecting CYP expression via miRNA-dependent mechanisms. For example, NSC-156306 and NSC-642957 can induce the expression of miR-29a and consequently downregulate a target of miR-29a, CYP2C19.[15] Anti-miRNA antisense oligonucleotides (AMOs) are designed with altered internucleotide linkages and complementary sequences to targeted miRNAs to inhibit the activity of an abnormally overexpressed miRNA. AMOs are expected to be the most commonly used anti-miRNA agents due to their resistance to nuclease, high binding affinity, and low toxicity.[83] Other types of miRNA antagonists include peptide nucleic acids, miRNA sponges, and miRNA masks.[82, 84] In cases where miRNAs are downregulated and require restoration of miRNA function, delivering miRNA mimics is a potential therapeutic solution. Studies have focused on efficient delivery methods of synthetic miRNAs. Increasing research on miRNA therapeutics will likely put miRNA-based treatment in the market in the near future.

4.5 CONCLUSIONS

Among different patients, diversity in terms of drug safety and drug response may be attributed to several factors, including genetic variations, epigenetic regulation via DNA methylation or by noncoding RNAs, environmental stressors, and disease/health conditions. Variations in miRNA-mediated epigenetic regulation of CYP gene expression have an important impact on interindividual variability in therapeutic effects and adverse reactions. Although bioinformatic predictions show that many CYPs could be targeted by miRNAs, it is essential to combine various computational and experimental methods to demonstrate the regulatory effects of CYP-miRNA pairs. The functions of some less studied CYPs, such as CYP2W1 and CYP2S1, have not been well characterized; thus miRNA regulation of these CYP genes is a more challenging task. Moreover, the regulatory network of environmental stressors, miRNAs, CYPs, and NRs is highly complex. Elucidating the relationship between each component may improve understanding of drug-drug interactions and enable the effective use of miRNAs as biomarkers for drug-induced toxicity and as therapeutic agents for human diseases.

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CHAPTER

5

Pathoepigenetics: The Role of Epigenetic Biomarkers in Disease Pathogenesis

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5.1 INTRODUCTION

Over 4000 phenotypes have been characterized at the molecular level in close association with defects in primary DNA structure; however, epigenetic disruption is emerging as an important alternative mechanism in the etiology of a broad range of congenital and developmental conditions, including epigenetic defects caused by either localized (in *cis*) genetic alterations or more distant (in *trans*) genetic events in which environmental effects also participate.[1] Epigenetic aberrations (DNA methylation anomalies, abnormal chromatin remodeling, histone posttranslational modifications, and miRNA dysregulation) contribute to the pathogenesis of prevalent disorders including cardiovascular disease[2] and associated disorders such us hypertension[3] and diabetes;[4] most forms of cancer (colon, breast, lung, prostate, melanoma)[5]; different modalities of brain disorders (neurodegenerative disorders such as Alzheimer and Parkinson disease, autistic disorders, psychosis, depression);[6–8] multiple metabolic and endocrine disorders; infectious diseases; immunological disorders; and probably the vast majority of pathogenic phenotypes associated with human disease. Furthermore, mutations in the proteins and enzymes that configure the epigenetic machinery lead to epigenetic Mendelian disorders.[9]

Environmental stressors during developmental stages may increase the risk of developing diverse pathologies later in life. This theory, currently known as the developmental origins of health and disease (DOHaD), postulates that the accumulation of environmental stress is internalized as acquired information designated as epigenetic memory, which is reflected by DNA methylation and histone modifications in chromatin. The demethylation of CpG islands activates histone acetylation and changes from heterochromatin to euchromatin, enhancing transcriptional activation. Most of these changes are induced by the binding of transcription factors to *cis*-elements at promoter and enhancer regions and the associated binding of histone acetyltransferase and the transcription initiation complex.[10]

Understanding the physiological and pathological effects that epigenetic marks exert in health and disease, respectively, is essential for future epigenetic interventions in terms of prevention, symptomatic treatment, antipathogenic therapeutics, and pharmacoepigenetics.[11]

This chapter summarizes the most recent epigenetic findings in major problems of health, such as cardiovascular disorders, cancer, and metabolic disorders, which together with brain disorders represent 60%–80% of the morbidity and mortality registered in developed countries. Independent chapters are devoted to the epigenetics of brain disorders and neurodegenerative disorders. A seminal opus on the role of epigenetics in human pathology is the book *Medical Epigenetics*,[12] edited by Prof. Trygve Tollefsbol in 2016, belonging to Elsevier's Translational Epigenetics Series. The vast majority of novel findings cited in this chapter cover the period 2016–18, as an updating exercise to Tollefsbol's excellent contribution.[12]

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5.2 CARDIOVASCULAR DISORDERS

Cardiovascular disorders (CVDs) are the major cause of mortality and morbidity, representing 25%–30% of annual deaths in developed countries. Novel interventions for the management of CVDs in the postgenomic era are necessary considering the benefits and limitations.[13] Genomic, epigenomic, and environmental factors are associated with complex CVDs including inherited cardiomyopathies, valvular diseases, primary arrhythmogenic conditions, congenital heart syndromes, hypercholesterolemia, atherosclerotic heart disease, hypertensive syndromes, and heart failure with preserved/reduced ejection fraction.[14–17]

Different genetic variants expressing defective proteins represent important risk factors for CVD. Atrial natriuretic peptide (*ANP*) (variant rs5065 (T2238C)) is an example.[18] Furthermore, changes in brain natriuretic peptide (*BNP*) promoter DNA methylation represent a risk for rheumatic heart disease (RHD). *BNP* gene hypermethylation was found in CpG4 and CpG5 in RHD patients compared with non-RHD controls. *BNP* gene body hypermethylation is associated with risk for RHD.[19]

Epigenome-wide association studies to investigate the epigenetic basis of myocardial infarction (MI) revealed three methylated DNA sites (cg06642177, cg07786668, cg17218495) showing associations with MI. The cg07786668 and cg17218495 sites are located in *ZFHX3* (zinc finger homeobox 3) and *SMARCA4* (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4) genes, respectively. SNPs in *ZFHX3* or *SMARCA4* are associated with CVD.[20]

Hedman et al.[21] identified 193 CpGs associated with lipid levels, including 25 novel CpGs not previously associated with lipids. Genes at lipid-associated CpGs are enriched in lipid and amino acid metabolism processes. A differentially methylated locus associated with triglycerides and high-density lipoprotein cholesterol (HDL-C; cg27243685) is associated with *cis*-expression of a reverse cholesterol transporter (*ABCG1*) and incident cardiovascular disease events. *Cis*-methylation quantitative trait loci are present in 64% of the 193 CpGs with an enrichment of signals from genome-wide association studies of lipid levels and coronary heart disease. Genome-wide significant variants associated with low-density lipoprotein cholesterol and coronary heart disease at *APOB* are *cis*-methylation quantitative trait loci for a low-density lipoprotein cholesterol-related differentially methylated locus.[21]

An epigenome-wide association study (EWAS) for CVDs identified disease-specific alterations in DNA methylation. In patients with a history of a CVD-associated disorders (hypertension, myocardial infarction, stroke, thrombosis, cardiac arrhythmia), differential DNA methylation was observed at 211 CpG sites, representing 196 genes, of which 42 have been described in the scientific literature to be related to cardiac function, cardiovascular disease, cardiogenesis, and recovery after ischemic injury.[22]

Acute coronary syndrome (ACS) is a leading cause of death worldwide. Immune functions play a vital role in ACS development, and epigenetic modulation contributes to the regulation of blood immune cells in this disease. A novel association of ACS with blood methylation at 47 cytosine-phosphoguanine sites has recently been discovered. Blood methylation of 26 replicated cytosine-phosphoguanine sites correlate with expressions of the *IL6R*, *FASLG*, and *CCL18* genes.[23]

Pentraxin 3 (*PTX3*) is expressed in the heart under inflammatory conditions, influencing atherogenesis. Patients with increased PTX3 levels may suffer from higher rates of cardiac events. The level of *PTX3* promoter methylation in CAD (62.69% $\pm$ 20.57%) is lower than in controls (72.45% $\pm$ 11.84%). Lower *PTX3* promoter methylation levels in CAD are associated with higher plasma PTX3 concentrations, and lower *PTX3* promoter methylation levels are associated with higher neutrophil to lymphocyte ratio in men.[24]

Antisense noncoding RNA in the *INK4* locus (*ANRIL*) fixed genetic variants have consistently been linked with coronary heart disease (CHD) risk. Murray et al.[25] investigated the relationships between perinatal *ANRIL* promoter DNA methylation and CHD risk markers in children aged 9 years. Genetic variants in the noncoding RNA *ANRIL* identify it as an important CHD risk locus. The early life environment may act through epigenetic processes to influence later CHD risk markers such as increased arterial pulse wave velocity (PWV, a measure of arterial stiffness), blood pressure, or heart rate.

The class III deacetylase sirtuin 1 (SIRT1) confers cardioprotection. SIRT1 expression is downregulated in the heart by a number of stress stimuli that collectively drive the pathogenesis of MI. In primary rat neonatal ventricular myocytes, ischemia or oxidative stress lead to a rapid upregulation of SUV39H, the mammalian histone H3K9 methyltransferase, paralleling SIRT1 downregulation. *SUV39H* knockout mice are protected from MI. Suppression of SUV39H activity with chaetocin attenuates cardiac injury following MI. SUV39H cooperates with heterochromatin protein 1 gamma (HP1γ) to catalyze H3K9 trimethylation on the *SIRT1* promoter and represses *SIRT1* transcription. SUV39H augments intracellular ROS levels in a SIRT1-dependent manner.[26]

The H3K9 trimethyltransferase SUV39H1 binds to the *SIRT1* promoter and represses *SIRT1* transcription. *SUV39H1* expression is upregulated in the myocardium in mice following I/R insults and in H/R-treated cardiomyocytes paralleling SIRT1 downregulation. Silencing *SUV39H1* expression or suppression of SUV39H1 activity erases H3K9Me3 from the *SIRT1* promoter and normalizes SIRT1 levels in cardiomyocytes. SUV39H1 deficiency or inhibition attenuates I/R-induced infarction and improves heart function.[27]

Heart failure in human and animal models shares conserved transcriptome-remodeling events that lead to expression of genes normally silenced in the healthy adult heart. Studies to identify muscle-specific chromatin regulators in a mouse model of hypertrophy and heart failure detected upregulation of the histone methyltransferase Smyd1 during disease. Smyd1 is responsible for restricting growth in the adult heart, with its absence leading to cellular hypertrophy, organ remodeling, and fulminating heart failure. Smyd1 is a muscle-specific regulator of gene expression. Activation of Smyd1 can prevent pathological cell growth.[28]

Abnormal thyroid hormone (TH) metabolism is significantly associated with impaired left ventricular function and death. The genes required for TH biosynthesis are expressed in the human heart and show alterations in patients with ischemic cardiomyopathy (ICM). Altered expression of genes encoding thyroperoxidase (*TPO*) and dual oxidase 2, as well as differential methylation patterns in *TPO*, have been observed in ICM.[29]

Adult cardiac progenitor cells (CPCs) display a low capacity to differentiate into cardiomyocytes in injured hearts, strongly limiting the regenerative capacity of the mammalian myocardium. Downregulation of Wnt target genes correlates with increased expression of the Wnt antagonist, Wnt inhibitory factor 1 (Wif1), which is necessary to stimulate CPC differentiation. Expression of the *Wif1* gene is repressed by DNA methylation and regulated by the de novo DNA methyltransferase Dnmt3a. miR-29a is upregulated early during CPC differentiation and downregulates *Dnmt3a* expression, thereby decreasing *Wif1* gene methylation and increasing the efficiency of differentiation of Sca-1+ CPCs. Transient silencing of *Dnmt3a* in CPCs subsequently injected in the border zone of infarcted mouse hearts improves CPC differentiation in situ and remote cardiac remodeling. miR-29a and Dnmt3a epigenetically regulate CPC differentiation through Wnt inhibition.[30]

Rearranged L-Myc fusion (RLF) acts as an epigenetic modifier maintaining low levels of DNA methylation at CpG island shores and enhancers across the genome. RLF is expressed in a range of fetal mouse tissues, including the fetal heart. *Rlf* homozygous mutant mice rarely survive to adulthood, with the majority dying shortly after birth. *Rlf ENU* mutant lines at E11.5–E14.5 show heart defects resembling those present in humans with left ventricular noncompaction. *Rlf* is expressed in the endocardium and epicardium of embryonic and postnatal hearts, and transiently to cardiomyocytes during heart looping and early chamber formation stages. *Rlf* mutant hearts show defective NOTCH pathway signaling. Attenuated *JAGGED 1* expression and NOTCH signaling are contributors to these defects. These results reported by Bourke et al. indicate that RLF is required for normal heart development in the mouse.[31] Notch signaling is a critical regulator of metabolism and angiogenesis during development. Genetic and pharmacological manipulation of endothelial Notch signaling affects endothelial fatty acid transport, cardiac angiogenesis, and heart function. Inhibition of Notch signaling in the endothelium by genetic ablation of *Rbp-jκ* induces heart hypertrophy and failure. Endothelial Notch signaling controls the expression of endothelial lipase, Angptl4, CD36, and Fabp4, which are all needed for fatty acid transport across the vessel wall. Notch signaling is a novel regulator of fatty acid transport across the endothelium and is an essential repressor of angiogenesis in the adult heart.[32]

The stress-responsive epigenetic repressor histone deacetylase 4 (HDAC4) regulates cardiac gene expression. The levels of an N-terminal proteolytically derived fragment of HDAC4 (HDAC4-NT) are lower in failing mouse hearts than in healthy control hearts. NR4A1 negatively regulates contractile function depending on the hexosamine biosynthetic pathway and the calcium sensor STIM1.[33]

Histone deacetylase enzymes (HDACs) contribute to ischemia reperfusion (I/R) injury, and pan-HDAC inhibitors are cardioprotective when administered either before an ischemic insult or during reperfusion. Selective inhibition of class I HDACs provides superior cardioprotection when compared with pan-HDAC inhibition in a pretreatment model. HDAC1 is present within the mitochondria of cardiac myocytes, and selective inhibition of class I HDACs with the drug MS-275 (entinostat) during reperfusion improves recovery from I/R injury in the first hour of reperfusion.[34]

Histone deacetylases play vital roles in the pathophysiology of heart failure, which is associated with mitochondrial dysfunction. Tumor necrosis factor-α (TNFα) contributes to the genesis of heart failure and impairs mitochondria. TNFα increases class I and II (but not class IIa) HDAC activities with enhanced expressions of class I (HDAC1, HDAC2, HDAC3, and HDAC8) but not class IIb HDAC (HDAC6 and HDAC10) proteins in HL-1 cells. TNFα induces mitochondrial dysfunction with impaired basal, ATP-linked, and maximal respiration, decreased cellular ATP synthesis, and increased mitochondrial superoxide production, which can be rescued by inhibiting HDACs with MPT0E014 (a class I and IIb inhibitor), or MS-275 (a class I inhibitor). Class I HDAC effects contribute to TNFα-induced

mitochondrial dysfunction in cardiomyocytes with altered complex I and II enzyme regulation. HDAC inhibition improves dysfunctional mitochondrial bioenergetics with attenuation of TNFα-induced oxidative stress.[35]

Aortic valve stenosis is the most common cardiac valve disease. Activation of monocyte macrophage and circulatory osteoprogenitor cells, and osteogenic trans-differentiation of aortic valve interstitial cells, can lead to valvular inflammation, fibrosis, and calcification, as well as to maladaptive myocardial remodeling and left ventricular hypertrophy with the participation of epigenetic mechanisms.[36] Vascular smooth muscle cells (VSMCs) dedifferentiate in response to vascular damage and inflammation. VSMC dedifferentiation contributes to vascular repair and to ignite cardiovascular pathologies, such as intimal hyperplasia/restenosis in coronary artery or peripheral vascular diseases and arterial aneurysm. Ubiquitin-like containing PHD and RING finger domains 1 (UHRF1) is an epigenetic master regulator of VSMC plasticity. miR-145 regulates VSMC plasticity and controls *Uhrf1* mRNA translation. UHRF1 triggers VSMC proliferation by directly repressing the promoters of cell cycle inhibitor genes, such as *p21* and *p27*, and of key prodifferentiation genes via the methylation of DNA and histones. *Uhrf1* shRNAs prevents intimal hyperplasia in the carotid artery and decreases vessel damage aortic aneurysm.[37] A total of 15 circulating miRNAs are differentially expressed in patients with abdominal aortic aneurysm (AAA). miR-155 and miR-29b are the most relevant miRNAs differentially expressed in AAA.[38] miRNA-155 also participates in the pathogenesis of cardiovascular disease. CHD patients have higher levels of miRNA-155 than controls.[39]

Ischemic preconditioning (IPC) protects the heart from prolonged ischemic insult and reperfusion injury. Posttranslational modifications of histone residues can confer rapid and drastic switches in gene expression in response to various stimuli, including ischemia. H3K9me2 levels are increased in the area at risk compared with remote myocardium. About 237 genes are transcriptionally repressed and enriched in H3K9me2 in the area at risk. Knockdown of the major H3K9 methyltransferase G9a results in decrease in H3K9me2 levels across mTOR (mechanistic target of rapamycin), increased mTOR expression, and decreased autophagic activity in response to rapamycin and serum starvation. G9a has an important role in regulating cardiac autophagy and the cardioprotective effect of IPC.[40]

The most significantly associated genetic locus for atrial fibrillation (AF) is in chromosomal region 4q25, where four independent association signals have been identified. Altered *PITX2c* expression might underlie the association. A single associated SNP (rs2595104) shows reduced enhancer activity with the AF risk allele. Deletion of the rs2595104 region and editing of the rs2595104 risk allele in human stem cell-derived cardiomyocytes results in diminished *PITX2c* expression in comparison with that of the nonrisk allele. This differential activity is mediated by activating enhancer binding protein 2 alpha (TFAP2α), which binds robustly to the nonrisk allele at rs2595104, but not to the risk allele, in cardiomyocytes. It appears that the AF-associated SNP rs2595104 alters *PITX2c* expression via interaction with TFAP2α.[41]

Differential methylation of *KCNQ1OT1* is associated with the risk of symptomatic long QTc syndrome.[42]

5.3 ATHEROSCLEROSIS

Several epigenetic mechanisms are potentially involved in atherogenesis. Atherosclerosis-specific DNA methylation profiles are only partially known. A 29-bp DNA motif (differential methylation motif) (DMM) proximal to CpG islands (CGIs) that undergo demethylation in advanced human atheromas has been identified. The DMM overlaps with the RNA polymerase III-binding B box of *Alu* short interspersed nuclear elements and contains a DR2 nuclear receptor response element. LXRβ (liver X receptor β) binds the DMM in a DR2-dependent fashion, and LXR (liver X receptor) agonists induce significant hypermethylation of the bulk of *Alu* in THP-1 cells. Three intergenic lncRNAs that harbor a DMM are under transcriptional control by LXR agonists and are differentially expressed in normal vs. atherosclerotic aortas. CGIs adjacent to lncRNAs tend to be hypomethylated in symptomatic atheromas. LXRs are atheroprotective, and it has been proposed that LXR agonist-induced *Alu* hypermethylation is a compensatory rather than proatherogenic response.[43]

The histone deacetylase Sirtuin-1 (SIRT1) is a histone deacetylase that influences longevity and has an antiatherogenic effect by regulating the acetylation of some functional proteins. Hydrogen sulfide (H_2S) has a protective role in the pathogenesis of atherosclerosis. In *ApoE* knockout atherosclerosis mice, treatment with an H_2S donor (NaHS or GYY4137) reduces atherosclerotic plaque area, macrophage infiltration, aortic inflammation, and plasma lipid level. H_2S treatment increases aorta and liver *SIRT1* mRNA expression. Overexpression of cystathionine gamma lyase (CSE) modifies intracellular SIRT1 expression. CSE/H_2S treatment increases SIRT1 deacetylation in endothelium and hepatocytes and macrophages, inducing deacetylation of its target proteins (p53, p65, and sterol response element binding protein), reducing endothelial and macrophage inflammation, and inhibiting macrophage cholesterol uptake and cholesterol de novo synthesis. Furthermore, CSE/H_2S induces SIRT1 sulfhydration at its two Zinc finger domains and

reduces SIRT1 ubiquitination and degradation. These effects reported by Du et al. indicate that CSE/H_2S sulfhydrates SIRT1, enhances SIRT1 binding to Zinc ion, promotes SIRT1 deacetylation activity, and increases SIRT1 stability, all contributing to reduce atherosclerotic plaque formation.[44]

Hyperinsulinemia and insulin resistance contribute to causing atherosclerosis, and expression of the estrogen receptor is closely related to the incidence of atherosclerosis. Insulin promotes the expression of DNA methyltransferases and inhibits ERα expression. Methylation of the *ERα* second exon region increases in vascular smooth muscle cells treated with insulin, and ERα can inhibit VSMC proliferation.[45] Epigenetic changes are also associated with aneurysms.[46]

5.4 HYPERTENSION

Hypertension (HT) is among the major components of the metabolic syndrome (HT, obesity, dyslipidemia, diabetes/insulin resistance), with over 9 million deaths per year worldwide.

DNA methylation and histone modifications play an important role in gene regulation and are involved in alteration of the phenotype and function of vascular cells in response to environmental stresses.[47]

A challenge to understanding enhancer-gene relationships is that enhancers are not always sequentially close to the gene they regulate. Studies on physical proximity mapping through sequencing have provided a view of chromatin close to the proximal promoter of the renin gene.[48] The clustering of mapped reads produces a genome-wide map of chromatin in contact with the *Ren* promoter. The largest number of contacts are found on chromosome 13, where the renin locus is located, and contacts are found on all other chromosomes except chromosome X. These contacts are enriched with genes positively correlated with renin expression and with mapped quantitative trait loci associated with blood pressure, cardiovascular, and renal phenotypes.[48]

Kelch-like 3 (KLHL3) is a component of an E3 ubiquitin ligase complex that regulates blood pressure by targeting With-No-Lysine (WNK) kinases for degradation. Mutations in *KLHL3* cause constitutively increased renal salt reabsorption and impaired K^+ secretion, resulting in hypertension and hyperkalemia. The KLHL3 ubiquitin ligase complex is involved in the low-K^+-mediated activation of Na-Cl cotransporter (NCC) in the kidney. In the distal convoluted tubules of mice eating a low-K^+ diet, increased KLHL3 phosphorylation at S433 (KLHL3S433-P) has been found, a modification that impairs WNK binding and reduces total KLHL3 levels. These changes are accompanied by the accumulation of the target substrate WNK4, and activation of the downstream kinases SPAK (STE20/SPS1-related proline-alanine-rich protein kinase) and OSR1 (oxidative stress-responsive 1), resulting in NCC phosphorylation and its accumulation at the plasma membrane. The effect of KLHL3 in low K^+-mediated induction of NCC reduces distal electrogenic Na^+ reabsorption, preventing further renal K^+ loss but promoting increased blood pressure.[49]

Recent studies have also illustrated the role of miRNAs in the regulation of gene expression associated with HT and fetal programming-mediated susceptibility to HT in adulthood.[50]

5.5 VENOUS THROMBOEMBOLISM

Genome-wide association studies identified three intragenic SNPs (*LEMD3* rs138916004, *LY86* rs3804476, *LOC100130298* rs142143628) in patients with venous thromboembolism. *LEMD3* rs138916004 and *LOC100130298* rs142143628 are only present in Africans, with variable differential expression in different cohorts. *LEMD3* encodes for an antagonist of TGFβ-induced cell proliferation arrest. *LY86* encodes for MD-1 which downregulates the proinflammatory response to lipopolysaccharide. *LOC100130298* is a noncoding RNA gene with unknown regulatory activity in gene expression.[51]

5.6 CANCER

Human malignant tumors are characterized by pervasive changes in the patterns of DNA methylation. These changes include a globally hypomethylated tumor cell genome and the focal hypermethylation of numerous 5′-cytosine-phosphate-guanine 3′ (CpG) islands, many of which are associated with gene promoters. Promoter hypermethylation events can lead to silencing of genes functioning in pathways reflecting hallmarks of cancer, including DNA repair, cell cycle regulation, promotion of apoptosis, or control of key tumor-relevant signaling networks. Many of the most commonly hypermethylated genes encode developmental transcription factors, whose methylation may lead

to permanent gene silencing. Inactivation of such genes deprives cells from maintaining lineage differentiation and will lock them into a perpetuated stem cell-like state thus providing a window for cell transformation.[52]

Epigenetic changes are associated with most forms of human cancer.[53] The interaction between an individual's epigenetic makeup and exposure to environmental conditions (exposome) is a determinant factor for cancer.[54]

Common fragile sites (CFSs) may influence cancer pathogenesis. CFS loci might be hotspots of genomic instability leading to inactivation of genes encoded within them, and CFSs might also be functional units in which loss of the encoded genes confers selective pressure, leading to cancer development. CFS expression is associated with genome integrity, and inactivation of CFS-resident tumor suppressor genes leads to dysregulation of the DNA damage response (DDR) and increased genomic instability. When breaks at CFSs are not repaired accurately, this can lead to deletions by which cells acquire growth advantage because of loss of tumor suppressor activities.[55]

Cancer is a heterogeneous disease with a high degree of diversity between and within tumors. Cancerous cells carry multiple genetic and epigenetic aberrations that may disrupt pathways essential for cell survival. The discovery of synthetic lethality may help to create personalized antitumor strategies. According to Toma et al.[56] synthetic lethality occurs when simultaneous inactivation of two genes or their products causes cell death, whereas individual inactivation of either gene is not lethal. The effectiveness of numerous antitumor therapies depends on the induction of DNA damage; therefore, tumor cells expressing abnormalities in genes whose products are crucial for DNA repair pathways are promising targets for synthetic lethality.[56]

The p53 pathway is inactivated in the majority of human cancers. Overexpression of important p53 negative regulators, such as murine double minute 2 (MDM2) or murine double minute 4 (MDM4), epigenetic deregulation, or alterations in *TP53* mRNA splicing all contribute to attenuating the p53 pathway.[57] p53-Induced lncRNA *TP53TG1* undergoes cancer-specific promoter hypermethylation-associated silencing. In vitro and in vivo assays identified a tumor suppressor activity for TP53TG1 and a role in the p53 response to DNA damage. TP53TG1 binds to the multifaceted DNA/RNA binding protein YBX1 to prevent its nuclear localization and thus the YBX1-mediated activation of oncogenes. *TP53TG1* epigenetic inactivation in cancer cells releases the transcriptional repression of YBX1-targeted growth-promoting genes and creates a chemoresistant tumor. *TP53TG1* hypermethylation in primary tumors is shown to be associated with poor outcome.[58]

Aberrant DNA and histone modifications that silence tumor suppressor genes or promote oncogenes have been demonstrated in multiple cancer models. The role of epigenetics is well established in several solid tumor cancers.[59] Epigenetic modifications occur in key oncogenes, tumor suppressor genes, and transcription factors, leading to cancer initiation and progression. The most commonly observed epigenetic changes include DNA methylation, histone lysine methylation and demethylation, histone lysine acetylation and deacetylation. However, there are several other novel posttranslational modifications that have been observed in recent times such as neddylation, sumoylation, glycosylation, phosphorylation, poly-ADP ribosylation, ubiquitination, and transcriptional regulation.[60]

Cancer-specific perturbations of signaling, metabolism, and epigenetics can be a cause and/or consequence of malignant transformation. Evidence indicates that these regulatory systems interact with each other to form highly flexible and robust cybernetic networks that promote malignant growth and confer treatment resistance.[61]

The concept of cancer stem cells was first proposed in the late 1990s. Different epigenetic events produce cancer progenitor cells from predisposed cells by the influence of their environment. Every somatic cell possesses an epigenetic signature in terms of histone modifications and DNA methylation, which are obtained during lineage-specific differentiation of pluripotent stem cells. This signature is an epigenetic switch that changes depending on the predisposition of the cells of a particular tissue and their microenvironment, potentially leading to the generation of cancer progenitor cells.[62]

The induction of aberrant DNA methylation is one of the most important mechanisms mediating the effect of inflammation on cancer development. Aberrant methylation of promoter CpG islands of tumor suppressor genes can silence their downstream genes.[63]

Tumors comprise functionally diverse subpopulations of cells with distinct proliferative potential. Dynamic epigenetic states defined by the linker histone H1.0 determine which cells within a tumor can sustain long-term cancer growth. Numerous cancer types exhibit high inter- and intratumor heterogeneity of H1.0, with H1.0 levels correlating with tumor differentiation status, patient survival, and at the single-cell level cancer stem cell markers. Silencing of H1.0 promotes maintenance of self-renewing cells by inducing derepression of megabase-sized gene domains harboring downstream effectors of oncogenic pathways. Self-renewing epigenetic states are not stable, and reexpression of H1.0 in subsets of tumor cells establishes transcriptional programs that restrict the long-term proliferative potential of cancer cells and drive their differentiation.[64]

Different epigenetic mechanisms are involved in multiple oncogenic processes. Global DNA hypomethylation promoting genomic instability leads to cancer and deterioration of human health with age. The use of *Alu* siRNA to direct

Alu-interspersed repetitive sequence methylation in human cells revealed an inverse correlation between *Alu* element methylation and endogenous DNA damage in white blood cells. Cells transfected with *Alu* siRNA exhibited high *Alu* methylation levels, increased proliferation, reduced endogenous DNA damage, and improved resistance to DNA-damaging agents.[65]

DNA methylation loss occurs frequently in cancer genomes within late-replicating regions termed partially methylated domains (PMDs). A local CpG sequence context is associated with preferential hypomethylation in PMDs. PMD hypomethylation increases with age, beginning during fetal development, and appears to track the accumulation of cell divisions. In cancer, PMD hypomethylation depth correlates with somatic mutation density and cell cycle gene expression, consistent with its reflection of mitotic history and suggesting its application as a mitotic clock. Zhou et al.[66] proposed that late replication leads to lifelong progressive methylation loss, which acts as a biomarker for cellular aging and may contribute to oncogenesis.

Long noncoding RNAs are highly versatile players in the regulation of gene expression in development and cancer. Hundreds of lncRNAs become dysregulated across tumor types, and multiple lncRNAs have demonstrated functions as tumor suppressors or oncogenes. Dysregulation of lncRNAs results in alterations of the epigenome in cancer cells. lncRNAs participate in various epigenetic regulatory processes, including coordination of chromatin dynamics, regulation of DNA methylation, modulation of other noncoding RNAs and mRNA stability, and control of epigenetic substrate availability through altered tumor metabolism. lncRNAs represent attractive targets for future therapeutic strategies in cancer.[67] Genetic variations and differential expression of miRNA regulome components are associated with cancer. MicroRNAs can be involved in the regulation of oncogenes and tumor suppressors. miRNA targets a broad range of genes, and minor changes in miRNAs may have prominent effects on cell transformation. Different genetic variants of the miRNA regulome have been reported to be associated with cancer. Genetic variations show structural and short polymorphisms with changes in the epigenetic landscape that affect miRNA genes and their regulatory elements, processing machinery, degradation machinery, and targets, leading to changes in miRNA silencing.[68] Small ncRNAs or micro RNAs (miRNAs), transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), antisense RNAs (asRNAs), small nuclear RNAs (snRNAs), Piwi-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), competing endogenous RNAs (ceRNAs), lncRNAs, and long intergenic ncRNAs (lincRNAs) have been associated with different types of neoplasia.[69]

Naked mole rats (NMRs, *Heterocephalus glaber*) are exceptionally long-lived and cancer-resistant rodents. Jiang et al.[70] identified a total of 4422 lncRNAs across the NMR genome based on 12 published transcriptomes. NMR lncRNAs share many common characteristics with other vertebrate species, such as tissue specificity and low expression. Only five NMR lncRNAs displayed homology with 1057 human cancer-related lncRNAs, demonstrating the low sequence conservation between NMR lncRNAs and human cancer-related lncRNAs. A total of 1295 lncRNAs were intensively coexpressed with potential tumor suppressor genes in NMR, and 194 lncRNAs exhibited strong correlation with four high-molecular-mass hyaluronan-related genes that were previously identified to play key roles in cancer resistance of NMR. lncRNAs may have important effects on the anticancer mechanism in NMR.[70]

The highly conserved RAS-mitogen activated protein kinase (MAPK) signaling pathway is involved in a wide range of cellular processes including differentiation, proliferation, and survival. Somatic mutations in genes encoding RAS-MAPK components frequently occur in many tumors, making the RAS-MAPK a critical pathway in human cancer. Let-7 miRNA acts as a tumor suppressor by repressing the RAS oncogene. miRNAs targeting the RAS-MAPK pathway are relevant in oncogenesis. miRNA alterations in human cancers may act as a rheostat of the oncogenic RAS signal that is often amplified as cancers progress.[71]

lncRNAs influence epithelial-mesenchymal transition (EMT), a fundamental step in tumor metastasis. In MCF10A cells undergoing TGFβ-induced EMT, deep-sequencing analysis shows that the long RNA transcriptome of MCF10A undergoes global changes as early as 8 h after treatment with TGFβ. The expression of 3403 known and novel lncRNAs, and 570 known and novel circRNAs are altered during EMT. The junction node RP6-65G23.5 has been identified as a key regulator of EMT. About 216 clusters containing lncRNAs have been located in "gene desert" regions. The expressions of all lncRNAs in these clusters change during EMT.[72]

Trimethylation at histone H3 lysine 4 (H3K4me3) and lysine 27 (H3K27me3) controls gene activity during development and differentiation. H3K4me3 and H3K27me3 changes dynamically occur in response to altered microenvironmental conditions, including low-oxygen conditions commonly present in solid tumors. Demethylation of H3K4me3 and H3K27me3 is mediated by oxygen and 2-oxoglutarate dioxygenase enzymes, suggesting that oxygen deprivation (hypoxia) may influence histone trimethylation. H3K4me3 and H3K27me3 marks rapidly increase at specific locations throughout the genome and are largely reversed upon reoxygenation. The histone H3K27me3 demethylase KDM6B/JMJD3 is inactivated by limited oxygen. Dynamic regulation of the epigenetic state within the tumor environment may have important consequences for tumor plasticity and biology.[73]

Mitochondrial DNA replication is critical for maintaining mtDNA copy number to generate sufficient cellular energy that is required for development and for functional cells. In early development, mtDNA copy number is strictly regulated at different stages, and the establishment of the mtDNA set point is required for sequential cell lineage commitment. The failure to establish the mtDNA set point results in incomplete differentiation or embryonic arrest. The regulation of mtDNA copy number during differentiation is closely associated with cellular gene expression, especially with the pluripotency network, and DNA methylation profiles. The findings from cancer research highlight the relationship between mitochondrial function, mtDNA copy number, and DNA methylation in regulating differentiation. DNA methylation at exon 2 of DNA polymerase gamma subunit A (*POLGA*) has been shown to be a key factor, which can be modulated to change the mtDNA copy number and cell fate of differentiating and tumor cells.[74]

Recent advances of next generation sequencer (NGS) and computational analyses have explored somatic protein-altered mutations in most cancer types. In contrast, information on somatic mutations in noncoding regions including introns, regulatory elements, and noncoding RNAs, is still very limited. Despite its limitations and complexity in data interpretation, whole genome sequencing (WGS) approaches can help to understand the diversity of cancer genophenotypes, integrating genomic data with RNA-sequencing, epigenomics, and immunogenomic and phenotypic (clinical/pathogenic) information.[75]

5.6.1 Breast Cancer

At least six epigenome-wide association studies (EWAS) for breast cancer risk identified a global loss of methylation observed in breast cancer cases compared with controls, as well as EWAS signatures of cancer risk factors such as smoking, body mass index, age, and alcohol use with numerous validated CpG sites.[76] The transcription factor BCL11A is a driving force in triple negative breast cancer (TNBC), contributing to the maintenance of a chemoresistant breast cancer stem cell (BCSC) population. BCL11A suppresses γ-globin and p21 and induces MDM2 expression in the hematopoietic system. In transcriptional repression, BCL11A interacts with several corepressor complexes, such as histone methyltransferase (PRC2) and histone deacetylase (NuRD and SIN3A) complexes through their common subunit, RBBP4/7. BCL11A competes with histone H3 for binding to the negatively charged top face of RBBP4. BCL11A2-16 pulls down RBBP4, RBBP7, and other components of PRC2, NuRD, and SIN3A from the cell lysate of the TNBC cell line SUM149.[77] Acetylated H3 lysine 23 (H3K23ac) is a specific histone posttranslational modification recognized by oncoprotein TRIM24. TRIM24 expression is positively correlated with H3K23ac levels, and high levels of both TRIM24 and H3K23ac predict shorter overall survival of breast cancer patients. Both TRIM24 and H3K23ac are higher in HER2-positive patients, and their levels are positively correlated with HER2 levels in breast cancer. TRIM24 expression is associated with the estrogen receptor (ER) and progesterone receptor (PR).[78] Combinatorial patterns of distinct histone modification signals identified AFAP1-AS1 as a triple negative breast cancer-specific gene associated with cell proliferation and epithelial-mesenchymal transition.[79]

SMYD2, a SMYD (SET and MYND domain) family protein with lysine methyltransferase activity, has been identified as a novel breast cancer oncogene. *PTPN13* is a novel SMYD2 transcriptional target gene that links SMYD2 to other known breast cancer-associated signaling pathways, including ERK, mTOR, and Akt signaling via PTPN13-mediated phosphorylation. Silencing of *SMYD2* by RNAi in triple negative breast cancer (TNBC) cell lines or inhibition of SMYD2 with its specific inhibitor, AZ505, significantly reduces tumor growth.[80]

There is an association between BC and cg05370838-rs2230576, cg00956490-rs940453, and cg11340537-rs2640785 CpG-SNP pairs. These CpG-SNP pairs are strongly associated with differential expression of *ADAM8*, *CREB5*, and *EXPH5* genes, respectively. The SNPs rs10101376, rs140679, and rs1538146 may have some prognostic value.[81]

A genome-wide transcriptional survey to explore the lncRNA landscape across 995 breast tissue samples identified 215 lncRNAs whose genes are aberrantly expressed in breast tumors. Four breast cancer subgroups correlate tightly with PAM50-defined mRNA-based subtypes. About 210 lncRNAs are prognostic of clinical outcome, and 215 are dysregulated lncRNAs in connetion with the activation of phosphatidylinositol 3-kinase, fibroblast growth factor, transforming growth factor-β pathways, and the activation of epidermal growth factor receptor (EGFR)-dependent pathways. A specific lncRNA (CYTOR), which regulates genes involved in the EGFR/mammalian target of the rapamycin pathway and is required for cell proliferation, cell migration, and cytoskeleton organization, plays a role in breast cancer.[82]

The catalytic subunit of the telomerase complex, hTERT, ensures the unlimited proliferative potential of cancer cells by maintaining telomere function and protecting from apoptosis. miR-296-5p and miR-512-5p target hTERT in breast cancer cells. Ectopic miR-296-5p and miR-512-5p reduce telomerase activity, drive telomere shortening, and cause proliferation defects by enhancing senescence and apoptosis in breast cancer cells. miR-296-5p and miR-512-5p expression

is reduced in human breast cancer. Overexpression of miR-296-5p and miR-512-5p target genes including *hTERT* is linked with reduced distant metastasis-free survival and relapse-free survival of basal-type breast cancer patients. Epigenetic silencing of miR-296 and miR-512 encoding genes is responsible for the low levels of miR-296-5p and miR-512-5p expression in basal-type breast cancer cells. Disrupting gene silencing results in dramatic upregulation of miR-296-5p and miR-512-5p levels leading to reduced *hTERT* expression and increased sensitivity to the induction of apoptosis. These results reported by Dinami et al.[83] suggest that epigenetic regulatory circuits in basal-type breast cancer may contribute to high hTERT levels by silencing miR-296-5p and miR-512-5p expression, thereby contributing to the aggressiveness of basal-type breast cancer.

At least 173 miRNAs have been found to be dysregulated in breast cancer. Ethnic differences have been reported. For instance, Lebanese patients have 21 exclusively dysregulated miRNAs and 4 miRNAs with different expression compared with American patients.[84]

Zhao and Ren[85] studied the role of taurine-upregulated gene 1 (*TUG1*) in MCF-7 breast cancer cells and the molecular mechanism involved in the regulation of microRNA-9 (miR-9). Higher expression of *TUG1* was observed in breast cancer tissues and cell lines than in the corresponding controls. *TUG1* knockdown reduced proliferation, suppressed cell cycle progression, and promoted the apoptosis of MCF-7 cells. TUG1 can negatively regulate the expression of miR-9. miR-9 inhibition abrogates the effect of *TUG1* knockdown on the proliferation, cell cycle progression, and apoptosis of MCF-7 cells. TUG1 positively regulates the expression of MTHFD2 in breast cancer cells. *TUG1* knockdown is associated with decreased cell proliferation, promoting apoptosis of breast cancer cells through the regulation of miR-9.

Genome-wide miRNA expression may be useful for predicting breast cancer risk and/or for the early detection of breast cancer. Taslim et al.[86] reported a 41-miRNA model that distinguished breast cancer risk with an accuracy of 83.3%. Of the 41 miRNAs 20 were detectable in serum, and predicted breast cancer occurrence within 18 months of blood draw (accuracy 53%). These risk-related miRNAs were enriched for HER-2 and estrogen-dependent breast cancer signaling.

Increased expression of voltage-gated sodium channels (VGSCs) has been implicated in the strong metastatic potential of human breast cancer. Kamarulzaman et al.[87] studied the role of epigenetics via transcription repressor, repressor element silencing transcription factor (REST), and histone deacetylases (HDACs) in enhancing Nav1.5 and nNav1.5 expression in human breast cancer by assessing the effect of the HDAC inhibitor trichostatin A (TSA). mRNA expression levels of Nav1.5 and nNav1.5 were initially very low in MCF-7 compared with MDA-MB-231 cells, and mRNA expression levels of *REST*, *HDAC1*, *HDAC2*, and *HDAC3* were all greater in MCF-7 than MDA-MB-231 cells. Treatment with TSA increased the mRNA expression level of *Nav1.5* and *nNav1.5* in MCF-7 cells, and TSA reduced the mRNA expression level of *REST* and *HDAC2* in this cell line.[87]

Kynurenine formation by tryptophan-catabolic indoleamine-2,3-dioxygenase 1 (IDO1) plays a key role in tumor immune evasion, and inhibition of IDO1 is efficacious in preclinical models of breast cancer. Estrogen receptor α (ER) is a negative regulator of IDO1 expression. Serum kynurenine levels as well as tumoral IDO1 expression are lower in patients with ER-positive than ER-negative tumors, and an inverse relationship between IDO1 and estrogen receptor mRNA is observed in breast cancer. The *IDO1* promoter is hypermethylated in ER-positive compared with ER-negative breast cancer. Reduced induction of IDO1 is also observed in human ER-positive breast cancer cell lines. IDO1 induction is enhanced upon DNA demethylation in ER-positive but not in ER-negative cells, and methylation of an *IDO1* promoter construct reduces *IDO1* expression, suggesting that enhanced methylation of the *IDO1* promoter suppresses IDO1 in ER-positive breast cancer. According to Dewi et al.[88] the association of ER overexpression with epigenetic downregulation of IDO1 appears to be a particular feature of breast cancer, as IDO1 was not suppressed by *IDO1* promoter hypermethylation in the presence of high ER expression in cervical or endometrial cancer.

The expression of Tripartite motif-containing protein 28 (TRIM28)/Krüppel-associated box (KRAB)-associated protein 1 (KAP1) is elevated in at least 14 tumor types, and high levels of TRIM28 are associated with the triple negative subtype of breast cancer (TNBC), with higher aggressiveness and lower survival rates. TRIM28 is essential for maintaining the pluripotent phenotype in embryonic stem cells. Downregulation of TRIM28 expression in xenografts leads to deceased expression of pluripotency and mesenchymal markers, as well as inhibition of signaling pathways involved in the complex mechanism of CSC maintenance. TRIM28 depletion reduces the ability of cancer cells to induce tumor growth.[89]

A metaprediction study to examine the polymorphism-mutation risk subtypes of the methylenetetrahydrofolate reductase gene (*MTHFR*) and air pollution as contributing factors for breast cancer revealed that *MTHFR 677 TT* is a risk genotype for breast cancer in East Asian women. Most polymorphism-mutations on *MTHFR 677 TT* have been found in the Middle East, Europe, Asia, and the Americas, whereas most mutations on *MTHFR 1298 CC* were located in Europe and the Middle East for controls. *MTHFR 677 TT* mutations yield a higher risk for breast cancer in Australia,

East Asia, the Middle East, South Europe, Morocco, and the Americas, and *MTHFR 1298 CC* mutations pose a higher risk in Asia, the Middle East, South Europe, and South America. Metapredictive analysis revealed that the air pollution level was associated with the *MTHFR 677 TT* polymorphism-mutation genotype.[90]

A locus at 19p13 is associated with breast cancer (BC) and ovarian cancer (OC) risk. Lawrenson et al.[91] analyzed 438 SNPs in this region in 46,451 BC and 15,438 OC cases, 15,252 *BRCA1* mutation carriers, and 73,444 controls, and identified 13 candidate causal SNPs associated with serous OC, ER-negative BC, BRCA1-associated BC, and triple negative BC. Genotype-gene expression associations were identified for candidate target genes *ANKLE1* and *ABHD8*. There are interactions between four candidate SNPs and *ABHD8*, and six risk alleles increase transactivation of the *ADHD8* promoter. Targeted deletion of a region containing risk SNP rs56069439 in a putative enhancer induces *ANKLE1* downregulation. Multiple SNPs at 19p13 regulate *ABHD8* and perhaps *ANKLE1* expression, and indicate common mechanisms underlying breast and ovarian cancer risk.[91]

5.6.2 Colorectal Cancer

Colorectal cancer (CRC) is a major cause of cancer death. Approximately 20% of CRCs arise within serrated polyps that exhibit both oncogenic *BRAF* mutation and widespread DNA methylation changes important in silencing genes restraining neoplastic progression. In addition to multiple environmental factors, genome-wide copy number variations, single nucleotide mutations, and DNA methylation changes contribute to CRC pathogenesis.[92] The *BrafV637E* mutation in murine intestine on an FVB;C57BL/6J background revealed extensive intestinal hyperplasia, murine serrated adenomas with dysplasia and invasive cancer, and gene-specific increases in DNA methylation in a time-dependent fashion. Persistent oncogenic Braf signaling is sufficient to induce widespread DNA methylation changes. DNA methylation arises slowly in direct response to prolonged oncogenic Braf signaling in serrated polyps.[93] Studies on the epigenetic mechanisms of DNA methylation and hydroxymethylation in the carcinogenesis of colorectal cancer identified a genome-wide distinct hydroxymethylation pattern (59,249 DMRs, 187,172 DhMRs, and 948 DEGs) which might be used as an epigenetic biomarker for differentiating colorectal tumor tissues from normal tissues. Hypermethylation of the *HADHB* gene correlates with downregulation of its transcription in colorectal cancer. HADHB reduces cancer cell migration and invasiveness, probably acting as a tumor suppressor gene.[94]

DNA hypermethylation is central for the development of CRC. Hypermethylated promoter regions infer a poor prognosis. *RARB* and *RASSF1A* hypermethylation influence survival. The risk of metastasis increases with the number of cell-free hypermethylated promoter regions.[95]

Active regulatory elements (AREs) mapped using data from 127 tissues and cell types from NIH Roadmap Epigenomics and Encyclopedia of DNA Elements (ENCODE) projects have been found to be enriched for stronger CRC-variant associations and for rare variant sets of CR AREs compared with nondigestive AREs.[96]

Free fatty acid receptor 2 (FFAR2, GPR43) is activated by short chain fatty acids (SCFAs) produced in the fermentation of dietary fiber. FFAR2 regulates colonic inflammation epigenetic changes in colon carcinogenesis. FFAR2 deficiency promotes the development of colon adenoma and the progression of adenoma to adenocarcinoma in mice. When the FFAR2's downstream cAMP/PKA/CREB pathway is enhanced, there is an overexpression of histone deacetylases (HDACs) in *FFAR2*-deficient mice, with differential binding of H3K27me3 and H3K4me3 histone marks onto the promoter regions of the inflammation suppressors, resulting in decreased expression of *sfrp1*, *dkk3*, and *socs1*. FFAR2 is required for butyrate to suppress HDAC expression and hypermethylation of inflammation suppressors, indicating that FFAR2 is an epigenetic tumor suppressor that acts at multiple stages of colon carcinogenesis.[97]

RASGRF1 is a guanine nucleotide exchange factor, which promotes the release of GDP from inactive Ras and stabilizes apoprotein. Studies on the methylation status of *RASGRF1* promoter and its correlation with clinicopathological parameters in CRC indicate that *RASGRF1* hypermethylation is involved in an epigenetic field defect in CRC and that it might be a potential risk factor for CRC.[98]

Together with protein-coding genes identified as oncogenes or tumor suppressors in CRC a number of lncRNAs have also been found to be associated with CRC. One such lncRNA is *LINC00472*, which is downregulated in CRC cell lines and cancer tissues. The silencing of *LINC00472* is caused by DNA hypermethylation at its promoter region. *LINC00472* expression and promoter DNA methylation correlate with clinicopathological features of CRC, and DNA hypermethylation of *LINC00472* may serve as a diagnostic biomarker for CRC.[99]

N-BLR is a primate-specific long noncoding RNA that modulates epithelial-mesenchymal transition, facilitates cell migration, and increases colorectal cancer invasion. *N-BLR* is associated with tumor stage, invasion potential, and overall

patient survival. *N-BLR* facilitates migration primarily via crosstalk with E-cadherin and ZEB1 mediated by a pyknon, a short ~20 nucleotide-long DNA motif contained in the *N-BLR* transcript that is targeted by members of the miR-200 family.[100]

The ten-eleven translocation (TET) family of oxygenases oxidize 5-mC to 5-hydroxymethylcytosine (5-hmC), which is a prerequisite for active DNA demethylation. Both TET1 expression and global 5-hmC content are significantly reduced in CRC. The oncogenic miRNA miR-21-5p has been identified as a diagnostic and prognostic biomarker in CRC. TET1 is a novel target of miR-21-5p. The 3′-UTR region of the *TET1* gene contains a miR-21-5p-binding site. Loss of TET1 is associated with the progression of CRC to advance stages.[101]

The study of colorectal adenocarcinoma progression by O-GlcNAc focused on the O-GlcNAc-mediated epigenetic regulation of human colon cancer stem cells (CCSC) shows that xenograft tumors from colon tumor cells with O-linked N-acetylglucosamine transferase (*OGT*) knockdown grow slower than those formed from control cells, indicating a reduced proliferation of tumor cells due to the inhibition of *OGT* expression. O-GlcNAc levels regulate the CCSC compartment. There is chromatin enrichment of O-GlcNAc-modified proteins at the promoter of the transcription factor *MYBL1*, characterized by the presence of H3K27me3. Increased expression of *MYBL1* is present in tumor cells with *OGT* knockdown. Forced overexpression of *MYBL1* leads to a reduced population of CCSC and tumor growth, similar to the effects of *OGT* silencing. O-GlcNAc levels regulate the methylation status of two CpG islands near the transcription start site of *MYBL1*. O-GlcNAc epigenetically regulates MYBL1, functioning similarly to H3K27me3.[102]

5.6.3 Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the fastest rising cause of cancer-related death in some developed countries. Nonalcoholic steatohepatitis (NASH), a progressive form of nonalcoholic fatty liver disease (NAFLD), is a major risk factor for HCC. Hepatic dipeptidyl peptidase 4 (DPP4) expression is elevated in ectopic fatty liver. Studies on transcriptional regulation of hepatic Dpp4 in young mice prone to diet-induced obesity indicate that hepatic Dpp4 is increased in animals with high weight gain, independent of liver fat content. The methylation of four intronic CpG sites is decreased, amplifying glucose-induced transcription of hepatic *Dpp4*. Hepatic triglyceride content is increased only in animals with elevated *Dpp4* expression. Analysis of human liver biopsy specimens revealed a correlation between *DPP4* expression and DNA methylation to stages of hepatosteatosis and nonalcoholic steatohepatitis. Glucose-induced expression of Dpp4 in the liver is facilitated by demethylation of the *Dpp4* gene early in life.[103]

Mice with a deficiency in the histone H3K9 methyltransferase suppressor of variegation 39 homolog 2 (Suv39h2) exhibited a less severe form of NASH induced by feeding with a high-fat, high-carbohydrate diet. In hepatocytes, Suv39h2 binds to the *Sirt1* gene promoter and represses *Sirt1* transcription. Suv39h2 deficiency normalizes Sirt1 expression, allowing nuclear factor kappa B/p65 to become hypoacetylated and thus dampening nuclear factor kappa B-dependent transcription of proinflammatory mediators.[104]

Multiple genome-wide association studies identified the *I148M PNPLA3* variant as the major common genetic determinant of NAFLD. Variants with moderate effect size in *TM6SF2*, *MBOAT7*, and *GCKR* also contribute to NASH.[105]

miRNA alterations are involved in the pathogenesis of NASH-derived HCC. Altered miRNA expression is associated with activation of major hepatocarcinogenesis-related pathways, including the TGFβ, Wnt/β-catenin, ERK1/2, mTOR, and EGF signaling. Overexpression of miR-221-3p, miR-222-3p, and the oncogenic miR-106b~25 cluster is linked to reduced levels of their protein targets, including E2F transcription factor 1 (E2F1), phosphatase and tensin homolog (PTEN), and cyclin-dependent kinase inhibitor 1 (CDKN1A). miR-93-5p, miR-221-3p, and miR-222-3p are overexpressed in human HCC.[106]

Overexpressed c-Myc and EZH2 usually mean high malignancy in cancers. MicroRNA-26a (miR-26a) suppresses EZH2 and c-Myc by targeting EZH2 and CDK8 in the Wnt pathway. miR-26a is a well-known tumor suppressor miRNA in multiple cancers. miR-26a is epigenetically silenced in a c-Myc-mediated PRC2-dependent way in HCC. miR-26a suppresses the migration of HCC by targeting p21-activated kinase 2(PAK2), which is a critical effector linking Rho GTPases to cytoskeleton reorganization. miR-26a might be a regulon that suppresses progression and metastasis of c-Myc/EZH2 double high advanced HCC.[107]

N6-methyladenosine (m6A) is the most abundant chemical modification on eukaryotic mRNA and is important to the regulation of mRNA stability, splicing, and translation. METTL3 (methyltransferase like 3), a major RNA N6-adenosine methyltransferase, is upregulated in HCC and other solid tumors. Overexpression of METTL3 is associated with poor prognosis of HCC patients. Knockdown of *METTL3* reduces HCC cell proliferation, migration, and

colony formation, suppressing HCC tumorigenicity and lung metastasis. Overexpression of METTL3 promotes HCC growth. *SOCS2* (suppressor of cytokine signaling 2) is a target of METTL3-mediated m6A modification. Knockdown of *METTL3* abolishes *SOCS2* mRNA m6A modification and increases *SOCS2* mRNA expression. m6A-mediated *SOCS2* mRNA degradation relies on the m6A (reader) protein YTHDF2-dependent pathway.[108]

G9a is a frequently upregulated histone methyltransferase in human HCCs. Upregulation of G9a is associated with HCC progression and aggressive clinicopathological features. Inactivation of *G9a* by RNAi knockdown, CRISPR/Cas9 knockout, and pharmacological inhibition abolishes H3K9 dimethylation and suppresses HCC cell proliferation and metastasis. Upregulation of G9a in human HCCs is attributed to gene copy number gain at chromosome 6p21. miR-1 is a negative regulator of G9a. Loss of miR-1 relieves the posttranscriptional repression on G9a and contributes to its upregulation in human HCC. Tumor suppressor RARRES3 is a critical target of G9a. Epigenetic silencing of *RARRES3* contributes to the tumor-promoting function of G9a.[109]

The lncRNA glypican 3 antisense transcript 1 (*GPC3-AS1*) is a potential biomarker for HCC screening. There is a significant upregulation of *GPC3-AS1* in HCC. Increased expression of *GPC3-AS1* is associated with α-fetoprotein, tumor size, microvascular invasion, encapsulation, Barcelona Clinic Liver Cancer stage, and worse prognosis of HCC patients. GPC3-AS1 associates with P300/CBP-associated factor and recruits it to the *GPC3* gene body region, consequently inducing an increase in euchromatic histone marks and activating *GPC3* transcription. *GPC3-AS1* expression is strongly correlated with GPC3 in HCC tissues. Gain-of-function and loss-of-function analyses show that *GPC3-AS1* overexpression enhances HCC cell proliferation and migration and xenograft tumor growth. *GPC3-AS1* knockdown inhibits HCC cell proliferation and migration. The effects of GPC3-AS1 on HCC cell proliferation and migration are dependent on the upregulation of GPC3.[110]

Alterations in folate-dependent 1-carbon metabolism are involved in the pathogenesis of NASH. NASH and NASH-related liver carcinogenesis are characterized by a dysregulation of 1-carbon homeostasis, with diminished expression of key 1-carbon metabolism genes, inhibition of the S-adenosylhomocysteine hydrolase (*Ahcy*) gene, and increased levels of S-adenosyl-L-homocysteine (SAH). The reduction in *Ahcy* expression is associated with gene-specific cytosine DNA hypermethylation and enrichment of the gene promoter by transcription-inhibiting markers such as trimethylated histone H3 lysine 27 and deacetylated histone H4 lysine 16. Epigenetically mediated inhibition of *Ahcy* expression may cause SAH elevation and subsequent downstream disturbances in transsulfuration and transmethylation pathways during the development and progression of NASH.[111]

5.6.4 Gastric Cancer

Galanin is a 30 amino acid neuropeptide that acts via G protein-coupled receptors (GALR1, GALR2, GALR3). *GALR1* is a tumor suppressor gene frequently silenced in head and neck squamous cell carcinoma. Both galanin and GALR1 inhibit human oral cancer cell proliferation. In gastric cancer, galanin acts as an epigenetic silencing agent, and galanin hypermethylation impairs its tumor suppressor function in gastric carcinogenesis.[112]

Long noncoding RNAs play a critical role in tumorigenesis of gastric cancer. lncRNA *MAP3K20* antisense RNA 1 (*MLK7-AS1*) has been identified as a gastric cancer-specific lncRNA. lncRNA *MLK7-AS1* is increased in gastric cancer tissues. Gastric cancer patients with high MLK7-AS1 expression have a shorter survival and poorer prognosis. Knockdown of *MLK7-AS1* inhibits cell proliferation and induces apoptosis in HGC27and MKN-45 cells. miR-375 is a target of MLK7-AS1. MLK7-AS1 interacts with Dnmt1 and recruits it to miR-375 promoter, hypermethylating miR-375 promoter, and repressing miR-375 expression.[113]

5.6.5 Urogenital Carcinoma

Urothelial carcinoma (UC), the most common cancer of the urinary bladder, causes over 40,000 deaths per year in the European Union. Extensive aberrant DNA methylation is present in urothelial carcinoma, contributing to genetic instability, altered gene expression, and tumor progression. DNA hypermethylation of the 5′ regulatory regions of the *ODC1*, *AHCY*, and *MTHFR* genes occurs in early urothelial carcinoma. These hypermethylation events are associated with genome-wide DNA hypomethylation, which is commonly associated with genetic instability.[113]

5.6.6 Prostate Cancer

Prostate cancer (PC) is the second most commonly diagnosed cancer in men after lung cancer and the third leading cause of cancer-related mortality after lung and colon cancer. Studies on DNA methylation in transgenic *TRAMP* mice

show aberrant CpG hypermethylation and hypomethylation in over 2000 genes.[114] The cAMP response element-binding protein (CREB)-, histone deacetylase 2 (HDAC2)-, glutathione S-transferase pi (GSTP1)-, and polyubiquitin-C (UBC)-related pathways show significantly altered methylation profiles.[115]

Metabolic syndrome (MeS) is associated with increased PC aggressiveness and recurrence. Porreti et al.[116] proposed C-terminal binding protein 1 (CTBP1), a transcripcional corepressor, as a molecular link between these two conditions. CTBP1 depletion decreases PC growth in MeS mice. CTBP1 represses chloride channel accessory 2 (CLCA2) expression in prostate xenografts developed in MeS animals. CTBP1 binds to *CLCA2* promoter and represses its transcription and promoter activity in PC cell lines. CTBP1 forms a repressor complex with ZEB1, EP300, and HDACs, which modulates *CLCA2* promoter activity. Some 21 miRNAs modulated by CTBP1 are involved in angiogenesis, extracellular matrix organization, focal adhesion, and adherent junctions. miR-196b-5p directly targets CLCA2 by cloning CLCA2 3′ UTR.[116]

SIRT1 can promote PC progression. Androgen deprivation induces reactive oxygen species production, and reactive oxygen species activate SIRT1 expression. Increased SIRT1 expression induces neuroendocrine differentiation of PC cells by activating the Akt pathway. The interaction between Akt and SIRT1 is independent of N-Myc. SIRT1 facilitates tumor maintenance. Targeting SIRT1 may reduce the tumor burden during androgen deprivation.[117] miRNAs are globally downregulated in PC. Exposure of PC cell lines to the demethylating agent 5-Aza-CdR results in an increase in the expression levels of miRNAs. Downregulation of miR-130a has been associated with promoter hypermethylation. miR-130a methylation levels discriminate PC from nonmalignant tissues. Repressive histone marks are found in the promoter of miR-130a, and overexpression of miR-130a reduces cell viability and invasion capability, increasing apoptosis. Silencing *DEPD1C* and *SEC23B* resembles the effect of overexpressing miR-130a. miR-130a is an epigenetically regulated miRNA involved in the regulation of key molecular and phenotypic features of prostate carcinogenesis, acting as a tumor suppressor miRNA.[118] miR-141, one of the miR-200 family members, is underexpressed in several PC stem/progenitor cell populations. Enforced expression of miR-141 inhibits cancer stem cell properties including holoclone and sphere formation, as well as invasion, and suppresses tumor regeneration and metastasis. Whole genome RNA sequencing uncovers novel miR-141-regulated molecular targets in PC cells including Rho GTPase family members (CDC42, CDC42EP3, RAC1, ARPC5) and stem cell molecules (CD44, EZH2).[119]

Prostanoid thromboxane (TX) A2 and its T Prostanoid receptor (TP) are implicated in PC. Both TPα and TPβ form functional signaling complexes with members of the protein kinase C-related kinase (PRK) family, AGC-kinases essential for the epigenetic regulation of androgen receptor (AR)-dependent transcription and promising therapeutic targets for the treatment of castrate-resistant prostate cancer (CRPC). The activation of PRKs through the TXA2/TP signaling axis induces phosphorylation of histone H3 at Thr11 (H3Thr11), a marker of androgen-induced chromatin remodeling and transcriptional activation, raising the possibility that TXA2-TP signaling can mimic and/or enhance AR-induced cellular changes even in the absence of circulating androgens such as in CRPC. TXA2/TP-induced PRK activation can mimic and/or enhance AR-mediated cellular responses in the model androgen-responsive prostate adenocarcinoma LNCaP cell line. TXA2/TP signaling can act as a neoplastic and epigenetic regulator, promoting and enhancing both AR-associated chromatin remodeling (H3Thr11 phosphorylation, WDR5 recruitment, and acetylation of histone H4 at lysine 16) and AR-mediated transcriptional activation (KLK3/prostate-specific antigen and TMPRSS2 genes) through mechanisms involving TPα/TPβ-mediated PRK1 and PRK2 signaling complexes.[120]

5.6.7 Ovarian Cancer

Methylation of promoter CpG islands may suppress the function of miRNAs by inhibiting their expression. Promoter methylation may suppress the expression of 12 miRNAs associated with epithelial ovarian cancer (EOC) (miR-124-3p, miR-125b-5p, miR-127-5p, miR-129-5p, miR-132-3p, miR-137, miR-148a-3p, miR-191-5p, miR-193a-5p, miR-203a, miR-339-3p, and miR-375). There are aberrations in the methylation patterns of 11 miRNA genes; 8 novel hypermethylated miRNA genes (miR-124-1, miR-124-2, miR-124-3, miR-127, miR-132, miR-137, miR-193A, and miR-339) and 1 hypomethylated miRNA gene (miR-191) have been identified. There is a strong correlation between methylation status and alterations in expression levels of the 12 EOC-related miRNAs. There is also an association between hypermethylation of miR-124-2, miR-124-3, miR-125B-1, miR-127, miR-129-2, miR-137, miR-193A, miR-203A, miR-339, and miR-375 and EOC metastasis to lymph nodes, peritoneum, and distant organs.[121]

Ovarian fibrosarcomas are rare tumors in pediatrics with a paucity of mutations (0.77/Mb) and CNV alterations. A point mutation in the metal-binding site of the microRNA-processing *DICER1* enzyme and a frameshift alteration in the tumor suppressor gene *NF1* have been identified. A germinal truncating mutation in *DICER1* is consistent with a DICER1 syndrome diagnosis. This syndrome also presents as a global lncRNA deregulation, with decreased lncRNAs

transcripts expressed in the tumor, and concomitant upregulation of noncoding transcripts associated with cancer, such as *MALAT1*, *MIR181A1HG*, *CASC1*, *XIST*, and *FENDRR*.[122]

5.6.8 Cervical Cancer

Cervical cancer is one of the leading causes of death in women worldwide. Methylation changes have been detected in precancerous stages, and methylation biomarkers may have some value in cervical cancer screening. Methylation of CpG islands in the promoter region of the retinoic acid receptor beta (*RARβ*) gene, a tumor suppressor gene, are associated with cervical cancer. The frequency of *RARβ* promoter methylation correlates with the severity of cervical epithelium anomalies.[123]

Fragile histidine triad (*FHIT*) is a tumor suppressor gene that is frequently silenced in cervical cancer (CC) and preneoplastic lesions. Promoter hypermethylation is present in CC, and its epigenetic silencing has been observed at the mRNA or protein level. Folate may modulate DNA methylation and defects in the folate metabolic pathway may have a connection with carcinogenesis. The *FHIT* gene methylation rate increases with the severity of cervix lesions, where, as folate levels, the *FHIT* mRNA and protein expression levels are reduced. Folate deficiency and *FHIT* gene promoter hypermethylation may be associated with cervical carcinogenesis.[124]

5.6.9 Head and Neck Squamous Cell Carcinoma

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide with high recurrence, metastasis, and poor treatment outcome. Smoking, alcohol use, and betel quid chewing are the three major causes of head and neck cancers.[125]

miRNAs, such as short 20 to 25-nt ncRNAs, are responsible for posttranscriptional regulation of gene expression with effects in oncogenesis by acting as oncomiRNAs or tumor suppressor miRNAs.

miRNAs regulated by promoter DNA methylation and histone acetylation in human papilloma virus-positive head and neck squamous cell carcinoma have been identified. Among 10 miRNAs specifically upregulated in microarray analysis of AZA-treated SCC090 cells, decreased expression of hsa-miR-181c-5p, hsa-miR-132-5p, and hsa-miR-658 has been observed. Increased frequency of methylation at the promoter of hsa-miR-132-5p, negatively correlated with its expression, is also present in this type of cancer. In TSA-treated SCC090 cells, hsa-miR-129-2-3p and hsa-miR-449a are upregulated. The levels of enrichment by antiacetyl-H3 and antiacetyl-H4 are significantly low.[126] Differential expression of both miRNAs and lncRNAs, which include oncogenic ncRNAs (miR-21, miR-31, miR-155, miR-211, *HOTAIR*, and *MALAT1*) and tumor suppressor ncRNAs (let7d, miR-17, miR-375, miR-139, and MEG3), has been observed in HNSCC.[126]

SALL3 promoter methylation is associated with transcriptional inhibition and correlates with disease recurrence and reduced disease-free survival. *SALL3* hypermethylation is associated with expression of *TET1*, *TET2*, and *DNMT3A* genes.[127]

5.6.10 Lung Cancer

Lung cancer is the leading cause of cancer-related deaths with 1.8 million new cases each year and poor five-year prognosis. Smoking-associated DNA hypomethylation has been linked to lung cancer risk. A549 cell exposure to cigarette smoke stimulates expression of predicted target xenobiotic response-related genes *AHRR* and *CYP1B1*. Expression of both genes has been linked to smoking-related transversion mutations in lung tumors. Smoking-associated hypomethylation may be a consequence of enhancer activation, revealing environmentally-induced regulatory elements implicated in lung carcinogenesis.[128] Promoter hypermethylation of tumor suppressors leads to their inactivation and thereby can promote cancer development and progression. DNA methylation at sites cg11637544 in *KDM2A* and cg26662347 in *KDM1A* is a relevant biomarker for squamous cell carcinomas (SCC) and SCC survival, correlating with gene expression (cg11637544 for *KDM2A*; cg26662347 for *KDM1A*).[129] DNA methylation changes are associated with cigarette smoking. Six CpGs for which hypomethylation is associated with lung cancer risk have been identified, including cg05575921 in the *AHRR* gene, cg03636183 in the *F2RL3* gene, cg21566642 and cg05951221 in 2q37.1, cg06126421 in 6p21.33, and cg23387569 in 12q14.1. For cg05951221 and cg23387569 the strength of association was virtually identical in never smokers and current smokers.[129]

ZAR1 (zygote arrest 1) is a maternal effect gene with expression limited to certain reproductive tissues. *ZAR1* is also expressed in normal lung but inactivated by promoter methylation in lung cancer. *ZAR1* is hypermethylated in

primary lung cancer samples (22% in small-cell lung carcinoma (SCLC) and 76% in nonsmall-cell lung carcinoma (NSCLC)) versus normal control lung tissue (11%). Demethylation treatment of various lung cancer cell lines reversed *ZAR1* promoter hypermethylation and subsequently reestablished *ZAR1* expression.[130]

Alterations in fatty acid (FA) metabolism may be involved in the development of lung cancer. In six genome-wide association studies (GWASs) from the Transdisciplinary Research in Cancer of the Lung (TRICL) consortium, which included 12,160 cases with lung cancer and 16,838 cancer-free controls, a total of 30,722 SNPs from 317 genes relevant to FA metabolic pathways have been identified. A total of 26 SNPs were mapped to the *CYP4F3* gene in which a proxy SNP, *CYP4F3* rs4646904, was suggested to change splicing pattern/efficiency and to be associated with gene expression levels.[131]

Novel drivers are epigenetically altered through aberrant methylation in early-stage lung adenocarcinoma (LADC), regardless of the presence or absence of tobacco smoking-induced epigenetic field defects. Several candidate tumor suppressor genes (TSGs) have been found inactivated by hypermethylation in lung cancer. *TRIM58* is the most prominent candidate for TSG. *TRIM58* is robustly silenced by hypermethylation even in early-stage primary LADC. The restoration of *TRIM58* expression in LADC cell lines inhibits cell growth, suggesting that aberrant inactivation of *TRIM58* consequent to CGI hypermethylation might stimulate the early carcinogenesis of LADC regardless of smoking status.[132]

Differentially methylated (DM) loci associated with lncRNA/mRNA expression are present in nonsmall-cell lung cancer (NSCLC). A total of 113,644 DM loci have been identified between tumors and adjacent tissues. Some 26,310 DM loci were associated with 1685 differentially expressed genes, and 839 genes had significant correlations between methylation and expression, of which 26 hypermethylated loci in transcription start site 200 have been correlated with low gene expression. Correlations between methylation and expression have been validated in five genes (*CDO1*, *C2orf40*, *SCARF1*, *ZFP106*, and *IFFO1*).[133]

ADP ribosylation factor (ARF)-like 4c (*ARL4C*) expression, induced by a combination of Wnt/β-catenin and EGF/Ras signaling, contributes to epithelial morphogenesis and ARL4C overexpression as a result of Wnt/β-catenin and EGF/Ras signaling alterations. *ARL4C* is involved in tumorigenesis. *ARL4C* expression correlates with DNA hypomethylation in the 3′ untranslated region (UTR) of the *ARL4C* gene during lymphogenesis. In tissue specimens from patients with lung or tongue squamous cell carcinoma (SCC), *ARL4C* is highly expressed in tumor lesions. *ARL4C* DNA is hypomethylated in the 3′ UTR. The ten-eleven translocation methylcytosine dioxygenase (TET) enzyme, which mediates DNA demethylation, is highly expressed in NCI-H520 cells. Knockout of *TET* family proteins (TET1–3) in NCI-H520 cells reduces 5-hydroxymethylcytosine (5hmC) levels and promotes DNA methylation in the 3′ UTR, leading to decreased *ARL4C* expression and ARL4C-mediated cellular migration. 5hmC is frequently detected in tumor lesions of ARL4C-positive lung SCC, and DNA methylation in the 3′ UTR of the *ARL4C* gene is lower than in nontumor regions. *ARL4C* is expressed due to hypomethylation in the 3′ UTR for certain types of cancers, and *ARL4C* methylation status is involved in cancer cell function.[134]

5.6.11 Melanoma

Melanoma is a malignant tumor of melanocytes and is considered to be the most aggressive cancer of all skin diseases. Several genetic and epigenetic factors have been associated with the development and progression of melanoma. Loss of the DNA hydroxymethylation mark (5-hydroxymethylcytosine, 5-hmC), along with DNA hypermethylation at promoter regions of several tumor suppressor genes, are used as biomarkers for melanoma. 5-Aza-2′-deoxycytidine, an epigenetic modifier causing DNA demethylation, and ten-eleven translocation family dioxygenase (TET), which catalyzes the generation of 5-hmC, show therapeutic potential in melanoma treatment.[135]

In experimental melanoma the DNA damage marker γH2AX is found to be increased in melanocytes after 24 h of deadhesion, accompanied by increased SIRT1 expression and decreased levels of its target, H4K16ac. SIRT1 starts to be associated with DNMT3B during the stress condition, and this complex is maintained along malignant progression. *Mxd1* is a target of both SIRT1 and DNMT3B and is downregulated from premalignant melanocytes to metastatic melanoma cells. The DNMT inhibitor 5AzaCdR reverses *Mxd1* expression, and *Sirt1* stable silencing increases *Mxd1* mRNA expression, leading to downregulation of MYC targets, such as *Cdkn1a*, *Bcl2*, and *Psen2*, whose upregulation is associated with human melanoma aggressiveness and poor prognosis.[5]

Uveal melanoma (UM) is a severe human malignancy with a high mortality rate. Epigenetic dysregulation is present in UM tumorigenesis, progression, and metastasis, including microRNA expression, hypermethylation of genes, and histone modification.[136]

Mutations in the BRCA1-Associated-Protein 1 (*BAP1*), a dynamic tumor suppressor, are associated with an increased risk of uveal melanoma, cutaneous melanoma, mesothelioma, and other forms of cancer. Germline *BAP1* mutations cause hereditary cancer susceptibility.[137]

Studies on DNA methylation in the development and metastasis of uveal melanoma (UM) revealed the involvement of several genes, including tumor suppressor genes (TSGs), cyclin-dependent kinase genes, and others, including Ras and EF-hand domain containing (*RASEF*) gene, *RAB31*, *hTERT*, embryonal fyn-associated substrate, and deleted in split-hand/split-foot 1.

TSG genes (*RASSF1A*, *p16INK4a*) encode cyclin-dependent kinase inhibitors. Hypermethylation of *RASSF1A* is observed in UM. Promoter methylation of *RASSF1A* is also associated with the development of metastasis. Hypermethylation of *p16INK4a* is also present in UM cell lines.[138] *PIWIL3* expression is increased in primary melanomas and has a positive correlation between primary melanoma *PIWIL3* expression and tumor thickness.[139]

5.6.12 Esophageal Squamous Cell Carcinoma

Esophageal squamous cell carcinoma (ESCC) is widely regarded as one of the most lethal types of cancer worldwide. Barrett esophagus (BE), a metaplastic condition affecting the lower oesophagus as a result of long-standing gastroesophageal reflux and chronic inflammation, is a precursor lesion for esophageal adenocarcinoma.[140] The risk for developing Barrett esophagus and/or esophageal adenocarcinoma (EAC) is associated with specific demographic and behavioral factors, including gender, obesity/elevated body mass index (BMI), and tobacco use. Alterations in DNA methylation, an epigenetic modification that can affect gene expression and that can be influenced by environmental factors, is frequently present in both BE and EAC and is believed to play a role in the formation of BE and its progression to EAC.[141] Differentially methylated loci have been found in esophagus tissues when comparing males with females, obese with lean individuals, and smokers with nonsmokers.[141]

Human transmembrane protein 176A (TMEM176A) is involved in primary ESCC. *TMEM176A* is highly expressed in BIC1 cells, and loss of *TMEM176A* expression is found in TE1, TE3, TE13, KYSE140, KYSE180, KYSE410, KYSE450, KYSE520, Segl, KYSE150, YES2, and COLO680N cells. Complete methylation is detected in TE1, TE3, TE13, KYSE140, KYSE180, KYSE410, KYSE450, KYSE520, Segl, KYSE150, YES2, and COLO680N cells, while unmethylation is detected in BIC1 cells. Restoration of *TMEM176A* expression can be induced by 5-Aza-2′-deoxycytidine treatment in methylated cell lines. *TMEM176A* is methylated in 66.7% of ESCC cells, and promoter region methylation is associated with tumor differentiation and reduced expression of *TMEM176A*. Methylation of *TMEM176A* is associated with poor five-year overall survival. TMEM176A inhibits cell invasion and migration, induces apoptosis in esophageal cancer cells, and suppresses esophageal cancer cell growth. TMEM176A is a potential tumor suppressor in human ESCC.[142]

The lncRNA *LUCAT1* is involved in the carcinogenesis of ESCC. *LUCAT1* is upregulated in ESCC cell lines and cancer tissue. *LUCAT1* knockdown reduces cell proliferation, induces apoptosis, and upregulates tumor suppressor genes by reducing DNA methylation in KYSE-30 cells. *LUCAT1* siRNA reduces DNA methyltransferase 1 (DNMT1) protein levels without affecting transcription. Patients with high *LUCAT1* expression have lower survival rates than patients with low *LUCAT1* expression. LUCAT1 regulates the stability of DNMT1 and inhibits the expression of tumor suppressors through DNA methylation, leading to the formation and metastasis of ESCC.[143]

Circular RNAs (circRNAs) have emerged as a new type of noncoding RNA with significant RNase resistance, wide abundance, and remarkable internal diversity. In malignant esophageal epithelial cell lines 813 upregulated and 445 downregulated circRNA candidates have been identified. Differentially expressed circRNAs have been found to be associated with pathways involved in metabolism, cell apoptosis, proliferation, and migration. *circRNA9927-NBEAL1* is particularly relevant in ESCC.[144]

5.6.13 Myelodysplastic Syndromes

Myelodisplastic syndrome (MDS)-associated mutations include *JAK2*, *MPL*, *LNK*, *CBL*, *CALR*, *TET2*, *ASXL1*, *IDH1*, *IDH2*, *IKZF1*, and *EZH2*.[145] Epigenetic regulators are the largest group of genes mutated in MDS patients. Most mutated genes belong to one of three groups of genes with normal functions in DNA methylation, in H3K27 methylation/acetylation, or in H3K4 methylation. Mutations in the majority of epigenetic regulators disrupt their normal function and induce a loss-of-function phenotype.[146]

Over 80 mutant genes were found in patients with MDS. The most frequent mutations were associated with epigenetics (50%), followed by spliceosome (37%), signal transduction (34%), transcription factors (24%), and cell cycle/apoptosis (17%). About 90% of MDS cases have at least one gene mutation.[147]

The cancer risk-associated rs6983267 SNP and the accompanying long noncoding RNA *CCAT2* in the highly amplified 8q24.21 region have been implicated in cancer predisposition. *CCAT2* overexpression leads to spontaneous myeloid malignancies. *CCAT2* is overexpressed in bone marrow and peripheral blood of myelodysplastic/myeloproliferative neoplasms (MDS/MPN) patients. CCAT2 induces global deregulation of gene expression by downregulating EZH2 in an allele-specific manner.[148]

5.6.14 Leukemia

Acute myeloid leukemia (AML) is a heterogeneous group of hematopoietic malignancies due to sophisticated genetic mutations and epigenetic dysregulation. miRNAs are important regulators of gene expression in leukemogenesis. miR-375 has been reported to be a suppressive miRNA in multiple types of cancers and in AML. The expression of miR-375 is decreased in leukemic cell lines and primary AML blasts as a result of DNA hypermethylation of precursor-miR-375 (pre-miR-375) promoter. Lower expression of miR-375 predicts poor outcome in AML patients. Overexpression of miR-375 reduces *HOXB3* expression and represses the activity of a luciferase reporter through binding 3′ untranslated regions (3′ UTR) of *HOXB3* mRNA. Overexpression of *HOXB3* partially blocks miR-375-induced arrest of proliferation and reduction of colony number, suggesting that HOXB3 plays an important role in miR-375-induced antileukemia activity. HOXB3 induces DNA methyltransferase 3B (DNMT3B) expression to bind the pre-miR-375 promoter and enhances DNA hypermethylation of pre-miR-375, leading to lower expression of miR-375.[149]

At least 47 genes were found to be aberrantly methylated in Chinese patients with leukemia. A further subgroup metaanalysis by leukemia subtype demonstrated that hypermethylation of five genes, namely cyclin-dependent kinase (*CDKN*)*2A*, DNA-binding protein inhibitor-4, *CDKN2B*, glioma pathogenesis-related protein 1, and p73, contributed to the risk for various subtypes of leukemia. A strong association between *CDKN2A* and leukemia has been identified in Chinese but not in European patients.[150]

The somatic translocation *t(8;21)(q22;q22)/RUNX1-RUNX1T1* is one of the most frequent rearrangements found in children with standard-risk acute myeloid leukemia (AML). The DNA methylation status of patients who suffer from relapse is different from that of children maintaining complete remission. Cell-to-cell adhesion and cell-motility pathways are aberrantly activated in relapsed patients. Most of these factors are RUNX1-RUNX1T1 targets. Ras Homolog Family Member (*RHOB*) overexpression is a key player.[151]

CHD4 is essential for cell growth of leukemic cells. Loss of function of *CHD4* in acute myeloid leukemia cells causes an arrest in the G0 phase of the cell cycle as well as downregulation of MYC and its target genes involved in cell cycle progression. Inhibition of CHD4 confers antileukemic effects on primary childhood acute myeloid leukemia cells and prevents disease progression.[152]

Juvenile myelomonocytic leukemia (JMML) is an aggressive myeloproliferative disorder of early childhood characterized by mutations activating RAS signaling. Three JMML subgroups have been identified with unique molecular and clinical characteristics. The high methylation group (HM) is characterized by somatic *PTPN11* mutations and poor clinical outcome. The low methylation group is enriched for somatic *NRAS* and *CBL* mutations, as well as for Noonan patients, and has a good prognosis. The intermediate methylation group (IM) shows enrichment for monosomy 7 and somatic *KRAS* mutations. Hypermethylation is associated with repressed chromatin, genes regulated by RAS signaling, frequent cooccurrence of RAS pathway mutations, and upregulation of DNMT1 and DNMT3B, suggesting a link between activation of the DNA methylation machinery and mutational patterns in JMML.[153, 154]

Epigenetic markers are associated with survival after relapse of B cell precursor acute lymphoblastic leukemia (BCP-ALL). In pediatric T cell ALL, promoter-associated methylation alterations correlate with prognosis. The CpG island methylation phenotype (CIMP) classification has been proposed as a strong candidate for improved risk stratification of relapsed BCP-ALL.[155]

Ten-eleven translocation 2 (*TET2*) is frequently mutated somatically in both myeloid and lymphoid malignancies. Mutations tend to cluster in the C-terminal enzymatic domain and a cysteine-rich (CR) domain of unknown function. The CR domain binds chromatin preferentially at the histone H3 tail by recognizing H3 lysine 36 monomethylation and dimethylation (H3K36me1/2). Missense mutations in the CR domain perturb TET2 recruitment to the target locus and its enzymatic activities. This novel H3K36me recognition domain might represent a critical link between histone modification and DNA hydroxylation in leukemogenesis.[156]

TET2 mutations result in impairment of the dioxygenase activity of TET2 that interferes with 5-mC to 5-hmC conversion. *TET2* mutations are a driver of tumorigenesis in blood cells, and *TET2* mutations are often acquired at the hematopoietic stem/early progenitor cell stage. *TET2* is the second most frequently mutated gene in clonal

hematopoiesis in individuals with no apparent blood cancers, suggesting that while *TET2* mutations alone are insufficient to cause hematologic malignancy they represent an early event during tumorigenesis.[157]

The global methylation profile of high CpG-rich regions based on IGHV mutational status has been characterized in chronic lymphocytic leukemia (CLL). Subhash et al.[158] identified 5800 hypermethylated and 12,570 hypomethylated CLL-specific differentially methylated genes (cllDMGs) compared with normal controls. From cllDMGs 40% of hypermethylated and 60% of hypomethylated genes were mapped to noncoding RNAs. The major repetitive elements such as short interspersed elements (*SINE*) and long interspersed elements (*LINE*) have a high percentage of cllDMRs (differentially methylated regions) in IGHV subgroups compared with normal controls. Two novel lncRNAs (hypermethylated *CRNDE* and hypomethylated *AC012065.7*) were validated in an independent CLL sample cohort. The methylation levels showed an inverse correlation with gene expression levels, and survival analysis revealed that hypermethylation of *CRNDE* and hypomethylation of *AC012065.7* correlated with an inferior outcome.

High HDAC4 levels are detected in cases with *FLT3-ITD* mutations in AML. Decreasing expression levels of both HDAC4 and SIRT6 are observed during the induction treatment of FAB M5-type AML. There is a strong correlation between the expression levels of HDAC4 and SIRT6 in AML.[159]

5.6.15 Lymphoma

LSD1 is upregulated and H3K4me1 and H3K4me2 are downregulated in cases with mantle cell lymphoma (MCL), compared with those with proliferative lymphadenitis. *LSD1* silencing decreases the levels of apoptosis-related proteins Bcl-2, pro-caspase-3 and C-myc, and DNMT1, and increases p15, inducing apoptosis. *LSD1* silencing increases the expression of H3K4me1 and H3K4me2, and histone acetylated H3 in JeKo-1 and MOLT-4 cells. *LSD1* siRNA also decreases cyclin D1 expression. Overexpression of LSD1 may be associated with MCL pathogenesis.[160]

Sestrin1 is a tumor suppressor in follicular lymphoma that controls mTORC1 activity and is inactivated by chromosomal deletions or epigenetically silenced by mutant *EZH2Y641X*. EZH2 inhibition promotes Sestrin1 reexpression to restore its tumor suppressor activity.[161]

Waldenström macroglobulinemia (WM) is a low-grade B cell lymphoma, classified as a lymphoplasmacytic lymphoma, characterized by the presence of clonal lymphoplasmacytic cells in the bone marrow and serum monoclonal immunoglobulin-M in the circulation. WM cells present with aberrant histone hypoacetylation that may be explained, at least in part, via deregulated microRNAs.[162]

5.6.16 Multiple Myeloma

Multiple myeloma is characterized by clonal proliferation of plasma cells within the bone marrow resulting in anemia, lytic bone lesions, hypercalcemia, and renal impairment.[163] Genetic defects, tumor-microenvironment interactions, and epigenetic aberrations are associated with MM. Epigenetic mechanisms (DNA methylation, histone modifications, ncRNAs) are important contributing factors in MM with impact on disease initiation, progression, clonal heterogeneity, and response to treatment.[164]

miRNAs are key regulators of gene expression and have been reported to exert transcriptional control in multiple myelomas. The presence of lesions in genes encoding DNA methylation modifiers and the histone demethylase KDM6A/UTX are predictors of poor prognosis. The frequency of mutations in epigenetic modifiers appears to increase following treatment, most notably in genes encoding histone methyltransferases and DNA methylation modifiers.[165]

5.6.17 Other Modalities of Cancer

5.6.17.1 Oral Carcinoma

Oral tumors are a heterogeneous group of tumors with different histopathological and molecular features. Genetic and epigenetic alterations are often detected in the development of oral cancer.[166–168] A metaanalysis to clarify the effect of RAS association domain family protein 1a (*RASSF1A*), retinoic acid receptor beta (*RARβ*), and E-cadherin (*CDH1*) promoter hypermethylation on the risk of oral cancer confirmed that *RASSF1A*, *RARβ*, and *CDH1* promoter hypermethylation might increase the risk of oral cancer.[167]

Oral squamous cell carcinoma (OSCC) is the most common type of oral neoplasm, accounting for over 90% of all oral malignancies and 38% of head and neck tumors. Diverse genetic, epigenetic, and environmental factors are involved in the pathogenesis of this neoplasm of poor prognosis.[169] A total of 14 lncRNAs were found to be

upregulated and 13 downregulated in OSCC. The expression levels of *SOX21-AS1* are decreased in OSCC. The promoter activity of *SOX21-AS1* is suppressed by methylation, and aberrant promoter hypermethylation of *SOX21-AS1* is frequently found in OSCC tissues. SOX21-AS1 suppresses oral cancer cell growth and invasion, and the levels of SOX21-AS1 correlate with an advanced stage, large tumor size, and poor disease-specific survival in OSCC patients.[170]

5.6.17.2 Testicular Teratoma

Spontaneous testicular teratoma is a type of tumor that develops from primordial germ cells (PGCs) in embryos. Mutation in the dead-end 1 (*Dnd1*) gene, which encodes an RNA-binding protein, enhances teratoma formation in a strain of mice (129/Sv). The levels of histone H3 lysine 27 (H3K27) trimethylation (me3) and its responsible methyltransferase, enhancer of zeste homolog 2 (Ezh2), are decreased in the teratoma-forming cells of *Dnd1* mutant embryos. Dnd1 suppresses miR-26a-mediated inhibition of Ezh2 expression, and *Dnd1* deficiency results in decreased H3K27me3 of Ccnd1, a cell cycle regulator gene. Ezh2 expression or Ccnd1 deficiency repress the reprogramming of PGCs into pluripotent stem cells, which mimick the conversion of embryonic germ cells into teratoma-forming cells.[171]

TP53, the gene encoding the p53 protein, is the most frequently mutated gene among all human cancers, whereas tumors that retain the wild-type *TP53* gene often use alternative mechanisms to repress the p53 tumor suppressor function. Testicular teratocarcinoma cells rarely contain mutations in *TP53*, yet the transcriptional activity of wild-type *p53* is compromised, despite its high expression level. In the teratocarcinoma cell line NTera2, p53 is subject to lysine methylation at its carboxyl terminus, which has been shown to repress p53's transcriptional activity. Reduction of cognate methyltransferases reactivates p53 and promotes differentiation of the NTera2 cells. Reconstitution of methylation-deficient *p53* mutants into p53-depleted NTera2 cells results in elevated expression of p53 downstream targets and precocious loss of pluripotent gene expression compared with reexpression of wild-type *p53*. Lysine methylation of endogenous wild-type p53 represses its activity in cancer cells.[172]

5.6.17.3 Bladder Cancer

Bladder cancer is a modality of cancer with high frequency in males (male:female ratio 4.1:1; 3.6:1 for mortality). Bladder cancer is associated with mutations in over 150 genes, including fibroblast growth factor receptor 3 (*FGFR3*) (for low-grade, noninvasive papillary tumors) and tumor protein p53 (*TP53*) (for high-grade, muscle-invasive tumors). Altered gene expression might be present in up to 500 coding sequences in low-grade and up to 2300 in high-grade tumors, under the regulatory control of noncoding RNAs.[173, 174]

5.6.17.4 Neuroendocrine Tumors

Neuroendocrine tumors (NETs) are a very heterogeneous group of tumors that are thought to originate from the cells of the endocrine and nervous systems, with manifestation in the gastrointestinal and pulmonary systems.[175] Somatostatin receptors (SSTRs), as byproducts of *SSTR1-5* gene superfamily, are commonly expressed in NETs. Tumor SSTR expression status is associated with clinical outcomes in NET. High expression of SSTR2 is associated with longer overall survival.[176]

5.6.17.5 Parathyroid Carcinoma

Parathyroid carcinoma (PTC) is a rare malignancy representing approximately 0.005% of all cancers. PTC may present as part of a complex hereditary syndrome or as an isolated nonhereditary endocrinopathy. Hereditary and syndromic forms of PTC, such as hyperparathyroidism-jaw tumor syndrome (HPT-JT), multiple endocrine neoplasia types 1 and 2 (MEN1 and MEN2), and familial isolated primary hyperparathyroidism (FIHP) show a clear genetic component. Cell division cycle 73 (*CDC73*) germline mutations cause HPT-JT, and *CDC73* mutations occur in 70% of sporadic PC. Other genes involved in sporadic PTC include germline *MEN1* and rearranged during transfection (*RET*) mutations and somatic alterations of the retinoblastoma 1 (*RB1*) and tumor protein P53 (*TP53*) genes, as well as epigenetic aberrations.[177] Over 90% of PTCs are hormonally active hypersecreting parathormone (PTH). Somatic inactivating mutations of the *CDC73/HRPT2* gene, encoding parafibromin, are the most frequent genetic anomalies occurring in PTCs. Aberrant DNA methylation and microRNA dysregulation are present in PTC.[178]

5.6.17.6 Cholangiocarcinoma

The *LINE-1* methylation level tends to be lower in high-grade differentiation, lymphatic emboli, and higher T stage cholangiocarcinoma. *LINE-1* hypomethylation is also linked to lower cancer-specific survival. DNA methylation changes occurring in cancer cells are featured with both promoter CpG island hypermethylation and diffuse genomic hypomethylation. Long interspersed element-1 (*LINE-1*) is repeated in an interspersed manner with an estimated

500,000 copies per genome. *LINE-1* has its CpG sites in the 5′ untranslated region methylated heavily in normal cells and undergoes demethylation in association with oncogenesis. Tumor differentiation, lymphatic invasion, and T stage are associated with a low average methylation level of *LINE-1*.[179]

5.6.17.7 Pancreatic Cancer

miR-377 through targeting DNMT1 may reduce DNA methylation of some tumor suppressor genes and restore their expression in pancreatic cancer cells.[180]

5.6.17.8 Pheochromocytomas and Paragangliomas

Mutations in Krebs cycle genes are frequently found in patients with pheochromocytomas and paragangliomas. Disruption of SDH, FH, or MDH2 enzymatic activities lead to accumulation of specific metabolites, which give rise to epigenetic changes in the genome that cause a characteristic hypermethylated phenotype. Some tumors carry cancer-predisposing somatic mutations in the *IDH1* or *GOT2* variant c.357A > T associated with higher tumor mRNA and protein expression levels. A truncating germline *IDH3B* mutation was found in a patient with a single paraganglioma showing an altered α-ketoglutarate/isocitrate ratio.[181]

5.6.17.9 Pituitary Tumors

Genetic mutations involving oncogenes or tumor suppressor genes are infrequent in pituitary tumors where epigenetic changes accumulate.[182]

5.6.17.10 Ewing Sarcoma

Differential methylation analysis between Ewing sarcoma and reference samples revealed 1166 hypermethylated and 864 hypomethylated CpG sites corresponding to 392 and 470 genes, respectively. Differential hypermethylation of CpGs located in the body and S shore of the *PTRF* gene in Ewing sarcoma correlates with its repressed transcriptional state. Reintroduction of PTRF/Cavin-1 in Ewing sarcoma cells revealed a role of this protein as a tumor suppressor. Restoration of caveolae in the membrane of Ewing sarcoma cells disrupts the MDM2/p53 complex, which consequently results in the activation of p53 and the induction of apoptosis.[181]

5.6.17.11 Osteosarcoma

lncRNA *ZEB1-AS1* is upregulated in osteosarcoma. Increased expression of *ZEB1-AS1* correlates with larger tumor size, progressed Enneking stage, tumor metastasis, worse recurrence-free and overall survival of osteosarcoma patients. Enhanced expression of *ZEB1-AS1* promotes osteosarcoma cell proliferation and migration, and *ZEB1-AS1* knockdown inhibits osteosarcoma cell proliferation and migration. ZEB1-AS1 binds and recruits p300 to the *ZEB1* promoter region, induces an open chromatin structure, and activates *ZEB1* transcription. ZEB1 depletion abrogates the roles of ZEB1-AS1 on the proliferation and migration of osteosarcoma cells. ZEB1-AS1 functions as an oncogene in osteosarcoma via epigenetically activating ZEB1.[183]

5.6.17.12 Chondrosarcoma

Chondrosarcoma (CS) is the second most common primary malignant bone tumor. CS is highly resistant to conventional chemotherapy and radiotherapy. DNA methylation-associated epigenetic changes have been found to play a pivotal role in the initiation and development of CS.[184]

5.6.17.13 Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is a hormonal and metabolic disorder affecting 5%–20% of reproductive-aged women worldwide that results in androgen excess, menstrual dysfunction, and oligoovulatory subfertility, with increased risks for type 2 diabetes, endometrial adenocarcinoma,[185] and potentially vascular disease. PCOS is a complex genetic trait with strong heritability (70%) and an epigenetic component.[186] Nearly 100 different genes might be involved in PCOS pathogenesis. PCOS granulosa cells show 25% reduction in DNA methylation. Hypomethylated genes associated with the synthesis of lipids and steroids may exhibit an aberrant expression promoting the synthesis of androgens, which may partially explain the hyperandrogenism present in women with PCOS.[187]

5.7 METABOLIC DISORDERS

Prenatal exposure to stress is associated with adverse health outcomes later in life, and DNA methylation is considered one possible underlying mechanism.[188] Hypermethylation patterns have been identified in subjects with a particular constitutional profile (i.e., phlegm-dampness constitution), potentially prone to develop metabolic disorders. In the Chinese population, 288 differentially methylated probes in 256 genes revealed hypermethylation of the *SQSTM1*, *DLGAP2*, and *DAB1* genes associated with diabetes mellitus, *HOXC4* and *SMPD3* associated with obesity, and *GRWD1* and *ATP10A* associated with insulin resistance.[189]

5.7.1 Lipid Metabolism

DNA methylation is associated with blood lipid levels (i.e., triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol).

Five CpG sites in the *DHCR24*, *CPT1A*, *ABCG1*, and *SREBF1* genes have been identified. Four CpG sites were associated with triglycerides (*CPT1A* (cg00574958 and cg17058475), *ABCG1* (cg06500161), and *SREBF1* (cg11024682)), two CpG sites were associated with HDL-C (*ABCG1* (cg06500161) and *DHCR24* (cg17901584)), and no associations were detected for LDL-C or total cholesterol.[190] A genome-wide association study (GWAS) identified loci associated with the plasma triglyceride (TG) response to ω-3 fatty acid (FA) supplementation in *IQCJ*, *NXPH1*, *PHF17*, and *MYB* genes. Associations between 12 tagged SNPs of *IQCJ*, 26 of *NXPH1*, 7 of *PHF17*, and 4 of *MYB* and gene-specific CpG site methylation levels have been found, suggesting that the response of plasma TG to ω-3 FA supplementation may be modulated by the effect of DNA methylation on the expression levels of specific genes.[191]

Methylation of long interspersed nuclear element-1 (*LINE-1*) in leukocytes is associated with dyslipidemia in the Japanese population. Hypermethylation is observed in the high LDL cholesterol and high LDL/HDL ratio groups. Subjects with two or more lipid abnormalities exhibit higher hypermethylation levels, indicating that *LINE-1* DNA hypermethylation in leukocytes is associated with CVD risk profiles (high LDLC, high LDL/HDL ratio, and the degree of abnormal lipid metabolism).[192]

5.7.2 Obesity

Approximately 1.5 billion people worldwide are overweight or affected by obesity, and are at risk for developing type 2 diabetes, cancer, cardiovascular disease, and related metabolic and inflammatory disturbances. Obesity is a major problem of health in the Western population. The prevalence of obesity has increased substantially in the past decades, and the burden of obesity-related complications has been growing steadily. Major pathogenic risk factors for obesity include genomic defects, epigenetic aberrations, and multiple environmental factors.[193]

Adiposity may influence DNA methylation. Body mass index (BMI) is associated with widespread changes in DNA methylation (187 genetic loci). Alterations in DNA methylation are predominantly the consequence of adiposity, rather than the cause. Methylation loci are enriched for functional genomic features in multiple tissues, and sentinel methylation markers identify gene expression signatures at 38 loci. The methylation loci identify genes involved in lipid and lipoprotein metabolism, substrate transport, and inflammatory pathways. Disturbances in DNA methylation predict future development of type 2 diabetes and other adverse clinical consequences of obesity.[194] Genome-wide association studies (GWAS) identified 100 obesity-associated genetic variants.[195] The first EWAS for adiposity in Africans identified three epigenome-wide significant loci (*CPT1A*, *NLRC5*, and *BCAT1*) for both general adiposity and abdominal adiposity.[196] Studies on genome-wide DNA promoter methylation along with mRNA profiles in paired samples of human subcutaneous adipose tissue and omental visceral adipose tissue from nonobese vs. obese individuals identified a negative correlation between the methylation and expression of several obesity-associated genes. *HAND2*, *HOXC6*, *PPARG*, *SORBS2*, *CD36*, and *CLDN1* have been identified as adipose tissue depot-specific genes.[197]

MacroH2A1 is a variant of histone *H2A*, present in two alternately exon-spliced isoforms, macroH2A1.1 and macroH2A1.2, that regulate cell plasticity and proliferation, during pluripotency and tumorigenesis. MacroH2A1.1 protein levels in the visceral adipose tissue of obese humans positively correlate with BMI, while macroH2A1.2 is nearly absent. MacroH2A1.2 overexpression in mouse adipose tissue induces changes in the transcript levels of key adipogenic genes. MacroH2A1.2 overexpression inhibits adipogenesis, while overexpression of macroH2A1.1 has the opposite effect.[198]

Metabolic diseases may originate in early life, and epigenetic changes are implicated as key drivers of this early-life programming. Epigenetic marks present at birth may predict an individual's future risk for obesity and type 2

diabetes. Studies on epigenetic marks in the blood of newborn children did not find individual methylation sites at birth to be associated with obesity or insulin sensitivity measures at 5 years. However, DNA methylation in 69 genomic regions at birth was associated with BMI.[199] Maternal obesity and diabetes during pregnancy are strongly associated with altered fetal growth and development as well as with lifelong perturbations in metabolic tissues. Maternal obesity alters the epigenome of the next generation, with the consequent impact on growth, organ development, metabolic disorders, and cardiovascular disease.[200, 201] Male obesity is a major risk factor for serious chronic diseases, reproductive capacity, and offspring health. Obesity-related impaired spermatogenesis is associated with a decrease in microscopic and molecular sperm characteristics and pregnancy success. Epigenetics is an important mediator explaining interactions between an obesogenic environment and sperm/offspring outcomes. Father-to-child effects have been reported in relation to preconceptional nutritional and life style-related factors. The obesogenic environment of the father before conception is a potential origin of health or disease in the offspring.[202]

There is a link between DNA methylation, obesity, and adiposity-related diseases. An association study of body mass index (BMI) and differential methylation for over 400,000 CpGs in whole blood-derived DNA from 3743 participants in the Framingham Heart Study and the Lothian Birth Cohorts identified novel and previously reported BMI-related differential methylation at 83 CpGs that replicated across cohorts. BMI-related differential methylation has been associated with concurrent changes in the expression of genes in lipid metabolism pathways. Methylation at one of the 83 replicated CpGs, cg11024682 (intronic to sterol regulatory element binding transcription factor 1 (*SREBF1*)) demonstrated links to BMI, adiposity-related traits, and coronary artery disease.[203]

Neonatal adiposity is a risk factor for future obesity. Maternal prepregnancy BMI is associated with decreased methylation at five CpG sites near the *LEP* transcription start site. Maternal BMI and cord blood leptin are closely associated, and cord blood leptin positively correlates with neonatal adiposity measures including birth weight, fat mass, and percent body fat.[204] Alterations in genetics, epigenetics, and microbiota influence childhood obesity. Epigenetics plays a key role in transmitting obesity risk to offspring. SNPs at genetic loci for adipokines and their receptors are associated with obesity.[205]

An epigenome-wide association study (EWAS) on obesity in African American healthy youth and young adults identified 76 obesity-related CpG sites in leukocytes. Of the 54 validated CpG sites, 29 associations with obesity were novel and 37 were replicated (71.5%) in neutrophils. A total of 51 CpG sites were associated with at least one cardiometabolic risk factor, and 9 after adjustment for obesity. Some 16 CpG sites were associated with expression of 17 genes in *cis*, 5 of which displayed differential expression between obese cases and lean controls.[206]

DNA methylation changes in whole blood are strongly associated with obesity and insulin resistance. A total of 49 differentially methylated cytosines (DMCs) are altered in obese subjects; 2 sites (Chr.21:46,957,981 and Chr.21:46,957,915) in the 5′ untranslated region of solute carrier family 19 member 1 (*SLC19A1*) show decreased methylation in obesity. A differentially methylated region (DMR) analysis demonstrated a decrease in methylation of Chr.21:46,957,915-46,958,001 in *SLC19A1* of −34.9% (70.4% lean vs. 35.5% obese).[207] A total of 94 CpGs associated with body mass index (BMI) and 49 CpGs associated with waist circumference, located in 95 loci, have recently been validated, and 70 CpGs associated with BMI and 33 CpGs related to waist circumference have been newly discovered. These CpGs explained 25.94% and 29.22% of the variability of BMI and waist circumference, respectively, in the sample; 95 loci were validated in genome-wide association studies, 10 of which had Tag SNPs associated with BMI. Functional and pathway analysis identified neurologic, psychological, endocrine, and metabolic dysfunction associated with obesity.[208]

The study of body composition by bioimpedance analysis and genome-wide DNA methylation in preschool children from four European countries revealed specific DNA methylation variants associated with BMI, fat mass, fat-free mass, fat mass index, and fat-free mass index in *SNED1*(*IRE-BP1*), *KLHL6*, *WDR51A* (*POC1A*), *CYTH4-ELFN2*, *CFLAR*, *PRDM14*, *SOS1*, *ZNF643* (*ZFP69B*), *ST6GAL1*, *C3orf70*, *CILP2*, *MLLT4*, and ncRNA *LOC101929268*, linking DNA methylation with lipid and glucose metabolism, diabetes, and body size/composition in children.[209] Genome-wide analysis identified 734 CpGs (783 genes) differentially methylated in obese children. The DNA methylation levels of *VIPR2*, *GRIN2D*, *ADCYAP1R1*, *PER3*, and *PTPRS* regions correlated with the obesity trait.[210]

Tet methylcytosine dioxygenase (TET) is catalytically capable of oxidizing DNA 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) toward complete removal of methylated cytosine. Both *Tet1* and *Tet2* genes are upregulated in a time-dependent manner, accompanied by increased expression of hallmark adipogenic genes such as *Pparγ* and *Fabp4*. TET upregulation leads to reduced DNA methylation and elevated hydroxymethylcytosine at the *Pparγ* locus; knockdown of *Tet1* and *Tet2* blocks adipogenesis by repression of *Pparγ* expression.[211]

Genetic mutations in *SLC39A13/ZIP13*, a member of the zinc transporter family, are known to reduce adipose tissue mass in humans. *Zip13*-deficient mice show enhanced beige adipocyte biogenesis and energy expenditure, as well as ameliorated diet-induced obesity and insulin resistance. An accumulation of the CCAAT/enhancer binding

protein-β (C/EBP-β) protein, which cooperates with dominant transcriptional coregulator PR domain containing 16 (PRDM16) to determine brown/beige adipocyte lineage, is essential for the enhanced adipocyte browning caused by the loss of *ZIP13*. ZIP13-mediated zinc transport is a prerequisite for degrading the C/EBP-β protein to inhibit adipocyte browning.[212]

The accumulation of reactive oxygen species (ROS) promotes vascular disease in obesity. The adaptor p66Shc appears to be a key molecule responsible for ROS generation and vascular damage. ROS-driven endothelial dysfunction is observed in visceral fat arteries (VFAs) isolated from obese subjects. Gene profiling of chromatin-modifying enzymes in VFA revealed a significant dysregulation of methyltransferase SUV39H1, demethylase JMJD2C, and acetyltransferase SRC-1 in obese VFA. These changes are associated with reduced dimethylation (H3K9me2) and trimethylation (H3K9me3) as well as acetylation (H3K9ac) of histone 3 lysine 9 (H3K9) on *p66Shc* promoter. Reprogramming SUV39H1, JMJD2C, and SRC-1 in isolated endothelial cells suppresses p66Shc-derived ROS, restores nitric oxide levels, and rescues endothelial dysfunction. SUV39H1 is the upstream effector orchestrating JMJD2C/SRC-1 recruitment to *p66Shc* promoter. SUV39H1 overexpression in obese mice erases H3K9-related changes on *p66Shc* promoter, while *SUV39H1* genetic deletion in lean mice recapitulates obesity-induced H3K9 remodeling and *p66Shc* transcription.[213]

Failure in glucose response to insulin is a common pathology associated with obesity. A differential methylation pattern has been found between diabetic and nondiabetic samples in 24 CpGs that map to 16 genes. The *HOOK2* gene shows differentially hypermethylated regions and association with type 2 diabetes. Samples from females with diabetes were found hypermethylated at the cg04657146-region and cg 11738485-region of the *HOOK2* gene.[214]

Obesity is recognized as a major risk for colorectal cancer. Transcriptome analysis shows that obesity-related colonic cellular metabolic switch favoring long-chain fatty acid oxidation occurs in young mice, while obesity-associated downregulation of negative feedback regulators of proproliferative signaling pathways is present in older mice. Colonic DNA methylome is preprogrammed by obesity at a young age, priming for a tumor-prone gene signature after aging.[115]

Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear receptor with antineoplastic effects that is deregulated in obesity. miR-27b, 130b, and 138 are upregulated in obese and CRC patients, who also show low PPARγ levels. *PPARγ* promoter hypermethylation is present in CRC patients, correlating with low PPARγ levels. Upregulation of microRNAs 27b, 130b, and 138 is associated with susceptibility to CRC in obese subjects through PPARγ downregulation, and hypermethylation of the *PPARγ* gene promoter is associated with CRC through suppression of PPARγ regardless of BMI.[215] The study of *PPARγ* methylation across 23 CpG sites shows that *PPARγ* methylation levels tend to increase with age. Methylation at birth is inversely associated with birth weight, and methylation at 9 years is inversely associated with 9-year BMI.[215] Peroxisome proliferator-activated receptor γ coactivator 1α (Pgc-1α) is a critical regulator of brown adipose tissue (BAT) thermogenesis, which is highly inducible by environmental stimuli such as cold and diet. Interactions between histone modifications and transcription factors at the *Pgc-1α* promoter cause BAT *Pgc-1α* transcription in response to cold. Histone modifications also modulate BAT *Pgc-1α* transcription in response to nutrients. *Pgc-1α* DNA methylation and RNA expression correlate with indicators of adiposity and glucose homeostasis across numerous human tissues.[216]

Changes in the adipose tissue, miRNome, have been characterized in obesity and weight loss[217] to identify molecular pathways affected by obesity and weight changes. Next generation sequencing (NGS) studies identified miRNAs differentially expressed in 47 samples of visceral (VAT) and subcutaneous (SAT) adipose tissues from normal weight (N), obese (O), and obese after surgery-induced weight loss (PO) individuals. NGS identified 344 miRNAs expressed in adipose tissues with ≥5 reads per million. The expression of 54 miRNAs differed between VAT-O and SAT-O, 20 miRNAs differed between SAT-O and SAT-N, 79 miRNAs differed between SAT-PO and SAT-N, and 61 miRNAs differed between SAT-PO and SAT-O.[217]

5.7.3 Diabetes

Type 2 diabetes mellitus (T2D) is a metabolic disorder predisposing to diabetic cardiomyopathy, atherosclerotic cardiovascular disease (CVD), heart failure, and many other disturbing health consequences. Genetic, epigenetic, and environmental factors are involved in the pathogenesis of diabetes.[4] SNPs identified by GWAS explain less than 20% of the estimated heritability for T2D. Case-control studies and studies examining the impact of nongenetic and genetic risk factors on DNA methylation in humans have identified epigenetic changes in tissues from subjects with T2D vs. nondiabetic controls.[218] T2D genome-wide association studies (GWAS) in a cohort of 70,127 subjects identified 7 novel associated regions, including 5 common variants (*LYPLAL1*, *NEUROG3*, *CAMKK2*, *ABO*, and *GIP* genes), a low-frequency (*EHMT2*) locus, and a rare variant (rs146662057) in chromosome Xq23. rs146662057, associated with

a twofold increased risk for T2D in males, is located within an active enhancer associated with the expression of Angiotensin II Receptor type 2 gene (*AGTR2*), a modulator of insulin sensitivity.[219] Marked differences in the methylation status of CpG sites within *MHC* genes (cis-metQTLs) have been found between carriers of type 1 diabetes risk haplotypes HLA-DRB1*03-DQA1*0501-DQB1*0201 (*DR3-DQ2*) and HLA-DRB1*04-DQA1*0301-DQB1*0302 (*DR4-DQ8*). These differences were found in children and adults and were accompanied by reduced HLA-DR protein expression in immune cells with the *HLA-DR3-DQ2* haplotype.[220]

Intrauterine exposure to hyperglycemia confers increased metabolic risk in later life. Studies comparing pairwise DNA methylation differences between siblings whose intrauterine exposure to maternal gestational diabetes (GDM) were discordant identified 12 of 465,447 CpG sites with differential methylation. The most relevant were markers within genes associated with monogenic diabetes (*HNF4A*) or obesity (*RREB1*).[221]

DNA methyltransferase 1 (DNMT1) is associated with increased susceptibility to T2D in Han Chinese individuals. The nuclear receptor subfamily 4 group A member 1 (*NR4A1*) promoter is hypermethylated in patients with T2D and in a mouse model of T2D. DNA hypermethylation of the *NR4A1* promoter reduces *NR4A1* mRNA expression. Transient transfection of human *NR4A1* into RIN-m5F and 293T cells causes DNMT1 inhibition and induces insulin receptor activation. *NR4A1* knockdown by shRNA results in overexpression of DNMT1 and inhibition of insulin receptor, suggesting that the *NR4A1* gene is involved in the epigenetics pathway. T2D model mice treated with the DNMT1 inhibitor aurintricarboxylic acid (ATA) show reduced activation of DNMT1 in pancreatic β cells, reversing the changes in *NR4A1* expression and decreasing blood glucose. DNMT1 causes *NR4A1* DNA hypermethylation and blocks insulin signaling in patients with T2D.[222]

The development of insulin resistance in cardiac tissue decreases cellular glucose import and enhances mitochondrial fatty acid uptake. While triacylglycerol and cytotoxic lipid species begin to accumulate in the cardiomyocyte, the energy substrate utilization ratio of free fatty acids to glucose changes to almost entirely free fatty acids. miRNAs mediate this metabolic transition. Mitochondrial processes are regulated by miRNAs in the diabetic heart, and epigenetic changes, exosomal transport, and posttranslational sequestration contribute to regulating miRNA expression.[223]

In patients with latent autoimmune diabetes of the adult (LADA), Tregs are reduced and FOXP3 is downregulated in $CD4^+$ T cells. *STAT3*, *HDAC3*, *HDAC5*, *SIRT1*, *DNMT1*, and *DNMT3b* mRNAs are upregulated in LADA $CD4^+$ T cells, while *FOXP3* mRNA is decreased. p-STAT3 binds to the *Foxp3* promoter and histone H3 acetylation at K9 and K14 of the *FOXP3* promoter. Ectopic *STAT3* expression reduces *FOXP3* promoter activities. The *Foxp3* promoter is hypermethylated in LADA.[224]

Metabolic memory and epigenetic factors are important in the pathogenesis of diabetic complications and interact with genetic variants, metabolic factors, and clinical risk factors. miRNAs interact with epigenetic mechanisms and pleiotropically mediate the effects of hyperglycemia on the vasculature.[225]

5.7.3.1 Diabetic Retinopathy

Inhibition of the multifunctional deacetylase Sirtuin 1 (Sirt1) has been implicated in the pathogenesis of diabetic retinopathy.[226] SIRT6 deficiency causes major retinal transmission defects, changes in the expression of glycolytic genes, and elevated levels of apoptosis. High glucose levels induce retinal increase in vascular endothelial growth factor (VEGF) and loss of brain-derived neurotrophic factor (BDNF), accompanied by reduced levels of SIRT6 and increased acetylation levels of its substrates H3K9 and H3K56.[227]

5.7.3.2 Diabetic Vasculopathy

Cardiovascular disorders and renal disease are common complications in patients with diabetes, contributing to high morbidity and mortality. Hyperglycemia promotes tissue damage through reactive oxygen species (ROS) generation, which affects chromatin structure and gene expression leading to the upregulation of proinflammatory and profibrotic mediators.[228] ROS generated by upregulated NADPH oxidase (Nox) contribute to structural-functional alterations of the vascular wall in diabetes. HDACs mediate vascular Nox upregulation in diabetes, and HDAC inhibition reduces vascular ROS production in experimental diabetes.[229]

5.7.3.3 Gestational Diabetes

Gestational diabetes (GDM) is associated with increased risk of metabolic and neurological disorders in the offspring. Diabetes induces epigenetic changes in the transcription factor *Srebf2* (sterol regulatory element binding transcription factor 2), a master gene in regulation of cholesterol metabolism. Fetuses from diabetic mothers show growth restriction, decreased liver and brain weight, and decreased microglial activation in the hippocampus. CpG hypermethylation of the *Srebf2* promoter in the fetal liver and brain is associated with decreased *Srebf2* gene expression.[230]

miRNAs are implicated in type 2 diabetes, and circulating miRNAs affect pregnancy and may represent a risk for gestational diabetes. Of 10 miRNAs selected (miR-126-3p, miR-155-5p, miR-21-3p, miR-146b-5p, miR-210-3p, miR-222-3p, miR-223-3p, miR-517-5p, miR-518a-3p, and miR-29a-3p), the miR-155-5p and miR-21-3p levels were positively associated with GDM. miR-146b-5p and miR-517-5p were borderline. Associations of miR-21-3p and miR-210-3p with GDM have been observed among overweight/obese but not lean women; and associations of six miRNAs (miR-155-5p, miR-21-3p, miR-146b-5p, miR-223-3p, miR-517-5p, and miR-29a-3p) with GDM were present only among women carrying male fetuses. These results reported by Wander et al.[231] indicate that circulating early-mid-pregnancy miRNAs are associated with GDM, particularly among women who are overweight/obese prepregnancy or pregnant with male offspring.

5.7.4 Malnutrition and Starvation

Early-life malnutrition has been associated with neurodevelopment and adulthood neuropsychiatric disorders. Different epigenetic mechanisms can mediate this correlation.[232] Severe acute malnutrition (SAM) in infants may present as one of two distinct syndromic forms: nonedematous (marasmus), with severe wasting and no nutritional edema; or edematous (kwashiorkor) with moderately severe wasting. Significant differences in methylation of CpG sites from 63 genes have been observed in skeletal muscle DNA from survivors of marasmus and kwashiorkor, affecting genes in the immune, body composition, metabolic, musculoskeletal growth, neuronal function, and cardiovascular pathways.[233]

Starvation can cause long-term effects on homeostasis through activation of epigenetic mechanisms. Starvation in an early larval stage of *Caenorhabditis elegans* causes a depletion of AMP-activated protein kinase (AMPK), a master regulator of cellular energy homeostasis, which results in developmental defects following their recovery of food and sterility. *AMPK* loss in this quiescent period may be responsible for transgenerational phenotypes that can become progressively worse with each successive generation. *AMPK* might affect this process, and the initial transcription that occurs in germ cells may adversely affect subsequent germline gene expression and genomic integrity.[234]

The *Drosophila* histone methyltransferase G9a (dG9a) is key to acquiring tolerance to starvation stress. The depletion of dG9a leads to high sensitivity to starvation stress in adult flies, while its overexpression induces starvation stress resistance. dG9a plays an important role in maintaining energy reservoirs including amino acid, trehalose, glycogen, and triacylglycerol levels during starvation. Depletion of dG9a represses starvation-induced autophagy by controlling the expression level of *Atg8a*, a critical gene for the progression of autophagy, in a different manner from that in cancer cells.[235]

5.7.5 Metabolic Syndrome

Metabolic syndrome is a complex disorder in which obesity, diabetes, dyslipidemia, and high blood pressure converge to generate a global metabolic disorder. Factors altering the inherited maternal epigenome may affect fetal and neonatal growth, thus contributing to metabolic syndrome. Potential causes of metabolic syndrome by in utero epigenetic alterations of genes involved in energy metabolism (*PPARγ* and *PPARα*), microRNAs, arginine methyltransferases, lysine demethylases, and histone deacetylaces have been elucidated.[236] Two differentially methylated CpG sites in the *IGF2BP1* gene on chromosome 17 (cg06638433) and the *ABCG1* gene on chromosome 21 (cg06500161) have been found to be associated with metabolic syndrome.[237] The methylated status of three loci, cg18181703 (*SOCS3*), cg04502490 (*ZNF771*), and cg02988947 (*LIMD2*), is associated with body mass index percentile (BMI%), a clinical index for obesity in children, adolescents, and adults, as well as with multiple metabolic syndrome (MetS) traits, including central obesity, fat depots, insulin responsiveness, and plasma lipids. The *SOCS3* methylation locus is also associated with the clinical definition of MetS. *SOCS3* methylation status is inversely associated with BMI%, waist to height ratio, triglycerides, and MetS, and positively correlated with HDL-C. Epigenetic modulation of *SOCS3*, a gene involved in leptin and insulin signaling, may play an important role in obesity and MetS.[238]

5.8 IMMUNOLOGICAL AND INFLAMMATORY DISORDERS

Inflammation is a biological response to tissue injury, pathogen invasion, and irritants. During the inflammatory phase, cells of both the innate and the adaptive immune system are activated and recruited to the site of inflammation. These mediators are downstream targets for transcription factors such as activator protein-1 (AP1), nuclear factor

kappa-light-chain-enhancer (NFκB), signal transducers and activators of transcription factors (STAT1), and interferon regulatory factors (IRFs), which control the expression of most immunomodulatory genes. p38 mitogen-activated protein kinase (p38MAK) is activated in response to lipopolysaccharide (LPS) stimulation, which results in the activation of AP-1 transcription factor and expression of proinflammatory cytokines, IL-12 and IL-23. Toll-Interleukin 1 Receptor (TIR) Domain Containing Adaptor Protein (TIRAP) serves as an adaptor molecule that brings Protein kinase C delta type (PKCδ) and p38 in close proximity, facilitating the activation of p38MAPK by PKCδ.[239]

Chronic low-grade inflammation reflects a subclinical immune response implicated in the pathogenesis of complex diseases. Epigenome-wide association studies (EWAS) of serum C-reactive protein (CRP), a sensitive marker of low-grade inflammation, have revealed differential methylation at 218 CpG sites to be associated with CRP in Europeans and 58 CpG sites (45 unique loci) among African Americans. DNA methylation at 9 (16%) CpG sites was associated with whole blood gene expression in *cis*, 10 (17%) CpG sites were associated with a nearby genetic variant, and 51 (88%) were also associated with at least one related cardiometabolic entity. CpG sites accounted for up to 6% interindividual variation of age-adjusted and sex-adjusted CRP, independent of known CRP-related genetic variants.[240]

Regulatory T (Treg) cells expressing the transcription factor *FOXP3* play a pivotal role in maintaining immunologic self-tolerance. EZH2 is recruited to the *FOXP3* promoter and its targets in Treg cells. EZH2 deficiency in $FOXP3^+$ T cells results in lethal multiorgan autoimmunity. EZH2Δ/Δ$FOXP3^+$ T cells lack a regulatory phenotype in vitro and secrete proinflammatory cytokines. EZH2Δ/Δ$FOXP3^+$ mice develop spontaneous inflammatory bowel disease.[241]

Chronic inflammatory disorders are associated with higher tumor incidence through epigenetic and genetic alterations. There is an association between inflammation marker C-reactive-protein (CRP) and global DNA methylation levels in peripheral blood leukocytes, especially in individuals with the minor allele of the *MTHFR* missense SNP rs1801133.[242]

DNA hypermethylation occurs during human hematopoietic differentiation. T cell-specific hypermethylated regions are strongly associated with open chromatin marks, enhancer elements, and binding sites of specific key transcription factors involved in hematopoietic differentiation, such as PU.1 and TAL1.[243] The role of T cells is pivotal in immunity and immunopathology. Activated T cells undergo clonal expansion and differentiation followed by a contraction phase after pathogenic clearance. Cell survival and cell death are critical for controlling the number of naive T cells, effector, and memory T cells. Naive T cells are highly reliant on BCL-2 and sensitive to BCL-2 inhibition. Activated T cells show a different pattern in the regulation of apoptosis by pro- and antiapoptotic members of the BCL-2 family. A mechanism of epigenetic regulation of cell survival unique to activated T cells has been proposed.[244]

5.8.1 Asthma

Epigenome-wide association studies provide support for associations between epigenetic regulation of gene expression and asthma.[245] Some 27 methylated CpG sites have been identified, 14 of which were associated with asthma. Lower methylation levels have been observed at all associated loci across childhood. These CpG sites and their associated transcriptional profiles indicate activation of eosinophils and cytotoxic T cells in childhood asthma.[246]

Exposure prior to conception may represent potential risk factors for offspring asthma. Studies on grandmaternal smoking during pregnancy and the risk of asthma in grandchildren revealed that children have an increased risk of asthma in the first six years of life if their grandmothers smoked during early pregnancy, independent of maternal smoking.[247]

Eosinophilic asthma (EA) is the most frequent form of the four asthma phenotypes. Some lncRNAs act in the early differentiation of T helper cells, controlling gene transcription, protein expression, and epigenetic regulation. A total of 41 dysregulated lncRNAs and 762 dysregulated mRNAs have been found in EA compared with control samples. The pathways most enriched in EA are measles, T cell receptor signaling pathway, peroxisome proliferator-activated receptors (PPAR) signaling pathway, Fc gamma R-mediated phagocytosis, NF (nuclear factor) kappa B signaling pathway, chemokine signaling pathway, and primary immunodeficiency.[248]

Epithelial barrier dysfunction is a central feature in the pathogenesis of allergic disease; and epithelial-mesenchymal transition (EMT) has been proposed as one mechanism afflicting barrier in asthma. Epithelial differentiation has been observed to be suppressed in asthma with insufficiency of insulin and Notch signaling and absence of conventional EMT markers. *EFNB2*, *FGFR1*, *FGFR2*, *INSR*, *IRS2*, *NOTCH2*, *TLE1*, and *NTRK2* are markers of dysregulation in epithelial-mesenchymal signaling.[249]

5.8.2 Rheumatoid Arthritis

Differential miRNA expression is observed in patients with rheumatoid artritis (RA) (13 miRNAs), in which expression of miR-103a-3p, miR-155, miR-146a-5p, and miR-26b-3p is upregulated, whereas miR-346 is downregulated. mRNA expression of *DICER1*, *AGO1*, *CREB1*, *DAPK1*, and *TP53* is downregulated with miR-103a-3p expression in first-degree relatives.[250] *SIRT3* mRNA expression is increased, whereas *SIRT2* mRNA expression is decreased in RA. Conversely, *SIRT2* and *SIRT3* mRNA expression increases in active RA.[251]

Other inflammatory bone disorders in which epigenetics may play a pathogenic role are osteoarthritis[252] and autoimmune juvenile idiopathic arthritis (JIA). JIA results from a complex interplay between genetics and environment. The $CD4^+$ T cell DNA methylome of 68 polyarticular and extended oligoarticular JIA patients revealed several CpG modules, specifically those enriched in CpG sites belonging to genes that mediate T cell activation, uniquely correlated with clinical activity.[253]

5.8.3 Gout

Gouty arthritis is the most common type of inflammatory and immune disease. Zhong et al.[254] studied *DNMT1*, *DNMT3A*, and *DNMT3B* polymorphisms potentially associated with gout susceptibility. The distribution frequencies of *DNMT1* rs2228611 AA genotype and A allele have been found to be significantly increased in patients with gout. The rs1550117 in *DNMT3A* and rs2424913 in *DNMT3B* exhibited no significant associations with gout susceptibility.

5.8.4 Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder with heterogeneous presentation and complex pathogenesis in which genetic, epigenetic, and environmental factors are involved. In SLE cases, differentially methylated CpGs (DMCs) at 7245 CpG sites have been identified in the genome. Type I interferon-regulated genes are hypomethylated in SLE. Genetic associations with SLE include *PTPRC* (*CD45*), *MHC-class III*, *UHRF1BP1*, *IRF5*, *IRF7*, *IKZF3*, and *UBE2L3*.[255] DNA methylation alterations in cytokine genes, such as IFN-related gene and retrovirus gene, have been found in SLE. Histone modifications such as histone methylation and acetylation lead to transcriptional alterations of several genes such as *PTPN22*, *LRP1B*, and *TNFSF70*. *DNMT1*-related miRNAs, renal function-associated miRNAs, miRNAs involved in the immune system, and other miRNAs have been used for phenotype classification.[256]

DNA hypomethylation participates in the pathogenesis of SLE. 3-Hydroxy butyrate dehydrogenase 2 (BDH2), a modulator of intracellular iron homeostasis, is involved in the regulation of DNA hypomethylation and hyperhydroxymethylation in lupus $CD4^+$ T cells. BDH2 expression is decreased, intracellular iron is increased, and the global DNA hydroxymethylation level is augmented, whereas the methylation level is reduced in lupus $CD4^+$ T cells. BDH2 is the target gene of miR-21 which promotes DNA demethylation in $CD4^+$ T cells through inhibition of BDH2 expression. BDH2 deficiency-induced dysregulation of iron homeostasis in $CD4^+$ T cells contributes to DNA demethylation in SLE.[257]

5.8.5 Psoriasis

Psoriasis is a complex chronic inflammatory cutaneous disorder with aberrant immunopathogenetic mechanisms, involving the role of Th1 and the IL-23/Th17 axis, skin-resident immune cells, and major signal transduction pathways under the influence of environmental factors and epigenetic changes.[258] Epidemiological studies demonstrating excessive paternal transmission provided the earliest evidence of epigenetic deregulation in psoriatic disease.[259] 5-Hydroxymethylcytosine (5-hmC) and ten-eleven translocation-2 (TET2) are involved in psoriasiform dermatitis. TET2 and 5-hmC are highly expressed in imiquimod-induced psoriasiform skin lesions.[260]

DNA methylation and genetic makers are closely associated with psoriasis. Zhou et al.[261] identified 129 SNP-CpG pairs that constitute 28 unique methylation quantitative trait loci and 34 unique CpGs. 18 SNPs were associated with psoriasis, forming 93 SNP-CpG pairs with 17 unique CpG sites. Some 11 of 93 SNP-CpG pairs, composed of 5 unique SNPs and 3 CpG sites, showed a methylation-mediated relationship between SNPs and psoriasis. The 3 CpG sites are located on the body of *C1orf106*, the *TSS1500* promoter region of *DMBX1*, and the body of *SIK3*.[261]

5.8.6 Inflammatory Bowel Disease

Spondyloarthritis (SpA), inflammatory bowel diseases (IBD), and psoriasis are the most frequent chronic inflammatory diseases, resulting from a combination of genetic predisposition and environmental factors. Epigenetic modifications include DNA methylation, histone modifications, and small and long noncoding RNAs.[262] Some 92 of the 163 inflammatory bowel disease (IBD) loci colocalize with noncoding DNA regulatory elements (DREs). Mutations in DREs can contribute to IBD pathogenesis through dysregulation of gene expression. A total of 902 novel IBD candidate genes have been identified, including genes specific for IBD subtypes and many noteworthy genes including *ATG9A* and *IL10RA*.[263]

There is an association between IBD susceptibility and two polymorphisms of *DLG5 R30Q* (rs1248696) and *P1371Q* (rs2289310). R30Q is significantly associated with reduced susceptibility to IBD in Europeans by allelic and dominant comparisons, but not in the overall population. No significant association was found between R30Q and Crohn disease (CD) or ulcerative colitis (UC). P1371Q is associated with increased risk for IBD in Europeans and Americans. In contrast, a decreased risk for IBD is observed in the Asian population for P1371Q.[264]

Na^+/H^+ exchanger-3 (NHE3) plays a crucial role in intestinal Na^+ absorption; its reduction is implicated in infectious and IBD-associated diarrhea. DNA methylation is involved in the pathophysiology of IBD. In vitro methylation of *NHE3* promoter construct (p-1509/+127) cloned into a cytosine guanine dinucleotide-free lucia vector decreases promoter activity in Caco-2 cells. The DNA methyltransferase inhibitor, 5-azacytidine, decreases DNA methylation of the *NHE3* gene and increases *NHE3* expression, increases *NHE3* mRNA levels, and increases *NHE3* expression in the ileum and colon. Small interfering RNA knockdown of *GADD45b*, a protein involved in DNA demethylation, decreases *NHE3* mRNA expression. These studies show that Na^+/H^+ exchanger-3 gene expression is regulated by an epigenetic mechanism involving DNA methylation.[265]

5.8.7 Allergy

Epigenetic mechanisms are critical for normal immune development. DNA methylation in whole blood is associated with total and allergen-specific IgE levels. At least 15 CpG sites are associated with IgE, mapping to biologically relevant genes, including *ACOT7*, *ILR5A*, *KCNH2*, *PRG2*, and *EPX*. A total of 331 loci are associated with allergen-specific IgE.[266]

Cow's milk allergy (CMA) is one of the most common food allergies in children. DNA methylation of Th1/Th2 cytokine genes and *FoxP3* affects CMA disease course. The miRNome is also implicated in the pathogenesis of allergy. Among the miRNAs differently expressed in peripheral blood mononuclear cells, 2 are upregulated and 14 are downregulated in children with active CMA. miR-193a-5p is the most downregulated miRNA. The predicted targets of miR-193a-5p are upregulated in CMA patients. miR-193a-5p is a posttranscriptional regulator of IL-4 expression and may have a role in IgE-mediated CMA.[267]

5.8.8 Atopic Dermatitis

Atopic dermatitis (AD) is a chronic inflammatory disease caused by the complex interaction of genetic, immune, and environmental factors. Candidate gene association studies indicate that filaggrin (*FLG*) null gene mutations are the most significant known risk factor for AD, and genes in the type 2 T helper lymphocyte (Th2) signaling pathways are the second most replicated genetic risk factor for AD. GWAS studies identified 34 risk loci for AD. Gene-profiling assays demonstrated that AD is associated with decreased gene expression of epidermal differentiation complex genes and elevated Th2 and Th17 genes. Hypomethylation of *TSLP* and *FCER1G* has been reported in AD. miR-155 targets the immune suppressor CTLA-4 and is overexpressed in infiltrating T cells in AD skin lesions.[268]

5.9 OTHER PATHOEPIGENETIC DISORDERS

Epigenetic changes are probably involved in the vast majority of pathologies that afflict human beings, and it is very likely that epigenetic solutions may also help to treat some of them in the future. Examples of these, besides those already shown in previous sections, are given in the following subsections.

5.9.1 Bronchopulmonary Dysplasia

Early pulmonary oxygen exposure is one of the most important factors implicated in the development of bronchopulmonary dysplasia (BPD). Early hyperoxia induces permanent changes in histone signatures at the *NOS3* and *STAT3* gene locus that explain in part the altered vascular response patterns in children with BPD.[269]

5.9.2 Bone Disease

The physiological processes involved in bone remodeling are tightly regulated by epigenetic factors. Epigenetic modifications are currently seen in tumoral and nontumoral bone diseases, as well as in bone fracture healing.[270] In osteoporosis many epigenetic biomarkers have been associated with bone mineral density or suggested to predict osteoporotic fractures. Treatments designed to modulate bone remodeling by selectively targeting the function of specific miRNAs are being evaluated.[271, 272] Morris et al.[273] performed an epigenome-wide association study (EWAS) of bone mineral density (BMD) and found one CpG site (cg23196985) that was associated with femoral neck BMD.

5.9.3 Wilson Disease

Wilson disease (WD) is an autosomal recessive genetic disorder caused by mutations in the copper transporter gene *ATP7B*, leading to the accumulation of copper in the liver and brain, thus resulting in hepatic, neurological, and psychiatric symptoms. Over 300 disease-causing mutations in *ATP7B* have been identified. WD is accompanied by structural and functional abnormalities in mitochondria, potentially altering the production of metabolites that are required for epigenetic regulation of gene expression. Environmental hits and subsequent changes in epigenetic regulation may impact copper accumulation and ultimately the WD phenotype.[274] Global DNA methylation in liver is modified by dietary choline in tx-j mice, a spontaneous mutant model of WD. The WD phenotype and hepatic gene expression of tx-j offspring can be modified by maternal methyl supplementation during pregnancy.[275]

5.9.4 Kawasaki Disease

Kawasaki disease (KD) is the most common coronary vasculitis in children with anemia. KD has been associated with elevated plasma hepcidin levels. KD patients exhibit epigenetic hypomethylation of *HAMP* promoter, with an opposite tendency between DNA methylation of target CpG sites (cg23677000 and cg04085447) and hepcidin levels, indicating that *HAMP* promoter hypomethylation upregulates hepcidin expression in KD patients.[276]

5.9.5 Glaucoma

Epigenetic changes are associated with different visual disorders.[277] Genome-wide association studies have identified an association at the *CDKN2B/CDKN2B-AS1* locus on 9p21 with normal tension glaucoma (NTG). CpG sites in the *CDKN2B* promoter show an association with NTG. Methylation at CpG sites in *CDKN2B* also associates with the genotype at rs1063192, which is known to confer risk for NTG.[278]

5.9.6 Dyskeratosis Congenita

Dyskeratosis congenita (DC) is an inherited bone marrow failure and cancer susceptibility syndrome caused by germline mutations in telomere biology genes. Germline mutations in *DKC1*, which encodes the protein dyskerin, cause X-linked recessive DC. Because of skewed X chromosome inactivation, female *DKC1* mutation carriers do not typically develop clinical features of DC. All female *DKC1* mutation carriers have normal leukocyte subset telomere lengths and similarly skewed X chromosome inactivation in multiple tissue types. Dyskerin expression, telomerase RNA accumulation, and pseudouridylation are present in all mutation carriers at levels comparable with healthy wild-type controls. Other mechanisms, in addition to X chromosome inactivation, such as germline mosaicism or epigenetics, may contribute to DC-like phenotypes present in female *DKC1* mutation carriers.[279]

5.9.7 Organ Fibrosis

Fibrosis is a pathological wound-healing process in response to chronic injury. DNA methylation is critical in the pathogenesis of fibrosis involving multiple organ systems, contributing to significant morbidity and mortality. Aberrant DNA methylation can silence or activate gene expression patterns that drive the fibrosis process characterized by excessive extracellular matrix production and accumulation, which eventually affects the organ architecture and results in organ failure.[280]

5.9.8 Liver Fibrosis

Hepatic stellate cells (HSCs) are a major source of fibrogenesis in the liver, contributing to cirrhosis, and SIRT1 plays an essential role in guiding the transition of HSC phenotypes.[281]

5.9.9 Alcoholic Liver Disease

Alcoholic liver disease (ALD) comprises a spectrum of disorders ranging from simple steatosis to cirrhosis and hepatocellular carcinoma. Excessive alcohol consumption triggers a series of metabolic reactions that affect the liver by inducing lipogenesis, increasing oxidative stress, and causing abnormal inflammatory responses. Genetic and epigenetic factors can also affect the progression of liver diseases, promoting nonalcoholic fatty liver disease (NAFLD),[282] fibrogenesis, cirrhosis, and hepatocellular carcinoma.[283]

5.9.10 Vitamin D Deficiency

Vitamin D deficiency is one of the most common nutritional deficiencies worldwide. Maternal and paternal vitamin D deficiency is associated with increased susceptibility to hypertension in offspring. Genome-wide methylation analyses in offspring identified hypermethylation of the promoter region of the Pannexin-1 (*Panx1*) gene in F1-depl rats. *Panx1* encodes a hemichannel known to be involved in endothelial-dependent relaxation.[284]

5.9.11 Autoimmune Disorders

Autoimmune diseases are complex epigenetic disorders with heterogeneous phenotypes.[285] Typical examples are systemic lupus erythematosus (SLE) or Sjögren syndrome which share similar symptoms, immune markers, and autoantibodies.[252, 286] Autoimmune thyroid diseases (AITD) are a group of both B cell- and T cell-mediated organ-specific autoimmune diseases classically represented by Graves disease and Hashimoto thyroiditis. Genetic, epigenetic, and environmental factors are involved in the pathogenesis of AITD.[287–289]

Epigenetic factors contribute to discordance rates in monozygotic twins in different autoimmune disorders (11% in SLE; 64% in psoriasis; 77% in PBC). Other autoimmune diseases in which discordance is found among monozygotic twins include type 1 diabetes, multiple sclerosis, rheumatoid arthritis, dermatomyositis, and systemic sclerosis.[290]

Epigenetic studies in Behçet disease show alterations in the methylation level of IRS elements, histone modifications such as H3K4me27 and H3K4me3, upregulation of miR-182 and miR-3591-3p, and downregulation of miR-155, miR-638, and miR-4488.[291]

5.9.12 IgA Nephropathy and Henoch-Schönlein Purpura

IgA nephropathy (IgAN) is the most common cause of primary glomerulonephritis worldwide.

miRNAs play a role in IgAN pathogenesis.[292] *SPRY2* gene variants are associated with IgA nephropathy, and the MAPK/ERK pathway is defective in this disease and in cases of Henoch-Schönlein purpura (HSP). PARP1 and DNMT1, involved in DNA repair and in antibody class switching and methylation, are critically downregulated in IgAN and HSP patients.[293]

5.9.13 Systemic Sclerosis

Prolonged activation of fibroblasts is a central hallmark of systemic sclerosis (SSc). Fibroblasts are effector cells that differentiate into an activated myofibroblast phenotype. Myofibroblasts persist in fibrotic disorders. Profibrotic

cytokines might trigger epigenetic changes that contribute to the persistently activated fibroblast phenotype. Several epigenetic alterations have been described in SSc as potential pathogenic factors.[294]

5.9.14 Infectious Disease

Pathogens have developed sophisticated strategies to evade the immune response, among which manipulation of host cellular epigenetic mechanisms plays a prominent role. Modulation of histone acetylation in host cells is an efficient strategy of bacterial immune evasion. Virulence factors and metabolic products of pathogenic microorganisms alter the expression and activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs) to suppress transcription of host defense genes through epigenetic changes in histone acetylation marks.[295]

DNA methylation in bacteria affect gene regulation, generating isogenic cells with different phenotypes. Restriction modification (RM) systems contain prototypic methylases that are responsible for much bacterial DNA methylation. RM systems are often considered an evolutionary response to bacteriophages; however, multi-hsdS type I systems have shown the capacity to change bacterial phenotypes. Phase-variable DNA methylation acts as a global regulatory mechanism in bacteria.[296]

In pulmonary tuberculosis (TB) the inflammatory immune response against *Mycobacterium tuberculosis* (Mtb) is associated with tissue destruction and cavitation, which drives disease transmission, chronic lung disease, and mortality. Matrix metalloproteinase (MMP)-1 is a host enzyme critical for the development of cavitation. *MMP* expression is epigenetically regulated.

Mtb infection decreased class I HDAC gene expression by over 50% in primary human monocyte-derived macrophages but not in normal human bronchial epithelial cells (NHBEs). Nonselective inhibition of HDAC activity decreases *MMP-1/-3* expression by Mtb-stimulated macrophages and NHBEs, while class I HDAC inhibition increases MMP-1 secretion by Mtb-stimulated NHBEs. *MMP-3* expression, but not MMP-1, is downregulated by siRNA silencing of *HDAC1*. Inhibition of HAT activity also decreases MMP-1/-3 secretion by Mtb-infected macrophages. The *MMP-1* promoter region between −2001 and −2942 base pairs from the transcriptional start site is key to controlling Mtb-driven *MMP-1* gene expression. Histone H3 and H4 acetylation and RNA Pol II binding in the *MMP-1* promoter region are increased in stimulated NHBEs.[297]

Severe sepsis, septic shock, and related inflammatory syndromes are driven by the aberrant expression of proinflammatory mediators by immune cells. During the acute phase of sepsis, overexpression of chemokines and cytokines drives physiological stress leading to organ failure and mortality. Following recovery from sepsis the immune system exhibits profound immunosuppression, evidenced by an inability to produce the same proinflammatory mediators that are required for normal responses to infectious microorganisms. Gene expression in inflammatory responses is influenced by the transcriptional accessibility of chromatin, with histone posttranslational modifications determining whether inflammatory gene loci are set to transcriptionally active, repressed, or poised states. Histone modifications play a central role in governing the cytokine storm of severe sepsis, and aberrant chromatin modifications induced during the acute phase of sepsis may mediate chronic immunosuppression in sepsis survivors.[298]

Histone modifications in *Plasmodium falciparum* suggest the presence of pathogenic mechanisms linking epigenetic factors to transcription. H4K8ac is a potential regulator of chromatin-linked transcriptional changes during the *P. falciparum* life cycle, which is associated not only with euchromatin but also with the heterochromatin environment. This fact suggests a regulatory connection between growth and the parasite-host interaction that impacts malaria parasite virulence.[299]

Epigenetics is involved in the control of the virus life cycle and the transformation of a normal cell into an onco cell. DNA tumor viruses (polyomavirus, adenovirus, papillomavirus, herpes virus, and other virus families) suffer histone modification, nucleosome changes, and DNA methylation that potentially regulate the biological consequences of the infection. DNA methylation occurs as part of gene silencing during latent infection by herpesviruses. In the case of polyomaviruses, adenoviruses, and papillomaviruses, transformation of the cell occurs via integration of the virus genome with the unregulated expression of critical viral genes capable of redirecting cellular gene expression via direct or indirect epigenetic regulation.[300]

At least seven human DNA and RNA viruses are oncogenic viruses (oncoviruses) that contribute to the development of various cancer types. About 15%–20% of human cancers worldwide have viral etiology. Human oncoviruses have developed multiple molecular mechanisms to interfere with specific cellular pathways to promote viral replication and viral life cycle maintenance in the host. Viral oncoproteins and viral noncoding RNAs are the key factors that can affect multiple cellular processes at both the genetic and epigenetic level. Epigenetic reprogramming is an essential issue for virus-induced carcinogenesis.[301]

Hepatitis B virus (HBV) causes liver diseases, and a large proportion of hepatocellular carcinoma (HCC) cases are associated with chronic HBV infection. HBV infection interferes with apoptosis signaling to promote HCC progression and viral proliferation. The HBV-mediated alteration of apoptosis is achieved via interference with cellular signaling pathways and regulation of epigenetics. HBV X protein (HBX) plays a major role in the interference of apoptosis.[302]

Vitamin D receptor (VDR)-mediated toll-like receptor (TLR) 2/1 signaling produces antimicrobial peptides, which are critical as a first line of defense in innate immunity. Genetic variants (*TLR1*, *TIRAP*, *VDR* SNPs), vitamin D status, season, and epigenetics are factors contributing to infectious disease predisposition. VDR-mediated TLR2/1 signaling is influenced by a combination of environment, epigenetics, and genetics, collectively influencing differential innate immunity.[303]

5.9.15 Thyroid Disorders

The thyroid gland is essential for normal human growth and development. A pioneering study of the thyroid epigenome revealed that epigenetic features characterizing promoters and transcription elongation tend to be more consistent than regions characterizing enhancers or polycomb-repressed regions and that epigenetically active genes consistent across all epigenomes tend to have higher expression than those not marked as epigenetically active in all epigenomes. A set of 18 genes epigenetically active and consistently expressed in the thyroid gland have been identified as well as 6 histone modifications (H3K4me1, H3K4me3, H3K27ac, H3K36me3, H3K9me3, H3K27me3) and epigenomic maps of 19 chromatin states.[304]

5.9.16 Pseudohypoparathyroidism

Pseudohypoparathyroidism type 1B is a rare imprinting disorder (ID), associated with the *GNAS* locus, characterized by parathyroid hormone (PTH) resistance in the absence of other endocrine or physical abnormalities. Sporadic PHP1B cases might represent errors in early embryonic methylation with imprinted mosaicisms. Epimutation in early postzygotic phases are mosaics.[305]

5.9.17 Preeclampsia

Preeclampsia (PE) is a heterogeneous hypertensive disorder of pregnancy. Preeclampsia is known to be a leading cause of mortality and morbidity among mothers and their infants. Approximately 3%–8% of all pregnancies in the United States are complicated by preeclampsia and another 5%–7% by hypertensive symptoms. Epigenetics studies in different clusters of placentas identified DNA methylation alterations underlying a portion of the transcriptional development of canonical PE and immunological PE in different clusters. Integrated transcriptome and epigenome analysis revealed modifications in TGF-β signaling, cell adhesion, oxidative phosphorylation, and metabolism pathways in canonical PE, and aberrations in antigen presentation, allograft rejection, and cytokine-cytokine receptor interaction in immunological PE.[306]

Studies on associations between PE and the epigenetics of circadian clock and clock-controlled genes in placental and newborn tissues show that DNA methylation differs in early-onset PE compared with spontaneous preterm birth at 6 CpGs in placental tissue and at 21 CpGs in umbilical cord.[307]

Reduced IGF-1 has been observed in preeclampsia. IGF-1 is decreased in preeclamptic placentas and hypoxic trophoblasts. Preeclamptic placentas and hypoxic trophoblasts are hypermethylated and hypohydroxymethylated with higher 5mC, DNMT1, and DNMT3b, and lower DNMT3a, 5hmC, TET1, TET2, and TET3. There is a negative correlation between IGF-1 and DNMT1. 5-Aza-dc treatment and *DNMT1*-siRNA increase the expression of IGF-1 in HTR8 cells, reflecting the potential mechanism of DNMT1-mediated DNA methylation in IGF-1 regulation. Preeclampsia is associated with hypermethylation of the *IGF-1* promoter mediated by DNMT1.[308]

Nomura et al.[309] studied imprinting gene dysregulation caused by hypertension and its potential link between maternal preeclampsia and neurocognitive dysregulation in offspring leading to the conclusion that dysregulation of placental genomic imprinting might be an underlying mechanism.

5.9.18 Preterm Birth

Preterm birth (PTB) is one of the leading causes of neonatal mortality and morbidity around the world. Epigenetic alterations of the human placenta are involved in adverse pregnancy outcomes associated with PTB.[310] PTB affects one in six Black babies in the United States. Altered maternal 1-carbon metabolism influences placental DNA methylation

patterns and programs the fetus for noncommunicable diseases in adult life. *MTHFR* and *MTR* mRNA levels are higher, while protein levels are lower, and *MTR* CpG sites are hypermethylated in the preterm placenta. Methylated CpG sites are negatively associated with maternal plasma vitamin B12 levels.[311]

A total of 45 methylated DNA loci have been identified in maternal blood associated with early sPTB. Replication analyses confirmed sPTB associations for cg03915055 and cg06804705, located in the promoter regions of the *CYTIP* and *LINC00114* genes, respectively.[312] The heritability of preterm birth is estimated to be around 17%–36%, with many other factors contributing to this condition, including infectious disease, metabolic disorders, behavioral problems, and epigenetic changes.[313]

5.9.19 Periodontitis

Epigenetic mechanisms are involved in the regulation of the host immune response in periodontitis.[314] Epigenetics explains in part the role of smoking in the development and progress of periodontal disease. Smoking-related changes in DNA methylation patterns and subsequent alterations in the expression of genes coding for methylation states of extracellular matrix (ECM) components may be related to increased susceptibility to periodontitis in smokers.[315]

5.9.20 Ulcerative Colitis

Aberrant DNA methylation patterns have been reported in ulcerative colitis. About 577 differentially methylated sites mapping to 210 genes have been identified, and 62 differentially expressed genes with increased expression in the presence of inflammation have also been detected. *ROR1*, *GXYLT2*, *FOXA2*, and *RARB* show an inverse correlation between methylation and gene expression.[316] *RUNX3*, *MINT1*, *MYOD*, and p16 exon1 as well as the promoter regions of *EYA4* and *ESR* are highly methylated in colitis-associated cancer; however, patterns of DNA methylation differ between ulcerative colitis and sporadic CRC.[317]

5.9.21 Chronic Obstructive Pulmonary Disease

An epigenome-wide association study of chronic obstructive pulmonary disease (COPD) has identified 1 significant differentially methylated probe (DMP) (cg03559389, *DIP2C*) and 104 significant differentially methylated regions (DMRs). A total of 34 DMRs have been mapped to genes differentially expressed with respect to the same trait, and 5 genes (*CTU2*, *USP36*, *ZNF516*, *KLK10* and *CPT1B*) have been associated with more than 2 traits.[318]

Alveolar macrophages from COPD patients are defective in their ability to phagocytose apoptotic cells ("efferocytosis") and this defect is potentially linked to the sphingosine-1 phosphate (S1P) system—in particular, the sphingosine-1 phosphate receptor 5 (S1PR5). Increased *S1PR5* mRNA expression levels correlate with both lung function and efferocytosis. *S1PR5* shows changes in DNA methylation. Alveolar macrophages isolated from COPD patients show hypomethylation levels in the same region compared with macrophages from non/ex-smokers.[319]

5.9.22 Neural Tube Defects

Despite folate supplementation in pregnant women, neural tube defects (myelomeningocele, lipomeningocele, split cord malformation, and congenital dermal sinus tract) are relatively frequent, with an overall prevalence of family history of 16.9% (3.1% in first-degree relatives). Epigenetic aberrations may influence congenital neural tube anomalies.[320]

5.9.23 High Altitude-Associated Disorders

Over the past decade major technological and analytical advances have propelled efforts toward identifying the molecular mechanisms that govern human adaptation to high altitude. Epigenetic processes are involved in shaping patterns of adaptation to high altitude by influencing adaptive potential and phenotypic variability under conditions of limited oxygen supply.[321]

5.9.24 Bruxism

DNA methylation changes are present in different circadian forms of bruxism (sleep bruxism, awake bruxism, mixed bruxism), and patients undergoing bruxism treatment exhibit hypomethylated DNA levels when compared with controls.[322]

5.9.25 Rare Syndromes

5.9.25.1 Mayer-Rokitansky-Küster-Hauser Syndrome

Mayer-Rokitansky-Küster-Hauser syndrome (MRKHS) is a disease caused by congenital absence of the uterus and two-thirds of the upper vagina. Diverse genomic abnormalities and association of MRKHS with Wnt family member 4 (*Wnt4*) mutations have been reported. In the regions where deletions and duplications are frequently detected the involvement of LIM homeobox 1 (*LHX1*), HNF1 homeobox B (*HNF1B*), and T-box 6 (*TBX6*) seem to be apparent. Some case reports of MRKHS indicate that this syndrome may be also caused by chromosomal translocation and epigenetic disorders.[323]

5.9.25.2 Silver-Russell Syndrome

Silver-Russell syndrome (SRS) is a syndromic form of fetal growth retardation caused by epigenetic downregulation of the fetal growth factor IGF2. Mutations in the *PLAG1*, *HMGA2*, and *IGF2* genes are present in sporadic and familial cases of SRS. HMGA2 regulates IGF2 expression through PLAG1. Genetic defects of the HMGA2/PLAG1/IGF2 pathway can lead to fetal and postnatal growth restriction.[324]

5.9.25.3 Lipodystrophic Syndromes

Mutations in *LMNA* encoding A-type lamins are responsible for laminopathies including muscular dystrophies, lipodystrophies, and premature aging syndromes. *LMNA* mutations alter nuclear structure and stiffness, binding to partners at the nuclear envelope or within the nucleoplasm, gene expression, and/or prelamin A maturation. LMNA-associated lipodystrophic features, combining generalized or partial fat atrophy and metabolic alterations associated with insulin resistance, may result from altered adipocyte differentiation or from altered fat structure. Pathogenic A-type lamin variants trigger lipodystrophy, metabolic complications, and cardiovascular events. In probands, metabolic alterations occur at an earlier age across generations, which might result from epigenetic deregulation induced by *LMNA* mutations.[325]

5.9.25.4 Scleroderma

Scleroderma (SSc) is a multifactorial disorder characterized by immune activation, vascular complications, and excessive fibrosis in internal organs. Genetic studies show an association of SSc with the MHC region (*HLA-DRB1, HLA-DQB1*, *HLA-DPB1, HLA-DOA1*), *IRF5*, *STAT4*, and *CD247*, as well as SNPs rs35677470 in *DNASE1L3*, rs5029939 in *TNFAIP3*, and rs7574685 in *STAT4*. Extensive epigenetic changes have been described in SSc. Alteration in enzymes and mediators involved in DNA methylation and histone modification, as well as dysregulated noncoding RNA levels all contribute to fibrosis, immune dysregulation, and impaired angiogenesis in this disease.[326]

5.9.26 Organ Transplantation-Related Allograft Injury

Ischemia during kidney transplant causes chronic allograft injury and functional complications.

Oxygen shortage reduces the DNA-demethylating activity of ten-eleven translocation (TET) enzymes, yielding hypermethylated genomes that promote tumor progression. Ischemia similarly induces DNA hypermethylation in kidney transplants and contributes to chronic injury. Methylation increases drastically in all allografts on ischemia, and hypermethylation is caused by loss of 5-hydroxymethylcytosine, the product of TET activity. Hypermethylation reduces the expression of genes involved in suppressing kidney injury and fibrosis. CpG hypermethylation in preimplantation specimens predicts chronic injury, fibrosis, and glomerulosclerosis one year after transplant.[327]

5.10 GUT MICROBIOME

The coevolution of mammalian hosts and their commensal microbiota has led to the development of complex symbiotic relationships between resident microbes and mammalian cells.[328] Host interactions with their resident gut microbiota (GM) have been reported to be involved in the pathogenesis of many metabolic diseases, including obesity, diabetes, and CVD. Around 10^{14} microorganisms reside within the lower human intestine, and many of these microorganisms have developed mutualistic or commensal associations with the host, participating in many physiological processes fundamental for the health conditions of the mutualistic/symbiotic host. Dysbiosis (altered gut microbial composition) along with other predisposing genetic and environmental factors may contribute to host metabolic disorders resulting in many ailments.[329] The gut microbiome is a key constituent of the colonic environment with important repercussions for human health. The eukaryotic epigenome responds to environmental stimuli through alterations in chromatin features and gene expression. Different diets influence the microbiome leading to metabolic disorders, cancer, malnutrition, and obesity. For instance, obesogenic diets may shape the microbiome prior to the development of obesity. Altered bacterial metabolite production induces histone modifications that may predispose the host to obesity. Furthermore, alterations of histone methylation and acetylation are associated with signaling pathways integral to the development of colon cancer.[330, 331] The interaction between the environment and the gastrointestinal system influences gut microbial ecology, impacting the capacity for nutrient processing and absorption in a manner that may limit growth and induce disease.[332] The interplay of epigenetic processes and intestinal microbiota may play an important role in intestinal development and homeostasis. Microbiota regulate a large proportion of the intestinal epithelial transcriptome in the adult host. Microbiota-dependent and -independent processes act together to shape the postnatal development of the transcriptome and DNA methylation signatures of intestinal epithelial cells (IECs). The bacterial effect on the transcriptome increases over time, whereas most microbiota-dependent DNA methylation differences are detected early after birth. Microbiota-responsive transcripts can be attributed to stage-specific cellular programs during postnatal development. A total of 126 genomic loci with differential DNA methylation and RNA transcription are associated with the presence of intestinal microbiota. There are microbiota-dependent functional methylation sites that may impact long-term gene expression signatures in IECs.[333]

Choline is an essential nutrient and methyl donor required for epigenetic regulation. Choline-utilizing bacteria compete with the host for this nutrient, impacting plasma and hepatic levels of methyl donor metabolites and recapitulating biochemical signatures of choline deficiency. Mice with high levels of choline-consuming bacteria show increased susceptibility to metabolic disorders. Bacteria-induced reduction of methyl donor availability influences global DNA methylation patterns in adult mice and in their offspring.[334] Epigenomic modifications enable host cells to alter gene expression without modifying the genetic code, and therefore represent potent mechanisms by which mammalian cells can transcriptionally respond, transiently or stably, to environmental cues. Epigenetic changes represent a level of regulation by which the host integrates and responds to microbial signals. Bacterial-derived short chain fatty acids are a link between microbiota and host epigenomic pathways.[328]

The human microbiome may be involved in the "missing heritability" problem, which states that genetic variants in genome-wide association studies (GWAS) cannot completely explain the heritability of complex traits. The composition of the human microbiome is associated with many pathological conditions. The microbiome encodes a second genome with nearly 100 times more genes than the human genome, and this second genome may act as a rich source of genetic variation. Human genotypes interact with the composition and structure of the microbiome. Microbial genetic composition can be strongly influenced by the host's behavior and its environment or by vertical and horizontal transmissions from other hosts. All these considerations posed by Sandoval-Motta et al.[335] might indicate the genetic similarities assumed in familial studies may cause overestimations of heritability values.

5.11 PLURIPOTENT STEM CELLS

Pluripotent stem cells (PSCs) in mammals are defined as naive and primed states according to their cellular, molecular, epigenetic, and functional states. DNA methylation is closely associated with cell reprogramming, functional remodeling, and cell differentiation of PSCs. The pluripotency and naive characteristics of PSCs are closely associated with cell DNA methylation.[336] Human pluripotent stem cell (hPSC) lines show a bias in their differentiation. The propensity to preferentially differentiate toward one germ layer or cell type over others depends on many complex factors. Chromosomal aberrations, mitochondrial mutations, genetic diversity, and epigenetic variance are the main drivers of

this phenomenon, leading to a wide range of phenotypes.[337] Differentiation propensity is influenced by many epigenetic variants that can condition loss of differentiation capacity, preference toward certain cell types and pathogenic phenotypes. Mitochondria and inherent genetic diversity also affect differentiation propensity. Variability in differentiation capacity can bring benefits or deleterious consequences in the clinical translation of hPSC (i.e., residual undifferentiated stem cells, transplantation of potentially transformed cells).[337]

Embryonic stem cells (ESCs) are regulated by pluripotency-related transcription factors in concert with chromatin regulators. A novel potent regulator of pluripotency and early differentiation is SET, which displays a rapid isoform shift during early differentiation from the predominant isoform in ESCs (SETα) to the primary isoform in differentiated cells (SETβ) through alternative promoters.[338]

PR-domain containing protein 14 (PRDM14) is a site-specific DNA-binding protein that is required for establishment of pluripotency in ESCs and primordial germ cells (PGCs) in mice. The methylation status of DNA is regulated by the balance between de novo methylation and passive/active demethylation, and global DNA hypomethylation is closely associated with cellular pluripotency and totipotency. PRDM14 ensures hypomethylation in mouse ESCs and PGCs through two distinct layers, transcriptional repression of the DNA methyltransferases Dnmt3a/b/l and active demethylation by recruitment of TET proteins.[339]

Mammary stem cells (MaSCs) show a hierarchical organization of the mammary epithelia featuring morphogenetic variation during puberty, pregnancy, lactation, and regression. A single MaSC is capable of reconstituting an entirely functional mammary gland on orthotopic transplantation. The most primitive cells are the seeds of breast malignant transformation. Epigenetic changes are key players in the transformation process.[340]

Liu et al.[341] studied the role of DNA methylation during erythrocyte production by human embryonic stem cells (hESCs) and found a negative correlation between DNA methylation and gene expression during the later differentiation stage. Erythropoietic genes with differentially methylated CpG sites, which were primarily enriched in nonisland regions, have been found to be upregulated, and demethylation of their gene bodies has been associated with the presence of enhancers and DNase I-hypersensitive sites. The components of JAK-STAT-NFκB signaling targeting key genes for erythropoiesis have been found to be hypomethylated and upregulated.

Several stem cell markers (*OCT-4*, *NANOG*, *SALL4*, *TERT*) are induced during reprogramming into human-induced pluripotent stem cells (iPSCs). *OCT-4*, *NANOG*, and *SALL4* gene expression are regulated by DNA methylation, and their promoters are hypomethylated in iPSCs during reprogramming. A methylated region (DMR) at a distal region in the *TERT* promoter shows a differential methylation pattern between human iPSCs and their parental somatic cells. The *TERT*-DMR is hypermethylated in iPSCs and hypomethylated in their parental somatic cells. The methylated *TERT*-DMR upregulates the promoter activity in iPSCs. Lamin B1 accumulates at the *TERT*-DMR in iPSCs but not in somatic cells. *TERT* transcription is enhanced by DNA methylation at the *TERT*-DMR via binding to nuclear lamina during reprogramming.[342]

Cancer is the end result of the accumulation of cell divisions in stem cells. Cell division can lead to a variety of cancer-promoting errors, such as mutations and epigenetic aberrations firing during DNA replication, chromosome aberrations arising during mitosis, errors in the distribution of cell fate determinants between daughter cells, and failures to restore physical interactions with other tissue components. The accumulation of cell divisions in stem cells provokes the accumulation of DNA alterations required for carcinogenesis and the growth of abnormal cell populations.[343]

Epiblast stem cells (EpiSCs), which are pluripotent cells isolated from early postimplantation mouse embryos (E5.5), show both similarities and differences compared with mouse embryonic stem cells (mESCs) isolated earlier from the inner cell mass (ICM) of the E3.5 embryo. While chromatin is very dispersed in E3.5 ICM, compact chromatin domains and chromocenters appear in E5.5 epiblasts after embryo implantation. DNA methylation is dispensable for global chromatin reorganization but required for the compaction of pericentromeric chromatin into chromocenters.[344]

Through the histone methyltransferase EZH2 the polycomb complex PRC2 mediates H3K27me3 and is associated with transcriptional repression. PRC2 regulates cell fate decisions in model organisms. The characterization of *EZH2*-deficient human embryonic stem cells (hESCs) revealed that H3K27me3 is lost upon *EZH2* deletion, identifying an essential requirement for EZH2 in methylating H3K27 in hESCs, in contrast to its nonessential role in mouse ESCs. *EZH2*-deficient hESCs show strongly reduced self-renewal and proliferation, thereby identifying a more severe phenotype compared with mouse ESCs. *EZH2*-deficient hESCs can initiate differentiation toward developmental lineages; however, they cannot fully differentiate into mature specialized tissues. Thus EZH2 is required for stable ESC self-renewal, regulation of transcriptional programs, and for late-stage differentiation in models of early human development.[345]

Nono is a component of the para-speckle, which stores and processes RNA. Mouse embryonic stem cells (mESCs) lack para-speckles, leaving the function of Nono in mESCs unclear. Nono functions as a chromatin regulator cooperating with Erk to regulate mESC pluripotency. Nono loss results in robust self-renewing mESCs with epigenomic and

transcriptomic features resembling the 2i (GSK and Erk inhibitors)-induced "ground state." Erk interacts with and is required for Nono localization to a subset of bivalent genes that have high levels of poised RNA polymerase. Nono loss compromises Erk activation and RNA polymerase poising at its target bivalent genes in undifferentiated mESCs, thus disrupting target gene activation and differentiation. Nono collaborates with Erk signaling to regulate the integrity of bivalent domains and mESC pluripotency.[346]

Lefty is a member of the transforming growth factor-beta (TGFβ) superfamily and a potent antagonist of the TGFβ/nodal/activin signaling pathway. Lefty is critical in sustaining self-renewal/pluripotency status and is implicated in the differentiation of embryonic stem cells (ESCs). Lefty proteins (human Lefty A and B) are secreted glycoproteins encased in exosomes for extracellular release. The exosomal- and cell-associated Lefty diverge in their proteolytic processing, possessing N-glycan structures of high mannose and a complex nature. Differentiation of hESCs to mesenchymal cells (MSCs) or neuronal progenitor cells (NPCs) entails distinct changes in the Lefty A/Lefty B gene(s), and protein expression. The proteolytic cleavage and N-glycan composition of the cell-associated and exosomal Lefty differ in the progenies differentiated.[347]

Poly(A) tail length and mRNA deadenylation play important roles in gene regulation. *CNOT3*-dependent mRNA deadenylation governs the pluripotent state. CNOT3, a component of the Ccr4-Not deadenylase complex, is required for mouse epiblast maintenance. It is highly expressed in blastocysts and its deletion leads to periimplantation lethality. The epiblast cells in *Cnot3* deletion embryos are quickly lost during diapause and fail to outgrow in culture. A CNOT3 C-terminus is required for its interaction with the complex and its function in ESCs. *Cnot3* deletion results in increases in the poly(A) tail lengths, half-lives, and steady-state levels of differentiation gene mRNAs. The half-lives of CNOT3 target mRNAs are shorter in ESCs and become longer during normal differentiation. CNOT3 maintains the pluripotent state by promoting differentiation gene mRNA deadenylation and degradation, and poly(A) tail length regulation is a posttranscriptional mechanism that controls pluripotency.[348]

Mammalian CXXC finger protein 1 (Cfp1) is a DNA-binding protein that is a component of the Setd1 histone methyltransferase complexes and is a critical epigenetic regulator of both histone and cytosine methylation. Murine embryonic stem cells lacking *Cfp1* exhibit a loss of histone H3-Lys4 trimethylation (H3K4me3) at many CpG islands, and a mislocalization of this epigenetic mark to heterochromatic subnuclear domains. These cells fail to undergo cellular differentiation. Differentiation defects are rescued upon introduction of a *Cfp1* expression vector. Cfp1 contains an N-terminal plant homeodomain (PHD), a motif frequently observed in chromatin-associated proteins that functions as a reader module of histone marks. The Cfp1 PHD domain directly and specifically binds to histone H3K4me1/me2/me3 marks. Mutations in *Cfp1* PHD residues (Y28, D44, or W49) ablate this histone interaction. The W49A point mutation does not affect the ability of Cfp1 to rescue appropriate restriction of histone H3K4me3 to euchromatic subnuclear domains or in vitro cellular differentiation in *Cfp1*-null ES cells. A mutated form of *Cfp1* that lacks DNA-binding activity (C169A) rescues cellular differentiation. The rescue of *Cfp1*-null ES cells with a double mutant form of *Cfp1* (W49A, C169A) results in partially defective differentiation. The *Cfp1* PHD domain is a reader of histone H3K4me marks involved in the regulation of lineage commitment in ES cells.[349]

The functionality of stem cells declines during aging, and this decline contributes to aging-associated impairments in tissue regeneration and function. Alterations in developmental pathways have been associated with declines in stem cell function during aging. The epigenetic stress response in muscle stem cells differs between aged and young mice. In aged mouse stem cells, aberrant global and site-specific induction of active chromatin marks are found, resulting in the specific induction of Hoxa9. Hoxa9 activates several developmental pathways and represents a factor that separates satellite cell gene expression in aged mice. The activated pathways include most of the currently known inhibitors of satellite cell function in aging muscle, including Wnt, TGFβ, JAK/STAT, and senescence signaling. Inhibition of aberrant chromatin activation or deletion of *Hoxa9* improves satellite cell function and muscle regeneration in aged mice, whereas overexpression of *Hoxa9* mimics aging-associated defects in satellite cells from young mice, which can be rescued by the inhibition of Hoxa9-targeted developmental pathways.[350]

lncRNAs play an integral regulatory role in the determination of cellular identity. *LncPRESS1* is a p53-regulated transcript that maintains hESC pluripotency in concert with core pluripotency factors. RNA-seq of hESCs depleted of lncPRESS1 revealed that lncPRESS1 controls a gene network that promotes pluripotency. LncPRESS1 physically interacts with SIRT6 and prevents *SIRT6* chromatin localization, which maintains high levels of histone H3K56 and H3K9 acetylation at promoters of pluripotency genes. *LncPRESS1* is a p53-regulated, pluripotency-specific lncRNA that safeguards the hESC state by disrupting SIRT6 activity.[351]

In the perinatal and adult forebrain the regionalized neural stem cells lining the ventricular walls produce different types of olfactory bulb interneurons. These postnatal stem cells are lineage-related to their embryonic counterparts that produce cortical, septal, and striatal neurons. Zinc finger proteins, Zic1 and Zic2, are postnatally induced in the dorsal olfactory bulb neuron lineage. These factors confer a GABAergic and calretinin-positive phenotype to neural stem cells

while repressing dopaminergic fate. The identification of master transcription factors, that instruct the fate of postnatally generated neurons, can help in deciphering the mechanisms driving fate transition from embryonic to adult neuron production.[352]

Adult stem cells are able to self-renew and differentiate into several specialized cell types. The use of several stem cell lines has emerged as a potential alternative for repairing and/or regenerating damaged tissues with epigenetic intervention.[353] Human endothelial colony-forming cells (ECFCs) represent a promising source of adult stem cells for vascular repair. Proangiogenic pathways are repressed in ECFCs as a result of the presence of bivalent (H3K27me3/H3K4me3) epigenetic marks, which decreases the regenerative potential of cells. Some epigenetic drugs that modify active H3K4me3 states can activate multiple proangiogenic signaling pathways (VEGFR, CXCR4, WNT, NOTCH, SHH), resulting in improved capacity of ECFCs to form capillary-like networks. Thus epigenetic drugs are able to increase the vascular repair properties of ECFCs through transient activation of proangiogenic signaling pathways.[354]

The use of induced pluripotent stem cells (iPSCs) is a promising strategy in cell therapy. Genomic alterations exist in iPSCs; however, it appears that observed genomic alterations in iPSCs are inherited rare alterations from parental cells, and the reprogramming process might not activate additional mutagenic activity.[355]

5.12 EPIGENETIC BIOMARKERS

In the omics era there is a clear tendency to rely on molecular diagnosis beyond traditional clinical and symptom-based examinations. Genetic markers and transcription signatures were first introduced as potential biomarkers; however, clinical implementation of these biomarkers is very limited as a result of low reproducibility and accuracy. Recently, epigenetic markers have been considered an alternative approach to disease diagnosis.[356] The ideal biomarker should integrate the effects of both genetic and nongenetic factors in biologically stable and technically reproducible ways, generate a score from biological samples as a surrogate for the cell/tissue to be assessed, and enable the effectiveness of risk-reducing measures to be monitored. DNA methylation-based tests meet most of these requirements.[357]

Many epigenetic biomarkers have been proposed in recent times, especially for cancer and cardiovascular disorders.[358, 359] Ubiquitin-like containing PHD and Ring Finger domain 1 (UHRF1) has been proposed as a universal biomarker for cancers. Many studies have validated UHRF1 as a powerful diagnostic and prognostic tool to differentially diagnose cancer, predict the therapeutic response, and assess the risk for tumor progression and recurrence.[360]

Gene promoter hypermethylation detected in sputum may predict lung cancer (LC) risk in never smokers, correlating with a rapid decline of forced expiratory volume in 1 second (FEV1), a major driver for development of airway obstruction[361]; and DNA methylation at sites cg11637544 in *KDM2A* and cg26662347 in *KDM1A* is a biomarker for squamous cell carcinomas (SCC) and SCC survival.[362]

A potential predictive value of *HSD17B4* hypermethylation for pathological complete response after preoperative trastuzumab plus chemotherapy in HER2-positive breast cancer has been proposed.[363] A tissue miRNA signature (miR-183-5p, miR-194-5p, and miR-1285-5p) that predicts prognosis in young breast cancer patients has been reported.[364] From a panel of 161 miR-500a-associated genes profiled, 73 were significantly associated with breast cancer-specific mortality. A high level of SUSD3 is associated with reduced breast cancer-specific mortality, whereas the opposite is observed for TPX2.[365] Sphingosine kinase 1 (SPHK1) is a bioactive lipid mediator that has been identified as a biomarker in various cancers, including breast cancer.[366]

Methylation biomarker panels have been proposed for noninvasive detection of colorectal adenoma and cancer in plasma samples. Methylation of *SFRP1*, *SFRP2*, *SDC2*, and *PRIMA1* promoter sequences has been observed in 85.1%, 72.3%, 89.4%, and 80.9% of plasma samples from patients with CRC and 89.2%, 83.8%, 81.1%, and 70.3% from adenoma patients, respectively. When applied as a panel, CRC patients could be distinguished from controls with 91.5% sensitivity and 97.3% specificity, while adenoma samples could be differentiated with 89.2% sensitivity and 86.5% specificity.[367] The current TNM (tumor node metastasis) staging system is inadequate at identifying high-risk CRC patients. A miRNA recurrence classifier (MRC) has been proposed. This novel miRNA recurrence classifier seems to be superior to currently used clinicopathological features and NCCN criteria and works independent of adjuvant chemotherapy status in identifying high-risk stage II and III CRC patients.[368] Genome-wide hydroxymethylation patterns can also be used as an epigenetic biomarker for differentiating colorectal tumor tissues from normal tissues.[94]

SNPs in rs920778 and rs12826786 in the lncRNA *HOTAIR* have been associated with the susceptibility and prognosis of prostate cancer.[369]

Human riboflavin transporter-3 (encoded by *SLC52A3*) plays a prominent role in riboflavin absorption. Abnormal expression of *SLC52A3* is present in multiple types of human cancers. *SLC52A3* has two transcript variants that differ in the transcriptional start site and encode different proteins: SLC52A3a and SLC52A3b. Aberrant expressions of *SLC52A3* are associated with stepwise development of esophageal squamous cell carcinoma (ESCC) as well as the survival rates of ESCC patients. SLC52A3a strongly promotes the proliferation and colony formation of ESCC cells. *SLC52A3* 5′-flanking regions contain NFκB p65/Rel-B-binding sites, which are crucial for mediating *SLC52A3* transcriptional activity in ESCC cells. p65/Rel-B bind to 5′-flanking regions of *SLC52A3*. NFκB signaling upregulates *SLC52A3* transcription upon TNFα stimulation. SLC52A3 has been proposed as both a predictive and prognostic biomarker for ESCC.[370]

mRNA-, microRNA-, and lncRNA-based biomarkers have been reported for bladder cancer detection, diagnosis, prediction of recurrence and monitoring after treatment.[371] In pediatric T cell ALL the CpG island methylation phenotype (CIMP) classification has been proposed for improved risk stratification of relapsed BCP-ALL.[155] Some tools have been developed as valuable platforms for researchers to perform assessment of methylation-based cancer biomarkers. For instance, MethSurv includes 7358 methylomes from 25 different human cancers.[372]

Concerning cardiovascular disorders, multiple differentially methylated regions (DMR) can be identified in atherosclerosis, related to epigenetic control of cell adhesion, chemotaxis, cytoskeletal reorganization, cell proliferation, cell death, estrogen receptor pathways, and phagocytic immune responses. A subset of 34 DMRs related to impaired oxidative stress, DNA repair, and inflammatory pathways could be replicated in atherosclerotic human aorta tissue and human carotid plaque samples. *BRCA1* and *CRISP2* DMRs were identified as the most central disease-associated DNA methylation biomarkers. Methylation changes at *BRCA1* and *CRISP2*-specific CpG sites were consistently associated with subclinical atherosclerosis.[373]

Personalized medicine is impacting forensic sciences. The application of structural and functional genomics, transcriptomics, epigenetics/imprintomics, proteomics, and metabolomics is entering into the sophisticated world of "molecular autopsy."[374] Future forensic applications of DNA methylation analysis and other epigenetic markers will broaden DNA-based forensic intelligence.[375]

5.13 CONCLUSIONS

Over 80% of major health problems in developed societies, represented by cardiovascular disorders, cancer, brain disorders, metabolic disorders, and immune/inflammatory disorders, are the result of convergent genomic, epigenetic, and environmental factors configuring pathogenic events leading to a particular phenotype. It is becoming clear that epigenetic changes influence the conditions of health and disease from conception to death. The uterine environment affects embryo-fetal development and maturation, with potential effects in adult life and aging as well as with transgenerational repercusions. Aberrations in different constituents of the epigenetic machinery (DNA hypo/hypermethylation, abnormal chromatin remodeling, histone posttranslational modifications, miRNA dysregulation) contribute to the pathogenesis of major health problems, with special impact in cancer. The pathoepigenetics concept is invading the constellation of pathologies that affect human beings. Consequently, the available information (still very limited) on the role of epigenetics in the pathogenesis of specific diseases has to be integrated in comprehensive modules with the help of bioinformatics to aid physicians to incorporate epigenetic biomarkers in the clinical setting for optimizing predictive (presymptomatic) and symptomatic diagnosis. Epigenetic biomarkers will also contribute to developing epigenetic drugs, to monitoring drug efficacy and safety, and to personalizing therapeutics.

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CHAPTER

6

Pharmacoepigenetic Processors: Epigenetic Drugs, Drug Resistance, Toxicoepigenetics, and Nutriepigenetics

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6.1 INTRODUCTION

In recent decades important progress has been achieved in the field of pharmacogenomics. In 1959 Vogel introduced the term "pharmacogenetics," after the pioneering work of Bönicke and Reif, Carson and coworkers, Kalow and Staron, and Motulsky in the 1950s. Years later (1967–73) Sjöqvist and coworkers made clear that the metabolism of tricyclic antidepressants was genetically controlled.[1,2] Over 500 papers have corroborated this assumption during the past half-century. However, pharmacogenetics is still in its infancy and its concept has evolved into a broader spectrum subsequent to completion of the human genome project. At the present time pharmacogenomics relates to the application of genomic technologies, such as genotyping, gene sequencing, gene expression, genetic epidemiology, transcriptomics, proteomics, metabolomics, and bioinformatics, to drugs under clinical development and those on the market, applying the large-scale systematic approaches of genomics to speed up the discovery of drug response markers, whether they act at the level of drug target, drug metabolism, or disease pathways.[1] Over the past decade several books have been published on the field, culminating in the first *World Guide for Drug Use and Pharmacogenomics*.[3] Pharmacogenetics accounts for 30%–90% variability in pharmacokinetics and pharmacodynamics; however, pharmacogenetics alone does not predict all phenotypic variations in drug response. Individual differences in drug response are associated with genetic and epigenetic variability (DNA methylation, histone/chromatin modifications, miRNA regulation) in pathogenic, mechanistic, metabolic, transporter, and pleiotropic genes involved in the pharmacogenomic cascade.[4,5]

The emerging impact of epigenetics during the past few years is helping to configure the structural and functional framework of pharmacoepigenetics, integrating the components of the epigenetic machinery within the pharmacogenetic cascade of events responsible for drug efficacy, safety, and resistance.[6–9] Furthermore, the epigenetic properties of old drugs are being investigated and novel epigenetic drugs have been incorporated into the armamentarium of modern pharmacology with potential applicability in many medical conditions.[7]

Epigenetic regulators include writers, erasers, and readers of chromatin marks. Alterations in the normal function of these epigenetic regulators can lead to diverse human pathologies. Consequently, different therapeutic strategies have been developed to modify epigenetic changes and dysregulation based on the concept of epigenetic reversibility. Several small-molecule inhibitors targeting writers or erasers have been approved by the FDA for the treatment of malignancies, and others are currently being evaluated in clinical trials. In contrast, the targeting of epigenetic readers has lagged behind. Recent discoveries of selective inhibitors targeting the BET family of acetyl-lysine readers are opening new avenues in the field of pharmacoepigenetics.[10]

Important issues to be addressed in the field of pharmacoepigenetics are the following: (i) characterization of the components of the pharmacoepigenetic apparatus (pharmacoepigenetic processors); (ii) development of new

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epigenetic drugs capable of modulating and reversing epigenetic aberrations associated with human pathology; and (iii) characterization of epigenetic drug pharmacogenetics, including pathogenic, mechanistic, metabolic, transporter, and pleiotropic genes involved in epigenetic drug efficacy and safety.

Additional issues to be taken into consideration are the potential role of epigenetics in drug resistance and the potential impact of nutriepigenetics and toxicoepigenetics in health and/or disease.

6.2 PHARMACOEPIGENETICS APPARATUS

The genes involved in the pharmacogenomic response to drugs fall into five major categories: (i) genes associated with disease pathogenesis; (ii) genes associated with the mechanism of action of drugs (enzymes, receptors, transmitters, messengers, components of the epigenetic machinery); (iii) genes associated with drug metabolism (phase I–II reaction enzymes); (iv) genes associated with drug transporters; and (v) pleiotropic genes involved in multifaceted cascades and metabolic networks.[4,11–16] Epigenetic regulation is responsible for the tissue-specific expression of genes involved in pharmacogenetic processes; consequently, epigenetics plays a key role in drug efficacy and safety and in the development of drug resistance. Epigenetic changes affect cytochrome P450 enzyme expression, major transporter function, and nuclear receptor interactions.[16,17]

6.2.1 Pathogenic Genes

Pathogenic genes are those whose dysfunction is directly responsible for a particular disease phenotype. Different categories of pathogenic genes associated with pharmacoepigenetics can be distinguished: (i) genes encoding components of the epigenetic machinery with mutations that cause an epigenetic Mendelian disorder; (ii) mutant genes with abnormal expression leading to specific pathogenic events; and (iii) a combination of both types of defective genes: genomic defects in an abnormal epigenetic environment. Many examples of pathogenic genes can be found in different chapters in this book. Dramatic changes in epigenetic events are common in pathogenic genes associated with major problems of health and particularly in cancer. These genes and their products are fundamental targets for efficient antitumoral treatment and for biomarkers of diagnosis and prognosis.[18] The impact of epigenetic events in oncogenic genes has been documented in many different types of cancers.

The lysine methyltransferase KMT2C (MLL3), a subunit of the COMPASS complex, implements monomethylation of Lys4 on histone H3 (H3K4) at gene enhancers. KMT2C (*MLL3*) mutations are frequent in different human tumors. A cancer-mutational hotspot in *MLL3* within the region encoding its plant homeodomain (PHD) repeats mediates association of MLL3 with histone H2A deubiquitinase and tumor suppressor BAP1. Cancer-associated mutations in the sequence encoding *MLL3* PHD repeats disrupt the interaction between *MLL3* and BAP1 and correlate with poor patient survival. Cancer cells that had PHD-associated *MLL3* mutations or lacked BAP1 show reduced recruitment of MLL3 and the H3K27 demethylase KDM6A (UTX) to gene enhancers. Inhibition of the H3K27 methyltransferase activity of the polycomb repressive complex 2 (PRC2) in tumor cells harboring *BAP1* or *MLL3* mutations restores normal gene expression patterns and impairs cell proliferation. These data reported by Wang et al.[19] provide mechanistic insight into the oncogenic effects of PHD-associated mutations in *MLL3* and suggest that restoration of a balanced state of polycomb-COMPASS activity may have therapeutic efficacy in tumors that bear mutations in the genes encoding these epigenetic factors.

DNA methylation of *NFATC2IP* is related to the body mass index. There are interactions between fat intake with genetic (rs11150675) and transcriptional (ILMN_1725441) variations at the *NFATC2IP* locus on 2-year weight change. *cis*-DNA methylation at cg26663590 of the *NFATC2IP* locus shows an opposite impact on weight loss in response to a high-fat vs low-fat diet. Baseline methylation at cg26663590 causally mediated 52.8% of the effect of rs11150675 on 2-year weight loss in high-fat diet cases. These data reported by Sun et al.[20] might reflect causal effects of genetic, epigenetic, and transcriptional variations at the *NFATC2IP* locus on adiposity changes in response to dietary fat intake.

Classical pathogenic genes are those associated with epigenetic Mendelian disorders (EMDs), as stated in Chapter 1. Mutations in genes encoding different components of the epigenetic machinery cause multiple congenital anomalies and intellectual disability syndromes.[15,16,21] Genetic mutations may affect writers, erasers, or readers of epigenetic marks, and chromatin remodelers as well. Many EMDs fall within the category of neurodevelopmental and imprinting disorders, and some of them may manifest in adults. EMDs associated with writers and readers of the DNA methylation machinery include (i) Rett syndrome, an X-linked disorder resulting from loss-of-function mutations in *MeCP2*;

(ii) 2q23.1 microdeletion/microduplication syndrome and autosomal dominant syndrome with deletion/duplication in the *MBD5* locus; (iii) immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome, caused by homozygous or compound heterozygous hypomorphic mutations in the *DNMT3B* gene; (iv) hereditary sensory and autonomic neuropathy with dementia and hearing loss (mutations in *DNMT1* exon 20); and (v) autosomal-dominant cerebellar ataxia, deafness, and narcolepsy (mutations in *DNMT1* exon 21). EMDs associated with the histone machinery affect writers, erasers, readers, and chromatin remodelers, including: (i) Kabuki syndrome, with mutations in mixed lineage leukemia 2 (*MLL2*), a histone H3K4 methyltransferase, or lysine-specific demethylase 6A (*KDM6A*); (ii) Rubinstein-Taybi syndrome, caused by haploinsufficiency of histone acetyltransferase enzyme genes (*CREBBP* and *EP300*); (iii) genitopatellar syndrome and Say-Barber-Biesecker-Young-Simpson syndrome (mutations in the histone acetyltransferase *KAT6B*), (iv) Wiedeman-Steiner syndrome (mutations in the *MLL* gene, histone methyltransferase *H3K4*), (v) Kleefstra syndrome (mutations in *EHMT1*, histone methyltransferase H3K9), (vi) Weaver syndrome (mutations in *EZH2*, histone methyltransferase H3K27), (vii) Sotos syndrome (mutations in *NSD1*, histone methyltransferase H3K36 and H4K20); (viii) brachydactyly-mental retardation syndrome (haploinsufficiency of the histone deacetylase gene, *HDAC4*); (ix) Cornelia de Lange syndrome 5 (*CDLS5*) (X-linked) and Wilson-Turner syndrome (*WTS*) (X-linked) (mutations in histone deacetylase *HDAC8*), (x) Claes-Jensen syndrome (X-linked) (mutations in *KDMSC*, histone demethylase H3K4); (xi) Kabuki syndrome (X-linked) (mutations in *KDM6A*, histone demethylase H3K27); (xii) Siderius X-linked mental retardation syndrome (mutations in *PHF8*, plant homeodomain finger protein); (xiii) Börjeson-Forssman-Lehmann syndrome (X-linked recessive trait, missense mutations in the *PHF6* gene, plant homeodomain finger protein); and (xiv) X-linked mental retardation and macrocephaly (mutations in *BRWD3*, bromodomain-containing protein). EMDs associated with chromatin remodelers include the following: (i) alpha thalassemia/mental retardation X-linked (*ATRAX*) syndrome (mutations in *ATRAX*, SWI/SNF ATP-dependent chromatin remodeler); (ii) four variants of Coffin-Siris syndrome: mental retardation autosomal dominant 14 (MRD14) (mutations in *ARID1A*), mental retardation autosomal dominant 12 (MRD12) (mutations in *ARID1B*), mental retardation autosomal dominant 16 (MRD16) (mutations in *SMARCA4*), and mental retardation autosomal dominant 15 (MRD15) (mutations in *SMARCB1*); (iii) rhabdoid tumor predisposition syndrome 2 (mutations in *SMARCA4*); (iv) schwannomatosis (mutations in *SMARCB1*); (v) rhabdoid tumor predisposition syndrome 1 (mutations in *SMARCB1*); (vi) Nicolaides-Baraitser syndrome (mutations in *SMARCA2*); (vii) floating harbor syndrome (mutations in *SRCAP*, INO80/SWR1 ATP-dependent chromatin remodeler); (viii) CHARGE syndrome (mutations in *CHD7*, CHD ATP-dependent chromatin remodeler); and (ix) mental retardation autosomal dominant 21 (MRC21) (mutations in *CTCF*, chromatin-organizing zinc finger protein).[15,21] Some other EMDs, mainly affecting the CNS, have been reported (see Chapter 22).

Epigenetic events have also been reported in pathogenic genes associated with many other medical conditions, such as cardiovascular disease, atherosclerosis, obesity, diabetes, neurodegenerative disorders, drug abuse and alcohol dependence, psychosis and other neuropsychiatric disorders, cerebrovascular disorders, and metabolic disorders. Epigenetic modifications in pathogenic genes are the first therapeutic obstacle to any pharmacological intervention with subsequent consequences in drug metabolism, efficacy, and safety (see Chapters 5, 22, and 38).

6.2.2 Mechanistic Genes

Mechanistic genes encode receptors and their respective subunits, enzymes, and messengers involved in the mechanism of action of a particular drug. In the case of epigenetic drugs mechanistic genes are those encoding components of the epigenetic machinery: (i) DNA methyltransferases (DNMTs) (DNMT1, DNMT3A, DNMT3B), which are the targets of nucleoside analogs, small molecules, and natural products with DNA methyltransferase-inhibitory activity; (ii) DNA demethylases (the ten-eleven translocation (TET) family, the AID/APOBEC family, and the BER (base excision repair) glycosylase family); (iii) histone deacetylases, the target of HDAC inhibitors (short chain fatty acids, hydroxamic acids, cyclic peptides, benzamides, ketones, sirtuin modulators); (vi) histone acetyltransferases; (v) histone methyltransferases (lysine and arginine methyltransferase), (vi) histone demethylases; (vi) chromatin-associated proteins (ATP-dependent chromatin-remodeling complexes): the SWI/SNF (switching defective/sucrose nonfermenting) family, the ISWI (imitation SWI) family, the CHD (chromodomain, helicase, DNA binding) family, and the INO (inositol requiring 80) family, and associated proteins (DOT1L, EZH2, G9A, PRMTs); (vii) bromodomains; (viii) chromodomains; and (ix) other components of the epigenetic machinery (Table 6.1).

TABLE 6.1 Potential Drug Targets in Components of the Epigenetic Machinery

DNA methyltransferases (DNMTs)
DNMT1
DNMT2
DNMT3A
DNMT3B
DNMT3-like (DNMT3L)
DNMT3L/DNMT3A complex
DNMT1/PCNA/UHRF1 complex
Methyl-DNA-binding proteins: Methyl-CpG-binding domain (MBD)
C2H2 zinc finger domain
SET- and RING finger-associated (SRA) domain
ZBTB38
DNA demethylases
Ten-eleven translocation (TET) family
AID/APOBEC family
BER (base excision repair) glycosylase family
Histone methyltransferases
Histone lysine methyltransferases (HKMT)
G9a histone lysine methyltransferase (G9a) (KMT1C, EHMT2)
SUV39 subfamily of KMTs
SUV39H1, SUV39H2, GLP, SETDB1, SETDB2
SET and MYND domain-containing proteins (Smyd family) lysine methyltransferases
ESET protein (SETDB1)
SETD8/SET8/Pr-SET7/KMT5A lysine methyltransferase
Disruptor of telomeric silencing-1-like (DOT1L)
KMT2A/MLL1 lysine methyltransferase complex
EZH2 histone methyltransferase inhibitors
Histone arginine methyltransferases
Protein arginine methyltransferases (PRMT1–9)
Histone demethylases
Histone lysine demethylases
Lysine-specific demethylase 1 (LSD1) (KDM1A)
KDM1–8
Fe(II)/2-oxoglutarate-dependent dioxygenases
Jumonji C domain-containing histone lysine demethylases (JMJCs)
JMJD1B, H3K9me2 lysine demethylase
JMJD-1.2-KDM7 family
JMJD2A-ETV2 complex
JMJD2D-ETV2 complex
Prolyl hydroxylases
E26 transformation-specific (ETS) variant 2 (ETV2) protein
Histone acetyltransferases (HATs)
Histone lysine acetyltransferases (KATs)
General control nonderepressible 5 (GCN5) KAT2A/GCN5
KAT2B/PCAF
KAT6–8
K (lysine) acetyltransferase 8 (KAT8, MOF)
CREBBP/CBP
EP300-thymine DNA glycosylase (TDG)
Monocytic leukemia zinc finger protein-related factor (MORF)
PHF20
ATP citrate lyase (ACL)
Super elongation complex (SEC)
Multiprotein histone acetyltransferase (HAT) complex
HBO1
Inhibitor of growth family member (ING) 4/5
MYST/Esa1-associated factor (MEAF) 6
Jade family PHD finger (JADE) 1/2/3
Bromodomain and PHD finger-containing protein (BRPF) 2/3
Histone deacetylases (HDACs) (HDAC1–18)
– Class I HDACs (HDAC1, 2, 3, and 8)
HDAC1/HDAC2 transcriptional corepressor complexes (SIN3A, NuRD, CoREST)

TABLE 6.1 Potential Drug Targets in Components of the Epigenetic Machinery—cont'd

HDAC3-(SMRT/N-CoR) complexes
– Class II HDACs: class IIa (HDAC4, 5, 7, and 9); class IIb (HDAC6 and 10)
– Class III HDCAs: sirtuin family: nuclear (SIRT1, 2, 6, 7), mitochondrial (SIRT3, 4, 5), cytoplasmic (SIRT1, 2)
– Class IV HDAC (HDAC11)
Histone deacetylase RPD3
Other PTM modifiers
Acetylation, methylation, phosphorylation, crotonylation, propionylation, butyrylation, 2-hydroxyisobutyrylation, formylation, citrullination, SUMOylation, ubiquitylation, glycosylation, ADP ribosylation, biotinylation, succinylation, malonylation, glutarylation.
ATP-dependent chromatin-remodeling complexes
SWI/SNF (switching defective/sucrose nonfermenting) family
ISWI (imitation SWI) family
CHD (chromodomain, helicase, DNA binding) family
INO (inositol requiring 80) family
Other potential epigenetic targets (in alphabetical order)
2-Oxoglutarate (2OG)-dependent dioxygenases (2OGDDs)
5-Methylcytosine reader Mbd1: CXXC3 domain of Mbd1
8-Oxoguanine DNA glycosylase1 (OGG1)
ASF1: histone H3/H4 chaperone
BET (bromodomain and extraterminal domain) proteins (BRD1–60), (BDR I–VIII family), BRPF1, BRPF2, BRPF3, BRD7, BRD9, ATAD2, ATAD2b, SWI/SNF complex, HBO1 HAT complex, ATPase family AAA domain-containing protein 2 (ATAD2), BAZ2B
Canonical histones: H2A variants, H2A-H2B dimers, H3.1, H3.2, H3–H4 core.
CENP-A (centromere protein A)
Chaperones
Chromatin remodelers: ATRX, NUPR1, LSH, SMARCAD1
Chromatin-remodeling factor HDAC4
Chromodomains
circRNAs
Deubiquitinase (DUB) Ubp10
DNA alkylation repair enzyme ALKB2
E26 transformation-specific (ETS) variant 2 (ETV2) protein (ETS-related 71)
E3-ubiquitin ligase complex of H2B:RNF20/RNF40
Glycoside hydrolase *O*-GlcNAcase (OGA)
Histone acetylation-binding double PHD finger (DPF) domains
Histone chaperone chromatin assembly factor 1 (CAF-1)
Histone code reader Spin1
Histone deubiquitinases
Histone H1: replication dependent (H1.1–H1.5); replication independent (H1.10 and H1.0)
HOXA
HP1
Human histone cell cycle regulator (HIRA) complex: HIRA, ubinuclein-1, CABIN1, transiently antisilencing function 1
Intragenic enhancers: transcriptional coactivator Creb-binding protein (CBP), CBP/p300
Karyopherin protein Kap123
Lipid mediators
lncRNA n342419 (MANTIS): histone demethylase JARID1B
MDM2: E3 ubiquitin ligase
Men1, menin
miRNA-associated modifiers: (i) miRNA genes and upstream regulators, (ii) miRNA silencing machinery, and (iii) miRNA targets
Mitochondrial lncRNAs: (i) lncND5, lncND6, and lncCyt b RNA; (ii) chimeric mitochondrial DNA-encoded lncRNAs; and (iii) putative mitochondrial DNA-encoded lncRNAs
Mitochondrial transcription factor A (TFAM)
MOZ (KAT6A) and DPF2 (BAF45d)
mRNA modifiers: *N*6-methyladenosine, *N*6,2′-*O*-dimethyladenosine, 5-methylcytidine, 5-hydroxylmethylcytidine, inosine, pseudouridine, *N*1-methyladenosine
Multisubunit CCR4 (carbon catabolite repressor 4)-NOT (Negative on TATA) complex, CCR4-NOT subunits CNOT1, CNOT2, CNOT3, master regulator class II transactivator (CIITA)
N6-adenine DNA methylation modifiers: m6A demethylase, DNA polymerase κ
Nuclear activating miRNAs (namiRNAs)
Nuclear pore complex (NPC)
Nucleotide excision repair (NER) mediators: UV-RING1B complex, H2A-ubiquitin-binding protein ZRF1, NER factor XPA, endoribonuclease DICER, MMSET, SWI/SNF, INO80, PARP1, histone chaperone Asf1, chromatin assembly factor 1 (CAF-1), damage sensors Ddc1 and Ddc2, E3 ubiquitin ligase complex Rtt101Mms1, Mms22 substrate adaptor, repair genes EXO5, MGMT, ALKBH3, histone methyltransferase gene SET-domain

Continued

TABLE 6.1 Potential Drug Targets in Components of the Epigenetic Machinery—cont'd

containing 2 (SETD2), KAT5 (TIP60) histone acetyltransferase, RAD51 mediator proteins, Wwox protein, 53BP1-interacting chromatin-associated proteins, γH2AX, RING-less BRCA1 protein, *O*-GlcNAc transferase (OGT), ZBTB4
Nup93, Nup188, Nup205
Oligosaccharyltransferase complex (OST)
O-Linked β-*N*-acetylglucosamine (*O*-GlcNAc) transferase (OGT)
Pleiohomeotic (Pho), REPO domain of Pho, Spt5
Poly(ADP-ribose) polymerases (PARPs) PARP-1, PARP-2 and PARP-3
Polycomb group proteins: polycomb repressive complex 2 (PRC2), enhancer of zeste homolog 2 (EZH2), PHF1, MTF2, PHF19, heterochromatin protein 1 (LHP1), PR-DUB deubiquitination complexes, coactivator multiprotein bridging factor 1 (Mbf1), Friend of GATA-1 (FOG1), protein EPOP (elongin BC and polycomb repressive complex 2-associated protein; a.k.a. C17orf96, esPRC2p48, and E130012A19Rik), polycomb PRC1
Proteasome proteins
Pyruvate dehydrogenase (PDH) complex (PDC): E4 transcription factor 1 (E4F1), dihydrolipoamide acetlytransferase (Dlat), dihydrolipoyl dehydrogenase (Dld), mitochondrial pyruvate carrier 1 (Mpc1), solute carrier family 25 member 19 (Slc25a19)
RNA methylation modifiers: DNA replication regulators: TICRR/TRESLIN, acetyl-histone-binding bromodomain (BRD) and extraterminal (BET) proteins BRD2 and BRD4, replication licencing factor CDC6, MCM2–7 replicative helicase, CDC7-Dbf4 kinase and cyclin-dependent kinase, CDC45 and go-ichi-ni-san complex (GINS), CDC45·MCM2–7·GINS (CMG) helicase complex, TIMELESS (TIM)
RNA-binding proteins: HuR, GRSF1, SHARP, SLIRP, PPR, and PNPASE
RNA-induced silencing complex (RISC)
RNA-processing factor Nudt21
SAGA complex: USP22, DUB module, ATXN7L3, ENY2
SET complex
Small ubiquitin-like modifier (SUMO) proteins
SUMO isopeptidase SENP3
SUUR protein
SUV4–20 histone H4K20 methyltransferases
Tandem Tudor domain-plant homeodomain (TTD-PHD)
Target of rapamycin complex 1 (TORC1), high mobility group box (HMGB) proteins
Tousled-like kinases (TLK1–2)
Transcription factors and TF regulators: general transcription factor IID (TFIID), coactivator Spt-Ada-Gcn5 acetyltransferase (SAGA), SAGA/TREX-2 subunit Sus1, SAGA deubiquitinating enzyme Ubp8, novel binding site-directed DNA demethylation-inducing TFs (RUNX3, GATA2, CEBPB, MAFB, NR4A2, MYOD1, CEBPA, and TBX5), Hnf4a (major transcription factor of the DnaJ heat shock protein family (Hsp40) member C22 (Dnajc22)), ETS family of transcription factors, ETS-domain transcription factor GABPA, serum response factor (Srf), Sp1 (and the 26 members of strong Sp/KLF family of transcription factors), transcriptional regulators of transcription factors (MeCP2, multidomain CCCTC-binding factor (CTCF)), transcriptional silencers, EPHB2, SNAIL1, EPHB2 enhancer factors (FOXA1, MYB, CDX2, TCF7L2), motif ACTAYRNNNCCCR (M4), THAP11/HCF-1 complex
Transcriptional repressors: NuRD-interacting transcriptional regulator Ikaros
Transposable elements: RNA transposons, DNA transposons, human endogenous retroviruses (HERVs), LINE retrotransposons, SINE retrotransposons, long terminal repeat (LTR)-type retrotransposons (HERV/LTRs), HERV/LTR-shared regulatory element (HSRE), pluripotent TFs (SOX2, POU5F1, NANOG), embryonic endoderm/mesendoderm TFs (GATA4/6, SOX17, FOXA1/2), hematopoietic TFs (SPI1 (PU1), GATA1/2, TAL1), CTCF, DCCGTAGCCATTTTGGCTCAAG spliced leader (DinoSL), putative promoter motif from the DinoSL TTT(T/G), protein POF, Sleeping Beauty transposon (SB), long interspersed nuclear element-1 (LINE-1), intracisternal A particle (IAP), PIWI-interacting small RNAs (piRNAs), scan RNAs (scnRNAs)-Ptiwi01-Ptiwi09, iesRNAs-Ptiwi10-Ptiwi11, Alu elements-short interspersed nuclear element (SINE) family, RNA-editing enzyme ADAR, nuclear ribonucleoprotein HNRNPC, nuclear RNA helicase DHX9, ERV-9 LTR retrotransposon, TFs NF-Y and GATA-1-2, HERV-K(HML-10) endogenous retrovirus family, death-associated protein 3 (DAP3), SVA repressor BORIS
Trithorax group (trxG) of transcriptional activators
tRNA modifiers: queuosine, inosine, 5-methoxycarbonylmethyl-2-thiouridine, wybutosine, threonyl-carbamoyl-adenosine, 5-methylcytosine
Ubiquitin C-terminal hydrolase isozyme L1 (UCHL1)
Ubiquitin ligase RNF19A
UHRF1: histone- and DNA-binding RING E3 ubiquitin ligase
UHRF2: ubiquitin-protein ligase E3
USP7 (ubiquitin-specific protease 7), USP38, USP44, USP52 deubiquitinases
WD40 repeat-containing protein

6.2.3 Metabolic Genes

Metabolic genes (Table 6.2) play an essential role in drug biotransformation, and epigenetic changes in metabolic genes contribute to interindividual differences in drug response.[17] Genes associated with drug metabolism include genes encoding phase I–II reaction enzymes (Table 6.2). Drug-metabolizing enzymes (DMEs) exhibit dramatic inter-individual and intraindividual variability in expression and activity, in part due to DNA methylation. Highly variable DNA methylation was observed in 37 DME genes, 7 of which showed significant inverse correlations between DNA methylation and mRNA expression. Some DMEs may act as tumor suppressor or housekeeper enzymes based on their unique DNA methylation features.[22]

TABLE 6.2 Drug Metabolism-Related Genes

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
PHASE I ENZYMES						
Alcohol dehydrogenases	*ADH1A*	Alcohol dehydrogenase 1A (class I), alpha polypeptide	4q23	14.62 kb	103700	Susceptibility to alcohol dependence
	ADH1B	Alcohol dehydrogenase 1B (class I), beta polypeptide	4q23	15.05 kb	103720	Susceptibility to alcohol dependence Susceptibility to genotoxicity induced by alcohol drinking Susceptibility to esophageal squamous cell carcinoma
	ADH1C	Alcohol dehydrogenase 1C (class I), gamma polypeptide	4q23	16.27 kb	103730	Susceptibility to Parkinson disease Susceptibility to alcohol dependence
	ADH4	Alcohol dehydrogenase 4 (class II), pi polypeptide	4q22	20.62 kb	103740	Susceptibility to alcoholism Susceptibility to cluster headache
	ADH5	Alcohol dehydrogenase 5 (class III), chi polypeptide	4q23	17.80 kb	103710	Susceptibility to alcohol dependence Susceptibility to childhood asthma
	ADH6	Alcohol dehydrogenase 6 (class V)	4q23	16.61 kb	103735	
	ADH7	Alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide	4q23 q24	23.11 kb	600086	
	ADHFE1	Alcohol dehydrogenase, iron containing, 1	8q13.1	36.00 kb	611083	
Aldehyde dehydrogenases	*ALDH1A1*	Aldehyde dehydrogenase 1 family, member A1	9q21.13	52.38 kb	100640	
	ALDH1A2	Aldehyde dehydrogenase 1 family, member A2	15q21.3	112.28 kb	603687	
	ALDH1A3	Aldehyde dehydrogenase 1 family, member A3	15q26.3	36.82 kb	600463	Microphthalmia, isolated 8
	ALDH1B1	Aldehyde dehydrogenase 1 family, member B1	9p11.1	5.96 kb	100670	
	ALDH2	Aldehyde dehydrogenase 2 family (mitochondrial)	12q24.2	43.44 kb	100650	Alcohol sensitivity, acute myocardial infarction Susceptibility to hangover Susceptibility to alcohol-induced flushing Susceptibility to genotoxicity induced by alcohol drinking Susceptibility to esophageal squamous cell carcinoma Susceptibility to esophageal cancer in heavy drinking patients
	ALDH3A1	Aldehyde dehydrogenase 3 family, member A1	17p11.2	10.00 kb	100660	
	ALDH3A2	Aldehyde dehydrogenase 3 family, member A2	17p11.2	28.00 kb	609523	Sjogren-Larsson syndrome
	ALDH3B1	Aldehyde dehydrogenase 3 family, member B1	11q13	18.00 kb	600466	Susceptibility to paranoid schizophrenia

Continued

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
	ALDH3B2	Aldehyde dehydrogenase 3 family, member B2	11q13	19.05 kb	601917	
	ALDH4A1	Aldehyde dehydrogenase 4 family, member A1	1p36	31.00 kb	606811	Hyperprolinemia, type II
	ALDH5A1	Aldehyde dehydrogenase 5 family, member A1	6p22	42.24 kb	610045	Succinic semialdehyde dehydrogenase deficiency 4-Hydroxybutyric aciduria
	ALDH6A1	Aldehyde dehydrogenase 6 family, member A1	14q24.3	24.00 kb	603178	Methylmalonate semialdehyde dehydrogenase deficiency
	ALDH7A1	Aldehyde dehydrogenase 7 family, member A1	5q31	52.16 kb	107323	Epilepsy, pyridoxine dependent Susceptibility to low-trauma osteoporotic fracture
	ALDH8A1	Aldehyde dehydrogenase 8 family, member A1	6q23.2	32.00 kb	606467	
	ALDH9A1	Aldehyde dehydrogenase 9 family, member A1	1q23.1	36.00 kb	602733	
	AOX1	Aldehyde oxidase 1	2q33	85.00 kb	602841	
Aldo-keto reductases	*AKR1A1*	Aldo-keto reductase family 1, member A1 (aldehyde reductase)	1p33 p32	19.00 kb	103830	
	AKR1B1	Aldo-keto reductase family 1, member B1 (aldose reductase)	7q35	16.78 kb	103880	Susceptibility to diabetic retinopathy in type 2 diabetes
	AKR1C1	Aldo-keto reductase family 1, member C1	10p15 p14	13.00 kb	600449	
	AKR1D1	Aldo-keto reductase family 1, member D1	7q32 q33	41.87 kb	604741	Bile acid synthesis defect, congenital, 2 Cholestasis with Δ(4)-3-3oxosteroid 5β-reductase deficiency
Amine oxidases	*MAOA*	Monoamine oxidase A	Xp11.3	91.92 kb	309850	Brunner syndrome Mental retardation with impulsive behavior MAOA/B deletion syndrome Susceptibility to anorexia nervosa Susceptibility to Parkinson disease Susceptibility to attention-deficit/hyperactivity disorder (ADHD)
	MAOB	Monoamine oxidase B	Xp11.23	115.87 kb	309860	MAOA/B deletion syndrome Susceptibility to Parkinson disease
	SMOX	Spermine oxidase	20p13	38.00 kb	615854	
Carbonyl reductases	*CBR1*	Carbonyl reductase 1	21q22.13	3.00 kb	114830	
	CBR3	Carbonyl reductase 3	21q22.2	11.59 kb	603608	Susceptibility to diabetes, type II
	CBR4	Carbonyl reductase 4	4q32.3	22.68 kb		
Cytidine deaminase	*CDA*	Cytidine deaminase	1p36.2 p35	26.96 kb	123920	
Cytochrome P450 family	*CYP1A1*	Cytochrome P450, family 1, subfamily A, polypeptide 1	15q24.1	6.00 kb	108330	Susceptibility to lung squamous cell carcinoma

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
						Susceptibility to childhood acute lymphoblastic leukemia Susceptibility to osteoporosis Susceptibility to hypospadias Susceptibility to head and neck cancer Susceptibility to obstructive pulmonary disease
	CYP1A2	Cytochrome P450, family 1, subfamily A, polypeptide 2	15q24.1	7.76 kb	124060	Susceptibility to porphyria cutanea tarda, independent Susceptibility to myocardial infarction
	CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	2p22.2	8.58 kb	601771	Glaucoma 3A, primary open angle, congenital, juvenile, or adult onset Peters anomaly Susceptibility to endometrial cancer
	CYP2A6	Cytochrome P450, family 2, subfamily A, polypeptide 6	19q13.2	6.91 kb	122720	Susceptibility to nicotine addiction and smoking behavior Susceptibility to lung cancer
	CYP2A7	Cytochrome P450, family 2, subfamily A, polypeptide 7	19q13.2	7.00 kb	608054	
	CYP2A13	Cytochrome P450, family 2, subfamily A, polypeptide 13	19q13.2	7.00 kb	608055	Susceptibility to lung cancer
	CYP2B6	Cytochrome P450, family 2, subfamily B, polypeptide 6	19q13.2	27.10 kb	123930	
	CYP2C8	Cytochrome P450, family 2, subfamily C, polypeptide 8	10q23.33	32.00 kb	601129	Rhabdomyolysis, cerivastatin induced
	CYP2C9	Cytochrome P450, family 2, subfamily C, polypeptide 9	10q24	50.00 kb	601130	
	CYP2C18	Cytochrome P450, family 2, subfamily C, polypeptide 18	10q24	52.00 kb	601131	
	CYP2C19	Cytochrome P450, family 2, subfamily C, polypeptide 19	10q24	90.00 kb	124020	Susceptibility to essential tremor
	CYP2D6	Cytochrome P450, family 2, subfamily D, polypeptide 6	22q13.1	4.00 kb	124030	Susceptibility to acute lymphoid and myeloid leukemia
	CYP2D7P1	Cytochrome P450, family 2, subfamily D, polypeptide 7 pseudogene 1	22q13			
	CYP2E1	Cytochrome P450, family 2, subfamily E, polypeptide 1	10q26.3	11.00 kb	124040	Susceptibility to lung adenocarcinoma Susceptibility to head and neck cancer Susceptibility to acute lymphoblastic leukemia Susceptibility to oral clefts Susceptibility to alcoholism and alcoholic cirrhosis
	CYP2F1	Cytochrome P450, family 2, subfamily F, polypeptide 1	19q13.2	13.00 kb	124070	

Continued

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
	CYP2J2	Cytochrome P450, family 2, subfamily J, polypeptide 2	1p31.3 p31.2	33.44 kb	601258	Susceptibility to coronary artery disease Susceptibility to myocardial infarction
	CYP2R1	Cytochrome P450, family 2, subfamily R, polypeptide 1	11p15.2	14.32 kb	608713	Rickets due to defect in vitamin D 25 hydroxylation Susceptibility to diabetes, type I
	CYP2S1	Cytochrome P450, family 2, subfamily S, polypeptide 1	19q13.1	14.00 kb	611529	
	CYP2W1	Cytochrome P450, family 2, subfamily W, polypeptide 1	7p22.3	6.00 kb	615967	
	CYP3A4	Cytochrome P450, family 3, subfamily A, polypeptide 4	7q21.1	27.21 kb	124010	Susceptibility to prostate cancer Susceptibility to treatment-related leukemia
	CYP3A5	Cytochrome P450, family 3, subfamily A, polypeptide 5	7q21.1	31.00 kb	605325	Susceptibility to hypertension, salt sensitive essential Susceptibility to prostate cancer
	CYP3A7	Cytochrome P450, family 3, subfamily A, polypeptide 7	7q21 q22.1	30.00 kb	605340	
	CYP3A43	Cytochrome P450, family 3, subfamily A, polypeptide 43	7q21.1	37.00 kb	606564	
	CYP4A11	Cytochrome P450, family 4, subfamily A, polypeptide 11	1p33	12.00 kb	601310	
	CYP4A22	Cytochrome P450, family 4, subfamily A, polypeptide 22	1p33	11.00 kb	615341	
	CYP4B1	Cytochrome P450, family 4, subfamily B, polypeptide 1	1p34 p12	20.00 kb	124075	
	CYP4F2	Cytochrome P450, family 4, subfamily F, polypeptide 2	19p13.12	20.05 kb	604426	Susceptibility to coeliac disease Susceptibility to myocardial infarction
	CYP4F3	Cytochrome P450, family 4, subfamily F, polypeptide 3	19p13.2	19.86 kb	601270	Susceptibility to coeliac disease
	CYP4F8	Cytochrome P450, family 4, subfamily F, polypeptide 8	19p13.1	14.00 kb	611545	
	CYP4F11	Cytochrome P450, family 4, subfamily F, polypeptide 11	19p13.1	22.50 kb	611517	
	CYP4F12	Cytochrome P450, family 4, subfamily F, polypeptide 12	19p13.1	24.10 kb	611485	
	CYP4Z1	Cytochrome P450, family 4, subfamily Z, polypeptide 1	1p33	50.00 kb		
	CYP7A1	Cytochrome P450, family 7, subfamily A, polypeptide 1	8q11 q12	9.98 kb	118455	Cholesterol 7α-hydroxylase (CYP7A1) deficiency
	CYP7B1	Cytochrome P450, family 7, subfamily B, polypeptide 1	8q21.3	202.82 kb	603711	Bile acid synthesis defect, congenital, 3 Spastic paraplegia 5A, autosomal recessive Severe cholestasis
	CYP8B1	Cytochrome P450, family 8, subfamily B, polypeptide 1	3p22.1	3.00 kb	602172	

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
	CYP11A1	Cytochrome P450, family 11, subfamily A, polypeptide 1	15q23 q24	30.00 kb	118485	Adrenal insufficiency, congenital, with 46XY sex reversal, partial or complete Susceptibility to breast cancer
	CYP11B1	Cytochrome P450, family 11, subfamily B, polypeptide 1	8q21	7.46 kb	610613	Adrenal hyperplasia, congenital, due to 11β-hydroxylase deficiency Aldosteronism, glucocorticoid remediable Susceptibility to aldosterone-producing adenoma
	CYP11B2	Cytochrome P450, family 11, subfamily B, polypeptide 2	8q21 q22	7.29 kb	124080	Aldosterone to renin ratio raised Corticosterone methyloxydase type I deficiency Corticosterone methyloxydase type II deficiency Glucocorticoid-remediable aldosteronism Susceptibility to low-renin hypertension
	CYP17A1	Cytochrome P450, family 17, subfamily A, polypeptide 1	10q24.3	7.00 kb	609300	17,20-Lyase deficiency, isolated 17α-Hydroxylase/17,20-lyase deficiency
	CYP19A1	Cytochrome P450, family 19, subfamily A, polypeptide 1	15q21.1	130.54 kb	107910	Aromatase deficiency syndrome Aromatase excess syndrome
	CYP20A1	Cytochrome P450, family 20, subfamily A, polypeptide 1	2q33.2	58.00 kb		
	CYP21A2	Cytochrome P450, family 21, subfamily A, polypeptide 2	6p21.3	3.35 kb	613815	Adrenal hyperplasia III, female Pseudohermaphroditism
	CYP24A1	Cytochrome P450, family 24, subfamily A, polypeptide 1	20q13	20.53 kb	126065	Hypercalcemia, infantile
	CYP26A1	Cytochrome P450, family 26, subfamily A, polypeptide 1	10q23 q24	4.41 kb	602239	
	CYP26B1	Cytochrome P450, family 26, subfamily B, polypeptide 1	2p13.2	118.60 kb	605207	Craniosynostosis with radiohumeral fusions and other skeletal and craniofacial anomalies
	CYP26C1	Cytochrome P450, family 26, subfamily C, polypeptide 1	10q23.33	7.43 kb	608428	Focal facial dermal dysplasia 4
	CYP27A1	Cytochrome P450, family 27, subfamily A, polypeptide 1	2q33 qter	33.54 kb	606530	Cerebrotendinous xanthomatosis
	CYP27B1	Cytochrome P450, family 27, subfamily B, polypeptide 1	12q13.1 q13.3		609506	Vitamin D-dependent rickets, type I
	CYP39A1	Cytochrome P450, family 39, subfamily A, polypeptide 1	6p21.1 p11.2	103.07 kb	605994	
	CYP46A1	Cytochrome P450, family 46, subfamily A, polypeptide 1	14q32.1	42.88 kb	604087	Susceptibility to primary open-angle glaucoma Susceptibility to Alzheimer disease
	CYP51A1	Cytochrome P450, family 51, subfamily A, polypeptide 1	14q32.1	22.00 kb	601637	

Continued

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
	POR	P450 (cytochrome) oxidoreductase	7q11.2		124015	Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis Disordered steroidogenesis due to cytochrome P450 oxidoreductase POR deficiency
	TBXAS1	Thromboxane A synthase 1 (platelet)	7q34 q35	191.05 kb	274180	Ghosal hematodiaphyseal dysplasia Thromboxane A synthase deficiency
Cytochrome b5 reductase	*CYB5R3*	Cytochrome b5 reductase 3	22q13.2	30.59 kb	613213	Methemoglobinemia, type I Methemoglobinemia, type II
Dihydropyrimidine dehydrogenase	*DPYD*	Dihydropyrimidine dehydrogenase	1p22	843.32 kb	612779	5-Fluorouracil toxicity Dihydropyrimidine dehydrogenase deficiency.
Esterases	*AADAC*	Arylacetamide deacetylase	3q25.1	14.00 kb	600338	
	CEL	Carboxyl ester lipase	9q34.3	9.88 kb	114840	Diabetes-pancreatic exocrine dysfunction syndrome
	CES1	Carboxylesterase 1	16q22.2	30.31 kb	114835	
	CES1P1	Carboxylesterase 1 pseudogene 1	16q12.2			
	CES2	Carboxylesterase 2	16q22.1	11.00 kb	605278	
	CES3	Carboxylesterase 3	16q22.1		605279	
	CES5A	Carboxylesterase 5A	16q12.2			
	ESD	Esterase D	13q14.1 q14.2	25.00 kb	133280	
	GZMA	Granzyme A (granzyme 1, cytotoxic T lymphocyte-associated serine esterase 3)	5q11 q12	7.59 kb	140050	
	GZMB	Granzyme B (granzyme 2, cytotoxic T lymphocyte-associated serine esterase 1)	14q11.2	3.00 kb	123910	Susceptibility to autism spectrum disorder
	PON1	Paraoxonase 1	7q21.3	26.21 kb	168820	Susceptibility to coronary heart disease Susceptibility to atherosclerosis Susceptibility to pesticide poisoning Susceptibility to exsudative age-related macular degeneration Susceptibility to abdominal aortic aneurysm Susceptibility to Alzheimer disease Susceptibility to sporadic amyotrophic lateral sclerosis Susceptibility to attaining longevity Susceptibility to stroke
	PON2	Paraoxonase 2	7q21.3	30.21 kb	602447	Susceptibility to coronary artery disease
	PON3	Paraoxonase 3	7q21.3	36.00 kb	602720	Susceptibility to coronary heart disease Susceptibility to Alzheimer disease

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
	UCHL1	Ubiquitin carboxyl terminal esterase L1 (ubiquitin thiolesterase)	4p14	11.52 kb	191342	Neurodegeneration with optic atrophy, childhood onset Parkinson disease 5
	UCHL3	Ubiquitin carboxyl terminal esterase L3 (ubiquitin thiolesterase)	13q22.2	56.14 kb	603090	
Epoxidases	*EPHX1*	Epoxide hydrolase 1, microsomal (xenobiotic)	1q42.1	35.46 kb	132810	Fetal hydantoin syndrome
	EPHX2	Epoxide hydrolase 2, microsomal (xenobiotic)	8p21	53.84 kb	132811	Susceptibility to coronary heart disease; Susceptibility to heart failure; Susceptibility to ischemic stroke
Flavin-containing monooxygenases	*FMO1*	Flavin-containing monooxygenase 1	1q24.3	37.45 kb	136130	
	FMO2	Flavin-containing monooxygenase 2	1q24.3	23.00 kb	603955	
	FMO3	Flavin-containing monooxygenase 3	1q24.3	26.92 kb	136132	Trimethylaminuria
	FMO4	Flavin-containing monooxygenase 4	1q24.3	27.73 kb	136131	
	FMO5	Flavin-containing monooxygenase 5	1q21.1	39.00 kb	603957	
	FMO6P	Flavin-containing monooxygenase 6 pseudogene	1q24.3	24.08 kb		
Glutathione reductase/ peroxidases	*GPX1*	Glutathione peroxidase 1	3p21.3	1.18 kb	138320	Hemolytic anemia due to glutathione peroxidase deficiency Susceptibility to breast cancer Susceptibility to diabetes type II Susceptibility to Keshan disease (endemic dilated cardiomyopathy) Susceptibility to thoracic aortic aneurysm in hypertensive patients
	GPX2	Glutathione peroxidase 2 (gastrointestinal)	14q24.1	3.00 kb	138319	
	GPX3	Glutathione peroxidase 3 (plasma)	5q23	8.55 kb	138321	Susceptibility to cerebral venous thrombosis
	GPX4	Glutathione peroxidase 4	19p13.3	2.85 kb	138322	Hepoxilin A3 synthase-linked ichthyosis Susceptibility to infertility
	GPX5	Glutathione peroxidase 5	6p22.1	8.94 kb	603435	
	GPX6	Glutathione peroxidase 6 (olfactory)	6p22.1	12.00 kb	607913	
	GPX7	Glutathione peroxidase 7	1p32	6.00 kb	615784	
	GSR	Glutathione reductase	8p21.1	48.00 kb	138300	Hemolytic anemia due to glutathione reductase deficiency
Peptidases	*DPEP1*	Dipeptidase 1 (renal)	16q24.3	17.00 kb	179780	
	METAP1	Methionyl aminopeptidase 1	4q23	27.40 kb	610151	

Continued

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
Prostaglandin endoperoxide synthases	*PTGS1*	Prostaglandin endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	9q32 q33.3	24.00 kb	176805	
	PTGS2	Prostaglandin endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	1q25.2 q25.3	8.61 kb	600262	Susceptibility to diabetes type II Susceptibility to prostate cancer
Short chain dehydrogenases/ reductases	*DHRS1*	Dehydrogenase/reductase (SDR family) member 1	14q12	8.86 kb	610410	
	DHRS2	Dehydrogenase/reductase (SDR family) member 2	14q11.2	9.28 kb	615194	
	DHRS3	Dehydrogenase/reductase (SDR family) member 3	1p36.1	49.00 kb	612830	
	DHRS4	Dehydrogenase/reductase (SDR family) member 4	14q11.2	15.52 kb	611596	
	DHRS7	Dehydrogenase/reductase (SDR family) member 7	14q23.1	20.00 kb	612833	
	DHRS9	Dehydrogenase/reductase (SDR family) member 9	2q31.1	31.00 kb	612131	
	DHRS12	Dehydrogenase/reductase (SDR family) member 12	13q14.3	36.16 kb	616163	
	DHRS13	Dehydrogenase/reductase (SDR family) member 13	17q11.2	5.29 kb	616157	
	DHRSX	Dehydrogenase/reductase (SDR family) X linked	Xp22.33; Yp11.2	281.43 kb	600713	Cortisone reductase deficiency 2
	HSD11B1	Hydroxysteroid (11β) dehydrogenase 1	1q32 q41	48.75 kb	600713	Cortisone reductase deficiency 2
	HSD17B10	Hydroxysteroid (17β) dehydrogenase 10	Xp11.2	3.12 kb	300256	Choreoathetosis, abnormal behavior, and mental retardation Hydroxyacyl-CoA dehydrogenase, type II, deficiency Mental retardation mild, 17 Mental retardation, 31
	HSD17B11	Hydroxysteroid (17β) dehydrogenase 11	4q22.1	54.76 kb	612831	
	HSD17B14	Hydroxysteroid (17β) dehydrogenase 14	19q13.33	22.66 kb	612832	
Superoxide dismutase	*SOD1*	Superoxide dismutase 1, soluble	21q22.11	9.31 kb	147450	
	SOD2	Superoxide dismutase 2, mitochondrial	6q25.3	14.21 kb	177460	Microvascular complications of diabetes 6
Xanthine dehydrogenase	*XDH*	Xanthine dehydrogenase	2p23.1	80.42 kb	607633	Xanthinuria, type I
PHASE II ENZYMES						
Amino acid transferases	*AGXT*	Alanine glyoxylate aminotransferase	2q37.3	10.37 kb	604285	Hyperoxaluria, primary, type 1
	BAAT	Bile acid CoA: amino acid *N*-acyltransferase (glycine *N*-choloyltransferase)	9q22.3	24.00 kb	602938	Hypercholanemia, familial

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
	CCBL1	Cysteine conjugate β-lyase, cytoplasmic	9q34.11	48.90 kb	600547	
Dehydrogenases	*NQO1*	NAD(P)H dehydrogenase, quinone 1	16q22.1	17.23 kb	125860	Modifying the susceptibility to acute myeloid leukemia Susceptibility to cancer Susceptibility to breast cancer with poor survival Susceptibility to artery plaques, diabetic patients, type II
	NQO2	NAD(P)H dehydrogenase, quinone 2	6p25.2	19.00 kb	160998	Susceptibility to breast cancer
	XDH	Xanthine dehydrogenase	2p23.1	80.42 kb	607633	Xanthinuria type I
Esterases	*CES1*	Carboxylesterase 1	16q22.2	30.31 kb	114835	
	CES1P1	Carboxylesterase 1 pseudogene 1	16q12.2			
	CES2	Carboxylesterase 2	16q22.1	11.00 kb	605278	
	CES3	Carboxylesterase 3	16q22.1		605279	
	CES5A	Carboxylesterase 5A	16q12.2			
Glucuronosyl transferases	*DDOST*	Dolichyl diphosphooligosaccharide protein glycosyltransferase subunit (noncatalytic)	1p36.1	9.78 kb	602202	Congenital disorder of glycosylation, type Ir
	UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1	2q37	13.00 kb	191740	Crigler-Najjar syndrome 1 Crigler-Najjar syndrome 2 Gilbert syndrome
	UGT1A3	UDP glucuronosyltransferase 1 family, polypeptide A3	2q37	44.17 kb	606428	
	UGT1A4	UDP glucuronosyltransferase 1 family, polypeptide A4	2q37	54.51 kb	606429	
	UGT1A5	UDP glucuronosyltransferase 1 family, polypeptide A5	2q37	61.00 kb	606430	
	UGT1A6	UDP glucuronosyltransferase 1 family, polypeptide A6	2q37	81.63 kb	606431	Susceptibility to bladder cancer
	UGT1A7	UDP glucuronosyltransferase 1 family, polypeptide A7	2q37		606432	
	UGT1A8	UDP glucuronosyltransferase 1 family, polypeptide A8	2q37	13.00 kb	606433	
	UGT1A9	UDP glucuronosyltransferase 1 family, polypeptide A9	2q37	101.41 kb	606434	
	UGT1A10	UDP glucuronosyltransferase 1 family, polypeptide A10	2q37	136.83 kb	606435	Susceptibility to colon cancer
	UGT2A1	UDP glucuronosyltransferase 2 family, polypeptide A1, complex locus	4q13	64.78 kb	604716	
	UGT2A3	UDP glucuronosyltransferase 2 family, polypeptide A3	4q13.2	23.39 kb	616382	
	UGT2B10	UDP glucuronosyltransferase 2 family, polypeptide B10	4q13.2	14.00 kb	600070	

Continued

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
	UGT2B11	UDP glucuronosyltransferase 2 family, polypeptide B11	4q13.2	14.41 kb	603064	
	UGT2B15	UDP glucuronosyltransferase 2 family, polypeptide B15	4q13	24.06 kb	600069	
	UGT2B17	UDP glucuronosyltransferase 2 family, polypeptide B17	4q13	31.04 kb	601903	Bone mineral density QTL 12, osteoporosis Susceptibility to prostate cancer
	UGT2B28	UDP glucuronosyltransferase 2 family, polypeptide B28	4q13.2	14.55 kb	606497	
	UGT2B4	UDP glucuronosyltransferase 2 family, polypeptide B4	4q13	15.00 kb	600067	
	UGT2B7	UDP glucuronosyltransferase 2 family, polypeptide B7	4q13	16.00 kb	600068	
	UGT3A1	UDP glycosyltransferase 3 family, polypeptide A1	5p13.2	47.94 kb	616383	
	UGT8	UDP glycosyltransferase 8	4q26	78.59 kb	601291	
Glutathione transferases	*GSTA1*	Glutathione *S*-transferase alpha 1	6p12.1	12.27 kb	138359	
	GSTA2	Glutathione *S*-transferase alpha 2	6p12.1	13.00 kb	138360	
	GSTA3	Glutathione *S*-transferase alpha 3	6p12.1	13.06 kb	605449	
	GSTA4	Glutathione *S*-transferase alpha 4	6p12.1	17.39 kb	605450	
	GSTA5	Glutathione *S*-transferase beta 5	6p12.2	14.00 kb	607605	
	GSTK1	Glutathione *S*-transferase kappa 1	7q34	5.70 kb	602321	
	GSTM1	Glutathione *S*-transferase mu 1	1p13.3	5.95 kb	138350	Susceptibility to endometrial cancer Susceptibility to age-related hearing impairment Susceptibility to acute lymphoid and myeloid leukemia Susceptibility to vitiligo Susceptibility to head and neck cancer Susceptibility to leukopenia Susceptibility to non-Hodgkin lymphoma (NHL) Susceptibility to idiopathic male infertility Susceptibility to diabetes type II
	GSTM2	Glutathione *S*-transferase mu 2 (muscle)	1p13.3	7.22 kb	138380	
	GSTM3	Glutathione *S*-transferase mu 3 (brain)	1p13.3	4.00 kb	138390	
	GSTM4	Glutathione *S*-transferase mu 4	1p13.3	9.43 kb	138333	Susceptibility to lung cancer
	GSTM5	Glutathione *S*-transferase mu 5	1p13.3	6.00 kb	138385	

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
	GSTO1	Glutathione *S*-transferase omega 1	10q25.1	12.54 kb	605482	Susceptibility to hepatocellular carcinoma, cholangiocarcinoma, and breast cancer
	GSTO2	Glutathione *S*-transferase omega 2	10q25.1	36.53 kb	612314	Susceptibility to ovarian cancer Susceptibility to late onset Alzheimer disease
	GSTP1	Glutathione *S*-transferase pi 1	11q13	3.06 kb	134660	Susceptibility to esophagus carcinoma Susceptibility to chronic obstructive pulmonary disease Susceptibility to lung cancer Susceptibility to childhood asthma Susceptibility to diabetes type II
	GSTT1	Glutathione *S*-transferase theta 1	22q11.23	8.14 kb	600436	
	GSTT2	Glutathione *S*-transferase theta 2	22q11.23	3.79 kb	600437	
	GSTZ1	Glutathione *S*-transferase zeta 1	14q24.3	10.59 kb	603758	Tyrosinemia, type Ib
	GSTCD	Glutathione *S*-transferase, C terminal domain containing	4q24	138.94 kb	615912	
	MGST1	Microsomal glutathione *S*-transferase 1	12p12.3 p12.1	30.05 kb	138330	Susceptibility to colorectal cancer
	MGST2	Microsomal glutathione *S*-transferase 2	4q28.3	38.00 kb	601733	Susceptibility to psoriasis
	MGST3	Microsomal glutathione *S*-transferase 3	1q23	25.00 kb	604564	
	PTGES	Prostaglandin E synthase	9q34.3		605172	
Methyl transferases	*AS3MT*	Arsenic (+3 oxidation state) methyltransferase	10q24.32	32.45 kb	611806	Susceptibility to arsenic-dependent carcinogenesis
	ASMT	Acetylserotonin *O*-methyltransferase	Xp22.3/ Yp11.3	47.63 kb	402500	
	COMT	Catechol *O*-methyltransferase	22q11.21	28.24 kb	116790	Susceptibility to schizophrenia Susceptibility to panic disorder Susceptibility to obesity
	GNMT	Glycine *N*-methyltransferase	6p12	3.12 kb	606628	Persistent isolated hypermethioninaemia
	GAMT	Guanidinoacetate *N*-methyltransferase	19p13.3	4.46 kb	601240	Guanidinoacetate *N*-methyltransferase deficiency Cerebral creatine deficiency syndrome 2
	HNMT	Histamine *N*-methyltransferase	2q22.1	50.00 kb	605238	Susceptibility to asthma
	INMT	Indolethylamine *N*-methyltransferase	7p14.3	5.00 kb	604854	
	NNMT	Nicotinamide *N*-methyltransferase	11q23.1	16.70 kb	600008	Modification of plasma homocysteine levels Susceptibility to venous thrombosis, myocardial

Continued

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
						infarction, stroke, congestive heart failure Susceptibility to abdominal aortic aneurysm Susceptibility to osteoporotic fractures Susceptibility to Alzheimer disease
	PNMT	Phenylethanolamine *N*-methyltransferase	17q21.1	2.00 kb	171190	Susceptibility to multiple sclerosis
	TPMT	Thiopurine *S*-methyltransferase	6p22.3	26.83 kb	187680	
N-Acetyl transferases	*ACSL1*	Acyl-CoA synthetase long chain family, member 1	4q35	70.47 kb	152425	
	ACSL3	Acyl-CoA synthetase long chain family, member 3	2q34 q35	42.00 kb	602371	
	ACSL4	Acyl-CoA synthetase long chain family, member 4	Xq22.3 q23	92.05 kb	300157	AMME complex Mental retardation, 63 Mental retardation, 68
	ACSM1	Acyl-CoA synthetase medium chain family, member 1	16p12.3	67.00 kb	614357	
	ACSM2B	Acyl-CoA synthetase medium chain family, member 2B	16p12.3	39.00 kb	614359	
	ACSM3	Acyl-CoA synthetase medium chain family, member 3	16p13.11	22.00 kb	145505	Hypertension, essential
	AANAT	Aryalkylamine *N*-acetyltransferase	17q25	2.55 kb	600950	Susceptibility to delayed sleep phase syndrome
	GLYAT	Glycine *N*-acyltransferase	11q12.1	23.22 kb	607424	
	NAA20	*N*(α)-Acetyltransferase 20, NatB catalytic subunit	20p11.23	23.22 kb	610833	Susceptibility to variation of bone size and body lean mass
	NAT1	*N*-Acetyltransferase 1 (arylamine *N*-acetyltransferase)	8p22	13.00 kb	108345	Susceptibility to gastric adenocarcinoma
	NAT2	*N*-Acetyltransferase 2 (arylamine *N*-acetyltransferase)	8p22	9.97 kb	612182	*N*-Acetyltransferase 2 (arylamine *N*-acetyltransferase) deficiency Susceptibility to bladder cancer Susceptibility to squamous-cell carcinoma Susceptibility to idiopathic talipes equinovarus (clubfoot) Susceptibility to age-related hearing impairment
	SAT1	Spermidine/spermine N1-acetyltransferase 1	Xp22.1	3.02 kb	313020	Keratosis follicularis spinulosa decalvans Chromosome Xp duplications
Thioltransferase	*GLRX*	Glutaredoxin (thioltransferase)	5q14	9.02 kb	600443	
Sulfotransferases	*CHST1*	Carbohydrate (keratan sulfate GAL-6) sulfotransferase 1	11p11.2	16.00 kb	603797	
	CHST2	Carbohydrate (*N*-acetylglucosamine-6-*O*) sulfotransferase 2	3q24	2.00 kb	603798	

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
	CHST3	Carbohydrate (chondroitin 6) sulfotransferase 3	10q22.1	49.00 kb	603799	Larsen syndrome 2 Spondyloepiphyseal dysplasia, Omani type
	CHST4	Carbohydrate (*N*-acetylglucosamine-6-*O*) sulfotransferase 4	16q22.2	12.00 kb		
	CHST5	Carbohydrate (*N*-acetylglucosamine-6-*O*) sulfotransferase 5	16q22.3	6.64 kb	604817	
	CHST6	Carbohydrate (*N*-acetylglucosamine-6-*O*) sulfotransferase 6	16q22	21.00 kb	605294	Macular dystrophy, corneal 1 Susceptibility to Parkinson disease
	CHST7	Carbohydrate (*N*-acetylglucosamine-6-*O*) sulfotransferase 7	Xp11.23	24.00 kb	300375	
	CHST8	Carbohydrate (*N*-acetylgalactosamine-4-*O*) sulfotransferase 8	19q13.1	88.98 kb	610190	Peeling skin syndrome 3
	CHST9	Carbohydrate (*N*-acetylgalactosamine-4-*O*) sulfotransferase 9	18q11.2	269.67 kb	610191	
	CHST10	Carbohydrate sulfotransferase 10	2q11.2	25.00 kb	606376	
	CHST11	Carbohydrate (chondroitin 4) sulfotransferase 11	12q	305.10 kb	610128	
	CHST12	Carbohydrate (chondroitin 4) sulfotransferase 12	7p22	31.00 kb	610129	
	CHST13	Carbohydrate (chondroitin 4) sulfotransferase 13	3q21.3	18.96 kb	610124	
	GAL3ST1	Galactose 3-*O*-sulfotransferase 1	22q12.2	10.25 kb	602300	
	SULT1A1	Sulfotransferase family, cytosolic, 1A, phenol preferring, member 1	16p12.1	17.95 kb	171150	
	SULT1A2	Sulfotransferase family, cytosolic, 1A, phenol preferring, member 2	16p12.1	5.13 kb	601292	
	SULT1A3	Sulfotransferase family, cytosolic, 1A, phenol preferring, member 3	16p11.2	9.91 kb	600641	
	SULT1B1	Sulfotransferase family, cytosolic, 1B, member 1	4q13.3	28.00 kb	608436	
	SULT1C1	Sulfotransferase family, cytosolic, 1C, member 1	2q12.3		602385	
	SULT1C2	Sulfotransferase family, cytosolic, 1C, member 2	2q12.3	21.00 kb	608357	
	SULT1C3	Sulfotransferase family, cytosolic, 1C, member 3	2q12.3	18.16 *kb*		
	SULT1C4	Sulfotransferase family, cytosolic, 1C, member 4	2q12.3	11.21 kb		

Continued

TABLE 6.2 Drug Metabolism-Related Genes—cont'd

Cluster	Gene	Name	Locus	Size	OMIM	Related diseases
	SULT1E1	Sulfotransferase family 1E, estrogen preferring, member 1	4q13.1	18.94 kb	600043	Susceptibility to endometrial cancer
	SULT2A1	Sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA) preferring, member 1	19q13.3	15.00 kb	125263	
	SULT2B1	Sulfotransferase family, cytosolic, 2B, member 1	19q13.3	47.00 kb	604125	
	SULT4A1	Sulfotransferase family 4A, member 1	22q13.2	38.00 kb	608359	
	SULT6B1	Sulfotransferase family, cytosolic, 6B, member 1	2p22.2	28.78 kb		

Interethnic variability is an important issue in pharmacoepigenetics. Chu and Yang[23] performed the first systematic study to examine the population differentiation effect of DNA methylation on treatment response and drug absorption, distribution, metabolism, and excretion in multiple tissue types and cancer types. The authors analyzed the whole methylome and transcriptome data of primary tumor tissues of four cancer types (breast, colon, head and neck, and uterine corpus) and lymphoblastoid cell lines from African and European ancestry populations. Ethnicity-associated CpG sites exhibited similar methylation patterns in the two studied populations, but the patterns differed between tumor tissues and lymphoblastoid cell lines. Ethnicity-associated CpG sites may have triggered gene expression, influenced drug absorption, distribution, metabolism, and excretion, and showed tumor-specific patterns of methylation and gene regulation.

CYPs are a major source of variability in drug pharmacokinetics and pharmacodynamics. The highest expressed forms in liver are CYPs 3A4, 2C9, 2C8, 2E1, and 1A2, while 2A6, 2D6, 2B6, 2C19, and 3A5 are less abundant and CYPs 2J2, 1A1, and 1B1 are mainly expressed extrahepatically. Expression of each CYP is influenced by (i) genetic polymorphisms, (ii) ethnicity, (iii) induction by xenobiotics, (iv) regulation by endogenous factors, (v) health/disease states, (vi) age, (vii) sex, (viii) nutrition, and (ix) epigenetic regulation. Multiallelic genetic polymorphisms define CYP function and pharmacogenetic phenotypes (extensive, intermediate, poor, and ultrarapid metabolizers). Polymorphic variants in some CYP genes are associated with particular diseases, and CYP enzymes are involved in the metabolism of approximately 80% of current drugs.[3,24] The most relevant CYPs involved in the metabolism of common drugs are CYP2D6, CYP2C9, CYP2C19, and CYP3A4/5.

Over 70% of the Caucasian population are deficient metabolizers for the *CYP2D6/2C19/2C9* trigenic cluster; and for the *CYP2D6/2C19/2C9/3A4* tetragenic cluster more than 80% of subjects exhibit a deficient metabolizer genophenotype.[25] These four *CYP* genes encode enzymes responsible for the metabolism of 60%–80% of drugs currently used, showing ontogenic-, age-, sex-, circadian-, and ethnic-related differences.[4,26,27] According to the database of the *World Guide for Drug Use and Pharmacogenomics*,[3] 982 drugs are CYP2D6 related, 371 drugs are substrates, over 300 drugs are inhibitors, and 18 drugs are CYP2D6 inducers. Over 600 drugs are CYP2C9 related, 311 acting as substrates (177 are major substrates, 134 are minor substrates), 375 as inhibitors (92 weak, 181 moderate, and 102 strong inhibitors), and 41 as inducers of the CYP2C9 enzyme.[3] Nearly 500 drugs are CYP2C19 related, 281 acting as substrates (151 are major substrates, 130 are minor substrates), 263 as inhibitors (72 weak, 127 moderate, and 64 strong inhibitors), and 23 as inducers of the CYP2C19 enzyme.[3] The CYP3A4/5 enzyme metabolizes over 1900 drugs, 1033 acting as substrates (897 are major substrates, 136 are minor substrates), 696 as inhibitors (118 weak, 437 moderate, and 141 strong inhibitors), and 241 as inducers of the CYP3A4 enzyme.[3]

The distribution and frequency of *CYP2D6* genophenotypes in the Caucasian population are the following: CYP2D6 extensive metabolizers (EMs) account for 58.85%, whereas intermediate metabolizers (IMs) account for 31.11%, poor metabolizers (PMs) 4.49%, and ultrarapid metabolizers (UMs) 5.55%.[4,28] CYP2C9-PMs represent 4.82%, IMs 33.83%, and EMs 61.35%.[3,4,28] The frequencies of *CYP2C19* genophenotypes are: CYP2C19-EMs 74.11%, CYP2C19-IMs 24.43%, and CYP2C19-PMs 1.46%.[3,4,28] Concerning *CYP3A4/5* polymorphisms, 82.17% of cases are EMs (*CYP3A5*3/*3*), 16.48% are IMs (*CYP3A5*1/*3*), and 1.35% are RMs (*CYP3A5*1/*1*). Tetragenic haplotypes integrating *CYP2D6*, *CYP2C9*, *CYP2C19*, and *CYP3A4/5* variants yield 156 genotypes. The most frequent haplotype is H3 (1/1-1/1-1/1-3/3) (20.87%) representing fully extensive metabolizers, and only 17 haplotypes exhibit a frequency higher than 1% in the Spanish population. In addition to H3, the most frequent haplotypes (>2%) are H55 (1/4-1/1-1/1-1/3)(8.41%),

H26 (1/1-1/2-1/1-3/3)(8.07%), H4 (1/1-1/1-1/2-3/3)(8.07%), H58 (1/4-1/1-1/2-3/3)(3.99%), H72 (1/4-1/2-1/1-3/3) (3.82%), H2 (1/1-1/1-1/1-1/3)(3.74%), H9 (1/1-1/1-1/3-3/3) (3.57%), and H38 (1xN/1-1/1-1/1-3/3) (2.46%).[14] This indicates that in Caucasians about 80% of the population are deficient for the biotransformation of current drugs that are metabolized via CYP2D6-2C9-2C19-3A4 enzymes.

By analyzing the methylomes and transcriptomes of fetal and adult livers, Bonder et al.[29] identified 657 differentially methylated genes with adult-specific expression. These genes were enriched for the transcription factor binding sites of HNF1A and HNF4A. About 1000 genes specific to fetal liver were enriched for the GATA1, STAT5A, STAT5B, and YY1 binding sites.

Nuclear receptors including the aryl hydrocarbon receptor (AhR), orphan nuclear receptors, and nuclear factor erythroid 2 p45-related factor 2 (Nrf2) have been shown to be the key mediators of drug-induced changes in phase I, phase II metabolizing enzymes, and phase III transporters involved in efflux mechanisms. The expression of *CYP1* genes can be induced by AhR, which dimerizes with the AhR nuclear translocator (Arnt), in response to many polycyclic aromatic hydrocarbons (PAHs). The steroid family of orphan nuclear receptors, the constitutive androstane receptor (CAR) and pregnane X receptor (PXR), both heterodimerize with the retinoid X receptor (RXR) and transcriptionally activate the promoters of *CYP2B* and *CYP3A* gene expression by xenobiotics. The peroxisome proliferator-activated receptor (PPAR) also dimerizes with RXR and is activated by fibrates leading to transcriptional activation of the promoters on the *CYP4A* gene. *CYP7A* is a target gene of the liver X receptor (LXR), in which the elimination of cholesterol depends on CYP7A. *CYP7A* is also a target gene of the farnesoid X receptor (FXR), a bile acid receptor, whose activation results in the inhibition of hepatic acid biosynthesis and increased transport of bile acids from intestinal lumen to the liver. Transcriptional activation of these receptors on binding to promoters located at the 5′ flanking region of these *CYP* genes generally leads to induction of their mRNA gene expression. Phase II gene inducers possess an electrophilic-mediated stress response, resulting in the activation of bZIP transcription factor Nrf2, which dimerizes with Mafs and binds to the antioxidant/electrophile response element (ARE/EpRE) promoter, which is located in many phase II DMEs as well as in many cellular-defensive enzymes such as heme oxygenase-1 (HO-1), with the subsequent induction of the expression of these genes.[30]

A number of nuclear receptors including xenobiotic-sensing receptors such as CAR, PXR, and glucocorticoid receptor (GR) as well as liver-enriched receptors such as hepatic nuclear factor 4α (HNF4α) and the estrogen receptor α (ERα) regulate *CYP2C8* and *CYP2C9* expression.[31] CYP2C8 is a member of the CYP2C subfamily, which metabolizes both endogenous compounds and xenobiotics. Induction of P450 enzymes by drugs can result in tolerance as well as drug-drug interactions. CYP2C8 is the most strongly inducible member of the CYP2C subfamily in human hepatocytes. A distal PXR/CAR-binding site in the *CYP2C8* promoter confers inducibility of CYP2C8 via the PXR agonist/ligand rifampicin and the CAR agonist/ligand CITCO [6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime]. A glucocorticoid-responsive element has been identified that mediates dexamethasone induction via the GR. An HNF4α-binding site within the *CYP2C8* basal promoter region is cis-activated by cotransfected HNF4α.[31]

Both CAR and PXR transcriptionally upregulate the *CYP2C9* promoter. Two proximal HNF4α-binding sites at −152 and −185 bp of the *CYP2C9* promoter bind HNF4α and transcriptionally upregulate this promoter in response to HNF4α. Mutation of the two HNF4α-binding sites differentially prevented upregulation of *CYP2C9* promoter by both CAR as well as HNF4α, synergy between the two receptors, and essentially abolished induction by rifampicin in HepG2 cells transfected with PXR.[32] HNF4α controls the expression of many critical metabolic pathways, and the Mediator complex occupies a central role in recruiting RNA polymerase II (pol II) to these gene promoters. An impaired transcriptional HNF4α network in human liver is responsible for many pathological conditions, such as altered drug metabolism, fatty liver, and diabetes. Med25, an associated member of the Mediator complex, is required for the association of HNF4α with Mediator, its several cofactors, and RNA pol II.[33] The Mediator complex is vital for the transcriptional regulation of eukaryotic genes. Mediator binds to nuclear receptors at target response elements and recruits chromatin-modifying enzymes and RNA polymerase II. The Mediator subunit MED25 is involved in the transcriptional activation of *CYP2C9*. MED25 is recruited to the *CYP2C9* promoter through association with liver-enriched HNF4α, and MED25 influences the H3K27 status of the HNF4α binding region. This region is enriched for the activating marker H3K27ac and histone acetyltransferase CREBBP after MED25 overexpression, but it is trimethylated when MED25 expression is silenced. The epigenetic regulator polycomb repressive complex (PRC2), which represses expression by methylating H3K27, plays an important role in target gene regulation. Formaldehyde-assisted isolation of regulatory elements (FAIRE) revealed chromatin conformation changes that were reliant on MED25, indicating that MED25 induced a permissive chromatin state that reflected increases in *CYP2C9* mRNA.[34] Med25 also enhances ligand-dependent ERα-mediated transcriptional activation of *CYP2C9* promoter and interacts with activated ERα by 17β-estradiol through its C-terminal LXXLL motif.[35]

The effect of 5-Aza-2′-deoxycytidine (5AzaDC), a DNA methyltransferase inhibitor, and trichostatin A (TSA), an inhibitor of histone deacetylases, on the expression of *CYP2C19* and five of its known transcription factors (TFs)

has been assessed in cell lines derived from neoplastic liver and intestine. *CYP2C19* mRNA was substantially upregulated after treatment with 5azaDC despite the fact that the two intronic CpG islands in this gene remained substantially methylated (>50%). The TF NR1I3 was also consistently upregulated after treatment with 5AzaDC. *NR1I3* lacks CpG islands in the proximal promoter region and is therefore not likely to be directly regulated by DNA methylation. 5azaDC treatment affects an unidentified upstream regulator of both *CYP2C19* and/or NR1I3. The relationships between TF for *CYP2C19* and the expression of this target gene in human liver samples only accounted for ~70% of the variability of *CYP2C19* mRNA levels, suggesting that a yet unidentified master regulator of *CYP2C19* transcription might itself be a target of epigenetic control.[36]

The pregnane X receptor (PXR), which is a member of the nuclear receptor family of ligand-activated transcription factors, is an integral component of the body's defense mechanism against toxic xenobiotics. PXR is activated by a broad spectrum of lipophilic xenobiotics including prescription drugs, herbs, pesticides, endocrine disruptors, and other environmental contaminants. PXR binds to DNA as a heterodimer with the 9-cis-retinoic acid receptor (RXR) and regulates a large number of genes involved in the detoxification and excretion of toxic substances.[37] PXR is a key transcription factor that regulates drug-metabolizing enzymes such as CYP3A4 and plays important roles in intestinal first-pass metabolism. DNA methylation of the CpG-rich sequence of the *PXR* promoter was more densely detected in low-expression colon cancer cells than in high-expression cells. This methylation was reversed by treatment with 5-aza-dC, in association with reexpression of *PXR* and *CYP3A4* mRNA, but not *VDR* mRNA. *PXR* transcription was silenced by promoter methylation in low-expression cells, which most likely led to downregulation of *CYP3A4* transactivation. A lower level of *PXR* promoter methylation was observed in colorectal cancer tissues compared with adjacent normal mucosa, suggesting upregulation of *PXR/CYP3A4* mRNAs during carcinogenesis.[38]

CYP2D6 is responsible for the metabolism of approximately 25% of marketed drugs. The metabolism of CYP2D6 substrates is increased during pregnancy. Seven transcription factors—activating transcription factor 5 (ATF5), early growth response 1 (EGR1), forkhead box protein A3 (FOXA3), JUNB, Krüppel-like factor 9 (KLF9), KLF10, and REV-ERBα—were found to be upregulated in liver during pregnancy. KLF9 itself is a weak transactivator of *CYP2D6* promoter but significantly enhances *CYP2D6* promoter transactivation by HNF4α, a known transcriptional activator of *CYP2D6* expression. A KLF9 putative binding motif in the −22/−14 region is critical in the potentiation of HNF4α-induced transactivation of *CYP2D6*. Increased KLF9 expression is in part responsible for *CYP2D6* induction during pregnancy via the potentiation of HNF4α transactivation of *CYP2D6*.[39]

Little is known about the potential modulation of *CYP2D6* expression by miRNAs. Zeng et al.[40] screened 38 miRNA candidates that may interact with the transcript of *CYP2D6*. An inverse correlation between the expression of miRNA hsa-miR-370-3p and the expression of *CYP2D6* was observed in human liver tissue samples. hsa-miR-370-3p was able to directly bind to its cognate target within the coding region of the *CYP2D6* transcript. The transfection of hsa-miR-370-3p mimics into the HepG2CYP2D6 cell line, a genetically modified cell line that overexpresses exogenous *CYP2D6*, was able to suppress the expression of *CYP2D6* at both the mRNA and protein level. The transfection of hsa-miR-370-3p mimics was also able to inhibit endogenous mRNA expression and/or protein production of *CYP2D6* in HepaRG cells. Dexamethasone-induced expression of *CYP2D6* was inhibited by hsa-miR-370-3p mimics. hsa-miR-370-3p mimics facilitated the degradation of *CYP2D6* mRNA. A group of multifunctional proteins facilitated the interaction between hsa-miR-370-3p and *CYP2D6*, thereby promoting mRNA degradation.

Two putative degenerate CCAAT/enhancer-binding protein (C/EBP)-binding sites and an imperfect DR1 element (direct repeats of the hexamer AGGTCA separated by a 1-nucleotide spacer motif) within regions −296/−274, −274/−226, and −226/−183 may play critical roles in the transcriptional activation of the *CYP2D49* gene. The putative C/EBP boxes and DR1 element in the *CYP2D49* promoter are functional motifs that bind to C/EBPα and HNF4α, respectively. Both C/EBPα and HNF4α contribute significantly to sustaining a high level of *CYP2D49* transcription.[41]

Park et al.[42] studied the epigenetic regulation of *CYP* genes (*CYP1A1, CYP1A2, CYP1B1, CYP2D6, CYP2E1*) in human pluripotent stem cell-derived hepatocytes and in primary hepatocytes. Transcript levels of major *CYP* genes were much lower in human embryonic stem cell-derived hepatocytes (hESC-Hep) than in human primary hepatocytes (hPH). CpG islands of *CYP* genes were hypermethylated in hESC-Hep, whereas they had an open chromatin structure, as represented by hypomethylation of the CpG sites and permissive histone modifications, in hPH. Inhibition of DNMTs during hepatic maturation induced demethylation of the CpG sites of *CYP1A1* and *CYP1A2*, leading to the upregulation of their transcription. Combinatorial inhibition of DNMTs and histone deacetylases (HDACs) increased the transcript levels of *CYP1A1*, *CYP1A2*, *CYP1B1*, and *CYP2D6*. According to these data, it is likely that the limited expression of *CYP* genes in hESC-Hep is modulated by epigenetic regulatory factors such as DNMTs and HDACs.

Cytochrome P450 1A2 (CYP1A2) is one of the most abundant and important drug-metabolizing enzymes in human liver. The levels of two miRNAs, hsa-miR-132-5p and hsa-miR-221-5p, are inversely correlated with the expression of

CYP1A2 mRNA transcripts in normal human liver tissue samples represented in The Cancer Genome Atlas (TCGA) dataset. hsa-miR-132-5p suppresses the endogenous and lansoprazole-induced expression of *CYP1A2* at the biological activity, protein production, and mRNA transcript level. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays show that hsa-miR-132-5p attenuates CYP1A2-mediated lansoprazole-enhanced and flutamide-induced hepatic cell toxicity. hsa-miR-132-5p suppresses the expression of *CYP1A2*, and this suppression is able to decrease the extent of an adverse drug-drug interaction involving lansoprazole and flutamide.[43]

Many enzymes involved in xenobiotic metabolism, including *CYP1A1*, are regulated by the aryl hydrocarbon receptor (AhR). 3,3′,4,4′,5-Penta chlorobiphenyl (PCB 126) is a potent ligand for AhR and can thus induce the expression of *CYP1A1*. Vorrink et al.[44] studied the epigenetic determinants of *CYP1A1* induction in carcinoma cell lines. In contrast to HepG2 hepatocarcinoma cells, HeLa cervical carcinoma cells showed lower levels of *CYP1A1* mRNA expression following PCB 126 exposure. The two cell lines maintained differences in the chromatin architecture along the *CYP1A1* promoter region. Treatment with the epigenetic modifiers trichostatin A (TSA) and 5-aza-2′-deoxycytidine (5-Aza-dC), significantly increased the expression of *CYP1A1* after PCB 126 treatment in HeLa cells. No apparent differences were observed in methylation levels or specific location of CpG DNA methylation between the two cell lines in the *CYP1A1* promoter region. The differences in *CYP1A1* expression between HepG2 and HeLa cells might be the result of differences in the chromatin architecture of the *CYP1A1* promoter, and thus establish a role of epigenetic regulation in cell-specific *CYP1A1* expression.

Li et al.[45] studied the relationship between *CYP1A1* and *CYP1B1* promoter CpG island methylation and isoniazid-induced liver injury in rats. Whole genome methylation levels are reduced by isoniazid, and *CYP1A1* and *CYP1B1* promoter CpG island methylation levels are increased. *CYP1A1* and *CYP1B1* mRNA expression levels are reduced after treatment. CpG island hypermethylation of the *CYP1A1* and *CYP1B1* promoters regulate the low expression of genes involved in the occurrence of isoniazid-induced liver injury. As a result of alterations in *CYP1A1* and *CYP1B1* expression the mRNA expression levels of *TLR4*, *ERK*, *MDA*, *IL-6*, and *TNF-α* are upregulated and the expression of *SOD* and *PPAR-γ* are downregulated. Alterations in methylation patterns may involve changes in the TLR4/ERK signaling pathway and PPAR-γ, which may alter the expression of *MDA*, *SOD*, *IL-6*, and *TNF-α*, leading to liver injury.

Naselli et al.[46] studied the polymorphisms and methylation of sites contained in the 5′ flanking region of the metabolizing enzyme *CYP2E1* in correlation with its expression in both tumor and nonneoplastic liver cell lines. In treated cells reduced DNA methylation was not consistently associated with the increase of enzyme expression. The Rsa/Pst haplotype differentially influenced *CYP2E1* enzyme expression. Cells with the *VNTR A4/A4* genotype showed a reduced (20%–30%) inhibition of expression compared with the *A2/A2* genotype. Cells with the *A2/A3* genotype showed an increased expression (25%). *A2* and *A3 CYP2E1* alleles may play a more important role in the expression of the enzyme, compared with other epigenetic factors, since they are binding sites for trans-acting proteins.

Sexual differences are only partially attributable to hormones. Cultured male or female cells, even from embryos before sexual differentiation, differ in gene expression and sensitivity to toxins, and these differences persist in isolated primary cells. Male and female cells from Swiss Webster CWF mice manifest sex-distinct patterns of DNA methylation for X-ist and for cytochrome P450 (CYP; family members 1a1, 2e1m, and 7b1). *Dnmt3l* is differentially expressed but not differentially methylated, and *Gapdh* is neither differentially methylated nor expressed. *CYP* family genes differ in expression in whole tissue homogenates and cell cultures, with female *Cyp* expression 2- to 355-fold higher and *Dnmt3l* 12- to 32-fold higher in males. DNA methylation in the promoters of these genes is sex dimorphic; reducing methylation differences reduces differences in the expression of these genes by 1- to 6-fold. Stress or estradiol alters both methylation and gene expression. As reported by Penaloza et al.[47] sex-differential methylation may have medical effects, and different methylation patterns partially explain the sex-based differences in expression of *CYP* family members and X-ist, which potentially lead to inborn differences between males and females and their different responses to chronic and acute changes.

Growth hormone (GH) exerts sex-dependent effects on the liver in many species, with many hepatic genes, most notably genes coding for CYP enzymes, being transcribed in a sex-dependent manner. Sex differences in *CYP* expression are most striking in rats and mice (up to 500-fold male-female differences), but are also seen albeit to a much smaller degree in humans, where they are an important determinant of the sex dependence of hepatic drug and steroid metabolism. GH, via its sex-dependent temporal patterns of pituitary release, activates intracellular signaling, leading to the sexually dimorphic transcription of *CYPs* and other liver-expressed genes. GH-regulated transcription factor STAT5b (signal transducer and activator of transcription 5b), hepatocyte nuclear factors 3beta, 4alpha, and 6, and sex differences in DNA methylation and chromatin structure are involved in the sex-dependent actions of GH.[48]

LPS inhibits *CYP19A1* expression and 17β-estradiol (E2) production in granulosa cells (GCs). This is one of the major causes of infertility underlying postpartum uterine infections. GCs exposed to LPS transiently increased expression of proinflammatory cytokine genes (*IL-1β, TNF-α, IL-6*), followed by the inhibition of *CYP19A1* expression and E2 production. The transient increase in proinflammatory cytokines was associated with HDACs. Trichostatin A (TSA), an

HDAC inhibitor, can attenuate LPS-induced proinflammatory cytokine gene expression and can prevent LPS-mediated downregulation of *CYP19A1* expression and E2 in GCs.[49]

Cytochrome P450 (CYP) epoxygenases metabolize arachidonic acids to form epoxyeicosatrienoic acids (EETs), which exert beneficial roles in the cardiovascular system. Zhou et al.[50] evaluated the effects and mechanisms of *CYP2J2* gene delivery on ethanol-induced myocardial dysfunction with focus on autophagy and apoptosis. Chronic ethanol intake leads to cardiac dilation, contractile dysfunction, cardiomyocyte hypertrophy, oxidative stress, and cardiomyocyte apoptosis, and *CYP2J2* overexpression ameliorates these effects. Ethanol triggers myocardial autophagosome formation and impairs autophagic flux via disrupting autophagosome-lysosome fusion, as evidenced by increased LC3 II/I, Beclin-1, and SQSTM1 levels, accompanied by reduced *LAMP-2* expression. rAAV9-CYP2J2 treatment exerts cardioprotection via restoring autophagic flux in the alcoholic myocardium, and exogenous 11,12-EET addition restores ethanol-induced neonatal rat cardiomyocyte autophagic flux impairment and inhibits apoptosis via the AMPK/mTOR signaling pathway.

UDP-glucuronosyltransferases (UGTs) are a class of phase II drug-metabolizing enzymes (DMEs), playing essential roles in the homeostasis of endobiotics and metabolism of xenobiotics. The expression and enzyme activity of UGTs are regulated by multiple mechanisms and can be influenced by diverse factors. UGTs can be regulated at the epigenetic level via DNA methylation and histone modification. Various nuclear receptors can influence the mRNA levels of UGTs in a ligand-dependent manner. Some transcription factors (AP-1, NF-κB, p53, HFN1α, HNF4α) can also regulate UGTs at the transcriptional level. Multiple miRNAs have been found to be involved in the regulation of UGTs at the posttranscriptional level.[51]

Human UDP-glucuronosyltransferase (UGT) 1A10 is exclusively expressed in the intestine, contributing to presystemic first-pass metabolism. HNF4α and Sp1, as well as an intestine-specific transcription factor, caudal type homeobox (Cdx) 2, are involved in the constitutive expression of *UGT1A10*. UGT1A10 is not expressed in the liver, where HNF1α and Sp1 are abundantly expressed. Oda et al.[52] demonstrated that the CpG-rich region (−264 to +117) around the *UGT1A10* promoter was hypermethylated (89%) in hepatocytes, whereas the *UGT1A10* promoter was hypomethylated (11%) in the epithelium of the small intestine. The methylation of the *UGT1A10* promoter by SssI methylase abrogated transactivity even with overexpressed Cdx2 and HNF1α. The *UGT1A10* promoter was highly methylated (86%) in liver-derived HuH-7 cells, where *UGT1A10* is not expressed. In contrast, the *UGT1A10* promoter was hardly methylated (19%) in colon-derived LS180 cells, where *UGT1A10* is expressed. Treatment with 5-Aza-dC, an inhibitor of DNA methylation, resulted in an increase in *UGT1A10* expression only in HuH-7 cells. Overexpression of HNF1α and Cdx2 further increased *UGT1A10* expression only in the presence of 5-Aza-dC. According to these results, DNA hypermethylation would interfere with the binding of HNF1α and Cdx2, resulting in the defective expression of *UGT1A10* in human liver. Epigenetic regulation could be one of the mechanisms that determine the tissue-specific expression of *UGT1A10*.[22,52]

Wang et al.[53] investigated the role of miRNAs in UGT1A regulation. miR-298 overexpression reduces the mRNA level of *UGT1A1* and *UGT1A4* in HepG2 and LS174T cells, and that of *UGT1A3* and *UGT1A9* in LS174T cells. miR-298 repression increases the mRNA level of *UGT1A4* in HepG2 and LS174T cells, and that of *UGT1A1* and *UGT1A3* in LS174T cells. Inverse correlations between miR-298, as well as miR-491-3p, and *UGT1A3* and *1A4* mRNA levels have been observed in livers, indicating that miR-298 and miR-491-3p downregulate *UGT1A* expression.

Uridine diphosphate-glucuronosyltransferase (UGT) 2B7 is responsible for the glucuronidation of abundant endobiotics or xenobiotics. UGT2B7 is markedly repressed in colorectal carcinoma (CRC). Morphine stimulates the expression of *UGT2B7* during tolerance generation by activating signals in histone 3, especially for trimethylated lysine 27 (H3K4Me3) and acetylated lysine 4 (H3K27Ac). Brain-derived neutrophilic factor (BDNF), a secretory neurotrophin, enriched in CRC can interact and inhibit UGT2B7 by primarily blocking the positive signals of H3K4Me3 and by activating H3K27Ac on the promoter region of *UGT2B7*. BDNF repression is related to polycomb repressive complex (PRC) 1.[54]

6.2.4 Transporter Genes

Phase III transporters (Table 6.3) are expressed in many tissues such as the liver, intestine, kidney, and brain, and play crucial roles in drug absorption, distribution, and excretion. The orphan nuclear receptors PXR and CAR have been shown to be involved in the regulation of these transporters. Along with phase I and phase II enzyme induction, pretreatment with several kinds of inducers has been shown to alter the expression of phase III transporters, and alter the excretion of xenobiotics, which implies that phase III transporters may also be similarly regulated in a coordinated fashion.[30] Efflux pumps of the ABC (ATP-binding cassette) transporter family are also subject to miRNA-mediated gene regulation.[55]

TABLE 6.3 Transporter Genes

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
ATPASES							
P type	*ATP1A1*	ATPase, Na^+/K^+ transporting, alpha 1 polypeptide		182310	1p21	31.56 kb	Bipolar disorder
	ATP1A2	ATPase, Na^+/K^+ transporting, alpha 2 polypeptide	FHM2, MHP2	182340	1q23.2	27.85 kb	Alternating hemiplegia of childhood Migraine familial hemiplegic, 2 Migraine, familial basilar
	ATP1A3	ATPase, Na^+/K^+ transporting, alpha 3 polypeptide	AHC2, CAPOS, DYT12, RDP	182350	19q13.31	27.65 kb	Alternating hemiplegia of childhood 2 CAPOS syndrome Dystonia 12
	ATP1A4	ATPase, Na^+/K^+ transporting, alpha 4 polypeptide	ATP1A1, ATP1AL2	607321	1q23.2	35.00 kb	
	ATP1B1	ATPase, Na^+/K^+ transporting, beta 1 polypeptide	ATP1B	182330	1q24	26.00 kb	Susceptibility to essential hypertension
	ATP1B2	ATPase, Na^+/K^+ transporting, beta 2 polypeptide	AMOG	182331	17p13.1	6.84 kb	
	ATP1B3	ATPase, Na^+/K^+ transporting, beta 3 polypeptide	ATPB-3, CD298	601867	3q23	50.00 kb	
	ATP1B4	ATPase, Na^+/K^+ transporting, beta 4 polypeptide			Xq24	20.26 kb	
	ATP2A1	ATPase, Ca^{++} transporting, cardiac muscle, fast twitch 1	ATP2A, SERCA1	108730	16p12.1	26.02 kb	Brody myopathy
	ATP2A2	ATPase, Ca^{++} transporting, cardiac muscle, slow twitch 2	ATP2B, DAR, DD, SERCA2	108740	12q24.11	69.87 kb	Acrokeratosis verruciformis; Darier disease
	ATP2A3	ATPase, Ca^{++} transporting, ubiquitous	SERCA3	601929	17p13.3	40.57 kb	
	ATP2B1	ATPase, Ca^{++} transporting, plasma membrane 1	PMCA1, PMCA	108731	12q21.3	68.19 kb	Susceptibility to hypertension
	ATP2B2	ATPase, Ca^{++} transporting, plasma membrane 2	PMCA2, PMCA2a, PMCA2i	108733	3p25.3	181.56 kb	Modifier of the severity of sensorineural hearing loss
	ATP2B3	ATPase, Ca^{++} transporting, plasma membrane 3	CFAP39, CLA2, OPCA, PMCA3, PMCA3a, SCAX1	300014	Xq28	46.80 kb	Spinocerebellar ataxia, X linked 1
	ATP2B4	ATPase, Ca^{++} transporting, plasma membrane 4	ATP2B2, MXRA1, PMCA4, PMCA4b, PMCA4x	108732	1q32.1	117.28 kb	

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	ATP2C1	ATPase, Ca^{++} transporting, type 2C, member 1	ATP2C1A, BCPM, HHD, PMR1, SPCA1, hSPCA1	604384	3q22.1	34.34 kb	Hailey-Hailey disease
	ATP2C2	ATPase, Ca^{++} transporting, type 2C, member 2	SPCA2	613082	16q24.1	95.66 kb	
	ATP4A	ATPase, H^+/K^+ exchanging, alpha polypeptide		137216	19q13.1	13.47 kb	
	ATP4B	ATPase, H^+/K^+ exchanging, beta polypeptide	AV080843	137217	13q34	9.00 kb	
	ATP7A	ATPase, Cu^{++} transporting, alpha polypeptide	DSMAX, MK, MNK, SMAX3	300011	Xq21.1	139.70 kb	Menkes disease Occipital horn syndrome Spinal muscular atrophy, distal, X linked 3
	ATP7B	ATPase, Cu^{++} transporting, beta polypeptide	PWD, WC1, WD, WND	606882	13q14.3	78.83 kb	Wilson disease
	ATP8A1	ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1	ATPASEII, ATPIA, ATPP2	609542	4p13	248.73 kb	Susceptibility to multiple sclerosis
	ATP8A2	ATPase, aminophospholipid transporter, class I, type 8A, member 2	ATP, ATPIB, CAMRQ4, IB, ML-1	605870	13q12	649.21 kb	Cerebellar ataxia, mental retardation, and disequilibrium syndrome 4
	ATP8B1	ATPase, class I, type 8B, member 1	ATPIC, BRIC, FIC1, ICP1, PFIC, PFIC1	602397	18q21.31	85.38 kb	Benign recurrent intrahepatic cholestasis Cholestasis, progressive familial intrahepatic 1 Cholestasis, benign recurrent intrahepatic Cholestasis, intrahepatic, of pregnancy, 1
	ATP8B2	ATPase, class I, type 8B, member 2	ATPID	605867	1q21.3	25.00 kb	
	ATP8B3	ATPase, aminophospholipid transporter, class I, type 8B, member 3	ATPIK	605866	19p13.3	32.00 kb	
	ATP8B4	ATPase, class I, type 8B, member 4	ATPIM, KIAA1939	609123	15q21.2	255.00 kb	
	ATP9A	ATPase, class II, type 9A	ATPIIA	609126	20q13.2	171.59 kb	
	ATP9B	ATPase, class II, type 9B	ATPASEP, ATPIIB, HUSSY-20, NEO1L, hMMR1	614446	18q23	308.87 kb	
	ATP10A	ATPase, class V, type 10A	ATP10C, ATPVA, ATPVC	605855	15q11.2	184.49 kb	Angelman syndrome

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	ATP10B	ATPase, class V, type 10B	ATPVB		5q34	289.10 kb	
	ATP10D	ATPase, class V, type 10D	ATPVD, KIAA1487		4p12	108.10 kb	
	ATP11A	ATPase, class VI, type 11A	ATPIH, ATPIS	605868	13q34	59.00 kb	
	ATP11B	ATPase, class VI, type 11B	ATPIF, ATPIR	605869	3q27	128.13 kb	
	ATP11C	ATPase, class VI, type 11C	ATPIG, ATPIQ	300516	Xq27.1	105.94 kb	
	ATP12A	ATPase, H^+/K^+ transporting, nongastric, alpha polypeptide	ATP1AL1	182360	13q12.1-q12.3	31.00 kb	
	ATP13A1	ATPase type 13A1	ATP13A, CGI-152		19p13.11	16.00 kb	
	ATP13A2	ATPase type 13A2	CLN12, HSA9947, KRPPD, PARK9	610513	1p36	25.97 kb	Ceroid-lipofuscinosis, neuronal, 12 Parkinson disease 9 Kufor-Rakeb syndrome
	ATP13A3	ATPase type 13A3	AFURS1	610232	3q29	49.00 kb	
	ATP13A4	ATPase type 13A4	MGC126545, DKFZp761I1011	609556	3q29	114.00 kb	
	ATP13A5	ATPase type 13A5	FLJ16025		3q29	104.13 kb	
V type (vacuolar H^+-ATPase subunit)	*ATP6V1A*	ATPase, H^+ transporting, lysosomal 70 kDa, V1 subunit A	ATP6A11, HO68, VA68, VPP2, Vma1	607027	3q13.31	65.04 kb	
	ATP6V1B1	ATPase, H^+ transporting, lysosomal 56/58 kDa, V1 subunit B, isoform 1 (renal tubular acidosis with deafness)	VPP3, VATB, ATP6B1, VAM2, RTA1B	192132	2p13.1	29.00 kb	Renal tubular acidosis with deafness
	ATP6V1B2	ATPase, H^+ transporting, lysosomal 56/58 kDa, V1 subunit B, isoform 2	VPP3, ATP6B2, M057, VATB, ATP6B1B2	606939	8p21.3	24.00 kb	Zimmermann-Laband syndrome 2
	ATP6V1C1	ATPase, H^+ transporting, lysosomal 42 kDa, V1 subunit C1	ATP6D, ATP6C, VATC, Vma5, FLJ20057, ATP6C	603097	8q22.3	52.04 kb	
	ATP6V1C2	ATPase, H^+ transporting, lysosomal 42 kDa, V1 subunit C2	VMA5, ATP6C2		2p25.1	63.45 kb	
	ATP6V1D	ATPase, H^+ transporting, lysosomal 34 kDa, V1 subunit D	ATP6M, VATD, VMA8	609398	14q23-q24.2	22.00 kb	

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	ATP6V1E1	ATPase, H^+ transporting, lysosomal 31 kDa, V1 subunit E isoform 1	ATPE, ATP6E, ATP6V1E, ATP6E2	108746	22q11.1	36.00 kb	
	ATP6V1E2	ATPase, H^+ transporting, lysosomal 31 kDa, V1 subunit E isoform 2	ATP6EL2, MGC9341, ATP6V1EL2, ATP6E1		2p21	7.00 kb	
	ATP6V1F	ATPase, H^+ transporting, lysosomal 14 kDa, V1 subunit F	ATP6S14, VATF, Vma7	607160	7q32	3.00 kb	
	ATP6V1G1	ATPase, H^+ transporting, lysosomal 13 kDa, V1 subunit G1	ATP6GL, ATP6J, ATP6G, VMA10, ATP6G1, VAG1, ATP6G, DKFZp547P234	607296	9q32	11.16 kb	
	ATP6V1G2	ATPase, H^+ transporting, lysosomal 13 kDa, V1 subunit G isoform 2	NG38, ATP6G, VMA10, ATP6G2	606853	6p21.3	2.00 kb	
	ATP6V1G3	ATPase, H^+ transporting, lysosomal 13 kDa, V1 subunit G3	VMA10, ATP6G3, MGC119810, MGC119813		1q31.3	17.72 kb	
	ATP6V1H	ATPase, H^+ transporting, lysosomal 50/57 kDa, V1 subunit H	VMA13, CGI-11, SFD beta, SFD alpha, NBP1, VATH, V-ATPase	608861	8q11.2	127.77 kb	
	ATP6V0A1	ATPase, H^+ transporting, lysosomal V0 subunit a1	VPP1, ATP6N1, ATP6N1A, a1, Vph1, Stv1, DKFZp781J1951, voa1	192130	17q21	63.74 kb	
	ATP6V0A2	ATPase, H^+ transporting, lysosomal V0 subunit a2	A2, ARCL, ARCL2A, ATP6A2, ATP6N1D, J6B7, RTF, STV1, TJ6, TJ6M, TJ6S, VPH1, WSS	611716	12q24.31	47.58 kb	Cutis laxa, autosomal recessive, type IIA; Wrinkly skin syndrome
	ATP6V0A4	ATPase, H^+ transporting, lysosomal V0 subunit a4	A4, ATP6N1B, ATP6N2, RDRTA2, RTA1C, RTADR, STV1, VPH1, VPP2	605239	7q34	91.90 kb	Renal tubular acidosis, distal, autosomal recessive
	ATP6V0B	ATPase, H^+ transporting, lysosomal 21 kDa, V0 subunit b	ATP6F, MATPL, VMA16, HATPL	603717	1p32.3	3.90 kb	
	ATP6V0C	ATPase, H^+ transporting, lysosomal 16 kDa, V0 subunit c	ATPL, VATL, VMA3, ATP6C, ATP6L	108745	16p13.3	1.00 kb	

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	ATP6V0D1	ATPase, H^+ transporting, lysosomal 38 kDa, V0 subunit d1	P39, VATX, VMA6, ATP6D, ATP6DV, VPATPD	607028	16q22.1	43.17 kb	
	ATP6V0D2	ATPase, H^+ transporting, lysosomal 38 kDa, V0 subunit d2	VMA6, ATP6D2, FLJ38708, v-ATPase		8q21.3	55.32 kb	
	ATP6V0E1	ATPase, H^+ transporting, lysosomal 9 kDa, V0 subunit e1	ATP6H, M9.2, ATP6H, ATP6V0E	603931	5q35.1	51.14 kb	
	ATP6V0E2	ATPase, H^+ transporting V0 subunit E	C7orf32, ATP6V0E2L	611019	7q36.1	7.00 kb	
	TCIRG1	T cell, immune regulator 1, ATPase, H^+ transporting, lysosomal V0 protein a isoform 3	TIRC7, OC116, OPTR, A3, OPTB1, STV1, ATP6N1C, ATP6I, ATP6N1D, ATP6V0A3	604592	11q13.2	11.88 kb	Osteopetrosis, lethal B1 Osteopetrosis, autosomal recessive 1
F type	*ATP5A1*	ATP synthase, H^+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, cardiac muscle	ATP5A, ATP5AL2, ATPM, OMR, ORM, hATP1, MOM2	164360	18q21	14.18 kb	Combined oxidative phosphorylation deficiency 22 Mitochondrial complex (ATP synthase) deficiency, nuclear type 4
	ATP5B	ATP synthase, H^+ transporting, mitochondrial F1 complex, beta polypeptide	ATPSB, ATPMB, MGC5231	102910	12q13.13	7.89 kb	
	ATP5C1	ATP synthase, H^+ transporting, mitochondrial F1 complex, gamma polypeptide 1	ATP5C, ATP5CL1	108729	10p15.1	19.67 kb	
	ATP5D	ATP synthase, H^+ transporting, mitochondrial F1 complex, delta subunit		603150	19p13.3	3.00 kb	
	ATP5E	ATP synthase, H^+ transporting, mitochondrial F1 complex, epsilon subunit	ATPE, MGC104243, MC5DN3	606153	20q13.32	3.69 kb	Mitochondrial complex V (ATP synthase) deficiency nuclear type 3
	ATP5F1	ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit B1	PIG47, MGC24431	603270	1p13.2	12.00 kb	
	ATP5G1	ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit C1 (subunit 9)	ATP5G, ATP5A	603192	17q21.32	3.09 kb	

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	ATP5G2	ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit C2 (subunit 9)	ATP5A	603193	12q13.13	11.57 kb	
	ATP5G3	ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit C3 (subunit 9)	AT93, P3	602736	2q31.1	3.00 kb	
	ATP5H	ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit d	ATPQ, ATP5JD, My032		17q25	8.12 kb	
	ATP5I	ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit E	ATP5K, MGC12532	601519	4p16.3	1.90 kb	
	ATP5J	ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit F6	ATP5, ATPM, ATP5A	603152	21q21.1	11.00 kb	
	ATP5J2	ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit F2	ATP5JL, F1FO-ATPASE		7q22.1	8.02 kb	
	ATP5L	ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit G	ATP5JG		11q23.3	8.46 kb	
	ATP5L2	ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit g, isoform 2	ATP5K2, dJ222E13.5		22q13.2	0.80 kb	
	ATP5O	ATP synthase, H^+ transporting, mitochondrial F1 complex, subunit O	ATPO, HMC08D05, OSCP	600828	21q22.11	12.00 kb	
ATP-BINDING CASSETTE TRANSPORTERS							
Subfamily A (ABC1)	*ABCA1*	ATP-binding cassette, subfamily A (ABC1), member 1	ABC1, TGD, CERP, MGC164864, MGC165011, FLJ14958, HDLDT1	600046	9q31.1	96.81 kb	Familial hypoalphalipoproteinemia Tangier disease
	ABCA2	ATP-binding cassette, subfamily A (ABC1), member 2	ABC2, KIAA1062	600047	9q34	3.00 kb	
	ABCA3	ATP-binding cassette, subfamily A (ABC1), member 3	ABCC, ABC3, ABC-C, LBM180, EST111653	601615	16p13.3	64.33 kb	Surfactant metabolism dysfunction, pulmonary, 3

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	ABCA4	ATP-binding cassette, subfamily A (ABC1), member 4	ABC10, ABCR, ARMD2, CORD3, DKFZp781N1972, FFM, FLJ17534, RMP, RP19, STGD, STGD1	601691	1p22	128.31 kb	Age-related macular degeneration 2 Retinal cone-rod dystrophy 3 Retinitis pigmentosa 19 Stargardt disease 1 Macular degeneration, age related, 2 Fundus flavimaculatus
	ABCA5	ATP-binding cassette, subfamily A (ABC1), member 5	ABC4, ABC13, KIAA1888, FLJ16381	612503	17q24.3	68.00 kb	
	ABCA6	ATP-binding cassette, subfamily A (ABC1), member 6	EST155051, FLJ43498	612504	17q24.3	63.17 kb	
	ABCA7	ATP-binding cassette, subfamily A (ABC1), member 7	ABCX, ABCA-SSN	605414	19p13.3	25.47 kb	Susceptibility to Alzheimer disease
	ABCA8	ATP-binding cassette, subfamily A (ABC1), member 8	KIAA0822	612505	17q24	88.00 kb	
	ABCA9	ATP-binding cassette, subfamily A (ABC1), member 9	EST640918	612507	17q24.2	86.00 kb	
	ABCA10	ATP-binding cassette, subfamily A (ABC1), member 10	EST698739	612508	17q24	96.00 kb	
	ABCA12	ATP-binding cassette, subfamily A (ABC1), member 12	FLJ41584, DKFZp434G232, ABC12	607800	2q34	206.89 kb	Ichthyosis, congenital, autosomal recessive 4A Harlequin fetus type of congenital ichthyosis Ichthyosiform erythroderma, congenital, nonbullous, 4
	ABCA13	ATP-binding cassette, subfamily A (ABC1), member 13	FLJ33876, FLJ33951, FLJ16398, DKFZp313D2411	607807	7p12.3	449.25 kb	Susceptibility to schizophrenia Susceptibility to bipolar disorder
Subfamily B (MDR/TAP)	*ABCB1*	ATP-binding cassette, subfamily B (MDR/TAP), member 1	MDR1, PGY1, CLCS, P-gp, ABC20, CD243, GP170, MGC163296, IBD13	171050	7q21.12	210.00 kb	Susceptibility to inflammatory bowel disease Susceptibility to renal epithelial tumors
	TAP1	Transporter 1, ATP-binding cassette, subfamily B (MDR/TAP)	CIM, HAM1, MTP1, RING4, ABCB2, PSF1, ABC17, ATP1, TAP1N, D6S114E	170260	6p21.3	8.00 kb	Bare lymphocyte syndrome type IB Susceptibility to ankylosing spondytitis
	TAP2	Transporter 2, ATP-binding cassette, subfamily B (MDR/TAP)	APT2, PSF2, ABC18, ABCB3, RING11, D6S217E	170261	6p21.3	16.00 kb	Bare lymphocyte syndrome, type I Wegener-like granulomatosis

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	ABCB4	ATP-binding cassette, subfamily B (MDR/TAP), member 4	PGY3, ABC21, MDR3, MDR2, PFIC-3, GBD1, MDR2/3	171060	7q21.1	73.66 kb	Low phospholipid-associated cholelithiasis Cholestasis progressive familial intrahepatic 3 Gallbladder disease 1
	ABCB5	ATP-binding cassette, subfamily B (MDR/TAP), member 5	ABCB5alpha, ABCB5beta, EST422562	611785	7p21.1	41.39 kb	
	ABCB6	ATP-binding cassette, subfamily B (MDR/TAP), member 6	MTABC3, ABC14, UMAT, FLJ22414, ABC, EST45597, PRP	605452	2q36	10.68 kb	Isolated ocular coloboma 2 Microphthalmia, isolated, with coloboma 7 Langereis blood group Dyschromatosis universalis hereditaria 3
	ABCB7	ATP-binding cassette, subfamily B (MDR/TAP), member 7	MDR5, ABC7, ATM1P, MOAT-C, EST140535, ASAT	300135	Xq13.3	103.03 kb	Anemia, sideroblastic, and spinocerebellar ataxia
	ABCB8	ATP-binding cassette, subfamily B (MDR/TAP), member 8	MDR7, MABC1, M-ABC1, EST328128	605464	7q36	19.36 kb	
	ABCB9	ATP-binding cassette, subfamily B (MDR/TAP), member 9	MDR4, TAPL, KIAA1520, EST122234	605453	12q24	37.52 kb	
	ABCB10	ATP-binding cassette, subfamily B (MDR/TAP), member 10	INFA, M-ABC2, MTABC2, EST20237, ABC06B092	605454	1q42.13	42.11 kb	
	ABCB11	ATP-binding cassette, subfamily B (MDR/TAP), member 11	ABC16, PGY4, SPGP, BSEP, PFIC-2, BRIC2	603201	2q24	108.39 kb	Cholestasis progressive familial (severe) intrahepatic 2 Susceptibility to intrahepatic cholestasis of pregnancy (ICP) Susceptibility to drug-induced cholestasis
Subfamily C (CFTR/MRP)	*ABCC1*	ATP-binding cassette, subfamily C (CFTR/MRP), member 1	MRP, MRP1, MOAT, ABC29, GS-X, MRP-1, DKFZp781G125, DKFZp686N04233	158343	16p13.1	193.50 kb	
	ABCC2	ATP-binding cassette, subfamily C (CFTR/MRP), member 2	KIAA1010, MRP2, CMOAT, ABC30, DJS, CMOAT1, CMRP	601107	10q24	69.20 kb	Dubin-Johnson syndrome Susceptibility to nonalcoholic fatty liver disease Susceptibility to intrahepatic cholestasis of pregnancy
	ABCC3	ATP-binding cassette, subfamily C (CFTR/MRP), member 3	MRP3, CMOAT2, ABC31, MOAT-D	604323	17q22	57.00 kb	

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	ABCC4	ATP-binding cassette, subfamily C (CFTR/MRP), member 4	CFTRL1, MRP4, MOATB, EST170205, MOAT-B	605250	13q32	281.60 kb	
	ABCC5	ATP-binding cassette, subfamily C (CFTR/MRP), member 5	MRP5, MOATC, SMRP, ABC33, MOAT-C, pABC11	605251	3q27	97.00 kb	
	ABCC6	ATP-binding cassette, subfamily C (CFTR/MRP), member 6	ARA, MRP6, ABC34, MOATE, MLP1, EST349056, URG7	603234	16p13.1	73.91 kb	Angioid streaks Pseudoxanthoma elasticum Susceptibility to premature coronary artery disease Arterial calcification, generalized, of infancy, 2
	CFTR	Cystic fibrosis transmembrane conductance regulator (ATP-binding cassette subfamily C, member 7)	ABCC7, MRP7, CFTR/MRP, TNR-CFTR, ABC35, dJ760C5.1	602421	7q31.2	188.70 kb	Congenital bilateral absence of vas deferens Cystic fibrosis Sweat chloride elevation without CF Hypertrypsinemia, neonatal Pancreatitis, idiopathic Susceptibility to sarcoidosis
	ABCC8	ATP-binding cassette, subfamily C (CFTR/MRP), member 8	SUR1, ABC36, MRP8, HRINS, PHHI, SUR, HI, TNDM2	600509	11p15.1	83.95 kb	Diabetes mellitus, noninsulin dependent Diabetes mellitus, permanent neonatal Diabetes mellitus, transient neonatal 2 Hyperinsulinemic hypoglycemia, familial, 1 Hypoglycemia of infancy, leucine sensitive
	ABCC9	ATP-binding cassette, subfamily C (CFTR/MRP), member 9	SUR2, ABC37, ATFB12, CMD1O	601439	12p12.1	139.31 kb	Atrial fibrillation, familial, 12 Cardiomyopathy, dilated, 1O Hypertrichotic osteochondrodysplasia
	ABCC10	ATP-binding cassette, subfamily C (CFTR/MRP), member 10	MRP7, SIMRP7	612509	6p21.1	22.00 kb	
	ABCC11	ATP-binding cassette, subfamily C (CFTR/MRP), member 11	MRP8	607040	16q12.1	65.00 kb	Susceptibility to earwax type
	ABCC12	ATP-binding cassette, subfamily C (CFTR/MRP), member 12	MRP9	607041	16q12.1	63.00 kb	

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	ABCC13	ATP-binding cassette, subfamily C (CFTR/MRP), member 13	PRED6, C21orf73	608835	21q11.2	70.00 kb	
Subfamily D (ALD)	*ABCD1*	ATP-binding cassette, subfamily D (ALD), member 1	ALDP, AMN, ABC42, X-ALD	300371	Xq28	19.89 kb	Adrenoleukodystrophy Adrenomyeloneuropathy, adult Deafness, dystonia, and central hypomyelination
	ABCD2	ATP-binding cassette, subfamily D (ALD), member 2	ALDRP, ALDL1, ALDR, ABC39, ALD1	601081	12q12	68.82 kb	
	ABCD3	ATP-binding cassette, subfamily D (ALD), member 3	PMP1, PMP70, PXMP1, ABC43, ABD3	170995	1p21.3	100.29 kb	Bile acid synthesis defect, congenital, 5 Zellweger cerebrohepatorenal syndrome, variant type 1
	ABCD4	ATP-binding cassette, subfamily D (ALD), member 4	P70R, PMP69, PXMP1L, ABC45, ABC41, EST352188, MAHCJ, P79R	603214	14q24.3	17.79 kb	Methylmalonic aciduria and homocystinuria, cblJ type
Subfamily E (OABP)	*ABCE1*	ATP-binding cassette, subfamily E (OABP), member 1	RLI, RNS4I, RNASELI, OABP, ABC38, RNASEL1	601213	4q31	31.52 kb	
Subfamily F (GCN20)	*ABCF1*	ATP-binding cassette, subfamily F (GCN20), member 1	ABC50, GCN20R, ABC27, EST123147	603429	6p21.33	20.14 kb	
	ABCF2	ATP-binding cassette, subfamily F (GCN20), member 2	GCN20RL1, ABC28	612510	7q36	19.00 kb	
	ABCF3	ATP-binding cassette, subfamily F (GCN20), member 3	GCN20RL2, FLJ11198		3q27.1	7.00 kb	
Subfamily G (WHITE)	*ABCG1*	ATP-binding cassette, subfamily G (WHITE), member 1	WHL, ABC8, WHITE1, ABG1, MGC34313, WHT1	603076	21q22.3	97.56 kb	
	ABCG2	ATP-binding cassette, subfamily G (WHITE), member 2	ABCP, WHITE2, MXR, BCRP, MXR1, ABC15, BCRP, BCRP1, BMDP, CDw338, EST157481, CD338	603756	4q22	68.60 kb	Susceptibility to resistance to chemotherapy Susceptibility to gout Uric acid concentration, serum, QTL1
	ABCG4	ATP-binding cassette, subfamily G (WHITE), member 4	WHITE2	607784	11q23.3	13.00 kb	
	ABCG5	ATP-binding cassette, subfamily G (WHITE), member 5 (sterolin 1)	sterolin 1	605459	2p21	26.00 kb	Sitosterolemia
	ABCG8	ATP-binding cassette, subfamily G (WHITE), member 8 (sterolin 2)	MGC142217	605460	2p21	39.00 kb	Gallbladder disease Sitosterolemia

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
SOLUTE CARRIERS							
High-affinity glutamate and neutral amino acid transporter family (SLC1)	*SLC1A1*	Solute carrier family 1 (neuronal/epithelial high-affinity glutamate transporter, system Xag), member 1	EAAC1, EAAT3	133550	9p24	97.03 kb	Dicarboxylic aminoaciduria Susceptibility to schizophrenia Susceptibility to obsessive-compulsive disorder
	SLC1A2	Solute carrier family 1 (glial high-affinity glutamate transporter), member 2	EAAT2, GLT1, EAA2, GLT-1	600300	11p13-p12	168.35 kb	Susceptibility to idiopathic epilepsy (generalized or absence) Susceptibility to autism spectrum disorder
	SLC1A3	Solute carrier family 1 (glial high-affinity glutamate transporter), member 3	EAAT1, GLAST, FLJ25094, GLAST1	600111	5p13	81.98 kb	Episodic ataxia, type 6
	SLC1A4	Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	ASCT1, SATT	600229	2p15-p13	34.00 kb	
	SLC1A5	Solute carrier family 1 (neutral amino acid transporter), member 5	AAAT, ASCT2, ATBO, M7 V1, M7VS1, R16, RDRC	109190	19q13.3	13.00 kb	
	SLC1A6	Solute carrier family 1 (high-affinity aspartate/glutamate transporter), member 6	EAAT4, MGC33092, MGC43671	600637	19p13.12	22.74 kb	Susceptibility to schizophrenia
	SLC1A7	Solute carrier family 1 (glutamate transporter), member 7	AAAT, EAAT5	604471	1p32.3	55.00 kb	
Facilitative GLUT transporter family (SLC2)	*SLC2A1*	Solute carrier family 2 (facilitated glucose transporter), member 1	GLUT, GLUT1, MGC141895, MGC141896, PED, GLUT1DS	138140	1p34.2	33.80 kb	Dystonia 9 Glucose transporter type 1 deficiency syndrome 1 Glucose transporter type 1 deficiency syndrome 2 Susceptibility to epilepsy, idiopathic generalized 12 Susceptibility to diabetic nephropathy
	SLC2A2	Solute carrier family 2 (facilitated glucose transporter), member 2	GLUT2, GTR2	138160	3q26.1-q26.2	30.63 kb	Fanconi-Bickel syndrome; Diabetes mellitus, noninsulin dependent
	SLC2A3	Solute carrier family 2 (facilitated glucose transporter), member 3	GLUT3, GLUTL, FLJ90380	138170	12p13.3	17.07 kb	Susceptibility to myelomeningocele
	SLC2A4	Solute carrier family 2 (facilitated glucose transporter), member 4	GLUT4	138190	17p13	6.31 kb	Susceptibility to diabetes mellitus, noninsulin dependent

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC2A5	Solute carrier family 2 (facilitated glucose/fructose transporter), member 5	GLUT5, D1S274E	138230	1p36.2	32.00 kb	
	SLC2A6	Solute carrier family 2 (facilitated glucose transporter), member 6	GLUT9, GLUT6, HSA011372	606813	9q34	8.00 kb	
	SLC2A7	Solute carrier family 2 (facilitated glucose transporter), member 7	GLUT7	610371	1p36.2	23.05 kb	
	SLC2A8	Solute carrier family 2 (facilitated glucose transporter), member 8	GLUTX1, GLUT8	605245	9q33.3	11.49 kb	
	SLC2A9	Solute carrier family 2 (facilitated glucose transporter), member 9	GLUT9, GLUTX, GTR9, URATv1, UAQTL2	606142	4p16.1	214.03 kb	Hypouricemia, renal, 2 Susceptibility to gout
	SLC2A10	Solute carrier family 2 (facilitated glucose transporter), member 10	GLUT10, GT10	606145	20q13.1	26.71 kb	Arterial tortuosity syndrome Susceptibility to diabetes mellitus, noninsulin dependent
	SLC2A11	Solute carrier family 2 (facilitated glucose transporter), member 11	GLUT11, GLUT10, GT11	610367	22q11.23	28.00 kb	
	SLC2A12	Solute carrier family 2 (facilitated glucose transporter), member 12	GLUT8, GLUT12	610372	6q23.2	65.00 kb	
	SLC2A13	Solute carrier family 2 (facilitated glucose transporter), member 13	HMIT, MYCT, MGC48624	611036	12q12	350.84 kb	
	SLC2A14	Solute carrier family 2 (facilitated glucose transporter), member 14	GLUT14, SLC2A3P3, DKFZp564K1672, GLUT3	611039	12p13.31	59.10 kb	
Heavy subunits of heteromeric amino acid transporters (SLC3)	*SLC3A1*	Solute carrier family 3 (cystine, dibasic, and neutral amino acid transporters, activator of cystine, dibasic, and neutral amino acid transport), member 1	BATR, CDNAT, D2H, ATR1, rBAT, NBAT, FLJ34681	104614	2p16.3	45.37 kb	Cystinuria Hypotonia-cystinuria syndrome
	SLC3A2	Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	MIC8, S2, CD98, 4F2HC, MDU1, 4FZHC, CD98HC, NACAE, 4T2HC, 4F2, CD98HC	158070	11q13	32.84 kb	

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
Bicarbonate transporter family (SLC4)	*SLC4A1*	Solute carrier family 4, anion exchanger, member 1 (erythrocyte membrane protein band 3, Diego blood group)	AE1, EPB3, BND3, CD233, EMPB3, RTA1A, MGC116753, MGC126619, DI, FR, SW, WD, WR, WD1, BND3, MGC116750, MGC126623, KAE1	109270	17q21.31	18.42 kb	Ovalocytosis Blood group (Waldner, Diego, Swann, Froese, and Wright types) Renal tubular acidosis, distal Renal tubular acidosis, distal, spherocytosis, type 4 Hereditary elliptocytosis, 4 Hereditary hydrocytosis 2 Hereditary spherocytosis, type V
	SLC4A2	Solute carrier family 4, anion exchanger, member 2 (erythrocyte membrane protein band 3-like 1)	AE2, HKB3, BND3L, NBND3, EPB3L1	109280	7q36.1	18.31 kb	
	SLC4A3	Solute carrier family 4, anion exchanger, member 3	AE3, SLC2C	106195	2q36	13.00 kb	
	SLC4A4	Solute carrier family 4, sodium bicarbonate cotransporter, member 4	NBC1, SLC4A5, PNQBC, HNBC1, KNBC, NBC2, hhNMC, pNBC, NBCe1, NBC2, DKFZp781H1314, KNBC	603345	4q21	384.80 kb	Renal tubular acidosis, proximal, with ocular abnormalities
	SLC4A5	Solute carrier family 4, sodium bicarbonate cotransporter, member 5	NBC4, MGC129662	606757	2p13	127.17 kb	
	SLC4A7	Solute carrier family 4, sodium bicarbonate cotransporter, member 7	NBC3, NBC2, SBC2, SLC4A6, BT, DKFZp686H168, NBC2B, NBCN1	603353	3p22	111.07 kb	Susceptibility to breast cancer Susceptibility to addictions
	SLC4A8	Solute carrier family 4, sodium bicarbonate cotransporter, member 8	NBC3, NDCBE1, KIAA0739, DKFZp761B2318, NBC, FLJ46462, NDCBE	605024	12q13.13	124.45 kb	
	SLC4A9	Solute carrier family 4, sodium bicarbonate cotransporter, member 9	AE4, SBC5	610207	5q31	14.94 kb	
	SLC4A10	Solute carrier family 4, sodium bicarbonate transporter-like, member 10	NCBE, NBCn2	605556	2q24.2	360.94 kb	Susceptibility to autism spectrum disorder

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC4A11	Solute carrier family 4, sodium borate transporter, member 11	BTR1, MGC126418, MGC126419, NABC1, dJ794I6, RP4-794I6.3	610206	20p12	10.31 kb	Corneal dystrophy associated with teenage perceptive deafness Congenital hereditary endothelial dystrophy of the cornea 2 Corneal dystrophy, Fuchs endothelial 4 Keratoconus 9
Sodium glucose cotransporter family (SLC5)	*SLC5A1*	Solute carrier family 5 (sodium/glucose cotransporter), member 1	SGLT1, NAGT, D22S675	182380	22q12.3	70.00 kb	Glucose-galactose malabsorption
	SLC5A2	Solute carrier family 5 (sodium/glucose cotransporter), member 2	SGLT2, SL52, Na	182381	16p11.2	7.65 kb	Renal glucosuria
	SLC5A3	Solute carrier family 5 (inositol transporters), member 3	SMIT, SMIT1, SMIT2	600444	21q22.12	69.83 kb	
	SLC5A4	Solute carrier family 5 (low-affinity glucose cotransporter), member 4	SAAT1, SGLT3, DJ90G24.4, SGLT2		22q12.3	36.86 kb	
	SLC5A5	Solute carrier family 5 (sodium iodide symporter), member 5	NIS	601843	19p13.11	22.00 kb	Thyroid hormonogenesis, genetic defect in, I
	SLC5A6	Solute carrier family 5 (sodium-dependent vitamin transporter), member 6	SMVT	604024	2p23	12.72 kb	
	SLC5A7	Solute carrier family 5 (choline transporter), member 7	CHT, CHT1	608761	2q12	27.44 kb	Neuronopathy, distal hereditary motor, type VIIA Susceptibility to heart rate variability
	SLC5A8	Solute carrier family 5 (iodide transporter), member 8	AIT, SMCT1, SMCT	608044	12q23.1	54.02 kb	
	SLC5A9	Solute carrier family 5 (sodium/glucose cotransporter), member 9	SGLT4		1p33	26.00 kb	
	SLC5A10	Solute carrier family 5 (sodium/glucose cotransporter), member 10	SGLT5, FLJ25217		17p11.2	70.00 kb	
	SLC5A11	Solute carrier family 5 (sodium/glucose cotransporter), member 11	RKS,RKSL,RKST1, SMIT2,KST1	610238	16p12.1	65.00 kb	

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC5A12	Solute carrier family 5 (sodium/glucose cotransporter), member 12	MGC52019, SMCT2, DKFZp564G223	612455	11p14.2	55.01 kb	
Sodium- and chloride-dependent neurotransmitter transporter family (SLC6)	*SLC6A1*	Solute carrier family 6 (neurotransmitter transporter, GABA), member 1	GAT1, GABATHG, GABATR, GABT1, MCT1	137165	3p25.3	46.52 kb	Myoclonic-astatic epilepsy syndrome
	SLC6A2	Solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2	NET, NAT1, NET1, SLC6A5	163970	16q12.2	50.56 kb	Orthostatic intolerance Susceptibility to anorexia nervosa Susceptibility to attention deficit hyperactivity disorder (ADHD)
	SLC6A3	Solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	DAT1, DAT	126455	5p15.3	52.64 kb	Parkinsonism-dystonia, infantile Peak tic severity in Tourette syndrome Attention deficit hyperactivity disorder Susceptibility to bipolar affective disorder Susceptibility to psychiatric disorders Susceptibility to cocaine dependence Susceptibility to migraine with aura
	SLC6A4	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	5HTT, SERT, 5-HTT, OCD1, HTT, 5-HTTLPR	182138	17q11.2	39.58 kb	Anxiety-related personality traits Obsessive-compulsive disorder Susceptibility to sudden infant death Susceptibility to major depression Susceptibility to attention deficit hyperactivity disorder Susceptibility to autism
	SLC6A5	Solute carrier family 6 (neurotransmitter transporter, glycine), member 5	GLYT2, NET1	604159	11p15.1	55.67 kb	Startle disease, hyperekplexia 3 Susceptibility to schizophrenia
	SLC6A6	Solute carrier family 6 (neurotransmitter transporter, taurine), member 6	TAUT	186854	3p25.1	86.75 kb	
	SLC6A7	Solute carrier family 6 (neurotransmitter transporter, L-proline), member 7	PROT	606205	5q32	21.12 kb	Susceptibility to asthma

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC6A8	Solute carrier family 6 (neurotransmitter transporter, creatine), member 8	CRTR, CTSP, CT1, CRTRD, MGC87396, CRT	300036	Xq28	8.30 kb	Cerebral creatine deficiency syndrome 1
	SLC6A9	Solute carrier family 6 (neurotransmitter transporter, glycine), member 9	GLYT1, DKFZp547A1118	601019	1p33	34.98 kb	Susceptibility to methamphetamine dependence Susceptibility to essential hypertension
	SLC6A11	Solute carrier family 6 (neurotransmitter transporter, GABA), member 11	GAT4, GABT4, GABT3, GAT3	607952	4q42	122.23 kb	
	SLC6A12	Solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12	BGT1, GAT2, BGT-1	603080	12p13	24.50 kb	
	SLC6A13	Solute carrier family 6 (neurotransmitter transporter, GABA), member 13	GAT2, GAT-2	615097	12p13.3	42.00 kb	
	SLC6A14	Solute carrier family 6 (neurotransmitter transporter), member 14	ATB(0+), OBX, RP3-452H17.1, BMIQ11	300444	Xq23	24.88 kb	Susceptibility to obesity
	SLC6A15	Solute carrier family 6 (neutral amino acid transporter), member 15	V7.3, NTT6, NTT73, FLJ10316, SBAT1	607971	12q21.3	53.34 kb	
	SLC6A16	Solute carrier family 6 (neurotransmitter transporter), member 16	NTT5	607972	19q13.33	21.00 kb	
	SLC6A17	Solute carrier family 6, member 17	NTT4, XT1	610299	1p13.3	51.69 kb	Mental retardation, autosomal recessive 48
	SLC6A18	Solute carrier family 6, member 18	XTRP2, FLJ31236, B(0)AT3	610300	5p15.33	20.84 kb	Hyperglycinuria Iminoglycinuria
	SLC6A19	Solute carrier family 6 (neurotransmitter transporter), member 19	B0AT1, HND, FLJ20680, FLJ34635	608893	5p15.33	23.52 kb	Hartnup disorder Hyperglycinuria Iminoglycinuria, digenic Susceptibility to arterial hypertension
	SLC6A20	Solute carrier family 6 (neurotransmitter transporter), member 20	XT3, Xtrp3, SIT1	605616	3p21.3	41.00 kb	Hyperglycinuria Iminoglycinuria, digenic
Cationic amino acid transporter/glycoprotein-associated family (SLC7)	*SLC7A1*	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 1	ATRC1, MERR, CAT-1, HCAT1, ERR, REC1L, CTR1	104615	13q12.3	86.17 kb	Susceptibility to essential hypertension
	SLC7A2	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 2	ATRC2, HCAT2, CAT-2, CTR2	601872	8p22	73.48 kb	Infantile encephalopathy with severe infantile anorexia

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC7A3	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 3	ATRC3, CAT3, FLJ14541, CAT-3, MGC20687	300443	Xq13.1	5.55 kb	
	SLC7A4	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 4	CAAT3, CAT-4, CTR4, HCAT3, MGC129976, MGC129977, VH	603752	22q11.21	3.00 kb	
	SLC7A5	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 5	D16S469E, MPE16, LAT1, CD98LC, 4F2LC, CD98, E16, CD98LC	600182	16q24.3	39.47 kb	
	SLC7A6	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 6	KIAA0245, LAT2, LAT3	605641	16q22.1	37.30 kb	
	SLC7A7	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 7	LPI, Y+LAT1, LAT3	603593	14q11.2	46.58 kb	Lysinuric protein intolerance
	SLC7A8	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 8	LAT2, LPI-PC1	604235	14q11.2	58.35 kb	
	SLC7A9	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 9	CSNU3, BAT1, FLJ94301, B(0,+)AT	604144	19q13.1	39.27 kb	Cystinuria-lysinuria, type 3 Susceptibility to chronic kidney disease
	SLC7A10	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 10	asc-1, ASC1, FLJ20839	607959	19q13.1	17.14 kb	
	SLC7A11	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 11	XCT, CCBR1	607933	4q28-q32	71.00 kb	
	SLC7A13	Solute carrier family 7 (cationic amino acid transporter, y system), member 13	AGT1, XAT2, AGT-1		8q21.3	107.09 kb	
	SLC7A14	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 14	KIAA1613	615720	3q26.2	121.00 kb	Retinitis pigmentosa 68
Na^+/Ca^{2+} exchanger family (SLC8)	*SLC8A1*	Solute carrier family 8 (sodium/calcium exchanger), member 1	NCX1, CNC, MGC119581, DKFZp779F0871, FLJ37694, FLJ43417	182305	2p23-p22	400.30 kb	
	SLC8A2	Solute carrier family 8 (sodium/calcium exchanger), member 2	NCX2, KIAA1087	601901	19q13.3	43.20 kb	

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TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC8A3	Solute carrier family 8 (sodium/calcium exchanger), member 3	NCX3	607991	14q24.1	144.85 kb	
	SLC8B1	Solute carrier family 8 (sodium/lithium/calcium exchanger), member B1	NCLX, NCKX6, SLC24A6	609841	12q24.13	36.35 kb	
Na^+/H^+ exchanger family (SLC9)	*SLC9A1*	Solute carrier family 9 (sodium/hydrogen exchanger), member 1	APNH, APNH1, ASA, NHE1, FLJ42224, NHE-1	107310	1p36.1-p35	56.15 kb	·Lichtenstein-Knorr syndrome
	SLC9A2	Solute carrier family 9 (sodium/hydrogen exchanger), isoform 2	NHE2	600530	2q11.2	91.64 kb	
	SLC9A3	Solute carrier family 9 (sodium/hydrogen exchanger), isoform 3	APNH3, NHE3, SLC9A3B, MGC126718, MGC126720	182307	5p15.3	51.22 kb	Susceptibility to sudden infant death syndrome Susceptibility to preeclampsia
	SLC9A4	Solute carrier family 9 (sodium/hydrogen exchanger), member 4	NHE4, DKFZp313B031	600531	2q12.1	60.69 kb	
	SLC9A5	Solute carrier family 9 (sodium/hydrogen exchanger), member 5	NHE5	600477	16q22.1	23.24 kb	
	SLC9A6	Solute carrier family 9 (sodium/hydrogen exchanger), member 6	NHE6, KIAA0267, MRSA	300231	Xq26.3	61.84 kb	Mental retardation, X linked, South African type
	SLC9A7	Solute carrier family 9 (sodium/hydrogen exchanger), isoform 7	NHE7	300368	Xp11.3	152.00 kb	
	SLC9A8	Solute carrier family 9 (sodium/hydrogen exchanger), member 8	NHE8, KIAA0939, DKFZp686C03237, FLJ42500, MGC138418	612730	20q13.13	79.52 kb	
	SLC9A9	Solute carrier family 9 (sodium/hydrogen exchanger), member 9	FLJ35613, NHE9, Nbla00118	608396	3q24	583.28 kb	Autism spectrum disorder, 13

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC9A10	Solute carrier family 9, isoform 10	SLC9C1	612738	3q13.2	154.00 kb	
	SLC9B1	Solute carrier family 9, subfamily B (NHA1, cation proton antiporter 1), member 1	NHEDC1	611527	4q24	134.69 kb,	
	SLC9B2	Solute carrier family 9, subfamily B (NHA2, cation proton antiporter 2), member 2	NHEDC2	611789	4q24	26.07 kb	
Sodium bile salt cotransport family (SLC10)	*SLC10A1*	Solute carrier family 10 (sodium/bile acid cotransporter family), member 1	NTCP,NTCP1	182396	14q24.1	21.00 kb	
	SLC10A2	Solute carrier family 10 (sodium/bile acid cotransporter family), member 2	ASBT, ISBT, NTCP2, IBAT	601295	13q33	22.85 kb	Primary bile acid malabsorption
	SLC10A3	Solute carrier family 10 (sodium/bile acid cotransporter family), member 3	P3,DXS253E	312090	Xq28	4.00 kb	
	SLC10A4	Solute carrier family 10 (sodium/bile acid cotransporter family), member 4	P4, MGC29802		4p11	6.00 kb	
	SLC10A5	Solute carrier family 10 (sodium/bile acid cotransporter family), member 5	P5, FLJ38489		8q21.13	1.00 kb	
	SLC10A6	Solute carrier family 10 (sodium/bile acid cotransporter family), member 6	MGC129575, MGC129576, SOAT	613366	4q21.3	25.43 kb	
	SLC10A7	Solute carrier family 10 (sodium/bile acid cotransporter family), member 7	P7, C4orf13	611459	4q31.22	267.90 kb	
Proton-coupled metal ion transporter family (SLC11)	*SLC11A1*	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	LSH, NRAMP1, NRAMP		2q35	14.87 kb	Susceptibility to Buruli ulcer Susceptibility to infection by *Mycobacterium tuberculosis*
	SLC11A2	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	NRAMP2, DMT1, DCT1, FLJ37416	600523	12q13	40.04 kb	Pseudoiron deficiency anemia
Electroneutral cation-coupled Cl cotransporter family (SLC12)	*SLC12A1*	Solute carrier family 12 (sodium/potassium/chloride transporters), member 1	NKCC2, BSC1, MGC48843	600839	15q15-q21.1	97.78 kb	Bartter syndrome, type 1

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC12A2	Solute carrier family 12 (sodium/potassium/chloride transporters), member 2	NKCC1, BSC2, BSC, MGC104233	600840	5q23.3	105.90 kb	
	SLC12A3	Solute carrier family 12 (sodium/chloride transporters), member 3	NCCT, TSC, NCC	600968	16q13	50.64 kb	Gitelman syndrome 1 Susceptibility to primary hypertension Susceptibility to end-stage renal disease in diabetic nephropathy
	SLC12A4	Solute carrier family 12 (potassium/chloride transporters), member 4	KCC1, FLJ17069, FLJ40489	604119	16q22.1	24.30 kb	
	SLC12A5	Solute carrier family 12 (potassium/chloride transporters), member 5	KCC2, KIAA1176	606726	20q13.12	38.46 kb	Susceptibility to febrile seizures
	SLC12A6	Solute carrier family 12 (potassium/chloride transporters), member 6	KCC3, KCC3A, KCC3B, ACCPN, DKFZp434D2135	604878	15q13	108.70 kb	Agenesis of the corpus callosum with peripheral neuropathy
	SLC12A7	Solute carrier family 12 (potassium/chloride transporters), member 7	KCC4, DKFZP434F076	604879	5p15	61.68 kb	
	SLC12A8	Solute carrier family 12 (potassium/chloride transporters), member 8	FLJ23188, CCC9, DKFZp686L18248	611316	3q21.2	130.13 kb	
	SLC12A9	Solute carrier family 12 (potassium/chloride transporters), member 9	CIP1, WO3.3, CCC6, FLJ46905		7q22	14.28 kb	
Human Na^+-sulfate/carboxylate cotransporter family (SLC13)	*SLC13A1*	Solute carrier family 13 (sodium/sulfate symporters), member 1	NASI1, NAS1, KIAA0881	606193	7q31.32	86.00 kb	
	SLC13A2	Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 2	NADC1, NaDC-1, CTR2	604148	17p13.2	23.00 kb	

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC13A3	Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 3	NADC3, SDCT2	606411	20q13.12	126.00 kb	
	SLC13A4	Solute carrier family 13 (sodium/sulfate symporters), member 4	SUT-1, SUT1, hNaS2	604309	7q33	46.00 kb	
	SLC13A5	Solute carrier family 13 (sodium-dependent citrate transporter), member 5	NACT, MGC138356, mindy	608305	17p13.1	28.71 kb	Epileptic encephalopathy, early infantile, 25
Urea transporter family (SLC14)	*SLC14A1*	Solute carrier family 14 (urea transporter), member 1 (Kidd blood group)	RACH1, HUT11, UT1, UT-B1, UTE, HsT1341, FLJ33745, FLJ41687, RACH2	613868	18q11-q12	28.37 kb	Susceptibility to urinary bladder cancer
	SLC14A2	Solute carrier family 14 (urea transporter), member 2	HUT2, MGC119566, MGC119567, UT-A2, UT2, UTR, hUT-A6, FLJ16167	601611	18q12.1-q21.1	68.31 kb	Susceptibility to variation in blood pressure
Proton oligopeptide cotransporter family (SLC15)	*SLC15A1*	Solute carrier family 15 (oligopeptide transporter), member 1	PEPT1, HPECT1, PET1	600544	13q32.3	68.00 kb	
	SLC15A2	Solute carrier family 15 (H^+/peptide transporter), member 2	PEPT2	602339	3q13.33	47.00 kb	
	SLC15A3	Solute carrier family 15, member 3	PHT2, PTR3, FLJ26631, OCTP	610408	11q12.2	14.70 kb	
	SLC15A4	Solute carrier family 15, member 4	PHT1, PTR4, FP12591	615806	12q24.32	30.80 kb	
Monocarboxylate transporter family (SLC16)	*SLC16A1*	Solute carrier family 16, member 1 (monocarboxylic acid transporter 1)	MCT1, FLJ36745, MCT, MGC44475	600682	1p12	44.51 kb	Erythrocyte lactate transporter defect Hyperinsulinemic hypoglycemia, familial Monocarboxylate transporter 1 deficiency
	SLC16A2	Solute carrier family 16, member 2 (monocarboxylic acid transporter 8)	DXS128E, MCT7, XPCT, DXS128, MCT8	300095	Xq13.2	112.67 kb	Allan-Herndon-Dudley syndrome Monocarboxylate transporter 8 deficiency
	SLC16A3	Solute carrier family 16 (monocarboxylic acid transporters), member 3	MCT3, MCT4, MGC138472, MGC138474	603877	17q25	11.08 kb	
	SLC16A4	Solute carrier family 16, member 4 (monocarboxylic acid transporter 5)	MCT4	603878	1p13.3	28.13 kb	

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC16A5	Solute carrier family 16, member 5 (monocarboxylic acid transporter 6)	MCT5	603879	17q25.1	18.19 kb	
	SLC16A6	Solute carrier family 16, member 6 (monocarboxylic acid transporter 7)	MCT6, MCT7	603880	17q24.2	23.07 kb	
	SLC16A7	Solute carrier family 16, member 7 (monocarboxylic acid transporter 2)	MCT2	603654	12q13	92.28 kb	
	SLC16A8	Solute carrier family 16, member 8 (monocarboxylic acid transporter 3)	MCT3, REMP	610409	22q12.3-q13.2	5.03 kb	
	SLC16A9	Solute carrier family 16 (monocarboxylic acid transporters), member 9	MCT9, C10orf36, FLJ43803	614242	10q21.2	85.00 kb	
	SLC16A10	Solute carrier family 16, member 10 (aromatic amino acid transporter)	TAT1, PRO0813, MCT10	607550	6q21-q22	135.41 kb	
	SLC16A11	Solute carrier family 16 (monocarboxylic acid transporters), member 11	MCT11, FLJ90193	615765	17p13.1	2.29 kb	Type 2 diabetes
	SLC16A12	Solute carrier family 16 (monocarboxylic acid transporters), member 12	MCT12, CJMG, DKFZp686E188	611910	10q23.31	105.00 kb	Juvenile cataract, microcornea, and renal glycosuria
	SLC16A13	Solute carrier family 16 (monocarboxylic acid transporters), member 13	MCT13		17p13.1	4.07 kb	
	SLC16A14	Solute carrier family 16 (monocarboxylic acid transporters), member 14	MCT14, FLJ30794		2q36.3	33.00 kb	
Vesicular glutamate transporter family (SLC17)	*SLC17A1*	Solute carrier family 17 (sodium phosphate), member 1	NAPI1, NPT1, MGC126794, MGC126796, NAPI-1, NPT-1	182308	6p22.2	49.16 kb	Susceptibility to gout
	SLC17A2	Solute carrier family 17 (sodium phosphate), member 2	NPT3, MGC138238	611049	6p21.3	17.86 kb	
	SLC17A3	Solute carrier family 17 (sodium phosphate), member 3	NPT4, NPT3	611034	6p21.3	29.14 kb	Susceptibility to high-serum uric acid levels Susceptibility to gout

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC17A4	Solute carrier family 17 (sodium phosphate), member 4	KIAA2138, KAIA2138, MGC129623	604216	6p22.2	25.47 kb	
	SLC17A5	Solute carrier family 17 (anion/sugar transporter), member 5	SASD, AST, FLJ22227, FLJ23268, ISSD, NSD, SD, SIALIN	604322	6q13	60.63 kb	Salla disease Free sialic acid storage disease, infantile
	SLC17A6	Solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6	DNPI, VGLUT2	607563	11p14.3	41.38 kb	
	SLC17A7	Solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7	BNPI, VGLUT1	605208	19q13	12.00 kb	
	SLC17A8	Solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 8	VGLUT3	607557	12q23.1	64.00 kb	Neurosensory deafness 25
	SLC17A9	Solute carrier family 17, member 9	FLJ23412, C20orf59, POROK8, VNUT	612107	20q13.33	15.95 kb	Porokeratosis 8, disseminated superficial actinic type
Vesicular amine transporter family (SLC18)	*SLC18A1*	Solute carrier family 18 (vesicular monoamine), member 1	CGAT, VAT1, VMAT1	193002	8p21.3	20.00 kb	
	SLC18A2	Solute carrier family 18 (vesicular monoamine), member 2	SVMT, VAT2, VMAT2, SVAT, MGC26538	193001	10q25	36.38 kb	Susceptibility to alcoholism Susceptibility to Parkinson disease Susceptibility to schizophrenia Susceptibility to bipolar disorder
	SLC18A3	Solute carrier family 18 (vesicular acetylcholine), member 3	VACHT, MGC12716	600336	10q11.2	2.42 kb	Chromosome 10q11.2 microdeletion/ microduplication
	SLC18B1	Solute carrier family 18, subfamily B, member	C6orf192, dJ55C23.6	613361	6q22.3-q23.3		
Folate/thiamine transporter family (SLC19)	*SLC19A1*	Solute carrier family 19 (folate transporter), member 1	FOLT, RFC1, CHMD, IFC1, REFC	600424	21q22.3	27.00 kb	Susceptibility to spina bifida; Susceptibility to neural tube defect

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TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC19A2	Solute carrier family 19 (thiamine transporter), member 2	THTR1, TC1, THT1, TRMA	603941	1q23.3	22.06 kb	Thiamine-responsive megaloblastic anemia
	SLC19A3	Solute carrier family 19, member 3	THTR2	606152	2q37	32.82 kb	Thiamine metabolism dysfunction syndrome 2 (biotin- or thiamine-responsive encephalopathy type 2)
Type III Na^+-phosphate cotransporter family (SLC20)	*SLC20A1*	Solute carrier family 20 (phosphate transporter), member 1	GLVR1, FLJ41426, Glvr-1, PIT1, PiT-1, DKFZp686J2397	137570	2q13	17.88 kb	
	SLC20A2	Solute carrier family 20 (phosphate transporter), member 2	D8S1915, GLVR2, MLVAR, PIT2, GLVR-2, IBGC1, IBGC3, PIT-2, RAM1	158378	8p11.21	123.38 kb	Basal ganglia calcification, idiopathic, 1
Organic anion transporter family (SLCO/ SLC21)	*SLCO1A2*	Solute carrier organic anion transporter family, member 1A2	OATP1, OATPA, OATP, SLC21A3, OATP-A, OATP1A2	602883	12p12	49.00 kb	
	SLCO1B1	Solute carrier organic anion transporter family, member 1B1	LST1, OATP2, OATPC, OAT6, SLC21A6, OATP1B1, MGC133282	604843	12p	108.60 kb	Hyperbilirubinemia, rotor type, digenic
	SLCO1B3	Solute carrier organic anion transporter family, member 1B3	OATP8, SLC21A8, LST-3TM13, OATP1B3, LST2	605495	12p12	106.00 kb	Hyperbilirubinemia, rotor type, digenic
	SLCO1C1	Solute carrier organic anion transporter family, member 1C1	OATP-F, SLC21A14, OATP1, OATP1C1, OAT-RP-5, OATP14	613389	12p12.2	57.92 kb	Fatigue and depression in patients suffering from hyperthyroidism
	SLCO2A1	Solute carrier organic anion transporter family, member 2A1	MATR1, OATP2A1, PGT, PHOAR2, SLC21A2	601460	3q21	97.38 kb	Hypertrophic osteoarthropathy, primary, autosomal recessive 2
	SLCO2B1	Solute carrier organic anion transporter family, member 2B1	DKFZp686E0517, KIAA0880, OATP-B, OATP2B1, OATPB, SLC21A9, OATP-RP2, OATPRP2	604988	11q13	55.41 kb	
	SLCO3A1	Solute carrier organic anion transporter family, member 3A1	OATP-D, OATP3A1, OATPD, SLC21A11	612435	15q26	309.00 kb	
	SLCO4A1	Solute carrier organic anion transporter family, member 4A1	OATPE, SLC21A12, OATP4A1, OATPRP1, POAT	612436	20q13.33	29.00 kb	

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLCO4C1	Solute carrier organic anion transporter family, member 4C1	OATPX, OATP-H, OATP-M1, OATP4C1, PRO2176, SLC21A20	609013	5q21.2	62.00 kb	
	SLCO5A1	Solute carrier organic anion transporter family, member 5A1	OATP-J, OATP-RP4, OATP5A1, OATPJ, OATPRP4, SLC21A15	613543	8q13.3	162.63 kb	Chromosome 8q13 microdeletion
	SLCO6A1	Solute carrier organic anion transporter family, member 6A1	OATPY, OATP6A1, MGC26949, GST, CT48, OATP-I, SLC21A19	613365	5q21.1	127.07 kb	
Organic cation/ anion/zwitterion transporter family (SLC22)	*SLC22A1*	Solute carrier family 22 (organic cation transporter), member 1	OCT1	602607	6q25.3	36.89 kb	
	SLC22A2	Solute carrier family 22 (organic cation transporter), member 2	OCT2, MGC32628	602608	6q25.3	42.17 kb	
	SLC22A3	Solute carrier family 22 (extraneuronal monoamine transporter), member 3	EMT, OCT3, EMTH	604842	6q25.3	106.59 kb	Susceptibility to coronary artery disease Susceptibility to toxicity of substances of abuse
	SLC22A4	Solute carrier family 22 (organic cation/ ergothioneine transporter), member 4	OCTN1, MGC34546, UT2H, MGC40524, ETT	604190	5q31.1	49.76 kb	Susceptibility to Crohn disease Susceptibility to rheumatoid arthritis
	SLC22A5	Solute carrier family 22 (organic cation/ carnitine transporter), member 5	CDSP, FLJ46769, OCTN2, OCTN2VT, SCD	603377	5q23.3	25.91 kb	Carnitine deficiency, systemic primary Susceptibility to Crohn disease
	SLC22A6	Solute carrier family 22 (organic anion transporter), member 6	ROAT1, PAHT, OAT1, HOAT1, MGC45260, FLJ55736,	607582	11q12.3	8.40 kb	
	SLC22A7	Solute carrier family 22 (organic anion transporter), member 7	OAT2, NLT, MGC45202, MGC24091	604995	6p21.1	7.28 kb	
	SLC22A8	Solute carrier family 22 (organic anion transporter), member 8	OATP3, OAT3, MGC24086, ROCT	607581	11q11	23.02 kb	
	SLC22A9	Solute carrier family 22 (organic anion/ cation transporter), member 9	HOAT4, OAT4, OAT7, UST3H, ust3	607579	11q13.1		

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC22A10	Solute carrier family 22 (organic anion/ cation transporter), member 10	OAT5, hOAT5	607580	11q12.3	21.82 kb	
	SLC22A11	Solute carrier family 22 (organic anion/ cation transporter), member 11	OAT4, MGC34282	607097	11q13.1	15.90 kb	Osteoporosis
	SLC22A12	Solute carrier family 22 (organic anion/ urate transporter), member 12	OAT4L, RST, URAT1	607096	11q13.1	11.71 kb	Renal hypouricemia 1 Susceptibility to obesity Susceptibility to metabolic syndrome
	SLC22A13	Solute carrier family 22 (organic anion transporter), member 13	OCTL1, OCTL3, ORCTL3, OAT10, ORCTL-3	604047	3p21.3	12.48 kb	
	SLC22A14	Solute carrier family 22 (organic cation transporter), member 14	OCTL2, OCTL4, ORCTL4	604048	3p21.3	12.00 kb	
	SLC22A15	Solute carrier family 22 (organic cation transporter), member 15	FLIPT1, DKFZp761G0313	608275	1p13.1	93.56 kb	
	SLC22A16	Solute carrier family 22 (organic cation/ carnitine transporter), member 16	CT2, OCT6, OKB1, FLIPT2, WUGSC: RG331P03.1, dJ261K5.1	608276	6q21-q22.1	51.94 kb	Bipolar disorder type I
	SLC22A17	Solute carrier family 22, member 17	BOIT, BOCT, NGALR, 24p3R, NGALR2, NGALR3, hBOIT	611461	14q11.2	6.86 kb	
	SLC22A18	Solute carrier family 22, member 18	ORCTL2, IMPT1, STF, TSSC5, BWSCR1A, SLC22A1L, BWR1A, HET	602631	11p15.5	25.53 kb	Beckwith-Wiedemann syndrome Breast cancer, somatic Lung cancer, somatic Rhabdomyosarcoma, somatic
	SLC22A20	Solute carrier family 22, member 20	FLJ16331, Oat6	611696	11q13.1	13.00 kb	
	SLC22A23	Solute carrier family 22 member 23	FLJ22174, C6orf85, DKFZP434F011	611697	6p25.2	175.00 kb	
	SLC22A24	Solute carrier family 22, member 24	MGC34821	611698	11q12.3	25.00 kb	
	SLC22A25	Solute carrier family 22, member 25	UST6, HIMTP, MGC120420	610792	11q12.3	65.00 kb	
Na^+-dependent ascorbic acid transporter family (SLC23)	*SLC23A1*	Solute carrier family 23 (nucleobase transporters), member 1	NBTL, SVCT1, YSPL3, SLC23A2	603790	5q31.2	16.15 kb	Susceptibility to preterm delivery

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC23A2	Solute carrier family 23 (nucleobase transporters), member 2	SVCT2, YSPL2, KIAA0238, NBTL1, SLC23A1	603791	20p13	158.00 kb	Susceptibility to preterm delivery
	SLC23A3	Solute carrier family 23 (nucleobase transporters), member 3	SVCT3,Yspl1, FLJ31168		2q35	6.00 kb	
$Na^+/(Ca^{2+}-K^+)$ exchanger family (SLC24)	*SLC24A1*	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 1	NCKX1, KIAA0702, RODX, NCKX, HsT17412	603617	15q22	34.33 kb	Night blindness, congenital stationary (complete), 1D, autosomal recessive
	SLC24A2	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 1	NCKX2	609838	9p22.1	70.95 kb	
	SLC24A3	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 3		609839	20p13	510.25 kb	
	SLC24A4	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 4	FLJ38852, NCKX4, SLC24A2, SHEP6	609840	14q32.12	178.90 kb	Amelogenesis imperfecta, type IIA5
	SLC24A5	Solute carrier family 24, member 5	JSX, NCKX5, OCA6, SHEP4	609802	15q21.1	21.00 kb	·Albinism, oculocutaneous, type VI
Mitochondrial carrier family (SLC25)	*SLC25A1*	Solute carrier family 25 (mitochondrial carrier, citrate transporter), member 1	CTP1, DGCR5, DGS-J, CTP, SLC20A3, ODC1, CIC	190315	22q11.21	3.25 kb	D-2- and L-2-Hydroxyglutaric aciduria
	SLC25A2	Solute carrier family 25 (mitochondrial carrier, ornithine transporter) member 2	ORNT2, ORC2, MGC119153, MGC119151	608157	5q31	1.42 kb	
	SLC25A3	Solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 3	OK/SW-cl.48, PHC, PTP	600370	12q23	8.00 kb	Mitochondrial phosphate carrier deficiency
	SLC25A4	Solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 4	ANT1, T1, PEO3, t-PA	103220	4q35	71.22 kb	Congenital cataract and mitochondrial myopathy 2 Mitochondrial DNA depletion syndrome 12 (cardiomyopathic type) Progressive external ophthalmoplegia 2

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC25A5	Solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 5	2F1, AAC2, ANT2, T2, T3	300150	Xq24	2.99 kb	Hartsfield syndrome
	SLC25A6	Solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 6	AAC3, ANT, ANT 2, ANT 3, ANT3, ANT3Y	300151	Xp22.32 and Yp11.3	5.00 kb	
	UCP1	Uncoupling protein 1 (mitochondrial, proton carrier)	SLC25A7, UCP	113730	4q28-q31	8.91 kb	Susceptibility to obesity Susceptibility to diabetes
	UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	SLC25A8, UCPH, BMIQ4	601693	11q13	8.17 kb	Susceptibility to obesity Susceptibility to hyperglycemia and insulin resistance in severe sepsis
	UCP3	Uncoupling protein 3 (mitochondrial, proton carrier)	SLC25A9	602044	11q13.4	8.79 kb	Severe obesity and diabetes mellitus noninsulin dependent
	SLC25A10	Solute carrier family 25 (mitochondrial carrier, dicarboxylate transporter), member 10	DIC	606794	17q25.3	8.00 kb	
	SLC25A11	Solute carrier family 25 (mitochondrial carrier, oxoglutarate carrier), member 11	SLC20A4,OGC	604165	17p13.3	2.00 kb	
	SLC25A12	Solute carrier family 25 (mitochondrial carrier, Aralar), member 12	ARALAR, ARALAR1	603667	2q24	109.00 kb	Hypomyelination, global cerebral Autism spectrum disorder, 5
	SLC25A13	Solute carrier family 25, member 13 (citrin)	ARALAR2, CITRIN, AGC2	603859	7q21.3	201.93 kb	Citrulinemia, type II
	SLC25A14	Solute carrier family 25 (mitochondrial carrier, brain), member 14	BMCP1,UCP5	300242	Xq24	33.00 kb	
	SLC25A15	Solute carrier family 25 (mitochondrial carrier, ornithine transporter) member 15	ORNT1, D13S327, ORC1	603861	13q14	20.65 kb	Hyperornithinemia-hyperammonemia-homocitrullinemia syndrome
	SLC25A16	Solute carrier family 25 (mitochondrial carrier; Graves disease autoantigen), member 16	D10S105E, HGT, GDA, GDC, ML7	139080	10q21.3	46.00 kb	Susceptibility to Graves disease

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC25A17	Solute carrier family 25 (mitochondrial carrier, peroxisomal membrane protein, 34 kDa), member 17	PMP34	606795	22q13.2	49.00 kb	
	SLC25A18	Solute carrier family 25 (mitochondrial carrier), member 18	GC2	609303	22q11.2	30.46 kb	
	SLC25A19	Solute carrier family 25 (mitochondrial deoxynucleotide carrier), member 19	DNC, MUP1, DNCHA	606521	17q25.3	16.47 kb	Microcephaly, Amish type Thiamine metabolism dysfunction syndrome 4, progressive polyneuropathy type
	SLC25A20	Solute carrier family 25 (carnitine/ acylcarnitine translocase), member 20	CAC, CACT	613698	3p21.31	42.05 kb	Carnitine-acylcarnitine translocase deficiency
	SLC25A21	Solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	ODC1	607571	3p21.31	492.00 kb	
	SLC25A22	Solute carrier family 25 (mitochondrial carrier, glutamate), member 22	GC1, FLJ13044, NET44	609302	11p15.5	5.75 kb	Epileptic encephalopathy, early infantile, 3
	SLC25A23	Solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 23	APC2, MCSC2, SCaMC-3, MGC2615	608746	19p13.3	19.71 kb	
	SLC25A24	Solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 24	APC1, SCAMC-1	608744	1p13.3	65.00 kb	
	SLC25A25	Solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25	MCSC, PCSCL, SCAMC-2, KIAA1896	608745	9q34.11	10.00 kb	
	SLC25A26	Solute carrier family 25, member 26	SAMC, FLJ77340, DKFZp434E079	611037	3p14.1	135.73 kb	
	SLC25A27	Solute carrier family 25, member 27	UCP4, FLJ33552, RP11-446F17.2, UNQ772/PRO1566	613725	6p12.3	24.00 kb	
	SLC25A28	Solute carrier family 25, member 28	MRS3/4, NPD016	609767	10q24.2	9.00 kb	
	SLC25A29	Solute carrier family 25, member 29	FLJ38975, C14orf69	615064	14q32.2	15.00 kb	
	SLC25A30	Solute carrier family 25, member 30	KMCP1	610793	13q14.13	22.00 kb	
	SLC25A31	Solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 31	AAC4, ANT4	610796	4q28.1	44.00 kb	

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC25A32	Solute carrier family 25, member 32	MFTC	610815	8q22.3	14.00 kb	
	SLC25A33	Solute carrier family 25, member 33	BMSC-MCP, MGC4399	610816	1p36.22	43.00 kb	
	SLC25A34	Solute carrier family 25, member 34	RP11-169K16.2, DKFZp781A10161	610817	1p36.21	5.08 kb	
	SLC25A35	Solute carrier family 25, member 35		610818	17p13.1	8.04 kb	
	SLC25A36	Solute carrier family 25 (pyrimidine nucleotide carrier), member 36	PNC2	616149	3q23	36.00 kb	
	SLC25A37	Solute carrier family 25, member 37	MSC, MSCP, PRO1278, PRO1584, PRO2217, MTFRN, MFRN1, MFRN, MSC	610387	8p21.2	43.70 kb	
	SLC25A38	Solute carrier family 25, member 38	FLJ20551, FLJ22703	610819	3p22.1	14.00 kb	Sideroblastic anemia, pyridoxine refractory
	SLC25A39	Solute carrier family 25, member 39	CGI-69, CGI69 FLJ22407	610820	17q12	5.00 kb	
	SLC25A40	Solute carrier family 25, member 40	MCFP	610821	7q21.12	42.81 kb	Susceptibility to high triglyceride level
	SLC25A41	Solute carrier family 25, member 41	FLJ40442, MGC34725, SCaMC-3L	610822	19p13.3	7.74 kb	
	SLC25A42	Solute carrier family 25, member 42	MGC26694	610823	19p13.11	48.89 kb	
	SLC25A43	Solute carrier family 25, member 43		300641	Xq24	55.00 kb	Hartsfield syndrome
	SLC25A44	Solute carrier family 25, member 44	FLJ90431, KIAA0446, RP11-54H19.3	610824	1q22	18.86 kb	
	SLC25A45	Solute carrier family 25, member 45		610825	11q13.1	7.00 kb	
	SLC25A46	Solute carrier family 25, member 46		610826	5q22.1	23.00 kb	
	SLC25A47	Solute carrier family 25, member 47	HDMCP, C14orf68, HMFN1655	609911	14q32.2	7.04 kb	
	SLC25A48	Solute carrier family 25, member 48		616150	5q31.1	54.13 kb	
	MTCH1	Mitochondrial carrier homolog 1 (*Caenorhabditis elegans*)	CGI-64, PIG60, PSAP, SLC25A49	610449	6p21.2	18.77 kb	
	MTCH2	Mitochondrial carrier homolog 2 (*Caenorhabditis elegans*)	2310034D24Rik, HSPC032, MIMP, SLC25A50	613221	11p11.2	25.35 kb	

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC25A51	Solute carrier family 25, member 51	CG7943, MCART1		9p13.3-p12	26.78 kb	
	SLC25A52	Solute carrier family 25, member 52	MCART2	616153	18q12.1	1.32 kb	
	SLC25A53	Solute carrier family 25, member 53	MCART6	300941	Xq22.2		
Multifunctional anion exchanger family (SLC26)	*SLC26A1*	Solute carrier family 26 (sulfate transporter), member 1	EDM4, SAT1, SAT-1	610130	4p16.3	14.36 kb	
	SLC26A2	Solute carrier family 26 (sulfate transporter), member 2	DTDST, MST153, D5S1708, MSTP157, DTD	606718	5q31-q34	26.66 kb	Achondrogenesis IB Atelosteogenesis, type II Diastrophic dysplasia Epiphyseal dysplasia multiple 4
	SLC26A3	Solute carrier family 26 (anion exchanger), member 3	CLD, DRA	126650	7q31	37.77 kb	Bartter syndrome Chloride diarrhea, familial
	SLC26A4	Solute carrier family 26, member 4	TDH2B, EVA	605646	7q31	57.18 kb	Deafness, autosomal recessive 4, with enlarged vestibular aqueduct *Pendred syndrome*
	SLC26A5	Solute carrier family 26, member 5 (prestin)	PRES, DFNB61, MGC118886, MGC118887, MGC118888, MGC118889	604943	7q22.1	93.45 kb	Deafness, autosomal recessive 61
	SLC26A6	Solute carrier family 26, member 6	CFEX, DKFZp586E1422	610068	3p21.3	9.77 kb	
	SLC26A7	Solute carrier family 26, member 7	SUT2, MGC126268	608479	8q23	148.86 kb	
	SLC26A8	Solute carrier family 26, member 8	TAT1, FLJ32714	608480	6p21	81.12 kb	Spermatogenic failure 3
	SLC26A9	Solute carrier family 26, member 9		608481	1q32.1	30.41 kb	
	SLC26A10	Solute carrier family 26, member 10			12q13	6.24 kb	
	SLC26A11	Solute carrier family 26, member 11			17q25.3		
Fatty acid transporter family (SLC27)	*SLC27A1*	Solute carrier family 27 (fatty acid transporter), member 1	ACSVL5, FATP, FATP-1, FATP1	600691	19p13.11	35.68 kb	Alteration in LDL particle distribution; Increased plasma triglycerides
	SLC27A2	Solute carrier family 27 (fatty acid transporter), member 2	VLCS, VLACS, FACVL1, FATP2, HsT17226, VLACS, hFACVL1	603247	15q21.2	54.20 kb	
	SLC27A3	Solute carrier family 27 (fatty acid transporter), member 3	FATP3, MGC4365, ACSVL3	604193	1q21.3	4.87 kb	

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TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC27A4	Solute carrier family 27 (fatty acid transporter), member 4	FATP4, ACSVL4	604194	9q34.11	20.66 kb	Ichthyosis prematurity syndrome 1
	SLC27A5	Solute carrier family 27 (fatty acid transporter), member 5	VLCSH2,VLACSR, FACVL2	603314	19q13.43	13.00 kb	
	SLC27A6	Solute carrier family 27 (fatty acid transporter), member 6	FATP6, VLCS-H1, FACVL2, ACSVL2, DKFZp779M0564	604196	5q23.3	68.13 kb	
Na^+-coupled nucleoside transport family (SLC28)	*SLC28A1*	Solute carrier family 28 (sodium-coupled nucleoside transporter), member 1	CNT1, HCNT1	606207	15q25.3	61.12 kb	
	SLC28A2	Solute carrier family 28 (sodium-coupled nucleoside transporter), member 2	CNT2, HCNT2, SPNT1, MGC138252	606208	15q15	23.00 kb	
	SLC28A3	Solute carrier family 28 (sodium-coupled nucleoside transporter), member 3	CNT3	608269	9q22.2	90.00 kb	
Facilitative nucleoside transporter family (SLC29)	*SLC29A1*	Solute carrier family 29 (nucleoside transporters), member 1	ENT1, MGC1465, MGC3778	602193	6p21.1	14.65 kb	
	SLC29A2	Solute carrier family 29 (nucleoside transporters), member 2	HNP36, ENT2, DER12	602110	11q13	9.30 kb	
	SLC29A3	Solute carrier family 29 (nucleoside transporters), member 3	ENT3, FLJ11160, UNQ717/ PRO1380, HCLAP, HJCD, PHID	612373	10q22.1	44.14 kb	Histiocytosis-lymphadenopathy plus syndrome Dysosteosclerosis H syndrome Pigmented hypertrichotic dermatosis with insulin-dependent diabetes
	SLC29A4	Solute carrier family 29 (nucleoside transporters), member 4	ENT4, PMAT, FLJ34923	609149	7p22.1	21.14 kb	
Zinc efflux family (SLC30)	*SLC30A1*	Solute carrier family 30 (zinc transporter), member 1	ZNT1, ZRC1	609521	1q32.3	3.72 kb	
	SLC30A2	Solute carrier family 30 (zinc transporter), member 2	ZNT2, ZnT-2, MGC11303, FLJ36708, PP12488, TNZD	609617	1p35.3	8.12 kb	Zinc deficiency, transient neonatal Susceptibility to breast cell dysfunction

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC30A3	Solute carrier family 30 (zinc transporter), member 3	ZNT3, ZnT-3	602878	2p23.3	8.52 kb	
	SLC30A4	Solute carrier family 30 (zinc transporter), member 4	ZNT4	602095	15q21.1	40.33 kb	
	SLC30A5	Solute carrier family 30 (zinc transporter), member 5	ZNT5, ZTL1, ZNTL1, ZnT-5	607819	5q12.1	37.12 kb	
	SLC30A6	Solute carrier family 30 (zinc transporter), member 6	ZNT6, FLJ31101, MGC45055, MST103, MSTP103	611148	2p22.3	58.27 kb	
	SLC30A7	Solute carrier family 30 (zinc transporter), member 7	ZNT7, ZnTL2, DKFZp686M0368	611149	1p21.2	85.68 kb	
	SLC30A8	Solute carrier family 30 (zinc transporter), member 8	ZnT-8	611145	8q24.11	41.62 kb	Susceptibility to type 2 diabetes
	SLC30A9	Solute carrier family 30 (zinc transporter), member 9	HUEL, ZNT9, C4orf1, GAC63	604604	4p13	97.00 kb	
	SLC30A10	Solute carrier family 30, member 10	ZNT8, ZnT-10, ZNT10, DKFZp547M236	611146	1q41	14.39 kb	Hypermanganesemia with dystonia, polycythemia, and cirrhosis
Copper transporter family (SLC31)	*SLC31A1*	Solute carrier family 31 (copper transporters), member 1	CTR1, COPT1, hCTR1, MGC75487	603085	9q32	42.91 kb	
	SLC31A2	Solute carrier family 31 (copper transporters), member 2	CTR2, COPT2	603088	9q32	13.18 kb	
Vesicular inhibitory amino acid transporter family (SLC32)	*SLC32A1*	Solute carrier family 32 (GABA vesicular transporter), member 1	bA122O1.1, VIAAT, VGAT	616440	20q11.23	4.00 kb	
Acetyl-CoA transporter family (SLC33)	*SLC33A1*	Solute carrier family 33 (acetyl-CoA transporter), member 1, SPG42	AT1, AT-1, ACATN	603690	3q25.31	27.95 kb	Congenital cataracts, hearing loss, and neurodegeneration Spastic paraplegia 42, autosomal dominant
Type II Na^+-phosphate cotransporter family (SLC34)	*SLC34A1*	Solute carrier family 34 (sodium phosphate), member 1	NPT2, NAPI3, SLC11, NPT2a, NPTIIa, NPHLOP1, SLC17A2, NaPi2A	182309	5q35	14.42 kb	Fanconi renotubular syndrome 2 Nephrolithiasis/ osteoporosis, hypophosphatemic 1
	SLC34A2	Solute carrier family 34 (sodium phosphate), member 2	NAPI3B, NAPI-IIb, NPTIIb, FLJ90534, Napi-2b, FLJ90534, Npt2b, MX35, NAPI2b	604217	4p15.2	22.94 kb	Testicular microlithiasis Pulmonary alveolar microlithiasis
	SLC34A3	Solute carrier family 34 (sodium phosphate), member 3	NPTIIc, FLJ38680, NAPI-IIc, NPT2c, NAPI-2C	609826	9q34	5.62 kb	Hypophosphatemic rickets with hypercalciuria

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
Nucleoside-sugar transporter family (SLC35)	*SLC35A1*	Solute carrier family 35 (CMP-sialic acid transporter), member A1	CST, CMPST, FLJ76955, CMP-Sia-Tr, CMP-SA-Tr	605634	6q15	39.42 kb	Congenital disorder of glycosylation, type 2F
	SLC35A2	Solute carrier family 35 (UDP-galactose transporter), member A2	UGTL, UGALT, UGAT, UGT, UGT1, UGT2	314375	Xp11.23-p11.22	8.78 kb	Congenital disorder of glycosylation, type IIm
	SLC35A3	Solute carrier family 35 (UDP-*N*-acetylglucosamine (UDP-GlcNAc) transporter), member A3	GCNTRP, NGT, AMRS	605632	1p21	58.02 kb	Arthrogryposis, mental retardation, and seizures
	SLC35A4	Solute carrier family 35, member A4	MGC2541		5q31.3	4.26 kb	
	SLC35A5	Solute carrier family 35, member A5	FLJ20730, DKFZp434E102, FLJ11130, FLJ25973		3q13.2	22.11 kb	
	SLC35B1	Solute carrier family 35, member B1	UGALTR, UGTREL1	610790	17q21.33	6.59 kb	
	SLC35B2	Solute carrier family 35, member B2	PAPST1, UGTrel4, NFKBIE,SLL	610788	6p12.1-p11.2	3.43 kb	
	SLC35B3	Solute carrier family 35, member B3	CGI-19, C6orf196, dJ453H5.1, PAPST2	610845	6p24.3	22.32 kb	
	SLC35B4	Solute carrier family 35, member B4	YEA, YEA4, FLJ14697, NST	610923	7q33	27.71 kb	PHACE syndrome
	SLC35C1	Solute carrier family 35, member C1	FLJ11320, FUCT1	610923	11p11.2	9.00 kb	Congenital disorder of glycosylation type 2C
	SLC35C2	Solute carrier family 35 (GDP-fucose transporter), member C2	BA394O2.1, CGI-15, OVCOV1, C20orf5, FLJ37039, MGC20633, MGC32079, MGC39183		20q13.12	14.89 kb	
	SLC35D1	Solute carrier family 35 (UDP-glucuronic acid/UDP-*N*-acetylgalactosamine dual transporter), member D1	NST, KIAA0260, UGTREL7, MGC138236	610804	1p32-p31	49.88 kb	Chondrodysplasia lethal neonatal, with snail-like pelvis; Schneckenbecken dysplasia
	SLC35D2	Solute carrier family 35, member D2	SQV7L, UGTrel8, hfrc, HFRC1, MGC117215, MGC142139	609182	9q22.32	62.00 kb	
	SLC35D3	Solute carrier family 35, member D3	FRCL1, bA55K22.3, MGC102873	612519	6q23.3	3.00 kb	
	SLC35E1	Solute carrier family 35, member E1	FLJ14251, DKFZp564G0462, FLJ36689, MGC44954		19p13.11	22.55 kb	

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC35E2	Solute carrier family 35, member E2	KIAA0447, DKFZp686M0869, FLJ34996, FLJ44537, MGC104754, MGC117254, MGC126715, MGC138494		1p36.33	13.75 kb	
	SLC35E3	Solute carrier family 35, member E3	BLOV1		12q15	19.92 kb	
	SLC35E4	Solute carrier family 35, member E4			22q12.2	11.00 kb	
	SLC35F1	Solute carrier family 35, member F1	C6orf169, dJ230I3.1, FLJ13018		6q22.31	410.14 kb	
	SLC35F2	Solute carrier family 35, member F2	HSNOV1, FLJ13018		11q22.3	68.00 kb	
	SLC35F3	Solute carrier family 35, member F3	FLJ37712		1q42.2	419.58 kb	
	SLC35F4	Solute carrier family 35, member F4	FLJ37712,c14_5373, c14orf36		14q22.2	32.00 kb	
	SLC35F5	Solute carrier family 35, member F5	FLJ22004, NS5ATP3		2q14.1	56.57 kb	
	SLC35F6	Solute carrier family 35, member F6	ANT2BP, C2orf18, TANGO9		2p23.3	16.96 kb	
	SLC35G1	Solute carrier family 35, member G1	C10orf60, FLJ33990, TMEM20, POST		10q23.33	8.76 kb	
	SLC35G2	Solute carrier family 35, member G2	TMEM22		3q22.3	37.24 kb	
	SLC35G3	Solute carrier family 35, member G3	AMAC1, TMEM21A		17q12	2.00 kb	
	SLC35G4	Solute carrier family 35, member G4	AMAC1L1P, SLC35G4		18p11.21		
	SLC35G5	Solute carrier family 35, member G5	AMAC, AMAC1L2	615199	8p23.1	1.32 kb	
	SLC35G6	Solute carrier family 35, member G6	AMAC1L3, TMEM21B		17p13.1	2.16 kb	
Proton-coupled amino acid transporter family (SLC36)	*SLC36A1*	Solute carrier family 36 (proton/amino acid symporter), member 1	FLJ10815, LYAAT1, PAT1, TRAMD3	606561	5q33.1	44.78 kb	
	SLC36A2	Solute carrier family 36 (proton/amino acid symporter), member 2	PAT2, TRAMD1, FLJ16051, MGC119658, MGC119660	608331	5q33.1	32.61 kb	Hyperglycinuria Iminoglycinuria, digenic
	SLC36A3	Solute carrier family 36 (proton/amino acid symporter), member 3	PAT3, TRAMD2, tramdorin2	608332	5q33.1	26.00 kb	

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TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC36A4	Solute carrier family 36 (proton/amino acid symporter), member 4	PAT4, FLJ38932	613760	11q21	53.80 kb	
Glycerol-3-phosphate transporter family (SLC37)	*SLC37A1*	Solute carrier family 37 (glycerol-3-phosphate transporter), member 1	G3PP, FLJ22340	608094	21q22.3	81.00 kb	
	SLC37A2	Solute carrier family 37 (glycerol-3-phosphate transporter), member 2	FLJ00171, MGC71430, SPX2, pp11662		11q24.2	25.90 kb	
	SLC37A3	Solute carrier family 37 (glycerol-3-phosphate transporter), member 3	DKFZp761N0624, MGC32939, SPX3		7q34	64.76 kb	
	SLC37A4	Solute carrier family 37 (glycerol-6-phosphate transporter), member 4	G6PT1, PRO0685, MGC15729, GSD1d, G6PT2, G6PT3, TRG19, G6PT, GSD1b, GSD1c, TRG-19	602671	11q23.3	6.53 kb	Glycogen storage disease, type IB Glycogen storage disease, type IC
System A and System N sodium-coupled neutral amino acid transporter family (SLC38)	*SLC38A1*	Solute carrier family 38, member 1	ATA1, SAT1, NAT2, EVI163, SNAT1	608490	12q13.11	86.37 kb	
	SLC38A2	Solute carrier family 38, member 2	ATA2, SAT2, PRO1068, KIAA1382, SNAT2	605180	12q13.11	14.58 kb	
	SLC38A3	Solute carrier family 38, member 3	G17, SN1, SNAT3, NAT1	604437	3p21.3	15.72 kb	
	SLC38A4	Solute carrier family 38, member 4	ATA3, NAT3, SNAT4, PAAT, MGC126876, FLJ10191	608065	12q13	61.24 kb	
	SLC38A5	Solute carrier family 38, member 5	SN2, JM24, pp7194, SNAT5	300649	Xp11.23	11.64 kb	
	SLC38A6	Solute carrier family 38, member 6	NAT-1, MGC102697, SNAT6	616518	14q23.1	71.84 kb	
	SLC38A7	Solute carrier family 38, member 7	FLJ10815, FLJ12724, SNAT7	614236	16q21	18.38 kb	
	SLC38A8	Solute carrier family 38, member 8	FVH2	615585	16q23.3	32.37 kb	Foveal hypoplasia 2, with or without optic nerve misrouting and/or anterior segment dysgenesis

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC38A9	Solute carrier family 38, member 9	FLJ90709, FLJ46104, MGC120544	616203	5q11.2	86.42 kb	
	SLC38A10	Solute carrier family 38, member 10	MGC15523, PP1744, FLJ35718	616525	17q25.3	50.30 kb	
	SLC38A11	Solute carrier family 38, member 11	AVT2, FLJ39822, MGC150450	616526	2q24.3	57.22 kb	
Metal ion transporter family (SLC39)	*SLC39A1*	Solute carrier family 39 (zinc transporter), member 1	ZIP1, ZIRTL	604740	1q21	8.60 kb	
	SLC39A2	Solute carrier family 39 (zinc transporter), member 2	ZIP2, MGC119190, Eti-1, 6A1	612166	14q11.2	2.00 kb	
	SLC39A3	Solute carrier family 39 (zinc transporter), member 3	ZIP3	612168	19p13.3	7.55 kb	
	SLC39A4	Solute carrier family 39 (zinc transporter), member 4	ZIP4, AEZ, FLJ20327, MGC74741	607059	8q24.3	4.48 kb	Acrodermatitis enteropathica
	SLC39A5	Solute carrier family 39 (metal ion transporter), member 5	LZT-Hs7, MGC34778, ZIP5	608730	12q13.3	7.00 kb	Myopia 24, autosomal dominant
	SLC39A6	Solute carrier family 39 (zinc transporter), member 6	LIV-1, ZIP6	608731	18q12.2	20.86 kb	
	SLC39A7	Solute carrier family 39 (zinc transporter), member 7	KE4, HKE4, RING5, H2-KE4, D6S115E, D6S2244E, RING5, ZIP7	601416	6p21.3	3.60 kb	
	SLC39A8	Solute carrier family 39 (zinc transporter), member 8	BIGM103, LZT-Hs6, PP3105, ZIP8	608732	4q22-q24	94.46 kb	
	SLC39A9	Solute carrier family 39 (zinc transporter), member 9	FLJ11274, MGC74989, ZIP9		14q24.1	61.32 kb	
	SLC39A10	Solute carrier family 39 (zinc transporter), member 10	FLJ90515, KIAA1265, DKFZp564L2123, LZT-Hs2, Zip10, DKFZp781L10106, MGC126565, MGC138428	608733	2q32.3	80.89 kb	
	SLC39A11	Solute carrier family 39 (metal ion transporter), member 11	C17orf26, ZIP11	616508	17q21.31	446.74 kb	
	SLC39A12	Solute carrier family 39 (zinc transporter), member 12	FLJ30499, LZT-Hs8, MGC43205, MGC51099, ZIP12, bA570F3.1	608734	10p12.33	91.39 kb	

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC39A13	Solute carrier family 39 (zinc transporter), member 13	FLJ25785, ZIP13, LZT-Hs9	608735	11p11.2	7.00 kb	Spondylocheirodysplasia, Ehlers-Danlos syndrome like
	SLC39A14	Solute carrier family 39 (zinc transporter), member 14	KIAA0062, LZT-Hs4, ZIP14, cig19, NET34	608736	8p21.3	66.88 kb	
Basolateral iron transporter family (SLC40)	*SLC40A1*	Solute carrier family 40 (iron-regulated transporter), member 1	IREG1, FPN1, MTP1, SLC11A3, MST079, MSTP079	604653	2q32	20.22 kb	Hematochromatosis, type 4
MgtE-like magnesium transporter family (SLC41)	*SLC41A1*	Solute carrier family 41, member 1	MgtE	610801	1q32.1	23.94 kb	Susceptibility to Parkinson disease
	SLC41A2	Solute carrier family 41, member 2	SLC41A1-L1, MGC125330, MGC125331	610802	12q23.3	125.20 kb	
	SLC41A3	Solute carrier family 41, member 3	FLJ20473, SLC41A1-L2	610803	3q21.2	77.00 kb	
Rh ammonium transporter family (SLC42)	*RHAG*	Rhesus blood group-associated glycoprotein	CD241, RH2, RH50A, Rh50, Rh50GP, SLC42A1	180297	6p12.3	31.00 kb	*Anemia, hemolytic, Rh-null, regulator type* *Rh-mod syndrome*
	RHBG	Rh family, B glycoprotein (gene/pseudogene)	SLC42A2	607079	1q21.3	16.01 kb	
	RHCG	Rhesus blood group, C glycoprotein	RHGK, C15orf6	605381	15q25	25.00 kb	
Na^+ independent, system-L-like amino acid transporter family (SLC43)	*SLC43A1*	Solute carrier family 43, member 1	LAT3, PB39, POV1, R00504	603733	11q12.1	31.00 kb	
	SLC43A2	Solute carrier family 43, member 2	LAT4, MGC34680, FLJ23848	610791	17p13.3	54.00 kb	
	SLC43A3	Solute carrier family 43, member 3	EEG1, FOAP-13, PRO1659, SEEEG-1, DKFZp762A227		11q11	20.63 kb	
Choline-like transporter family (SLC44)	*SLC44A1*	Solute carrier family 44, member 1	CTL1, CHTL1, CDW92, CD92, RP11-287A8.1	606105	9q31.2	157.00 kb	
	SLC44A2	Solute carrier family 44, member 2	CTL2, PP1292, FLJ44586, DKFZp666A071	606106	19p13.1	42.12 kb	Susceptibility to transfusion-related acute lung injury Susceptibility to venous thromboembolism
	SLC44A3	Solute carrier family 44, member 3	CTL3, MGC45474		1p21.3	74.43 kb	
	SLC44A4	Solute carrier family 44, member 4	CTL4, NG22, FLJ14491, C6orf29, NG22	606107	6p21.3	15.85 kb	Susceptibility to late-onset Alzheimer disease

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
	SLC44A5	Solute carrier family 44, member 5	CTL5, FLJ34081, MGC34032		1p31.1	404.49 kb	
Putative sugar transporter family (SLC45)	*SLC45A1*	Solute carrier family 45, member 1	DNB5, PAST-A, KIAA0458	605763	1p36.23	19.00 kb	
	SLC45A2	Solute carrier family 45, member 2	AIM1, MATP, AIM-1, 1A1, SHEP5	606202	5p13.2	40.06 kb	Oculocutaneous albinism, type 4
	SLC45A3	Solute carrier family 45, member 3	PRST, IPCA-6, PSTP, PCANAP6, IPCA-2, IPCA-8, IPCA6, PCANAP2, PCANAP8	605097	1q32.1	22.65 kb	
	SLC45A4	Solute carrier family 45, member 4	KIAA1126		8q24.3	47.00 kb	
Folate transporter family (SLC46)	*SLC46A1*	Solute carrier family 46 (folate transporter), member 1	HCP1, MGC9564, PCFT, G21, PCFT/HCP1	611672	17q11.2	11.57 kb	Folate malabsorption, hereditary
	SLC46A2	Solute carrier family 46, member 2	TSCOT, Ly110	608956	9q32	11.00 kb	Susceptibility to cervical cancer
	SLC46A3	Solute carrier family 46, member 3	FKSG16		13q12.3	18.93 kb	
Multidrug and Toxin Extrusion (MATE) family (SLC47)	*SLC47A1*	Solute carrier family 47, member 1	FLJ10847, MATE1, MGC64822	609832	17p11.2	45.18 kb	Susceptibility to drug-induced nephrotoxicity
	SLC47A2	Solute carrier family 47, member 2	FLJ31196, MATE2, MATE2-B, MATE2-K, MATE2K	609833	17p11.2	38.42 kb	Susceptibility to drug-induced nephrotoxicity
Heme transporter family (SLC48)	*SLC48A1*	Solute carrier family 48 (heme transporter), member 1	HRG-1, HRG1, hHRG-1	612187	12q13.11	28.84 kb	
FLVCR-related transporter family (SLC49)	*FLVCR1*	Feline leukemia virus subgroup C cellular receptor 1	AXPC1, FLVCR, MFSD7B, PCA, PCARP	609144	1q32.3	38.60 kb	Ataxia, posterior column with retinitis pigmentosa
	FLVCR2	Feline leukemia virus subgroup C cellular receptor family, member 2	C14orf58, CCT, EPV, FLVCRL14q, MFSD7C, PVHH	610865	14q24.3	69.57 kb	Proliferative vasculopathy and -hydrocephaly syndrome
Sugar efflux transporters (SLC50)	*SLC50A1*	Solute carrier family 50 (sugar transporter), member 1	RP11-540D14.5, HsSWEET1, RAG1AP1, SCP, SWEET1	613683	1q22	3.05 kb	
Transporters of steroid-derived molecules (SLC51)	*SLC51A*	Solute carrier family 51, alpha subunit	OSTA, OSTalpha	612084	3q29	31.69 kb	
	SLC51B	Solute carrier family 51, beta subunit	OSTB, OSTBETA	612085	15q22.31	8.03 kb	

Continued

TABLE 6.3 Transporter Genes—cont'd

Subfamily	Symbol	Title/gene	Aliases	OMIM	Locus	Size	Other related diseases
Riboflavin transporter family (RFVT/SLC52)	*SLC52A1*	Solute carrier family 52, riboflavin transporter, member 1	GPCR, PAR2, FLJ10060, GPR172B, GPCR42, PAR2, RBFVD, RFT1, RFVT1, hRFT1, HuPAR-2	607883	17p13.2	2.83 kb	Riboflavin deficiency
	SLC52A2	Solute carrier family 52, riboflavin transporter, member 2	FLJ11856, PAR1, GPCR41, D15Ertd747e, GPCR, GPR172a, RFT3, RFVT2, hRFT3	607882	8q24.3	2.73 kb	Brown-Vialetto-Van Laere syndrome 2
	SLC52A3	Solute carrier family 52, riboflavin transporter, member 3	MGC10698, bA371L19.1, RFT2, C20orf54, RFVT3	613350	20p13	8.51 kb	Brown-Vialetto-Van Laere syndrome 1 Fazio-Londe disease
MISCELLANEOUS							
Aquaporins	*AQP1*	Aquaporin 1 (Colton blood group)	CO, CHIP28, AQP-CHIP	107776	7p14.3	13.72 kb	Colton blood group
	AQP7	Aquaporin 7	AQP9, AQP7L, AQPAP, MGC149555, MGC149556	602974	9p13.3	17.57 kb	
	AQP9	Aquaporin 9	AQP-9, HsT17287, SSC1	602914	15q21.3	47.74 kb	
Major vault protein	*MVP*	Major vault protein	LRP, VAULT1	605088	16p11.2	27.00 kb	
Metallothioneins	*MT2A*	Metallothionein 2A	MT2, CES1, MT-II	156360	16q13	0.88 kb	
	MT3	Metallothionein 3	GRIF, GIF, GIFB	139255	16q13	1.00 kb	

Genes associated with drug transporters (Table 6.3) include *ABC* genes, especially *ABCB1*, *ABCC1 (MRP1), ABCC2 (MRP2), ABCG2 (BCRP)*, genes of the solute carrier superfamily (SLC) and solute carrier organic (SLCO) transporter family, responsible for the transport of multiple endogenous and exogenous compounds, including folate (SLC19A1), urea (SLC14A1, SLC14A2), monoamines (SLC29A4, SLC22A3), aminoacids (SLC1A5, SLC3A1, SLC7A3, SLC7A9, SLC38A1, SLC38A4, SLC38A5, SLC38A7, SLC43A2, SLC45A1), nucleotides (SLC29A2, SLC29A3), fatty acids (SLC27A1–6), neurotransmitters (SLC6A2 (noradrenaline transporter), SLC6A3 (dopamine transporter), SLC6A4 (serotonin transporter, SERT), SLC6A5, SLC6A6, SLC6A9, SLC6A11, SLC6A12, SLC6A14, SLC6A15, SLC6A16, SLC6A17, SLC6A18, SLC6A19), glutamate (SLC1A6, SLC1A7), and others (Table 6.3).[4,56]

ABC transporters constitute a large family of membrane proteins, which transport a variety of compounds through the membrane against a concentration gradient at the cost of ATP hydrolysis. Substrates of ABC transporters include lipids, bile acids, xenobiotics, and peptides for antigen presentation. As they transport exogenous and endogenous compounds, they reduce the body load of potentially harmful substances. One by-product of such a protective function is that they also eliminate various useful drugs from the body, causing drug resistance.[57]

In humans there are 49 *ABC* transporter genes and multidrug resistance-associated proteins (MRP1/ABCC1, MRP2/ABCC2, MRP3/ABCC3, MRP4/ABCC4, MRP5/ABCC5, MRP6/ABCC6, MRP7/ABCC10, MRP8/ABCC11, and MRP9/ABCC12) that belong to the ABCC family integrated by 13 members. ABCC7 is the cystic fibrosis transmembrane conductance regulator. ABCC8 and ABCC9 are sulfonylurea receptors that constitute the ATP-sensing subunits of a complex potassium channel. MRP10/ABCC13 is a pseudogene that encodes a truncated protein, which is highly expressed in fetal human liver and has the highest similarity to MRP2/ABCC2 but without transporting

activity. These transporters are localized to the apical and/or basolateral membrane of the hepatocytes, enterocytes, renal proximal tubule cells, and endothelial cells of the blood-brain barrier. MRP/ABCC members transport endogenous substances and xenobiotics and their metabolites. MRP/ABCC transporters, except MRP9/ABCC12, transport organic anions (drugs conjugated to glutathione, sulfate, or glucuronate). Some MRP/ABCC members may transport endogenous compounds (leukotriene C4 by MRP1/ABCC1), bilirubin glucuronides (MRP2/ABCC2 and MRP3/ABCC3), prostaglandins E1 and E2 (MRP4/ABCC4), cGMP (MRP4/ABCC4, MRP5/ABCC5, and MRP8/ABCC11), and several glucuronosyl-, or sulfatidyl steroids. MRP/ABCC transporters can confer resistance to natural product anticancer drugs and their conjugated metabolites, platinum compounds, folate antimetabolites, nucleoside and nucleotide analogs, arsenical and antimonial oxyanions, peptide-based agents, and in concert with alterations in phase II conjugating or biosynthetic enzymes, classical alkylating agents. Some MRP/ABCC members (MRP1–3) are associated with tumor resistance caused by increased efflux and decreased intracellular accumulation of natural product anticancer drugs. Drug targeting of these transporters to overcome MRP/ABCC-mediated multidrug resistance may play a role in cancer chemotherapy.[58]

The multidrug resistance 1 (*MDR1*) gene (*ABCB1*) encodes for an ATP-binding cassette transporter P-glycoprotein (P-gp) involved in chemoresistance to taxanes. P-gp is an efflux pump capable of activating the multidrug resistance (MDR) phenotype. *ABCB1* has several binding sites in its promoter region, along with CpG islands and GC boxes, involved in its epigenetic control. LRPPRC is a potential regulator of *ABCB1* transcription via an invMED1 binding site in ABCB1. This invMED1 binding site overlaps with the GC-100 box.[59] *MDR1* promoter methylation is frequent in prostate carcinoma (PC), suggesting epigenetic regulation. *MDR1* promoter methylation and P-gp expression assessed in PC, high-grade prostatic intraepithelial neoplasia (HGPIN), benign prostatic hyperplasia (BPH), and morphologically normal prostate tissue (NPT) samples has shown that *MDR1* promoter methylation levels increase from NPTs to HGPIN and to PC. Histone active marks H3Ac, H3K4me2, H3K4me3, H3K9Ac, and H4Ac are increased at the *MDR1* promoter after exposure to TSA, suggesting that in prostate carcinogenesis *MDR1* downregulation is mainly due to histone posttranslational modifications. This occurs concomitantly with aberrant promoter methylation, substantiating the association with P-gp decreased expression.[60]

ABC transporters are drug efflux pumps responsible for the multidrug resistance phenotype causing hepatocellular carcinoma (HCC) treatment failure. A total of 12 ABC transporters (ABCA2, ABCB1, ABCB6, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCC10, ABCC11, ABCC12, and ABCE1) showed upregulation in HCC compared with adjacent healthy liver. Regulation of *ABC* expression in HCC might be mediated by miRNAs since dysregulation of 90 miRNAs has been demonstrated in HCC compared with healthy liver, including the upregulation of 11 and downregulation of 79. Some 13 cellular miRNAs have been confirmed as targeting *ABCA1*, *ABCC1*, *ABCC5*, *ABCC10*, and *ABCE1* genes and mediating changes in gene expression. *ABC* and miRNA expression showed an inverse relationship in HCC.[61]

ABCG2 is not only overexpressed in certain drug-resistant cancers but is also highly expressed in the mammary gland during lactation, carrying xenobiotics and nutrients into milk. Expression profiling of different mouse *Abcg2* mRNA isoforms (E1a, E1b, and E1c) revealed that *E1b* is predominantly expressed and induced in the lactating mouse mammary gland. *E1b* promoter sequences in the virgin gland are already hypomethylated and marked with the open chromatin histone mark H3K4me2. After 24 h of lactation blockade there was a significant reduction in *Abcg2* mRNA expression and a loss of signal transducer and activator of transcription-5 (STAT5) occupancy at the mouse *Abcg2* gene. Some of these STAT5-binding regions that contained interferon-γ-activated sequence (GAS) motifs function as an enhancer after prolactin treatment.[62]

The kidney plays an important role in the secretion of organic compounds including drugs, toxins, and endogenous metabolites. The renal elimination process of organic cations is mediated by two distinct transport systems expressed on the apical and basolateral membrane of proximal epithelial cells. Mammalian multidrug and toxin extrusion 1 (MATE1)/SLC47A1 is an ortholog of bacterial NorM. MATE1 is the H^+/organic cation antiporter at the apical membrane, which mediates the secretion of organic cations. Kidney-specific MATE2-K has been isolated from human kidney and localized at the brush border membrane of proximal tubules. Like MATE1, MATE2-K mediates the secretion of organic cations into urine.[63] OCT3 and MATE1 protein are important transporters in the placenta. Metformin is a substrate of both OCT3 and MATE1. A concentration-dependent transplacental clearance of metformin in both maternal-to-fetal and fetal-to-maternal directions has been observed. This transport was completely inhibited by MPP^+, a common OCT3 and MATE1 inhibitor.[64]

miRNAs target ABC transporters and are influential epigenetic regulators of drug metabolism, resistance, and toxicity.[61,65,66]

In skin, *ABCC3* is expressed at the highest levels, followed by *SLCO3A1*, *SLC22A3*, *SLC16A7*, *ABCA2*, *ABCC1*, and *SLCO2B1*. *ABCC3* accounts for 20.0% of total mean transporter mRNA content. *ABCC3* mRNA expression shows large interindividual variability. SNPs (−1767G > A, −1328G > A, −1213C > G, −897delC, −260T > A, and −211C > T) in the

ABCC3 promoter region do not affect *ABCC3* mRNA levels. ABCC3 expression levels negatively correlate with the methylation status of the CpG island (CGI) located 10 kb upstream of *ABCC3*. *ABCC3* mRNA is upregulated by the demethylating agent 5-Aza-2′-deoxycytidine. Deletion of the region surrounding CGI by CRISPR/Cas9 results in a decrease in *ABCC3* mRNA levels.[67]

High maternal gestational weight gain (GWG) is inversely correlated with offspring *ABCA1* methylation after accounting for ancestry, parental, and offspring exposures.[68]

The dopamine transporter (*DAT*) is the key regulator of dopaminergic transmission and is a target of several xenobiotics, including pesticides and pharmacological agents. Histone deacetylases play a prominent role in the regulation of *DAT* expression. Inhibition of histone deacetylases (HDACs) by valproate, butyrate, and trichostatin A lead to a 3- to 10-fold increase in *DAT* mRNA expression, a 50% increase in protein levels, and increased H3 acetylation levels, with increased enrichment of acetylation of histone 3 on lysines 9 and 14 (H3K9/K14ac) in the *DAT* promoter. Expression of Nurr1 and Pitx3, key regulators of DAT expression, are increased following valproate treatment, and Nurr1 binding is enriched in the *DAT* promoter. Histone acetylation and subsequent enhancement of transcription factor binding are plausible mechanisms for DAT regulation by valproate and perhaps by other xenobiotics.[69] Studies on the relationship between DNA methylation and expression of human dopamine transporter (*hDAT*) revealed hypomethylation of the two promoter regions (−1214 to −856 bp and −48 to 439 bp) of *hDAT* in different experimental models.[70]

SLC29A and SLC28A nucleoside transporters contribute to proper placental development and mediate uptake of nucleosides/nucleoside-derived drugs. The expression of *SLC28A1*, *SLC28A2*, and *SLC28A3* increases during gestation with great interindividual variability. SLC28A2 is a dominant subtype in the first trimester of the term human placenta, while SLC28A1 exhibits negligible expression in the term human placenta. mRNA of *SLC28A2* and *SLC28A3* are affected by 5-azacytidine, all-trans retinoic acids, and sodium valproate. The methylation status and activation of the retinoic acid receptor affect placental *SLC28A2* and *SLC28A3* transcription, and substrates of concentrative nucleoside transporter 2 might be taken up more in placentas with an overactivated cAMP/protein kinase A pathway.[71]

There is an apparent association between peripheral serotonin transporter gene (*SLC6A4*) methylation and function of frontal-limbic circuits and the brain's resting state. Blood-derived *SLC6A4* methylation is positively associated with superior frontal gray matter (GM) volume and with right lateral parietal area (RLP)-frontal pole regional resting state functional connectivity (rsFC). Saliva-derived *SLC6A4* methylation is positively associated with superior frontal GM volume. Buccal-derived *SLC6A4* methylation is positively associated with superior and inferior frontal and anterior cingulate cortical (ACC) GM volumes as well as with RLP-ACC, frontal pole, and medial prefrontal regional rsFC. Buccal cells seem to be highly sensitive when studying *SLC6A4* promoter methylation in brain disorders with potential pathogenic involvement of serotonin dysfunction.[72]

Monocarboxylate transporter 1 (MCT1) plays a crucial role in oligodendrocyte differentiation and myelination. *MCT1* is strongly expressed in oligodendrocyte but weakly expressed in oligodendrocyte precursors (OPCs). Histone deacetylase (HDAC) activity is required for induction of oligodendrocyte differentiation and maturation. The acetylation level of histone H3K9 (H3K9ac) is much higher in *mct1* gene (*Slc16a1*) promoter in OPCs than in oligodendrocytes. H3K9ac regulates *MCT1* expression, and there is a negative correlation between H3K9ac and *MCT1* expression in oligodendrocytes. The levels of HDAC1, 2, and 3 proteins in oligodendrocytes are higher than in OPCs, and knockdown of *HDAC2* decreases the expression of *MCT1* in oligodendrocytes. HDAC2 is involved in H3K9ac modifications that regulate the expression of *MCT1* during oligodendrocyte development.[73]

6.3 EPIGENETIC DRUGS

Epigenetic drugs (Tables 6.4–6.12) reverse epigenetic changes in gene expression and might open future avenues for the treatment of major problems of health.[6,7,74–77] Within this growing category of drugs, several inhibitors of histone deacetylation and DNA methylation have been approved by the US FDA for hematological malignancies,[74,78] and some epigenetic drugs are being evaluated in clinical trials for the treatment of several diseases (Table 6.11).

6.3.1 Classification of Epigenetic Drugs

According to their respective targets (Table 6.1), epigenetic drugs (Table 6.4) can be classified into the following categories: (i) DNA methyltransferase inhibitors (Table 6.5): DNMTs target DNA methyltransferases (DNMT1–3) and some DNMT complexes (DNMT3L/DNMT3A complex, DNMT1/PCNA/UHRF1 complex), and can be chemically distinguished as nucleoside analogs, small molecules, natural products, dual inhibitors, and other classes

TABLE 6.4 Classification of Potential Epigenetic Drugs

Potential targets	Categories	Drugs
DNA methyltransferases	**DNA methyltransferase inhibitors**	
DNMTs DNMT1 DNMT2 DNMT3A DNMT3B DNMT3-like (DNMT3L) DNMT3L/DNMT3A complex DNMT1/PCNA/UHRF1 complex	Nucleoside analogs	5-Aza-2′-deoxycytidine (Decitabine) 5-Azacytidine (Azacitidine) Zebularine Guadecitabine (SGI-110) 5-Aza-2′,2′-difluorodeoxycytidine (NUC013) 3′,5′-Di-trimethylsilyl-2′,2′-difluoro-5-azadeoxycytidine (NUC041) Oligodeoxyribonucleotide containing 2-amino-4-halopyridine-C-nucleoside (dXP)
	Small molecules	Hydralazine Compound 40 Procainamide RG108 [2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-3-(1*H*-indol-3-yl)propanoic acid]
	Natural products	Curcumin derivatives: RG-108, SGI-1027 Psammaplins Tea polyphenols: Epigallocatechin-3-gallate Catechins: Catechin, epicatechin bioflavonoids: quercetin, genistein, fisetin Procyanidin B2
	Replication foci targeting sequence (RFTS) domain	Histone H3 tail peptides
	Dual DNMT/G9a inhibitors	CM-272
	Other DNA methyltransferase inhibitors	Zebularine
Methyl-DNA-binding proteins: Methyl-CpG-binding domain (MBD) C2H2 zinc finger domain SET- and RING finger-associated (SRA) domain		Maleimide derivatives of RG108
DNA demethylases		
Ten-eleven translocation (TET) family AID/APOBEC family BER (base excision repair) glycosylase family	TET1 modulators Activation-induced cytidine deaminase (AID) regulators DNA glycosylase modulators	
Histone deacetylases (HDACs) **HDAC1–18** – Class I HDACs (HDAC1, 2, 3, and 8) HDAC1/HDAC2 transcriptional corepressor complexes (SIN3A, NuRD, CoREST) HDAC3-(SMRT/N-CoR) complexes – Class II HDACs: Class IIa (HDAC4, 5,7, and 9); Class IIb (HDAC6 and 10) – Class III HDCAs: Sirtuin family: nuclear (SIRT1, 2, 6, 7), mitochondrial (SIRT3, 4, 5), cytoplasmic (SIRT1, 2) – Class IV HDAC (HDAC11) Histone deacetylase RPD3	**Histone deacetylase (HDAC) inhibitors**	
	Short chain fatty acids	Sodium butyrate Sodium phenyl butyrate Valproic acid Magnesium valproate Pivaloyloxymethyl butyrate (AN-9, Pivanex)

Continued

TABLE 6.4 Classification of Potential Epigenetic Drugs—cont'd

Potential targets	Categories	Drugs
	Hydroxamic acids	Suberohydroxamic acid Suberoylanilide hydroxamic acid (SAHA, Vorinostat) Oxamflatin Pyroxamide Trichostatin A (TSA) *m*-Carboxycinnamic acid bis-hydroxamide (CBHA) Derivatives of the marine sponge *Psammaplysilla purpurea*: NVP-LAQ824, NVP-LBH589 LBH-589 (Panobinostat) M344 ITF2357 (Givinostat) PXD101 (Belinostat) JHJ-26481585 CHR-3996 CHR-2845 GC-1521 OSU-HDAC-42 PCI-24781 Tefinostat Abexinostat Tubastatin A Resminostat Dacinostat Quisinostat Ricolinostat Roclinostat Pracinostat Imidazo-ketopiperazine compounds
	Cyclic peptides	Romidepsin (Depsipeptide, FR901228) Apicidin Cyclic hydroxamic acid-containing peptides (CHAPS) Trapoxin A Trapoxin B Chlamydocin HC toxin Bacterial FK228 Plitidepsin (Aplidine)
	Benzamides	MS-275 (Entinostat) CI-994 RGFP136 RGFP966 MGCD0103 (Mocetinostat) Compound 60 Tacedinaline Chidamide
	Ketones	Trifluoromethyl ketone
	Small molecules	Droxinostat PTACH
	Quinoline-3-carboxamides	Tasquinimod
	Carbamates	Bufexamac (HDAC6i)
	Hybrid compounds	
	Pazopanib hybrids	Orthoaminoanilide 6d and hydroxamic acid

TABLE 6.4 Classification of Potential Epigenetic Drugs—cont'd

Potential targets	Categories	Drugs
	Dual indoleamine 2,3-dioxygenase 1 (IDO1) and histone deacetylase (HDAC) inhibitors	13f Compound 10
	Dual nicotinamide phosphoribosyltransferase (NAMPT) and histone deacetylase (HDAC) inhibitors	Thiazolocarboxamides (Compound 7f) Compound 35 Dinitrooxy compound 31 Furoxan derivative 16
	HDACi MS-275 + NO donors	Vorinostat-Tranylcypromine derivatives
	Polyamine-based HDACs-LSD1 dual binding inhibitors	Compound 14 Compound 47
	Dual G9a and HDAC inhibitors	
	Triple inhibitors	
	Sirtuin inhibitors	Nicotinamide/niacinamide Suramin Selisistat Inauhzin AGK-2 AK-7 Sirtinol Salermide MS3 Splitomycin Cambinol SEN-196 Dihydrocoumarin Tenovin-6 UVI5008 HR-73 SirReal2 5-Methylmellein Mellein Eurochevalierine 8-Bromo-1,2-dihydro-3*H*-naphth[1,2-e][1,3] oxazine-3-thione N-alkylated derivatives 2-((4,6-Dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide derivatives
	Sirtuin activators	Resveratrol SRT-501 SRT-1460 SRT-1720 SRT-2104 SRT-2183 GSK-184072 Quercetin Fisetin Butein Isoliquiritigenin Piceatannol Flutamide Hydrogen sulfide
	Other compounds	3-Deazaneplanocin A (DZNep) Tubacin EVP-0334 MOCPAC BATCP 6-([^{18}F]Fluoroacetamido)-1-hexanoicanilide Quinazolin-4-one derivatives: (*E*)-3-(2-Ethyl-7-fluoro-4-oxo-3-phenethyl-3,

Continued

TABLE 6.4 Classification of Potential Epigenetic Drugs—cont'd

Potential targets	Categories	Drugs
		4-dihydroquinazolin-6-yl)-*N*-hydroxyacrylamide *N*-Hydroxy-3-(2-methyl-4-oxo-3-phenethyl-3,4-dihydro-quinazolin-7-yl)-acrylamide Quinoline derivatives: SGI-1027 (*N*-(4-(2-amino-6-methylpyrimidin-4-ylamino)phenyl)-4-(quinolin-4-ylamino)benzamide) Carbamazepine APHA (*S*)-4-(2-(5-(Dimethylamino)naphthalene-1-sulfonamido)-2-phenylacetamido)-*N*-hydroxybenzamide (D17) HDAC3-inhibitor RGPF966 3′,4′-Dihydro-2′-*H*-spiro [imidazolidine-4,1′-naphthalene]-2,5-dione 1-(3-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamide α,β-Unsaturated carboxylic acid and urea-based derivatives *Schistosoma mansoni* histone deacetylase 8 (HDAC8) inhibitors: *N*-(2,5-Dioxopyrrolidin-3-yl)-*n*-alkylhydroxamate derivatives; non-hydroxamic acid benzothiadiazine dioxide derivatives Secondary and tertiary N-substituted 7-aminoheptanohydroxamic acid derivatives Polyoxometalates (PC-320) Macrocyclic nonribosomal peptide HDAC inhibitors Cd[L-proline]2 Tetrahydroisoquinoline-based HDAC inhibitors Dithienylethenes Fulgimides Isatin/*o*-phenylenediamine-based HDAC inhibitors JSL-1 Benzodiazepine (BZD) derivatives 7-Ureido-*N*-hydroxyheptanamide derivative (CKD5)
Histone acetyltransferase (HAT) **Histone lysine acetyltransferases (KATs)** General control nonderepressible 5 (GCN5) KAT2A/GCN5 KAT2B/PCAF KAT6–8 K (Lysine) acetyltransferase 8 (KAT8, MOF) CREBBP/CBP EP300-Thymine DNA glycosylase (TDG) Monocytic leukemia zinc finger protein-related factor (MORF) PHF20 ATP citrate lyase (ACL) Super elongation complex (SEC) Multiprotein histone acetyltransferase (HAT) complex: HBO1, inhibitor of growth family member (ING) 4/5, MYST/Esa1-associated factor (MEAF) 6, Jade family	**Histone acetyltransferase inhibitors**	Curcumin (Diferuloylmethane) Hydrazinocurcumin Lys-CoA H3-CoA-20 C646 CPTH2 CPTH6 CCT077791 CCT077792 L-45 Anacardic acid Garcinol Spermidinyl-CoA Compound 4e Compound 4g PU139 PU141

TABLE 6.4 Classification of Potential Epigenetic Drugs—cont'd

Potential targets	Categories	Drugs
PHD finger (JADE) 1/2/3, bromodomain and PHD finger-containing protein (BRPF) 2/3 FATp300 inhibitor		TH1834 NK13650A NK13650B HAti II MG149 I-CBP112 L002 MS7972 MS7867 A-485 GNE-272 EML 425 P300-IN-1 P300-IN-2 MOF-IN-1 MC1823 TTK21 CTPB SPV-106 Ginkgolic acid
	Histone acetyltransferase activators	*N*-(4-Chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide Pentadecylidenemalonate 1b (SPV-106)
Histone methyltransferases Histone lysine methyltransferases (HKMT) G9a histone lysine methyltransferase (G9a) (KMT1C, EHMT2) SUV39 subfamily of KMTs (SUV39H1, SUV39H2, G9a, GLP, SETDB1, SETDB2) SET and MYND domain-containing proteins (Smyd family) lysine methyltransferases ESET protein (SETDB1) SETD8/SET8/Pr-SET7/KMT5A lysine methyltransferase Disruptor of telomeric silencing-1-like (DOT1L) KMT2A/MLL1 lysine methyltransferase complex EZH2-polycomb repressive complex 2 (PRC2) Histone arginine methyltransferases Protein arginine methyltransferases (PRMT1–9)	**Histone methyltransferase inhibitors**	
	Lysine methyltransferase inhibitors	*S*-Adenosylmethionine (SAMe) SAMe analogs Chaetocin AMI-1 BIX-01294 BIX-01338 UNC0224 Deazaneplanocin A LLY-507 ®-PFI-2 Chaetocin Tazemetostat GSK126 GSK343 CPI-360 EI1 4-(4-benzyloxy)phenoxypiperidines *trans*-2-Phenylcyclopropylamine (2-PCPA) 5-hydroxypyrazole derivatives

Continued

TABLE 6.4 Classification of Potential Epigenetic Drugs—cont'd

Potential targets	Categories	Drugs
	G9a histone methyltransferase (G9a) (KMT1C, EHMT2) inhibitors	BIX-01294 BRD4770 NC0642 UNC0321 UNC0638
	Protein lysine methyltransferase (PKMT) inhibitors SUV420H1 and SUV420H2 inhibitors	A-196
	Arginine methyltransferase inhibitors	AMI-1 EPZ015666 (GSK3235025)
	Protein arginine methyltransferases (PRMT1–9) inhibitors	
	PRMT1 Alkyl bis(oxy) dibenzimidamide derivatives PRMT3 histone methyltransferase inhibitors PRMT4 (CARM1) histone methyltranferase inhibitors	14u Hexamidine 17b MethylGene 5-methylcytosine-adenosine compounds Bromomethylcytosine derivatives EZM2302 (GSK3359088)
	DOT1 histone methyltransferase inhibitors	EPZ004777 EPZ-5676 SGC0946
	EZH2 histone methyltransferase inhibitors	CPI-360 Deazaneplanocin A EI1 EPZ005687 EPZ-6438 GSK126 GSK343 Tazemetostat UNC1999 1o (*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide) ZRANB1-related peptides
	Polycomb repressive complex 2 (PRC2) inhibitors WD40 domain-containing protein EED	EED226, EED162
	Hybrid HAT/EZH2 inhibitors	MC2884
	SET7/9 histone methyltransferase inhibitors	Cyproheptadine Dibenzosuberene 2-Hydroxycyproheptadine (*R*)-PFI-2 DC-S239 DC-S285
	Other HMT inhibitors	Verticillin A Difluorinated propanediones
Histone demethylases Histone lysine demethylases Lysine-specific demethylase 1 (LSD1)(KDM1A) KDM1–8 Fe(II)/2-oxoglutarate-dependent dioxygenases Plant Homeodomain (PHD)	**Histone demethylase inhibitors** Lysine-specific demethylase 1 (LSD1) inhibitors	Tranylcypromine Fluorinated tranylcypromine analogs Parnate (*S*)-4-(2-(5-(Dimethylamino)naphthalene-1-sulfonamido)-2-phenylacetamido)-*N*-hydroxybenzamide (D17)

TABLE 6.4 Classification of Potential Epigenetic Drugs—cont'd

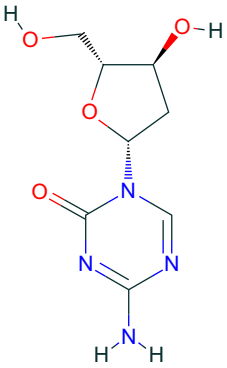
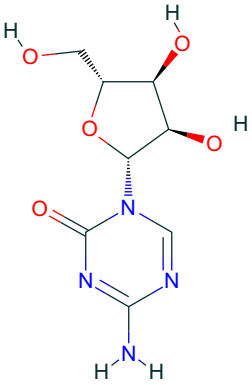
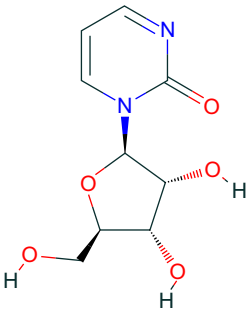
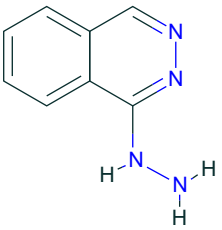
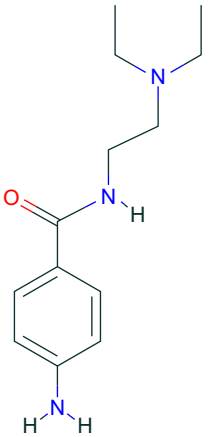
Potential targets	Categories	Drugs
Jumonji C domain-containing histone lysine demethylases (JMJCs) JMJD1B, H3K9me2 lysine demethylase JMJD-1.2-KDM7 family JMJD2A-ETV2 complex JMJD2D-ETV2 complex Prolyl hydroxylases E26 transformation-specific (ETS) variant 2 (ETV2) protein		Phenelzine Bizine SP2509 Namoline CBB1007 Pargyline Clorgyline Bizine GSK2879552 4-(Pyrrolidin-3-yl)benzonitrile derivatives GSK-690 S2157 Cyclic peptides H3K4M peptide derivatives
	Histone H3 peptide-based LSD1 inactivators	1-Aminocyclohexanecarboxylic acid-Lys-*trans*-2-phenylcyclopropylamine
	Fe(II)/2-oxoglutarate-dependent dioxygenase Jumonji C domain-containing histone lysine demethylase (JMJC) inhibitors	GSK-J4 KDM5-C70 JIB-04
	KDM1A inhibitors	ORY-1001
	KDM3 inhibitors	JIB-04 GSK-J4
	KDM4 inhibitors	Compound 6 (QC6352) Hydroxyquinoline derivatives Benzimidazole pyrazolone derivatives Pyrazolo[1,5-*a*]pyrimidine-3-carbonitrile derivatives PKF118–310
	KDM5A (KDM5A-PHD3) inhibitors	Amiodarone Amiodarone derivatives Pyrazole analog 35 JIB 04 CPI-455 KDOAM-25 3-Thio-1,2,4-triazole compounds YUKA1
	KDM6 histone lysine demethylase inhibitors JMJD3 (KDM6B) inhibitors	K18I variant of a histone H3-derived peptide R17 Myricetin Myricetin analogs
	PHF8 inhibitors	Cyclic peptides
ATP-dependent chromatin-remodeling complexes SWI/SNF (switching defective/sucrose nonfermenting) family ISWI (imitation SWI) family CHD (chromodomain, helicase, DNA binding) family INO (inositol requiring 80) family	**ATP-dependent chromatin remodelers** SWI/SNF remodelers	
	ISWI remodelers	
	CHD remodelers	
	INO remodelers	Chromatin remodeler INO80

Continued

TABLE 6.4 Classification of Potential Epigenetic Drugs—cont'd

Potential targets	Categories	Drugs
Chromatin-remodeling complex BAF		
JmjC domain-containing proteins		
Chromatin-associated proteins	TTD-PHD modulator (reader)	4-Benzylpiperidine-1-carboximidamide
Tandem Tudor domain-plant homeodomain (TTD-PHD)		
Polycomb repressive complex 1 (PRC1)	**BMI-1 inhibitor**	PTC-209
Bromodomains	**Bromodomain inhibitors** BET histone demethylase inhibitors	Apabetalone JQ1 IBET762 IBET151 PFI-1 CPI-203 CPI-0610 RVX-208 I-BET-151 I-BET-762 dBET1 OTX-015 ARV-771 ARV-825 MZ 1 UNC-669 UNC-1215 PFI-1 9F-913 MS436 Mivebresib BMS-986158 BAY 1238097
	BAZ2B histone demethylase inhibitors	GSK2801
	CECR2 bromodomain inhibitors	GNE-866
	TRIM24 bromodomain inhibitors	5H1T, 5H1U, and 5H1V
	Other bromodomain inhibitors	LP99 RVX-208 OXFBD02 (BDR4) OXFBD04 (BDR4) 4-Phenylisoquinolinone BET bromodomain inhibitors (BDR4) PLX51107 (BDR4) GNE-375 (BDR9)
Chromodomains	**Chromodomain inhibitors**	
Chromodomain Y-like protein (CDYL)-chromatin assembly factor 1 (CAF-1)-replicative helicase MCM complex	L3MBTL1 chromodomain inhibitors	UNC669
	L3MBTL3 chromodomain	UNC1215
Chromosome region maintenance 1 (CRM1, nuclear receptor exportin 1, XPO1)	CRM1 inhibitors	KPT-330 (Selinexor)
General control nonrepressed protein 5 (GCN5)	GCN5 inhibitors	1,8-Acridinedione derivatives (DC_G16-11)
Sumoylation	Sumoylation inhibitors	Ginkgolic acid

TABLE 6.5 Potential Epigenetic Drugs as Modulators of DNA Methylation

2D structure	Therapeutical agent
	**Name: Decitabine**; 5-aza-2′-deoxycytidine; dacogen; dezocitidine; 2′-deoxy-5-azacytidine **Molecular formula:** $C_8H_{12}N_4O_4$ **Molecular weight:** 228.21 g mol^{-1} **IUPAC name:** 4-Amino-1-[(2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3,5-triazin-2-one **Category:** Nucleoside analogs **Mechanism:** DNA methylation inhibitors **Targets:** DNMT1
	**Name: 5-Azacytidine**; azacitidine; azacytidine; ladakamycin; vidaza; mylosar; azacitidinum; 5-AZAC **Molecular formula:** $C_8H_{12}N_4O_5$ **Molecular weight:** 244.20 g mol^{-1} **IUPAC name:** 4-Amino-1-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3,5-triazin-2-one **Category:** Nucleoside analogs **Mechanism:** DNA methylation inhibitors **Targets:** DNMT1
	**Name: Zebularine**; 3690-10-6; pyrimidin-2-one ribonucleoside; pyrimidin-2-one beta-ribofuranoside; pyrimidin-2-one beta-D-ribofuranoside; NSC 309132 **Molecular formula:** $C_9H_{12}N_2O_5$ **Molecular weight:** 228.20 g mol^{-1} **IUPAC name:** 1-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]pyrimidin-2-one **Category:** Nucleoside analogs **Mechanism:** DNA methylation inhibitors **Targets:** DNMT1
	**Name: Hydralazine**; 1-hydrazinophthlazine; hydrazinophthalazine; apresolin; apresoline; hydralazin; hypophthalin; apressin; aprezolin **Molecular formula:** $C_8H_8N_4$ **Molecular weight:** 160.18 g mol^{-1} **IUPAC name:** Phthalazin-1-ylhydrazine **Category:** Small molecules **Mechanism:** DNA methylation inhibitors **Targets:** DNMT1
	**Name: Procainamide**; novocainamide; biocoryl; novocamid; procamide; procaine amide; pronestyl; novocainamid; novocaine amide; procan **Molecular formula:** $C_{13}H_{21}N_3O$ **Molecular weight:** 235.33 g mol^{-1} **IUPAC name:** 4-Amino-*N*-[2-(diethylamino)ethyl]benzamide **Category:** Small molecules **Mechanism:** DNA methylation inhibitors **Targets:** DNMT1

Continued

TABLE 6.5 Potential Epigenetic Drugs as Modulators of DNA Methylation—cont'd

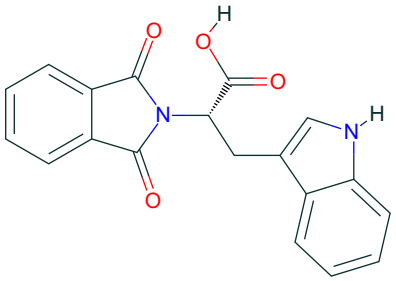
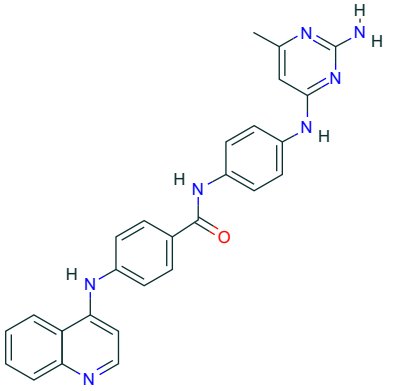
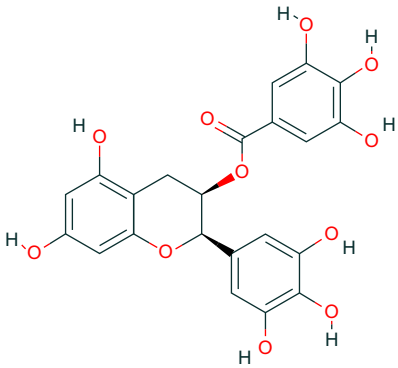
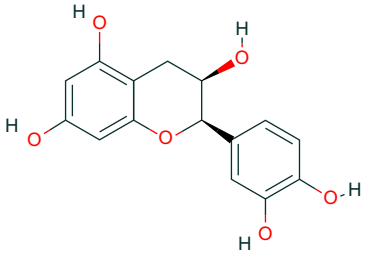
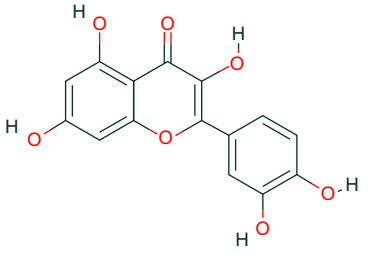
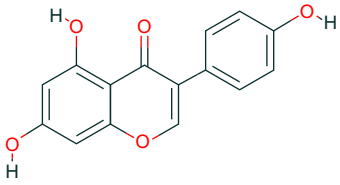
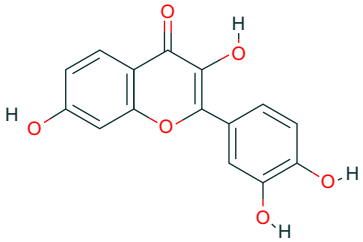
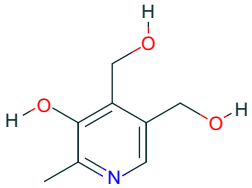
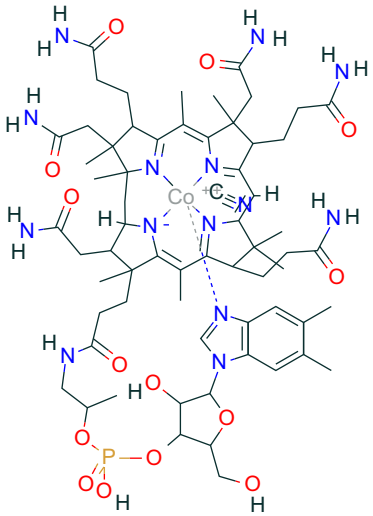
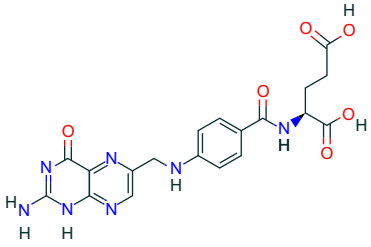
2D structure	Therapeutical agent
	**Name: RG108**; RG-108; *N*-phthalyl-L-tryptophan; (2*S*)-2-(1,3-dioxoisoindol-2-yl)-3-(1*H*-indol-3-yl) propanoic acid **Molecular formula:** $C_{19}H_{14}N_2O_4$ **Molecular weight:** 334.33 g mol^{-1} **IUPAC name:** (2*S*)-2-(1,3-dioxoisoindol-2-yl)-3-(1*H*-indol-3-yl)propanoic acid **Category:** Small molecules **Mechanism:** DNA methylation inhibitors **Targets:** DNMT1
	**Name: SGI-1027**; *N*-(4-(2-amino-6-methylpyrimidin-4-ylamino)phenyl)-4-(quinolin-4-ylamino) benzamide **Molecular formula:** $C_{27}H_{23}N_7O$ **Molecular weight:** 461.53 g mol^{-1} **IUPAC name:** *N*-[4-[(2-Amino-6-methylpyrimidin-4-yl)amino]phenyl]-4-(quinolin-4-ylamino) benzamide **Category:** Natural products **Mechanism:** DNA methylation inhibitors **Targets:** DNMT1
	**Name: (−)-Epigallocatechin gallate**; EGCG; epigallocatechin gallate; epigallocatechin 3-gallate; tea catechin; catechin derivative; teavigo **Molecular formula:** $C_{22}H_{18}O_{11}$ **Molecular weight:** 458.37 g mol^{-1} **IUPAC name:** [(2*R*,3*R*)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-chromen-3-yl] 3,4,5-trihydroxybenzoate **Category:** Natural products **Mechanism:** DNA methylation inhibitors **Targets:** DNMT1
	**Name: (−)-Epicatechin**; epicatechin; 490-46-0; L-epicatechin; (−)-epicatechol; epicatechol; (2*R*,3*R*)-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol **Molecular formula:** $C_{15}H_{14}O_6$ **Molecular weight:** 290.27 g mol^{-1} **IUPAC name:** (2*R*,3*R*)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol **Category:** Natural products **Mechanism:** DNA methylation inhibitors **Targets:** DNMT1
	**Name: Quercetin**; sophoretin; quercetol; meletin; xanthaurine; quercitin; 3,3′,4′,5,7-pentahydroxyflavone **Molecular formula:** $C_{15}H_{10}O_7$ **Molecular weight:** 302.24 g mol^{-1} **IUPAC name:** 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one **Category:** Natural products **Mechanism:** DNA methylation inhibitors **Targets:** DNMT1
	**Name: Genistein**; 446-72-0; prunetol; genisteol; 4′,5,7-trihydroxyisoflavone; 5,7,4′-trihydroxyisoflavone **Molecular formula:** $C_{15}H_{10}O_5$ **Molecular weight:** 270.24 g mol^{-1} **IUPAC name:** 5,7-Dihydroxy-3-(4-hydroxyphenyl)chromen-4-one **Category:** Natural products **Mechanism:** DNA methylation inhibitors **Targets:** DNMT1

TABLE 6.5 Potential Epigenetic Drugs as Modulators of DNA Methylation—cont'd

2D structure	Therapeutical agent
	**Name: Fisetin**; 528-48-3, 2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-4*H*-chromen-4-one; 5-desoxyquercetin; fisetholz; superfustel **Molecular formula:** $C_{15}H_{10}O_6$ **Molecular weight:** 486.24 g mol^{-1} **IUPAC name:** 2-(3,4-Dihydroxyphenyl)-3,7-dihydroxychromen-4-one **Category:** Natural products **Mechanism:** DNA methylation inhibitors **Targets:** DNMT1
	**Name: Vitamin B6**; pyridoxine; pyridoxol; vitamin B6; pyridoxin; gravidox **Molecular formula:** $C_8H_{11}NO_3$ **Molecular weight:** 169.18 g mol^{-1} **IUPAC name:** 4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol **Category:** Vitamins **Mechanism:** DNA methylation activators **Targets:** SAMe methyl donor
	**Name: Vitamin B12**; cyanocobalamin; cobalamin; crystamine; anacobin; berubigen **Molecular formula:** $C_{63}H_{88}CoN_{14}O_{14}P$ **Molecular weight:** 1355.39 g mol^{-1} **IUPAC name:** Cobalt(2+); [5-(5,6-dimethylbenzimidazol-1-yl)-4-hydroxy-2-(hydroxymethyl)oxolan-3-yl] 1-[3-[(4*Z*,9*Z*,14*Z*)-2,13,18-tris(2-amino-2-oxoethyl)-7,12,17-tris(3-amino-3-oxopropyl)-3,5,8,8,13,15,18,19-octamethyl-2,7,12,17-tetrahydro-1*H*-corrin-21-id-3-yl]propanoylamino] propan-2-yl hydrogen phosphate; cyanide **Category:** Vitamins **Mechanism:** DNA methylation activators **Targets:** SAMe methyl donor
	**Name: Vitamin B9**; folic acid; folate; 59-30-3; folacin; pteroylglutamic acid **Molecular formula:** $C_{19}H_{19}N_7O_6$ **Molecular weight:** 441.40 g mol^{-1} **IUPAC name:** (2*S*)-2-[[4-[(2-Amino-4-oxo-1*H*-pteridin-6-yl)methylamino]benzoyl]amino] pentanedioic acid **Category:** Vitamins **Mechanism:** DNA methylation activators **Targets:** SAMe methyl donor

DNMT, DNA methyltransferase; *SAMe*, *S*-adenosylmethionine.

(Tables 6.4 and 6.5); (ii) DNA demethylase modulators: no specific drugs have been developed targeting ten-eleven translocation (TET) family, AID/APOBEC family, and BER (base excision repair) glycosylase family proteins; (iii) histone deacetylase (HDAC) inhibitors (Table 6.6): these drugs target HDAC1–18, specifically class I HDACs (HDAC1, 2, 3, and 8), HDAC1/HDAC2 transcriptional corepressor complexes (SIN3A, NuRD, CoREST), HDAC3-(SMRT/N-CoR) complexes, class II HDACs: IIa (HDAC4, 5, 7, and 9), class IIb (HDAC6 and 10), class III HDACs (sirtuin family: nuclear (SIRT1, 2, 6, 7), mitochondrial (SIRT3–5), cytoplasmic (SIRT1, 2)) (Table 6.7), class IV HDAC (HDAC11), and histone deacetylase RPD3; HDAC inhibitors are classified into short chain fatty acids, hydroxamic acids, cyclic peptides,

TABLE 6.6 Potential Histone Deacetylase (HDAC) Inhibitors

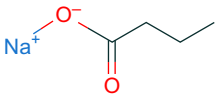
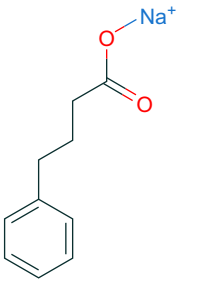
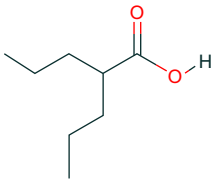
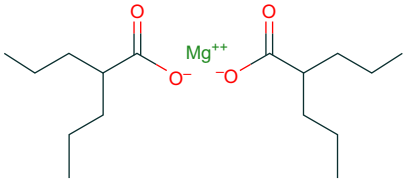
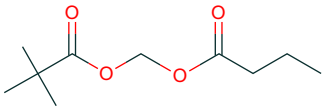
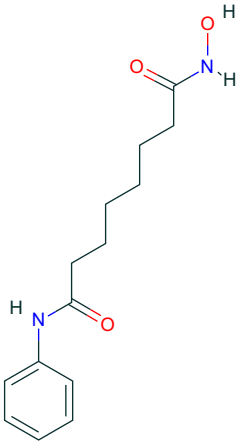
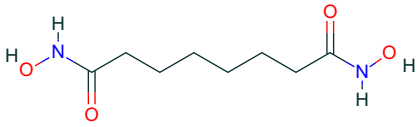
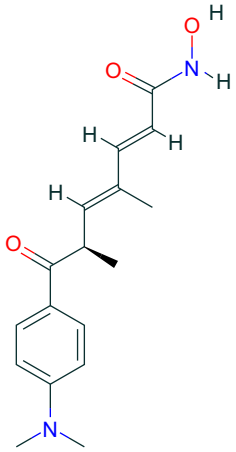
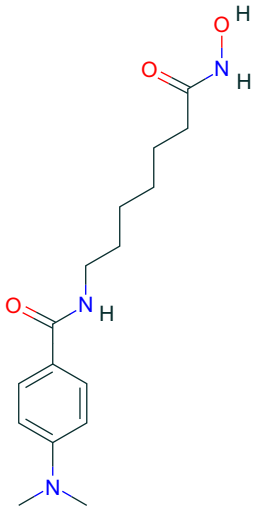
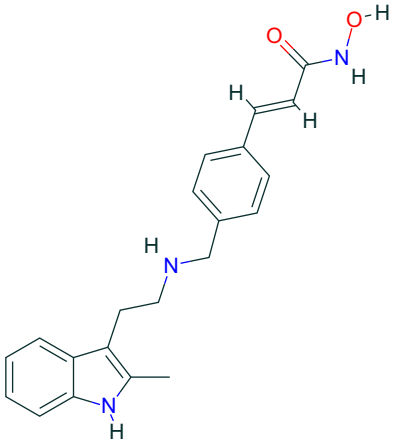
2D structure	Therapeutical agent
	**Name: Sodium butyrate**; sodium butanoate;156-54-7; butanoic acid sodium salt; butyric acid sodium salt; butyrate sodium **Molecular formula:** $C_4H_7NaO_2$ **Molecular weight:** 110.09 g mol^{-1} **IUPAC name:** Sodium butanoate **Category:** Short chain fatty acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs
	**Name: Sodium phenylbutyrate**; buphenyl; 4PBA; 4-phenylbutyric acid; 4-phenylbutonic acid; benzenebutyric acid; benzenebutanoic acid; sodium salt **Molecular formula:** $C_{10}H_{11}NaO_2$ **Molecular weight:** 164.20 g mol^{-1} **IUPAC name:** Sodium 4-phenylbutanoate **Category:** Short chain fatty acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: Valproic acid**; 2-propylpentanoic acid; 99-66-1; depakine; dipropylacetic acid; depakene; ergenyl; mylproin; mylproic acid; convulex **Molecular formula:** $C_8H_{16}O_2$ **Molecular weight:** 144.21 g mol^{-1} **IUPAC name:** 2-Propylpentanoic acid **Category:** Short chain fatty acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: Magnesium valproate**; valproate magnesium; 62959-43-7; magnesium dipropylacetate; magnesium 2-propylvalerate; magnesium 2-propylpentanoate **Molecular formula:** $C_{16}H_{30}MgO_4$ **Molecular weight:** 310.71 g mol^{-1} **IUPAC name:** Magnesium 2-propylpentanoate **Category:** Short chain fatty acids **Mechanism:** HDAC inhibitors **Targets:** HDAC2
	**Name: Pivanex**; pivaloyloxymethyl butyrate; AN-9; 122,110-53-6; pivaloyloxymethyl butyrate; ((2,2-dimethylpropanoyl)oxy)methyl butanoate **Molecular formula:** $C_{10}H_{18}O_4$ **Molecular weight:** 202.25 g mol^{-1} **IUPAC name:** Butanoyloxymethyl 2,2-dimethylpropanoate **Category:** Short chain fatty acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs
	**Name: Vorinostat**; suberoylanilide hydroxamic acid, SAHA; zolinza; suberanilohydroxamic acid; *N*-hydroxy-*N*′-phenyloctanediamide; 149647-78-9; SAHA cpd **Molecular formula:** $C_{14}H_{20}N_2O_3$ **Molecular weight:** 264.33 g mol^{-1} **IUPAC name:** *N*′-Hydroxy-*N*-phenyloctanediamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs

TABLE 6.6 Potential Histone Deacetylase (HDAC) Inhibitors—cont'd

2D structure	Therapeutical agent
	**Name: Suberohydroxamic acid**; SBHA; 38,937-66-5; *N*1,*N*8-dihydroxyoctanediamide; *N*,*N*′-dihydroxyoctanediamide; Suberic bishydroxamate **Molecular formula:** $C_8H_{16}N_2O_4$ **Molecular weight:** 204.23 g mol^{-1} **IUPAC name:** *N*,*N*′-Dihydroxyoctanediamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** HDAC1; HDAC3
	**Name: Trichostatin A**; TSA; 58,880-19-6; CHEBI:46024; (2*E*,4*E*,6*R*)-7-[4-(dimethylamino) phenyl]-*N*-hydroxy-4,6-dimethyl-7-oxohepta-2,4-dienamide **Molecular formula:** $C_{17}H_{22}N_2O_3$ **Molecular weight:** 302.37 g mol^{-1} **IUPAC name:** (2*E*,4*E*,6*R*)-7-[4-(dimethylamino)phenyl]-*N*-hydroxy-4,6-dimethyl-7-oxohepta-2,4-dienamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: M344**; 251456-60-7; M 344; 4-(dimethylamino)-*N*-[7-(hydroxyamino)-7-oxoheptyl] benzamide; histone deacetylase inhibitor III; *N*-hydroxy-7-(4-dimethylaminobenzoyl) aminoheptanamide **Molecular formula:** $C_{16}H_{25}N_3O_3$ **Molecular weight:** 307.39 g mol^{-1} **IUPAC name:** 4-(Dimethylamino)-*N*-[7-(hydroxyamino)-7-oxoheptyl]benzamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** HDAC6
	**Name: Panobinostat**; 404,950-80-7; LBH-589; faridak; farydak; NVP-LBH589; S1030_Selleck; AC1OCFY8; (*E*)-*N*-hydroxy-3-[4-[[2-(2-methyl-1*H*-indol-3-yl)ethylamino]methyl]phenyl] prop-2-enamide **Molecular formula:** $C_{21}H_{23}N_3O_2$ **Molecular weight:** 349.43 g mol^{-1} **IUPAC name:** (*E*)-*N*-Hydroxy-3-[4-[[2-(2-methyl-1*H*-indol-3-yl)ethylamino]methyl]phenyl] prop-2-enamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs

Continued

TABLE 6.6 Potential Histone Deacetylase (HDAC) Inhibitors—cont'd

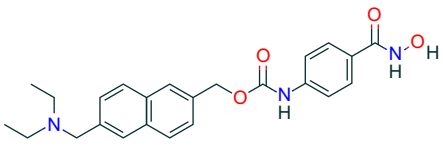
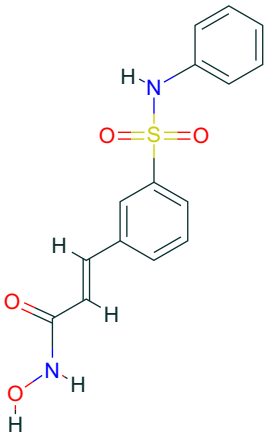
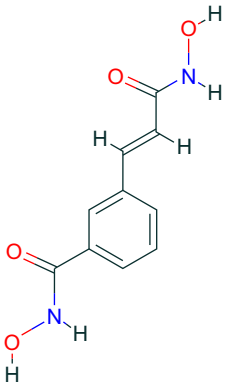
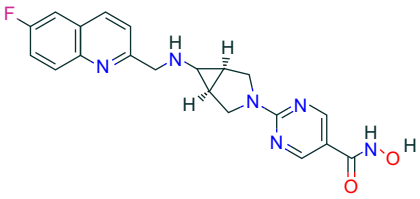
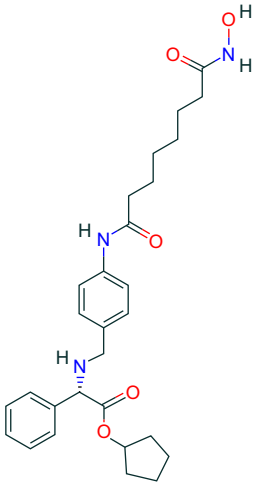
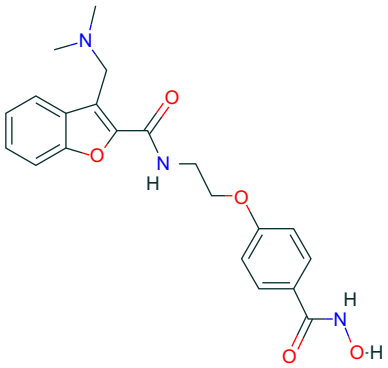
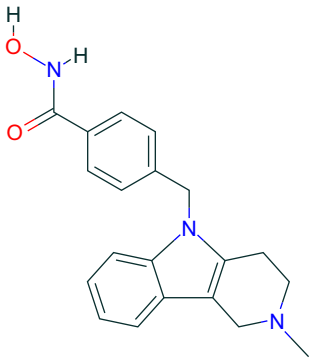
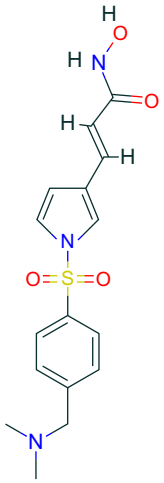
2D structure	Therapeutical agent
	**Name: Givinostat**; ITF2357; ITF-2357; UNII-5P60F84FBH; 497,833-27-9; CHEMBL1213492; [6-(Diethylaminomethyl)-2-naphthyl]methyl-*N*-[4-(hydroxycarbamoyl)phenyl]carbamate **Molecular formula:** $C_{24}H_{27}N_3O_4$ **Molecular weight:** 421.50 g mol^{-1} **IUPAC name:** [6-(Diethylaminomethyl)naphthalen-2-yl]methyl *N*-[4-(hydroxycarbamoyl) phenyl]carbamate **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: Belinostat**; 414,864-00-9; PXD101; PXD-101; belinostat (PXD101); UNII-F4H96P17NZ; (*E*)-*N*-Hydroxy-3-(3-(*N*-phenylsulfamoyl)phenyl)acrylamide **Molecular formula:** $C_{15}H_{14}N_2O_4S$ **Molecular weight:** 318.35 g mol^{-1} **IUPAC name:** (*E*)-*N*-Hydroxy-3-[3-(phenylsulfamoyl)phenyl]prop-2-enamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: *m*-Carboxycinnamic acid bis-hydroxamide**; Cbha; histone deacetylase inhibitor II; 174,664-65-4; *N*-hydroxy-3-[(*E*)-3-(hydroxyamino)-3-oxoprop-1-enyl]benzamide; *m*-carboxycinnamic acid bis-hydroxamide **Molecular formula:** $C_{10}H_{10}N_2O_4$ **Molecular weight:** 222.2 g mol^{-1} **IUPAC name:** *N*-Hydroxy-3-[(*E*)-3-(hydroxyamino)-3-oxoprop-1-enyl]benzamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: CHR-3996**; BDBM50347385; GTPL8391; 2-[(1*R*,5*S*,6*R*)-6-{[(6-fluoroquinolin-2-yl) methyl]amino}-3-azabicyclo[3.1.0]hexan-3-yl]-*N*-hydroxypyrimidine-5-carboxamide **Molecular formula:** $C_{20}H_{19}FN_6O_2$ **Molecular weight:** 394.41 g mol^{-1} **IUPAC name:** 2-[(1*R*,5*S*)-6-[(6-Fluoroquinolin-2-yl)methylamino]-3-azabicyclo[3.1.0]hexan-3-yl]-*N*-hydroxypyrimidine-5-carboxamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	Name: Tefinostat; CHR-2845; UNII-ZAU91150SB; 914382-60-8; Tefinostat (INN); SCHEMBL1491466; ZAU91150SB; cyclopentyl (2*S*)-2-(((4-(8-(hydroxyamino)-8-oxooctanamido)phenyl)methyl)amino)-2-phenylacetate **Molecular formula:** $C_{28}H_{37}N_3O_5$ **Molecular weight:** 495.62 g mol^{-1} **IUPAC name:** Cyclopentyl (2*S*)-2-[[4-[[8-(hydroxyamino)-8-oxooctanoyl]amino]phenyl] methylamino]-2-phenylacetate **Category:** Hydroxamic acids

TABLE 6.6 Potential Histone Deacetylase (HDAC) Inhibitors—cont'd

2D structure	Therapeutical agent
	**Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: Abexinostat**; PCI-24781; 783355-60-2; CRA-024781; CRA 024781; 3-((dimethylamino) methyl)-*N*-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl)benzofuran-2-carboxamide **Molecular formula:** $C_{21}H_{23}N_3O_5$ **Molecular weight:** 397.43 g mol^{-1} **IUPAC name:** 3-[(Dimethylamino)methyl]-*N*-[2-[4-(hydroxycarbamoyl)phenoxy]ethyl]-1-benzofuran-2-carboxamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: Tubastatin A**; tubastatin-A; 1252003-15-8; *N*-hydroxy-4-((2-methyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5(2*H*)-yl)methyl)benzamide; CHEMBL2018302; tubastatin A BASE **Molecular formula:** $C_{20}H_{21}N_3O_2$ **Molecular weight:** 335.41 g mol^{-1} **IUPAC name:** *N*-Hydroxy-4-[(2-methyl-3,4-dihydro-1*H*-pyrido[4,3-*b*]indol-5-yl)methyl] benzamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** HDAC6
	**Name: Resminostat**; 864814-88-0; 4SC-201; resminostat (RAS2410); UNII-1578EUB98L; RAS2410; (*E*)-3-(1-((4-((dimethylamino)methyl)phenyl)sulfonyl)-1*H*-pyrrol-3-yl)-*N*-hydroxyacrylamide **Molecular formula:** $C_{16}H_{19}N_3O_4S$ **Molecular weight:** 349.40 g mol^{-1} **IUPAC name:** (*E*)-3-[1-[4-[(Dimethylamino)methyl]phenyl]sulfonylpyrrol-3-yl]-*N*-hydroxyprop-2-enamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs

Continued

TABLE 6.6 Potential Histone Deacetylase (HDAC) Inhibitors—cont'd

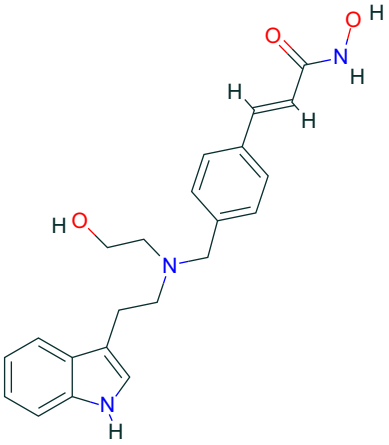
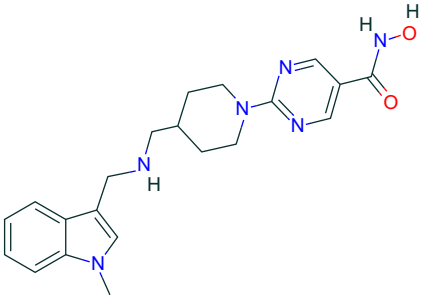
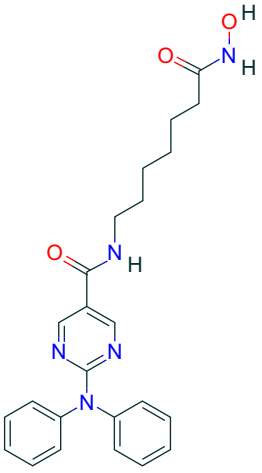
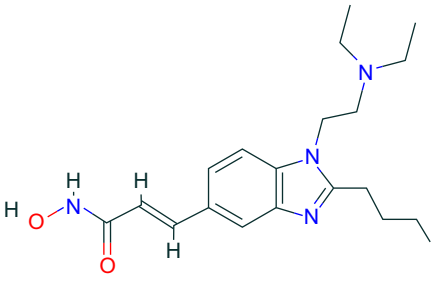
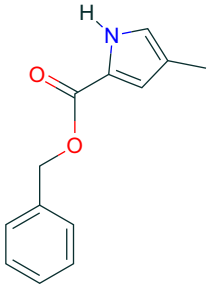
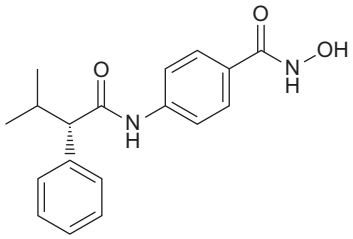
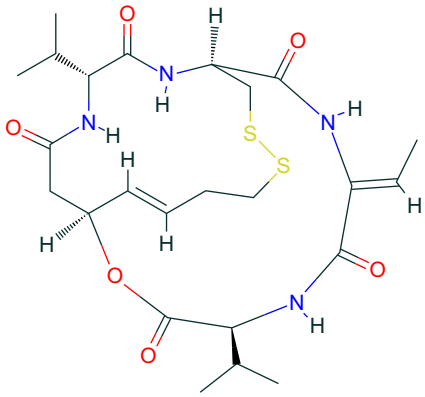
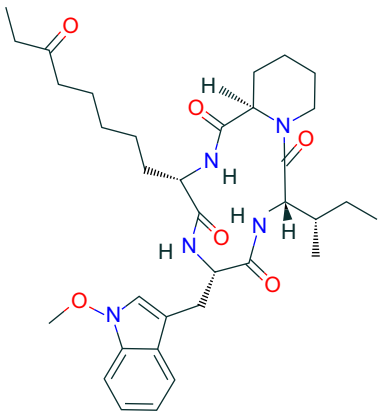
2D structure	Therapeutical agent
	**Name: Dacinostat**; 404951-53-7; NVP-LAQ824; LAQ824; LAQ-824; NVP-LAQ 824; (*E*)-*N*-hydroxy-3-[4-[[2-hydroxyethyl-[2-(1*H*-indol-3-yl)ethyl]amino]methyl]phenyl]prop-2-enamide **Molecular formula:** $C_{22}H_{25}N_3O_3$ **Molecular weight:** 379.45 g mol^{-1} **IUPAC name:** (*E*)-*N*-Hydroxy-3-[4-[[2-hydroxyethyl-[2-(1*H*-indol-3-yl)ethyl]amino]methyl]phenyl]prop-2-enamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: Quisinostat**; 875,320-29-9; JNJ-26481585; *N*-hydroxy-2-(4-((((1-methyl-1*H*-indol-3-yl)methyl)amino)methyl)piperidin-1-yl)pyrimidine-5-carboxamide; UNII-9BJ85K1J8S; JNJ26481585 **Molecular formula:** $C_{21}H_{26}N_6O_2$ **Molecular weight:** 394.48 g mol^{-1} **IUPAC name:** *N*-Hydroxy-2-[4-[[(1-methylindol-3-yl)methylamino]methyl]piperidin-1-yl]pyrimidine-5-carboxamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs; Class IV HDACs
	**Name: Ricolinostat**; ACY-1215; 1316214-52-4; rocilinostat; 2-(diphenylamino)-*N*-(7-(hydroxyamino)-7-oxoheptyl)pyrimidine-5-carboxamide; UNII-WKT909C62B **Molecular formula:** $C_{24}H_{27}N_5O_3$ **Molecular weight:** 433.51 g mol^{-1} **IUPAC name:** *N*-[7-(Hydroxyamino)-7-oxoheptyl]-2-(*N*-phenylanilino)pyrimidine-5-carboxamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** HDAC6
	**Name: Pracinostat**; 929016-96-6; SB 939; SB939; SB-939; pracinostat (SB939); UNII-GPO2JN4UON; (*E*)-3-(2-butyl-1-(2-(diethylamino)ethyl)-1*H*-benzo[*d*]imidazol-5-yl)-*N*-hydroxyacrylamide **Molecular formula:** $C_{20}H_{30}N_4O_2$ **Molecular weight:** 358.49 g mol^{-1} **IUPAC name:** (*E*)-3-[2-Butyl-1-[2-(diethylamino)ethyl]benzimidazol-5-yl]-*N*-hydroxyprop-2-enamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs; Class IV HDACs **Name: GC-1521**; 7-phenyl-2,4,6-hepta-trienoyl hydroxamic acid **Molecular formula:** $C_{13}H_{13}NO_2$ **Molecular weight:** 215.25 g mol^{-1} **IUPAC name:** Benzyl 4-methyl-1*H*-pyrrole-2-carboxylate **Category:** Hydroxamic acids

TABLE 6.6 Potential Histone Deacetylase (HDAC) Inhibitors—cont'd

2D structure	Therapeutical agent
	**Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: OSU-HDAC-42**; 935881-37-1; AR-42; AR 42; OSU-HDAC42; (*S*)-*N*-hydroxy-4-(3-methyl-2-phenylbutanamido)benzamide; (*S*)-HDAC-42 **Molecular formula:** $C_{18}H_{20}N_2O_3$ **Molecular weight:** 312.37 g mol^{-1} **IUPAC name:** *N*-Hydroxy-4-[[(2*S*)-3-methyl-2-phenylbutanoyl]amino]benzamide **Category:** Hydroxamic acids **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: Romidepsin**; depsipeptide; chromadax; FK228; antibiotic FR 901228; istodax; FK-228; NSC-630176; romidepsinum **Molecular formula:** $C_{24}H_{36}N_4O_6S_2$ **Molecular weight:** 540.69 g mol^{-1} **IUPAC name:** (1*S*,4*S*,7*Z*,10*S*,16*E*,21*R*)-7-Ethylidene-4,21-di(propan-2-yl)-2-oxa-12,13-dithia-5,8,20,23-tetrazabicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-pentone **Category:** Cyclic peptides **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs; Class IV HDACs
	**Name: Apicidin**; CHEMBL430060; OSI-2040; (3*S*,6*S*,9*S*,12*R*)-3-[(2*S*)-butan-2-yl]-6-[(1-methoxyindol-3-yl)methyl]-9-(6-oxooctyl)-1,4,7,10-tetrazabicyclo[10.4.0]hexadecane-2,5,8,11-tetrone; acipidin; AC1OCFAM **Molecular formula:** $C_{34}H_{49}N_5O_6$ **Molecular weight:** 623.80 g mol^{-1} **IUPAC name:** Cyclo[(2*S*)-2-amino-8-oxodecanoyl)-N1-methoxy-L-tryptophyl-L-isoleucyl-D-homoprolyl] **Category:** Cyclic peptides **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs
	Name: Trapoxin A; 133155-89-2; CTK8F0363; RT-016108 **Molecular formula:** $C_{34}H_{42}N_4O_6$ **Molecular weight:** 602.73 g mol^{-1} **IUPAC name:** (3*S*,6*S*,9*S*,12*R*)-3,6-Dibenzyl-9-[6-[(2*S*)-oxiran-2-yl]-6-oxohexyl]-1,4,7,10-tetrazabicyclo[10.4.0]hexadecane-2,5,8,11-tetrone **Category:** Cyclic peptides

Continued

TABLE 6.6 Potential Histone Deacetylase (HDAC) Inhibitors—cont'd

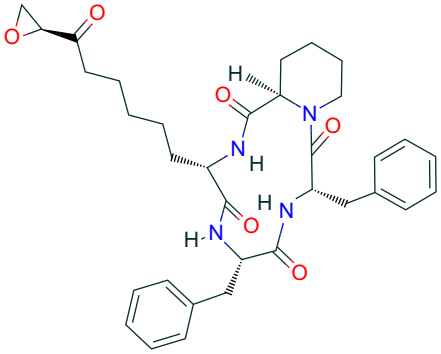
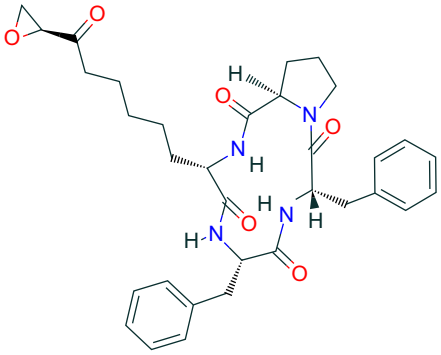
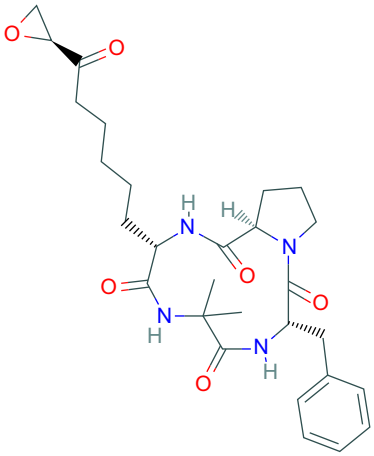
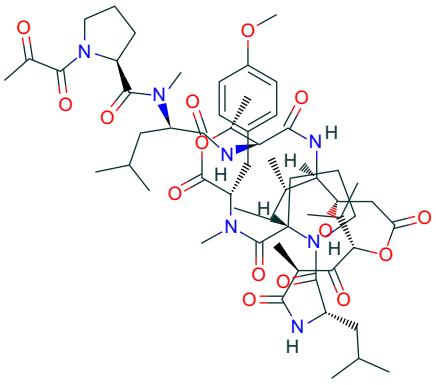
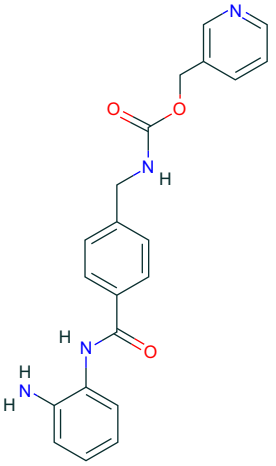
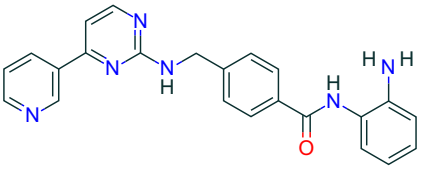
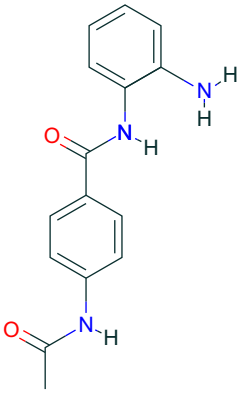
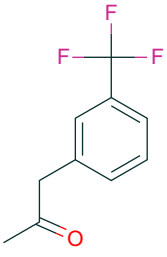
2D structure	Therapeutical agent
	**Mechanism:** HDAC inhibitors **Targets:** Class I HDACs
	**Name: Trapoxin B**; 133155-90-5; AC1L9B5C; CHEMBL2000089; CTK4B8393; cyclo[(a*S*,2*S*)-a-amino-h-oxo-2-oxiraneoctanoyl-L-phenylalanyl-L-phenylalanyl-D-prolyl] **Molecular formula:** $C_{33}H_{40}N_4O_6$ **Molecular weight:** 588.71 g mol^{-1} **IUPAC name:** (3*S*,6*S*,9*S*,12*R*)-3,6-dibenzyl-9-[6-[(2*S*)-oxiran-2-yl]-6-oxohexyl]-1,4,7,10-tetrazabicyclo[10.3.0]pentadecane-2,5,8,11-tetrone **Category:** Cyclic peptides **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs
	**Name: Chlamydocin**; (3s,9s,14ar)-9-benzyl-6,6-dimethyl-3-{6-[(2s)-oxiran-2-yl]-6-oxohexyl} decahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetrone; 53342-16-8; AmbotzLS-1109; HDInhib_000038 **Molecular formula:** $C_{28}H_{38}N_4O_6$ **Molecular weight:** 526.63 g mol^{-1} **IUPAC name:** (3*S*,9*S*,12*R*)-3-Benzyl-6,6-dimethyl-9-[6-[(2*S*)-oxiran-2-yl]-6-oxohexyl]-1,4,7,10-tetrazabicyclo[10.3.0]pentadecane-2,5,8,11-tetrone **Category:** Cyclic peptides **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs
	**Name: Plitidepsin**; aplidine; aplidin; 137219-37-5; dehydrodidemnin B; plitidepsina; plitidepsium; UNII-Y76ID234HW **Molecular formula:** $C_{57}H_{87}N_7O_{15}$ **Molecular weight:** 1110.34 g mol^{-1} **IUPAC name:** (2*S*)-*N*-[(2*R*)-1-[[(3*S*,6*S*,8*S*,12*S*,13*R*,16*S*,17*R*,20*S*,23*S*)-13-[(2*S*)-Butan-2-yl]-12-hydroxy-20-[(4-methoxyphenyl)methyl]-6,17,21-trimethyl-3-(2-methylpropyl)-2,5,7,10,15,19,22-heptaoxo-8-propan-2-yl-9,18-dioxa-1,4,14,21-tetrazabicyclo[21.3.0]hexacosan-16-yl]amino]-4-methyl-1-oxopentan-2-yl]-*N*-methyl-1-(2-oxopropanoyl) pyrrolidine-2-carboxamide **Category:** Cyclic peptides **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs

TABLE 6.6 Potential Histone Deacetylase (HDAC) Inhibitors—cont'd

2D structure	Therapeutical agent
	**Name: Entinostat**; MS-275; 209783-80-2; SNDX-275; MS 275; MS-27-275; histone deacetylase inhibitor I: S1053_selleck; pyridin-3-ylmethyl 4-((2-aminophenyl)carbamoyl) benzylcarbamate **Molecular formula:** $C_{21}H_{20}N_4O_3$ **Molecular weight:** 376.42 g mol^{-1} **IUPAC name:** Pyridin-3-ylmethyl *N*-[[4-[(2-aminophenyl)carbamoyl]phenyl]methyl] carbamate **Category:** Benzamides **Mechanism:** HDAC inhibitors **Targets:** HDAC1; HDAC3
	**Name: Mocetinostat**; 726169-73-9; MGCD0103; MGCD-0103; MGCD 0103; *N*-(2-aminophenyl)-4-([[4-(pyridin-3-yl)pyrimidin-2-yl]amino]methyl)benzamide; CHEMBL272980 **Molecular formula:** $C_{23}H_{20}N_6O$ **Molecular weight:** 396.45 g mol^{-1} **IUPAC name:** *N*-(2-Aminophenyl)-4-[[(4-pyridin-3-ylpyrimidin-2-yl)amino]methyl] benzamide **Category:** Benzamides **Mechanism:** HDAC inhibitors **Targets:** Class IV HDACs
	**Name: Tacedinaline**; CI-994; 112522-64-2; acetyldinaline; 4-acetamido-*N*-(2-aminophenyl) benzamide; CI 994; tacedinalina; *N*-acetyldinaline; 4-acetylamino-*N*-(2′-aminophenyl) benzamide **Molecular formula:** $C_{15}H_{15}N_3O_2$ **Molecular weight:** 269.30 g mol^{-1} **IUPAC name:** 4-Acetamido-*N*-(2-aminophenyl)benzamide **Category:** Benzamides **Mechanism:** HDAC inhibitors **Targets:** HDAC1; HDAC3; HDAC6; HDAC8
	**Name: Trifluoromethyl ketone**; ethyl 3-(trifluoromethyl)phenyl ketone-3′-(trifluoromethyl) propiophenone; 3-(trifluoromethyl)propiophenone **Molecular formula:** $C_{10}H_9F_3O$ **Molecular weight:** 202.18 g mol^{-1} **IUPAC name:** 1-[3-(Trifluoromethyl)phenyl]propan-2-one **Category:** Ketones **Mechanism:** HDAC inhibitors **Targets:** Class IIa HDACs; Class IIb HDACs

Continued

TABLE 6.6 Potential Histone Deacetylase (HDAC) Inhibitors—cont'd

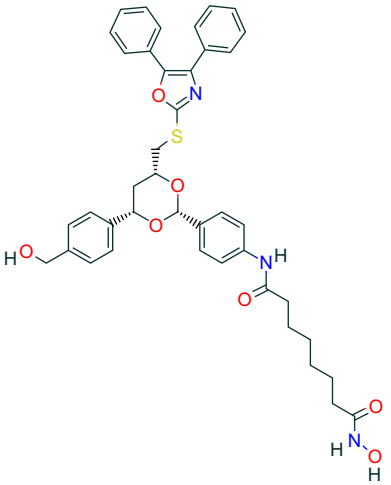
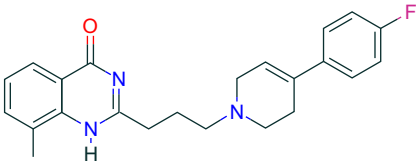
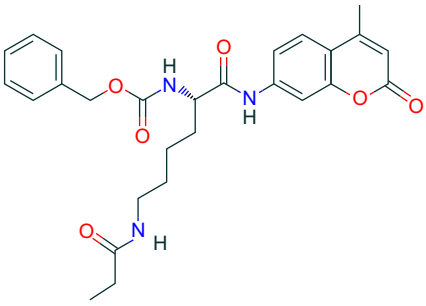
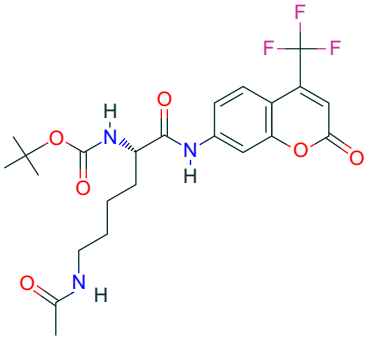
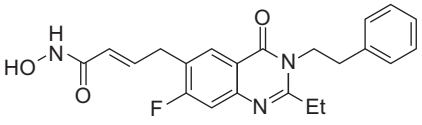
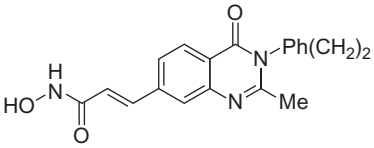
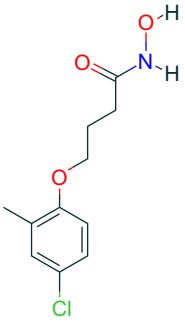
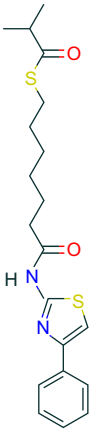
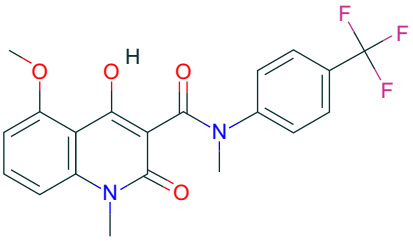
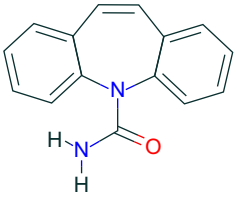
2D structure	Therapeutical agent
	**Name: Tubacin**; AC1O7Y2P; CHEMBL356769; 537049-40-4; *N*1-(4-((2*R*,4*R*,6*S*)-4-(((4,5-diphenyloxazol-2-yl)thio)methyl)-6-(4-(hydroxymethyl)phenyl)-1,3-dioxan-2-yl)phenyl)-*N*8-hydroxyoctanediamide; GTPL7374 **Molecular formula:** $C_{41}H_{43}N_3O_7S$ **Molecular weight:** 721.87 g mol^{-1} **IUPAC name:** *N*-[4-[(2*R*,4*R*,6*S*)-4-[(4,5-Diphenyl-1,3-oxazol-2-yl)sulfanylmethyl]-6-[4-(hydroxymethyl)phenyl]-1,3-dioxan-2-yl]phenyl]-*N*′-hydroxyoctanediamide **Category:** Miscellaneous **Mechanism:** HDAC inhibitors **Targets:** HDAC6
	**Name: EVP-0334**; FRM-0334; 2-{3-[4-(4-fluorophenyl)-3,6-dihydro-1(2*H*)-pyridinyl]propyl}-8-methyl-4(3*H*)-quinazolinone; AC1L9MGN; quinazolinone analog, 1e **Molecular formula:** $C_{23}H_{24}FN_3O$ **Molecular weight:** 377.46 g mol^{-1} **IUPAC name:** 2-[3-[4-(4-Fluorophenyl)-3,6-dihydro-2*H*-pyridin-1-yl]propyl]-8-methyl-1*H*-quinazolin-4-one **Category:** Miscellaneous **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: MOCPAC**; 787549-26-2; benzyl (*S*)-[1-(4-methyl-2-oxo-2*H*-chromen-7-ylcarbamoyl)-5-propionylaminopentyl]carbamate; AC1Q2RTN; CHEMBL184857; DTXSID10462150 **Molecular formula:** $C_{27}H_{31}N_3O_6$ **Molecular weight:** 493.56 g mol^{-1} **IUPAC name:** Benzyl *N*-[(2*S*)-1-[(4-methyl-2-oxochromen-7-yl)amino]-1-oxo-6-(propanoylamino)hexan-2-yl]carbamate **Category:** Miscellaneous **Mechanism:** HDAC inhibitors **Targets:** HDAC1
	**Name: BATCP**; 787549-23-9; Cpd 3b; (*S*)-[5-acetylamino-1-(2-oxo-4-trifluoromethyl-2*H*-chromen-7-ylcarbamoyl)pentyl]carbamic acid tert-butyl ester; Ba-tcp; AC1Q1L6D **Molecular formula:** $C_{23}H_{28}F_3N_3O_6$ **Molecular weight:** 499.49 g mol^{-1} **IUPAC name:** Tert-butyl *N*-[(2*S*)-6-acetamido-1-oxo-1-[[2-oxo-4-(trifluoromethyl)chromen-7-yl]amino]hexan-2-yl]carbamate **Category:** Miscellaneous **Mechanism:** HDAC inhibitors **Targets:** HDAC6
	**Name: (*E*)-3-(2-Ethyl-7-fluoro-4-oxo-3-phenethyl-3,4-dihydroquinazolin-6-yl)-*N*-hydroxyacrylamide** **Molecular formula:** $C_{21}H_{20}FN_3O_3$ **Molecular weight:** 381.40 g mol^{-1} **IUPAC name:** (*E*)-3-(2-Ethyl-7-fluoro-4-oxo-3-phenethyl-3,4-dihydroquinazolin-6-yl)-*N*-hydroxyacrylamide **Category:** Quinazolin-4-one derivatives **Mechanism:** HDAC inhibitors **Targets:** HDAC6 **Name: *N*-Hydroxy-3-(2-methyl-4-oxo-3-phenethyl-3,4-dihydro-quinazolin-7-yl)-acrylamide** **Molecular formula:** $C_{20}H_{19}N_3O_3$

TABLE 6.6 Potential Histone Deacetylase (HDAC) Inhibitors—cont'd

2D structure	Therapeutical agent
	**Molecular weight:** 349.38 g mol^{-1} **IUPAC name:** *N*-Hydroxy-3-(2-methyl-4-oxo-3-phenethyl-3,4-dihydro-quinazolin-7-yl)-acrylamide **Category:** Quinazolin-4-one derivatives **Mechanism:** HDAC inhibitors **Targets:** HDAC6
	**Name: Droxinostat**; 4-(4-Chloro-2-methylphenoxy)-*N*-hydroxybutanamide; 99873-43-5; MLS000109046; NS41080; NS-41080 **Molecular formula:** $C_{11}H_{14}ClN_3O_3$ **Molecular weight:** 243.69 g mol^{-1} **IUPAC name:** 4-(4-Chloro-2-methylphenoxy)-*N*-hydroxybutanamide **Category:** Small molecules **Mechanism:** HDAC inhibitors **Targets:** HDAC3; HDAC6; HDAC8
	**Name: PTACH**; 848354-66-5; NCH 51; Cpd 51; NCH-51; *S*-(7-oxo-7-((4-phenylthiazol-2-yl) amino)heptyl) 2-methylpropanethioate **Molecular formula:** $C_{20}H_{26}N_2O_2S_2$ **Molecular weight:** 390.56 g mol^{-1} **IUPAC name:** *S*-[7-oxo-7-[(4-phenyl-1,3-thiazol-2-yl)amino]heptyl] 2-methylpropanethioate **Category:** Small molecules **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	**Name: Tasquinimod**; 254964-60-8; ABR-215050; UNII-756U07KN1R; TASQ; 756U07KN1R; 4-hydroxy-5-methoxy-*N*,1-dimethyl-2-oxo-*N*-(4-(trifluoromethyl)-phenyl)-1,2-dihydroquinoline-3-carboxamide **Molecular formula:** $C_{20}H_{17}F_3N_2O_4$ **Molecular weight:** 406.36 g mol^{-1} **IUPAC name:** 4-Hydroxy-5-methoxy-*N*,1-dimethyl-2-oxo-*N*-[4-(trifluoromethyl)phenyl] quinoline-3-carboxamide **Category:** Quinoline-3-carboxamides **Mechanism:** HDAC inhibitors **Targets:** HDAC3; HDAC4
	**Name: Carbamazepine**; 298-46-4; tegretol; 5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide; carbamazepen; carbazepine **Molecular formula:** $C_{15}H_{12}N_2O$ **Molecular weight:** 236.27 g mol^{-1} **IUPAC name:** Benzo[*b*][1]benzazepine-11-carboxamide **Category:** Benzodiazepines **Mechanism:** HDAC inhibitors **Targets:** Class I HDACs; Class IIa HDACs; Class IIb HDACs
	Name: APHA; 3-(4-Aroyl-2-pyrrolyl)-*N*-hydroxy-2-propenamide; 3-(1-methyl-4-phenylacetyl-1*H*-2-pyrrolyl)-*N*-hydroxy-2-propenamide; SCHEMBL8131488; CHEBI:94735; 3f07 **Molecular formula:** $C_{16}H_{16}N_2O_3$ **Molecular weight:** 284.31 g mol^{-1} **IUPAC name:** (*E*)-*N*-Hydroxy-3-[1-methyl-4-(2-phenylacetyl)pyrrol-2-yl]prop-2-enamide **Category:** Aroyl-pyrrol hydroxyamides

Continued

TABLE 6.6 Potential Histone Deacetylase (HDAC) Inhibitors—cont'd

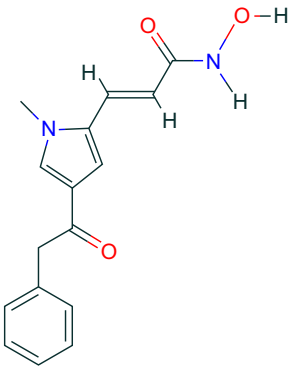
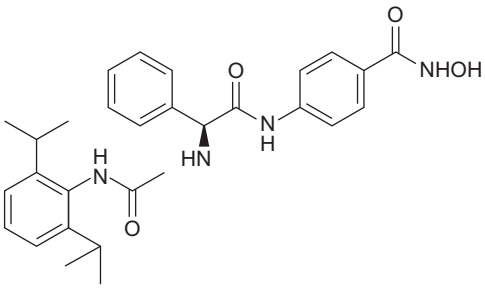
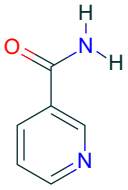
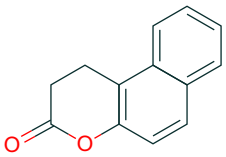
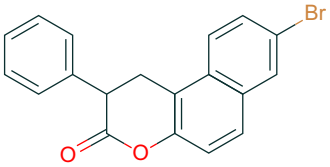
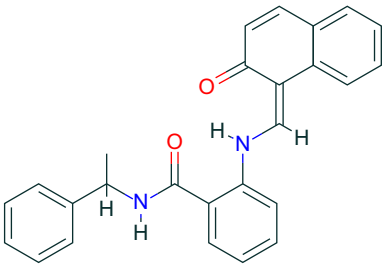
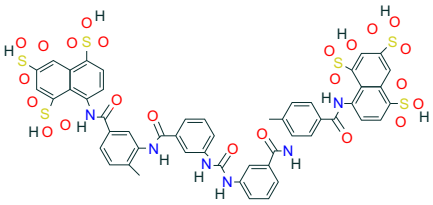
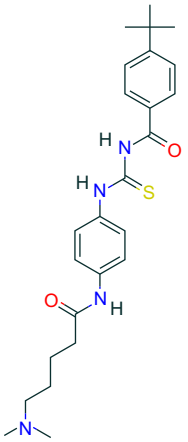
2D structure	Therapeutical agent
	**Mechanism:** HDAC inhibitors **Targets:** Class I HDACs
	**Name: (*S*)-4-(2-(5-(Dimethylamino)naphthalene-1-sulfonamido)-2-phenylacetamido)-*N*-hydroxybenzamide (D17)** **Molecular formula:** $C_{27}H_{27}N_4O_5S$ **Molecular weight:** 519.60 g mol^{-1} **IUPAC name:** (*S*)-4-(2-(5-(Dimethylamino)naphthalene-1-sulfonamido)-2-phenylacetamido)-*N*-hydroxybenzamide **Category:** Synthetic organic **Mechanism:** HDAC inhibitors **Targets:** HDAC1; HDAC2; HDAC3; HDAC6

TABLE 6.7 Potential Sirtuin (SIRT) Modulators

2D structure	Therapeutical agent
	**Name: Nicotinamide**; niacinamide, vitamin PP, aminicotin, nicotinic acid amide, amixicotyn, 3-pyridinecarboxamide, papulex, nicotylamide **Molecular formula:** $C_6H_6N_2O$ **Molecular weight:** 122.12 g mol^{-1} **IUPAC name:** Pyridine-3-carboxamide **Category:** Vitamins **Mechanism:** SIRT inhibitor **Targets:** SIRT1–7
	**Name: Splitomicin**; 1,2-dihydro-3*H*-naphtho[2,1-*b*]pyran-3-one; 5690-03-9; 1,2-dihydro-3*H*-benzo[*f*]chromen-3-one; CHEMBL86537 **Molecular formula:** $C_{13}H_{10}O_2$ **Molecular weight:** 198.22 g mol^{-1} **IUPAC name:** 1,2-Dihydrobenzo[*f*]chromen-3-one **Category:** Antibiotics **Mechanism:** SIRT inhibitor **Targets:** SIRT1; SIRT2
	**Name: HR-73**; 959571-93-8; SCHEMBL18134584; SCHEMBL18134584; AC1OCFZN; HR73; CHEMBL271761; 8-bromo-2-phenyl-1,2-dihydrobenzo[*f*]chromen-3-one **Molecular formula:** $C_{19}H_{13}BrO_2$ **Molecular weight:** 353.22 g mol^{-1} **IUPAC name:** 8-Bromo-2-phenyl-1,2-dihydrobenzo[*f*]chromen-3-one **Category:** Antibiotics **Mechanism:** SIRT inhibitor **Targets:** SIRT1; SIRT2
	**Name: Sirtinol**; Sir two inhibitor naphthol; 2-[(2-hydroxynaphthalen-1-ylmethylene) amino]-*N*-(1-phenethyl)benzamide; 2-{[(2-hydroxy-1-naphthyl)methylene]amino}-*N*-(1-phenylethyl)benzamide **Molecular formula:** $C_{26}H_{22}N_2O_2$ **Molecular weight:** 394.47 g mol^{-1} **IUPAC name:** 2-[[(*Z*)-(2-Oxonaphthalen-1-ylidene)methyl]amino]-*N*-(1-phenylethyl) benzamide **Category:** Heterocyclic compounds **Mechanism:** SIRT inhibitor **Targets:** SIRT1; SIRT2
	**Name: Suramin**; naphuride; germanin; naganol; belganyl; fourneau; farma; antrypol; suramine; naganin **Molecular formula:** $C_{51}H_{40}N_6O_{23}S_6$ **Molecular weight:** 1297.26 g mol^{-1} **IUPAC name:** 8-[[4-Methyl-3-[[3-[[3-[[2-methyl-5-[(4,6,8-trisulfonaphthalen-1-yl) carbamoyl]phenyl]carbamoyl]phenyl]carbamoylamino]benzoyl]amino]benzoyl] amino]naphthalene-1,3,5-trisulfonic acid **Category:** Polyanionic compounds **Mechanism:** SIRT inhibitor **Targets:** SIRT1; SIRT2
	**Name: Tenovin-6**; 011557-82-6; CHEMBL595354; CHEBI:77729; 4-tert-butyl-*N*-[[4-[5-(dimethylamino)pentanoylamino]phenyl]carbamothioyl]benzamide **Molecular formula:** $C_{25}H_{34}N_4O_2S$ **Molecular weight:** 454.63 g mol^{-1} **IUPAC name:** 4-Tert-butyl-*N*-[[4-[5-(dimethylamino)pentanoylamino]phenyl] carbamothioyl]benzamide **Category:** Small molecules **Mechanism:** SIRT inhibitor **Targets:** SIRT1; SIRT2; SIRT3

Continued

TABLE 6.7 Potential Sirtuin (SIRT) Modulators—cont'd

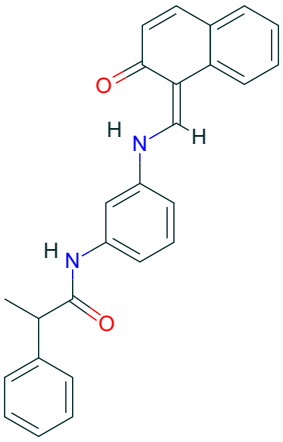
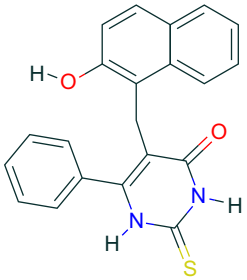
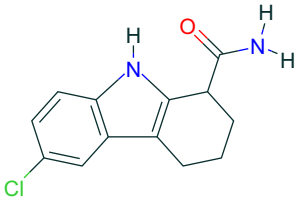
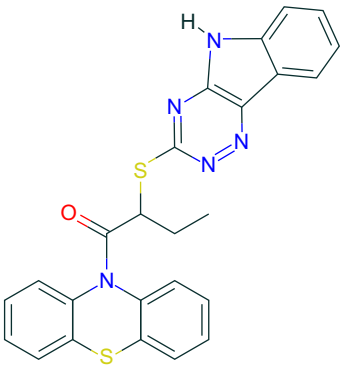
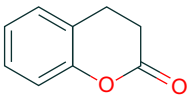
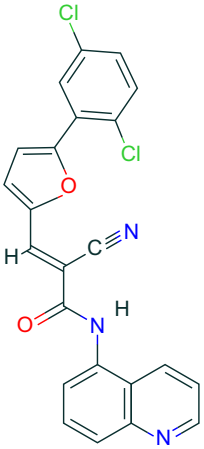
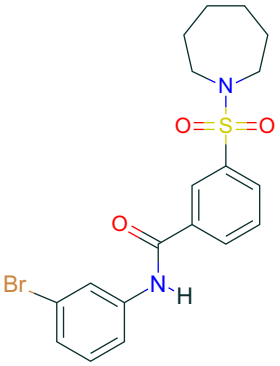
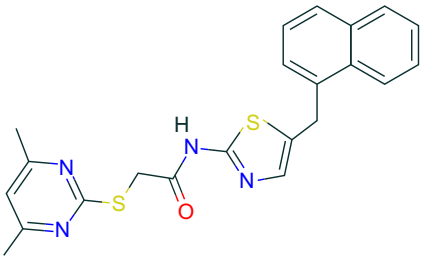
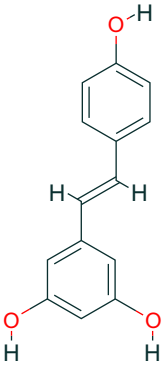
2D structure	Therapeutical agent
	**Name: Salermide**; (*E*)-*N*-(3-((2-Hydroxynaphthalen-1-yl)methyleneamino)phenyl)-2-phenylpropanamide; SCHEMBL8103931; HMS3648G04; 1105698-15-4 **Molecular formula:** $C_{26}H_{22}N_2O_2$ **Molecular weight:** 394.47 g mol^{-1} **IUPAC name:** *N*-[3-[[(*Z*)-(2-Oxonaphthalen-1-ylidene)methyl]amino]phenyl]-2-phenylpropanamide **Category:** Small molecules **Mechanism:** SIRT inhibitor **Targets:** SIRT1; SIRT2
	**Name: Cambinol**; NSC112546; NSC-112546; SIRT1/2 inhibitor IV, cambinol; NSC-1125476; 5-[(2-hydroxy-1-naphthyl)methyl]-2-mercapto-6-phenyl-4(3*H*)-pyrimidinone **Molecular formula:** $C_{21}H_{16}N_2O_2S$ **Molecular weight:** 360.43 g mol^{-1} **IUPAC name:** 5-[(2-Hydroxynaphthalen-1-yl)methyl]-6-phenyl-2-sulfanylidene-1*H*-pyrimidin-4-one **Category:** Small molecules **Mechanism:** SIRT inhibitor **Targets:** SIRT1; SIRT2
	**Name: Selisistat**; EX527; 49843-98-3; 6-chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide; SIRT1 inhibitor III; EX 527; SEN0014196 **Molecular formula:** $C_{13}H_{13}ClN_2O$ **Molecular weight:** 248.71 g mol^{-1} **IUPAC name:** 6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide **Category:** Small molecules **Mechanism:** SIRT inhibitor **Targets:** SIRT1
	**Name: Inauhzin**; 309271-94-1; AK175751; C25H19N5OS2; 1-phenothiazin-10-yl-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)butan-1-one; AC1NUV9U **Molecular formula:** $C_{25}H_{19}N_5OS_2$ **Molecular weight:** 459.58 g mol^{-1} **IUPAC name:** 1-Phenothiazin-10-yl-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)butan-1-one **Category:** Small molecules **Mechanism:** SIRT inhibitor **Targets:** SIRT1
	**Name: Dihydrocoumarin**; 3,4-dihydrocoumarin; hydrocoumarin; chroman-2-one; benzodihydropyrone; melilotin; melilotol; 1,2-benzodihydropyrone; 2-chromanone **Molecular formula:** $C_9H_8O_2$ **Molecular weight:** 148.16 g mol^{-1} **IUPAC name:** 3,4-Dihydrochromen-2-one **Category:** Small molecules **Mechanism:** SIRT inhibitor **Targets:** SIRT1

TABLE 6.7 Potential Sirtuin (SIRT) Modulators—cont'd

2D structure	Therapeutical agent
	**Name: AGK-2**; UNII-DDF0L8606A; sirtuin 2 inhibitor; 304896-28-4; 2-cyano-3-(5-(2,5-dichlorophenyl)furan-2-yl)-*N*-(quinolin-5-yl)acrylamide; CHEMBL224864 **Molecular formula:** $C_{23}H_{13}Cl_2N_3O_2$ **Molecular weight:** 434.28 g mol^{-1} **IUPAC name:** (*E*)-2-Cyano-3-[5-(2,5-dichlorophenyl)furan-2-yl]-*N*-quinolin-5-ylprop-2-enamide **Category:** Small molecules **Mechanism:** SIRT inhibitor **Targets:** SIRT2
	**Name: AK-7**; 420831-40-9; UNII-308B6B695N; CHEMBL3222141; 3-(azepan-1-ylsulfonyl)-*N*-(3-bromophenyl)benzamide; ZINC01159030 **Molecular formula:** $C_{19}H_{21}BrN_2O_3S$ **Molecular weight:** 437.35 g mol^{-1} **IUPAC name:** 3-(Azepan-1-ylsulfonyl)-*N*-(3-bromophenyl)benzamide **Category:** Small molecules **Mechanism:** SIRT inhibitor **Targets:** SIRT2
	**Name: SirReal2**; 2-(4,6-dimethyl-pyrimidin-2-ylsulfanyl)-*N*-(5-naphthalen-1-ylmethyl-thiazol-2-yl)-acetamide **Molecular formula:** $C_{22}H_{20}N_4OS_2$ **Molecular weight:** 420.55 g mol^{-1} **IUPAC name:** 2-(4,6-Dimethylpyrimidin-2-yl)sulfanyl-*N*-[5-(naphthalen-1-ylmethyl)-1,3-thiazol-2-yl]acetamide **Category:** Small molecules **Mechanism:** SIRT inhibitor **Targets:** SIRT2
	**Name: Resveratrol**; trans-resveratrol; 501-36-0; 3,4′,5-trihydroxystilbene; 3,4′,5-stilbenetriol; 3,5,4′-trihydroxystilbene; resvida; (*E*)-resveratrol **Molecular formula:** $C_{14}H_{12}O_3$ **Molecular weight:** 228.24 g mol^{-1} **IUPAC name:** 5-[(*E*)-2-(4-Hydroxyphenyl)ethenyl]benzene-1,3-diol **Category:** Natural polyphenols **Mechanism:** SIRT activator **Targets:** SIRT1

Continued

TABLE 6.7 Potential Sirtuin (SIRT) Modulators—cont'd

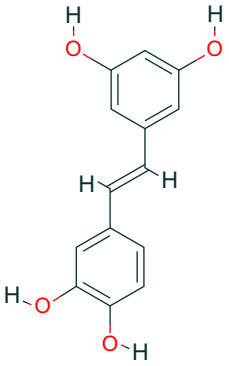
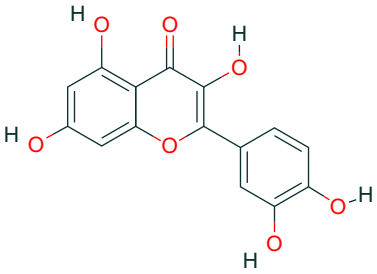
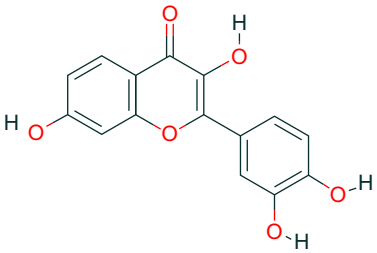
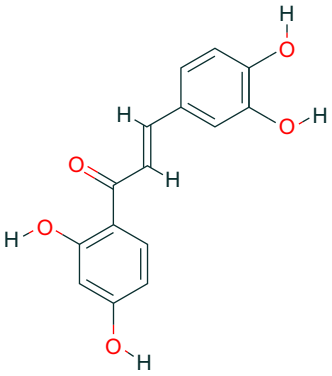
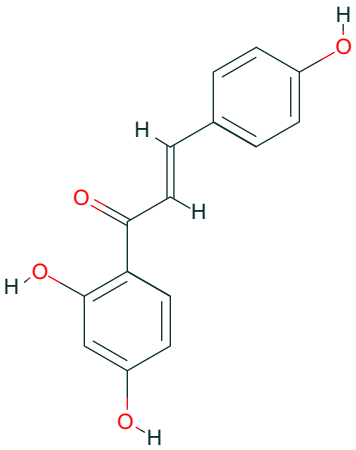
2D structure	Therapeutical agent
	**Name: Piceatannol**; 10083-24-6; 3-hydroxyresveratol; astringinin; piceatanol; (*E*)-4-(3,5-dihydroxystyryl)benzene-1,2-diol; 3,5,3′,4′-tetrahydroxystilbene; NSC-365798 **Molecular formula:** $C_{14}H_{12}O_4$ **Molecular weight:** 244.25 g mol^{-1} **IUPAC name:** 4-[(*E*)-2-(3,5-Dihydroxyphenyl)ethenyl]benzene-1,2-diol **Category:** Natural polyphenols **Mechanism:** SIRT activator **Targets:** SIRT1
	**Name: Quercetin**; sophoretin; quercetol; meletin; xanthaurine; quercitin; 3,3′,4′,5,7-pentahydroxyflavone **Molecular formula:** $C_{15}H_{10}O_7$ **Molecular weight:** 302.24 g mol^{-1} **IUPAC name:** 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one **Category:** Natural polyphenols **Mechanism:** SIRT activator **Targets:** SIRT1
	**Name: Fisetin**; 528-48-3; 2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-4*H*-chromen-4-one; 5-desoxyquercetin; 3,3′,4′,7-tetrahydroxyflavone; superfustel; cotinin; fietin; fustel; fustet **Molecular formula:** $C_{15}H_{10}O_6$ **Molecular weight:** 286.24 g mol^{-1} **IUPAC name:** 2-(3,4-Dihydroxyphenyl)-3,7-dihydroxychromen-4-one **Category:** Natural polyphenols **Mechanism:** SIRT activator **Targets:** SIRT1
	**Name: Butein**; 487-52-5; 2′,3,4,4′-tetrahydroxychalcone; 2′,4′,3,4-tetrahydroxychalcone; 3,4,2′,4′-tetrahydroxychalcone; EINECS 207-659-5 **Molecular formula:** $C_{15}H_{12}O_5$ **Molecular weight:** 272.26 g mol^{-1} **IUPAC name:** (*E*)-1-(2,4-Dihydroxyphenyl)-3-(3,4-dihydroxyphenyl)prop-2-en-1-one **Category:** Natural polyphenols **Mechanism:** SIRT activator **Targets:** SIRT1
	**Name: Isoliquiritigenin**; 961-29-5; 2′,4,4′-trihydroxychalcone; 4,2′,4′-trihydroxychalcone; isoliquirtigenin; (*E*)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl) prop-2-en-1-one **Molecular formula:** $C_{15}H_{12}O_4$ **Molecular weight:** 256.26 g mol^{-1} **IUPAC name:** (*E*)-1-(2,4-Dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one **Category:** Natural polyphenols **Mechanism:** SIRT activator **Targets:** SIRT1

TABLE 6.7 Potential Sirtuin (SIRT) Modulators—cont'd

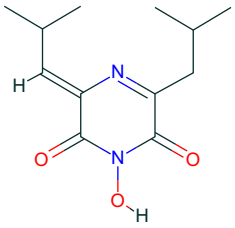
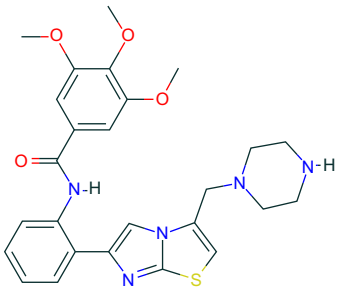
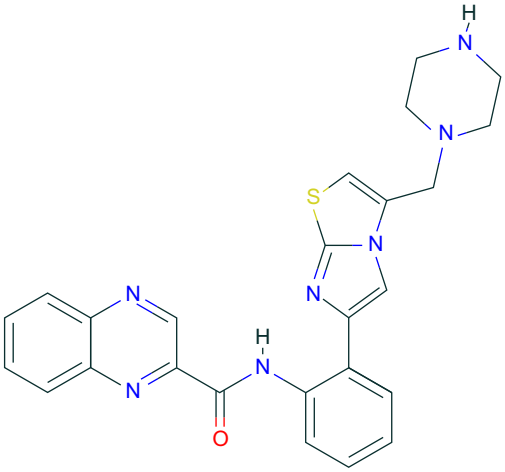
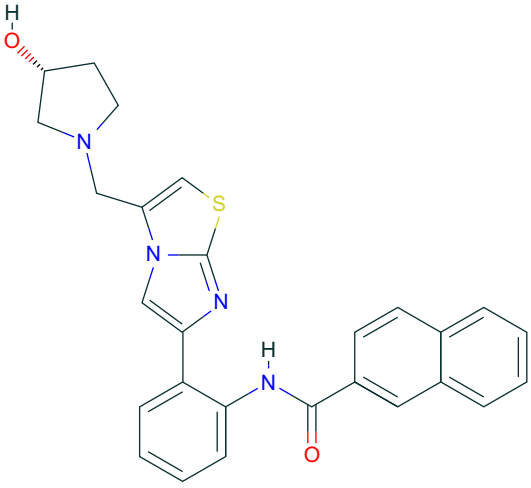
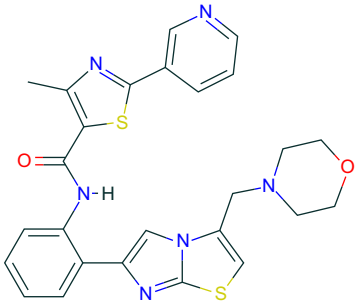
2D structure	Therapeutical agent
	**Name: Flutimide**; 162666-34-4; AC1O5YLM; DCL000372; DNC000657; GSK184072; (5*Z*)-1-hydroxy-3-isobutyl-5-(2-methylpropylidene)pyrazine-2,6-dione **Molecular formula:** $C_{12}H_{18}N_2O_3$ **Molecular weight:** 238.29 g mol^{-1} **IUPAC name:** (5*Z*)-1-Hydroxy-3-(2-methylpropyl)-5-(2-methylpropylidene)pyrazine-2,6-dione **Category:** Heterocyclic compounds **Mechanism:** SIRT activator **Targets:** SIRT1
	**Name: SRT-1460**; 3,4,5-trimethoxy-*N*-(2-(3-(piperazin-1-ylmethyl)imidazo[2,1-*b*]thiazol-6-yl)phenyl)benzamide; 925432-73-1; CHEMBL254156; AK-57112 **Molecular formula:** $C_{26}H_{29}N_5O_4S$ **Molecular weight:** 507.61 g mol^{-1} **IUPAC name:** 3,4,5-Trimethoxy-*N*-[2-[3-(piperazin-1-ylmethyl)imidazo[2,1-*b*][1,3]thiazol-6-yl]phenyl]benzamide **Category:** Small molecules **Mechanism:** SIRT activator **Targets:** SIRT1
	**Name: SRT-1720**; 925434-55-5; *N*-(2-(3-(piperazin-1-ylmethyl)imidazo[2,1-*b*]thiazol-6-yl)phenyl)quinoxaline-2-carboxamide; CHEMBL257991 **Molecular formula:** $C_{25}H_{23}N_7OS$ **Molecular weight:** 469.57 g mol^{-1} **IUPAC name:** *N*-[2-[3-(Piperazin-1-ylmethyl)imidazo[2,1-*b*][1,3]thiazol-6-yl]phenyl]quinoxaline-2-carboxamide **Category:** Small molecules **Mechanism:** SIRT activator **Targets:** SIRT1
	**Name: SRT-2183**; (*R*)-*N*-(2-(3-((3-hydroxypyrrolidin-1-yl)methyl)imidazo[2,1-*b*]thiazol-6-yl)phenyl)-2-naphthamide; CHEMBL403308; BDBM50376978; ZINC29043608 **Molecular formula:** $C_{27}H_{24}N_4O_2S$ **Molecular weight:** 468.56 g mol^{-1} **IUPAC name:** *N*-[2-[3-[[(3*R*)-3-Hydroxypyrrolidin-1-yl]methyl]imidazo[2,1-*b*][1,3]thiazol-6-yl]phenyl]naphthalene-2-carboxamide **Category:** Small molecules **Mechanism:** SIRT activator **Targets:** SIRT1
	**Name: SRT-2104**; 093403-33-8; sirtuin modulator; SRT 2104; UNII-4521NR0J09; SRT2104 (GSK2245840); SCHEMBL964014; ZINC43202455; DTXSID00648729 **Molecular formula:** $C_{26}H_{24}N_6O_2S_2$ **Molecular weight:** 516.64 g mol^{-1} **IUPAC name:** 4-Nethyl-*N*-[2-[3-(morpholin-4-ylmethyl)imidazo[2,1-*b*][1,3]thiazol-6-yl]phenyl]-2-pyridin-3-yl-1,3-thiazole-5-carboxamide **Category:** Small molecules **Mechanism:** SIRT activator **Targets:** SIRT1

TABLE 6.8 Potential Epigenetic Drugs Modulating Histone Acetylation

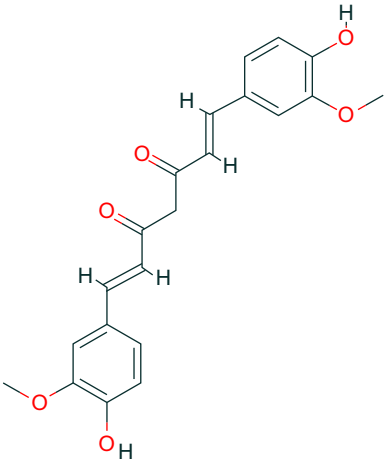
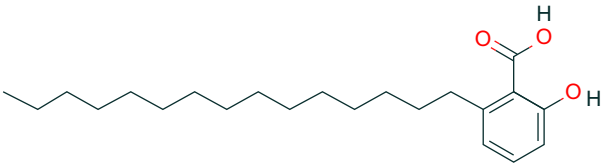
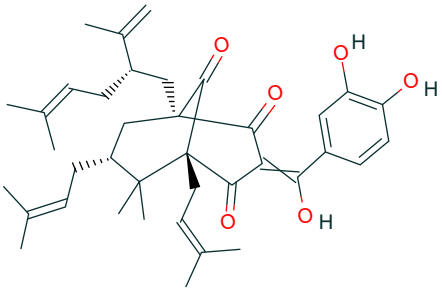
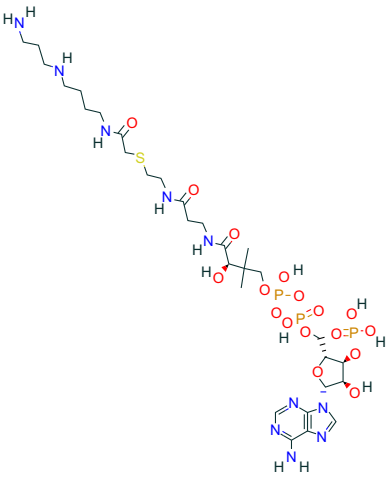
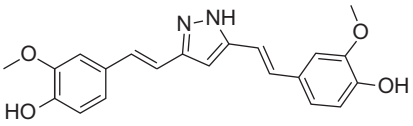
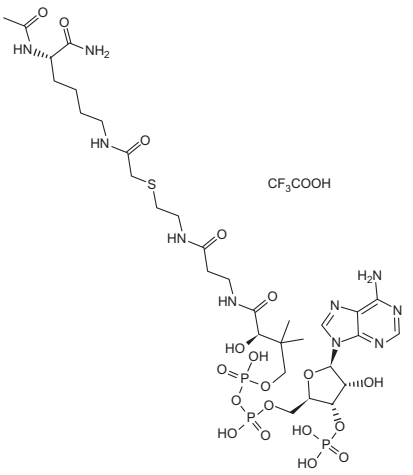
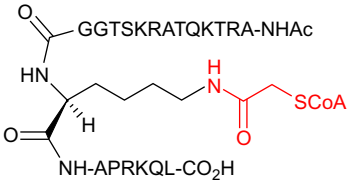
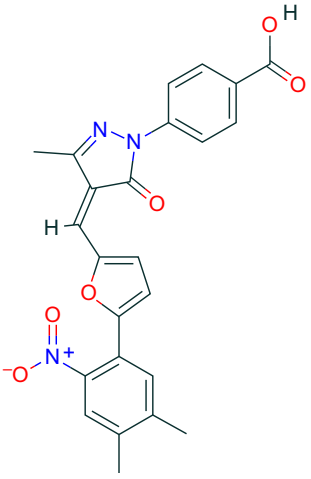
2D structure	Therapeutical agent
	**Name: Curcumin**; diferuloylmethane; natural yellow 3; 458-37-7; turmeric yellow; turmeric yellow; turmeric; kacha haldi; Gelbwurz; Curcuma; haldar; souchet **Molecular formula:** $C_{21}H_{20}O_6$ **Molecular weight:** 368.39 g mol^{-1} **IUPAC name:** (1*E*,6*E*)-1,7-bis(4-Hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione **Category:** Natural products **Mechanism:** HAT inhibitor **Targets:** p300/CBP
	**Name: Anacardic acid**; 6-pentadecylsalicylic acid; 16611-84-0; 2-hydroxy-6-pentadecylbenzoic acid; cyclogallipharic acid; 22:0-anacardic acid; ginkgolic acid C15:0 **Molecular formula:** $C_{22}H_{36}O_3$ **Molecular weight:** 348.53 g mol^{-1} **IUPAC name:** 2-Hydroxy-6-pentadecylbenzoic acid **Category:** Natural products **Mechanism:** HAT inhibitor **Targets:** p300/CBP
	**Name: Garcinol**; camboginol; 78824-30-3; AC1Q1NUM; GTPL7001; QDKLRKZQSOQWJQ-SMDXAGPFSA-N; ZINC4098424; AKOS025142024 **Molecular formula:** $C_{38}H_{50}O_6$ **Molecular weight:** 602.81 g mol^{-1} **IUPAC name:** (1*S*,5*R*,7*R*)-3-[(3,4-Dihydroxyphenyl)-hydroxymethylidene]-6,6-dimethyl-5,7-bis(3-methylbut-2-enyl)-1-[(2*S*)-5-methyl-2-prop-1-en-2-ylhex-4-enyl]bicyclo[3.3.1]nonane-2,4,9-trione **Category:** Natural products **Mechanism:** HAT inhibitor **Targets:** p300, PCAF
	**Name: Spermidinyl-CoA**; Spd(N1)-CoA; *N*-Coa-asp; *N*-(2-(*S*-Coa)acetyl)spermidine; *N*-(2-(*S*-coenzyme A)acetyl) spermidine; AC1L51EM; 83889-68-3; coenzyme A; *S*-(2-((4-((3-aminopropyl)amino)butyl)amino)-2-oxoethyl) **Molecular formula:** $C_{30}H_{55}N_{10}O_{17}P_3S$ **Molecular weight:** 952.80 g mol^{-1} **IUPAC name:** [[(3*R*)-4-[[3-[2-[2-[4-(3-aminopropylamino) butylamino]-2-oxoethyl]sulfanylethylamino]-3-oxopropyl] amino]-3-hydroxy-2,2-dimethyl-4-oxobutoxy]-hydroxyphosphoryl] [(2*R*,3*S*,4*R*,5*R*)-5-(6-aminopurin-9-yl)-4-hydroxy-3-phosphonooxyoxolan-2-yl]methyl hydrogen phosphate **Category:** Bisubstrate inhibitors **Mechanism:** HAT inhibitor **Targets:** p300/CBP

TABLE 6.8 Potential Epigenetic Drugs Modulating Histone Acetylation—cont'd

2D structure	Therapeutical agent
	**Name: Hydrazinocurcumin**; CTK7A **Molecular formula:** $C_{21}H_{20}N_2O_4$ **Molecular weight:** 364.39 g mol^{-1} **IUPAC name:** 4,4′-((1*E*,1′*E*)-(1*H*-Pyrazole-3,5-diyl)bis(ethene-2,1-diyl))bis(2-methoxyphenol) **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300
	**Name: Lys-CoA** **Molecular formula:** $C_{27}H_{48}N_9O_{17}P_3S$ **Molecular weight:** 895.71 g mol^{-1} **IUPAC name:** *S*-[2-[[(5*S*)-5-(Acetylamino)-6-amino-6-oxohexyl]amino]-2-oxoethyl]coenzyme A trifluoroacetate **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300
	**Name: H3-CoA-20** **Molecular formula:** H3-(Me)CoA-20 **Molecular weight:** 15 kDa **Peptide sequence:** ARTKQTARKSTGGKAPRKQLW **Category:** Synthetic peptides **Mechanism:** HAT inhibitor **Targets:** PCAF
	**Name: C646**; 328968-36-1; C-646; STK219801; 4-[(4*E*)-4-[[5-(4,5-dimethyl-2-nitrophenyl)furan-2-yl]methylidene]-3-methyl-5-oxopyrazol-1-yl]benzoic acid **Molecular formula:** $C_{24}H_{19}N_3O_6$ **Molecular weight:** 445.42 g mol^{-1} **IUPAC name:** 4-[(4*Z*)-4-[[5-(4,5-Dimethyl-2-nitrophenyl)furan-2-yl]methylidene]-3-methyl-5-oxopyrazol-1-yl]benzoic acid **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300/CBP
	Name: Compound 4e; CHEMBL336939; D06MMN; GTPL3090; BDBM50038138; (6*R*,7*R*)-1-((4*S*,5*R*)-4-acetoxy-5-methyl-3-methylene-6-phenyl-hexyl)-4,7-dihydroxy-6-(11-phenoxy-undecylcarbamoyloxy)-2,8-dioxa-bicyclo[3.2.1]octane-3,4,5-tricarboxylic acid **Molecular formula:** $C_{43}H_{57}NO_{15}$ **Molecular weight:** 827.92 g mol^{-1}

Continued

TABLE 6.8 Potential Epigenetic Drugs Modulating Histone Acetylation—cont'd

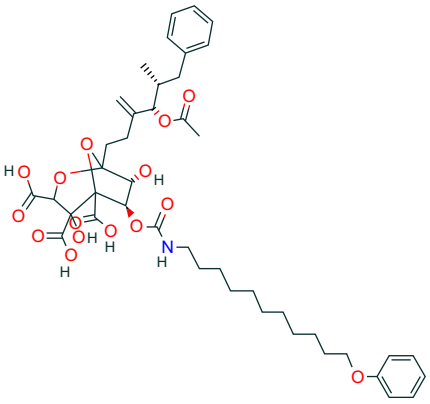
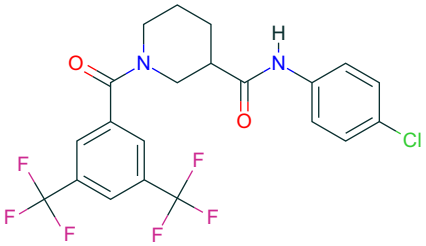
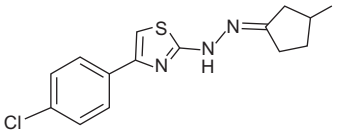
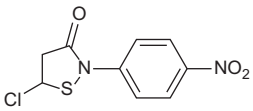
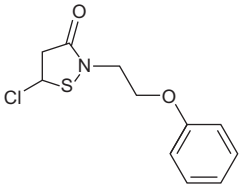
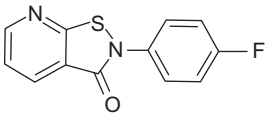
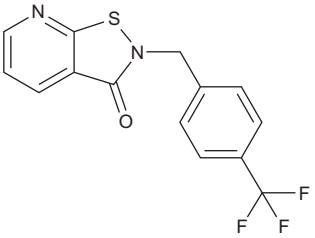
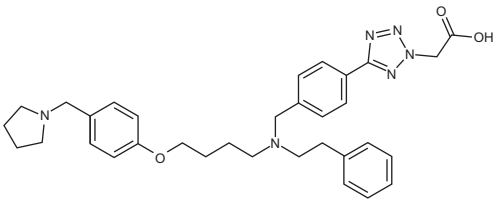
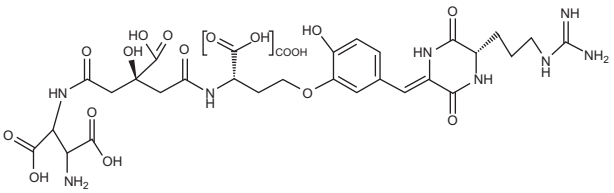
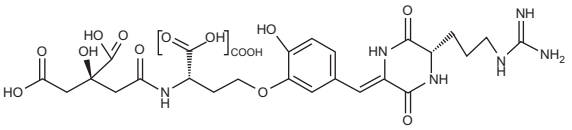
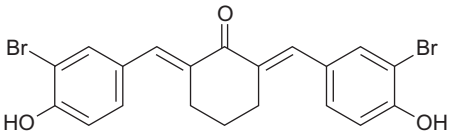
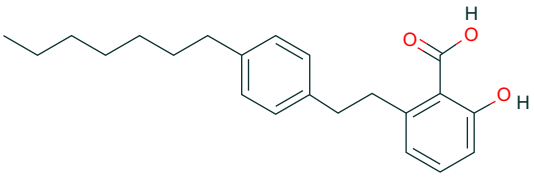
2D structure	Therapeutical agent
	**IUPAC name:** (6*R*,7*R*)-1-[(4*S*,5*R*)-4-Acetyloxy-5-methyl-3-methylidene-6-phenylhexyl]-4,7-dihydroxy-6-(11-phenoxyundecylcarbamoyloxy)-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300
	**Name: Compound 4g**; CCG-100602; Compound 4g (PMID: 19963382); CHEMBL603141; CCG100602; CCG 100602; 1207113-88-9; 1-(3,5-bis(trifluoromethyl)benzoyl)-*N*-(4-chlorophenyl)piperidine-3-carboxamide **Molecular formula:** $C_{21}H_{17}ClF_6N_2O_2$ **Molecular weight:** 478.82 g mol^{-1} **IUPAC name:** 1-[3,5-Bis(trifluoromethyl)benzoyl]-*N*-(4-chlorophenyl)piperidine-3-carboxamide **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300
	**Name: CPTH6**; 3-methylcyclopentylidene-[4-(4′-chlorophenyl)thiazol-2-yl]hydrazone **Molecular formula:** $C_{14}H_{14}ClN_3S$ **Molecular weight:** 291.80 g mol^{-1} **IUPAC name:** 3-Methyl-2-[4-(4-chlorophenyl)-2-thiazolyl] hydrazone cyclopentanone **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** PCAF, Gcn5
	**Name: CCT077791** **Molecular formula:** $C_9H_5ClN_2O_3S$ **Molecular weight:** 256.67 g mol^{-1} **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300, PCAF
	**Name: CCT077792** **Molecular formula:** $C_{11}H_8ClNO_2S$ **Molecular weight:** 253.70 g mol^{-1} **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300, PCAF
	**Name: PU139** **Molecular formula:** $C_{12}H_7FN_2OS$ **Molecular weight:** 246.26 g mol^{-1} **IUPAC name:** 2-(4-Fluorophenyl)isothiazolo[5,4-*b*]pyridin-3(2*H*)-one **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** PCAF, Gcn5, p300/CBP

TABLE 6.8 Potential Epigenetic Drugs Modulating Histone Acetylation—cont'd

2D structure	Therapeutical agent
	**Name: PU141** **Molecular formula:** $C_{14}H_9F_3N_2OS$ **Molecular weight:** 310.29 g mol^{-1} **IUPAC name:** 2-(4-(Trifluoromethyl)benzyl)isothiazolo[5,4-*b*]pyridin-3(2*H*)-one **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300/CBP
	**Name: TH1834** **Molecular formula:** $C_{33}H_{40}N_6O_3$ **Molecular weight:** 568.72 g mol^{-1} **IUPAC name:** 2-(5-(4-((Phenethyl(4-(4-(pyrrolidin-1-ylmethyl)phenoxy)butyl)amino)methyl)phenyl)-2*H*-tetrazol-2-yl)acetic acid **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** Tip60
	**Name: NK13650A** **Molecular formula:** $C_{29}H_{38}N_8O_{15}$ **Molecular weight:** 738.66 g mol^{-1} **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300
	**Name: NK13650B** **Molecular formula:** $C_{25}H_{32}N_6O_{12}$ **Molecular weight:** 608.55 g mol^{-1} **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300
	**Name: HATi II**; histone acetyltransferase inhibitor II; 2,6-bis[(3-bromo-4-hydroxyphenyl)methylene]cyclohexanone **Molecular formula:** $C_{20}H_{16}Br_2O_3$ **Molecular weight:** 464.15 g mol^{-1} **IUPAC name:** 2,6-Bis[(3-bromo-4-hydroxyphenyl) methylene]cyclohexanone **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300/CBP
	**Name: MG149**; 1243583-85-8;MG-149; CHEMBL1215739; 2-[2-(4-heptylphenyl)ethyl]-6-hydroxybenzoic acid **Molecular formula:** $C_{22}H_{28}O_3$ **Molecular weight:** 340.46 g mol^{-1} **IUPAC name:** 2-[2-(4-Heptylphenyl)ethyl]-6-hydroxybenzoic acid **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** Tip60, MOF
	Name: I-CBP112; CHEMBL3774655; 1640282-31-0; 1-[7-(3,4-dimethoxyphenyl)-9-[[(3*S*)-1 methylpiperidin-3-yl] methoxy]-2,3,4,5-tetrahydro-1,4-benzoxazepin-4-yl] propan-1-one; 1-[7-(3,4-dimethoxyphenyl)-9-{[(3*S*)-1-methylpiperidin-3-yl] methoxy}-2,3,4,5-tetrahydro-1,4-benzoxazepin-4-yl] propan-1-one **Molecular formula:** $C_{27}H_{36}N_2O_5$

Continued

TABLE 6.8 Potential Epigenetic Drugs Modulating Histone Acetylation—cont'd

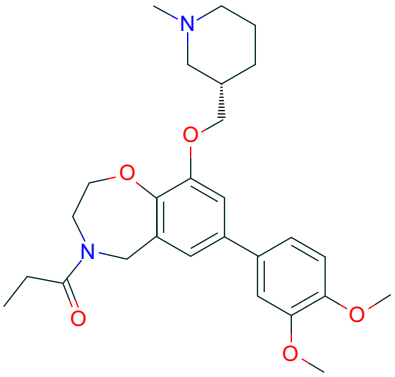
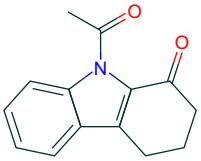
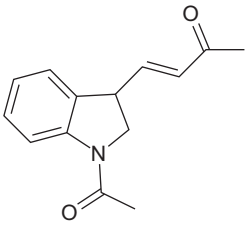
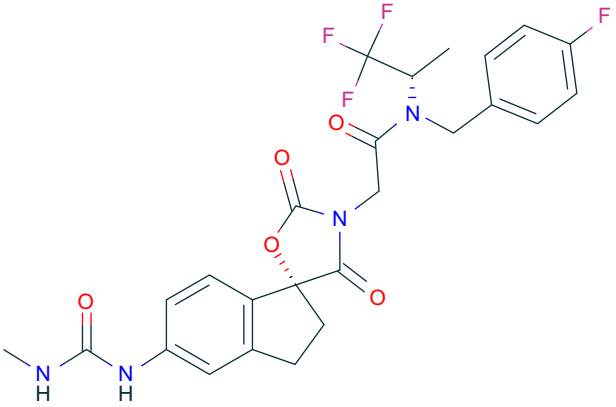
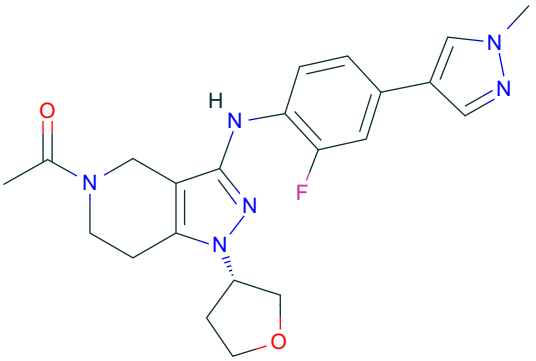
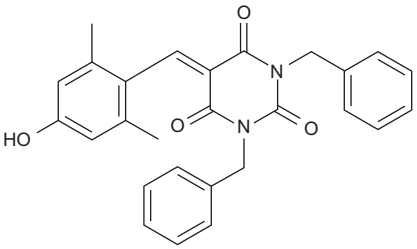
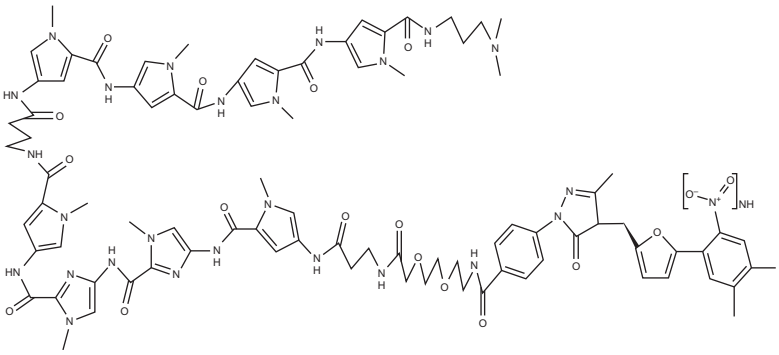
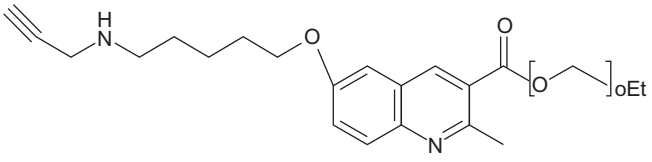
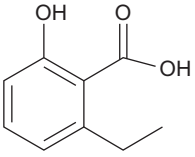
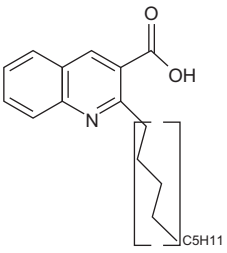
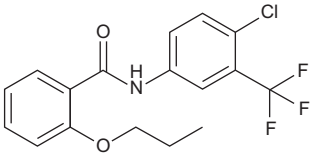
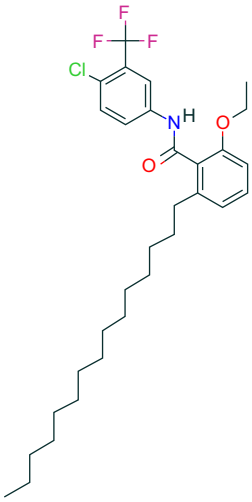
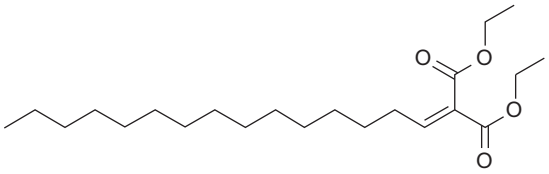
2D structure	Therapeutical agent
	**Molecular weight:** 468.59 g mol^{-1} **IUPAC name:** 1-[7-(3,4-Dimethoxyphenyl)-9-[[(3*S*)-1-methylpiperidin-3-yl]methoxy]-3,5-dihydro-2H-1,4-benzoxazepin-4-yl]propan-1-one **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300/CBP
	**Name: MS7972**; CHEMBL1236441; TTR; 9-acetyl-2,3,4,9-tetrahydro-1*H*-carbazol-1-one; 9-acetyl-3,4-dihydro-2*H*-carbazol-1-one; AC1LGXTL **Molecular formula:** $C_{14}H_{13}NO_2$ **Molecular weight:** 227.26 g mol^{-1} **IUPAC name:** 9-Acetyl-3,4-dihydro-2*H*-carbazol-1-one **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** CBP
	**Name: MS7867** **Molecular formula:** $C_{14}H_{15}NO_2$ **Molecular weight:** 229.27 g mol^{-1} **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** CBP
	**Name: A-485**; SCHEMBL17606148; CS-8185; HY-107455; 1889279-16-6; 6TF **Molecular formula:** $C_{25}H_{24}F_4N_4O_5$ **Molecular weight:** 536.48 g mol^{-1} **IUPAC name:** *N*-[(4-Fluorophenyl)methyl]-2-[(3*R*)-6-(methylcarbamoylamino)-2′,4′-dioxospiro[1,2-dihydroindene-3,5′,1,3-oxazolidine]-3′-yl]-*N*-[(2*S*)-1,1,1-trifluoropropan-2-yl]acetamide **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300/CBP
	Name: GNE-272; 1936428-93-1; 1-[3-[[2-fluoranyl-4-(1-methylpyrazol-4-yl)phenyl]amino]-1-[(3∼{s})-oxolan-3-yl]-6,7-dihydro-4∼{h}-pyrazolo[4,3-C]pyridin-5-yl]ethanone; 6XH; SCHEMBL17794706; AKOS032946256 **Molecular formula:** $C_{22}H_{25}FN_6O_2$ **Molecular weight:** 424.47 g mol^{-1}

TABLE 6.8 Potential Epigenetic Drugs Modulating Histone Acetylation—cont'd

2D structure	Therapeutical agent
	**IUPAC name:** 1-[3-[2-Fluoro-4-(1-methylpyrazol-4-yl)anilino]-1-[(3*S*)-oxolan-3-yl]-6,7-dihydro-4*H*-pyrazolo[4,3-*c*]pyridin-5-yl]ethanone **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300/CBP
	**Name: EML 425** **Molecular formula:** $C_{27}H_{24}N_2O_4$ **Molecular weight:** 440.49 g mol^{-1} **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300/CBP
	**Name: p300-IN-1** **Molecular formula:** $C_{88}H_{100}N_{26}O_{18}$ **Molecular weight:** 1809.90 g mol^{-1} **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300
	**Name: p300-IN-2** **Molecular formula:** $C_{21}H_{26}N_2O_3$ **Molecular weight:** 354.44 g mol^{-1} **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300
	**Name: MOF-IN-1** **Molecular formula:** $C_9H_{10}O_3$ **Molecular weight:** 166.17 g mol^{-1} **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** MOF
	**Name: MC1823** **Molecular formula:** $C_{15}H_{17}NO_2$ **Molecular weight:** 243.30 g mol^{-1} **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** Pan HAT

Continued

TABLE 6.8 Potential Epigenetic Drugs Modulating Histone Acetylation—cont'd

2D structure	Therapeutical agent
	**Name: TTK21**; *N*-[4-chloro-3-(trifluoromethyl)phenyl]-2-propoxy-benzamide **Molecular formula:** $C_{17}H_{15}ClF_3NO_2$ **Molecular weight:** 357.75 g mol^{-1} **IUPAC name:** *N*-[4-chloro-3-(trifluoromethyl)phenyl]-2-propoxy-benzamide **Category:** Synthetic compounds **Mechanism:** HAT inhibitor **Targets:** p300/CBP
	**Name: CTPB** (enzyme activator); *N*-(4-chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide; *N*-[4-chloro-3-(trifluoromethyl)phenyl]-2-ethoxy-6-pentadecylbenzamide **Molecular formula:** $C_{31}H_{43}ClF_3NO_2$ **Molecular weight:** 554.13 g mol^{-1} **IUPAC name:** *N*-[4-Chloro-3-(trifluoromethyl)phenyl]-2-ethoxy-6-pentadecylbenzamide **Category:** Small molecules **Mechanism:** HAT activator **Targets:** HAT
	**Name: SPV-106**; Pentadecylidenemalonate 1b; 2-pentadecylidene-propanedioic acid 1,3-diethyl ester **Molecular formula:** $C_{22}H_{40}O_4$ **Molecular weight:** 368.55 g mol^{-1} **IUPAC name:** Pentadecylidenemalonate 1b **Category:** Small molecules **Mechanism:** HAT activator **Targets:** HAT

Gcn5, histone acetyltransferase Gcn5; *HAT*, histone acetyltransferases; *MOF*, histone acetyltransferase MOF (from MYST family); *MYST*, Moz-Ybf2/Sas3-Sas2-Tip60 family of histone acetyltransferases; *p300/CBP*, p300/cyclic AMP-responsive element-binding protein; *PCAF*, p300/CBP-associated factor; *Tip60*, histone acetyltransferase Tip60 (from MYST family).

TABLE 6.9 Potential Epigenetic Drugs Modulating Histone Methylation

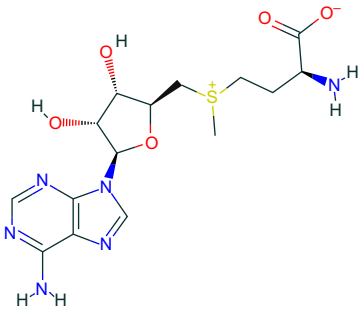
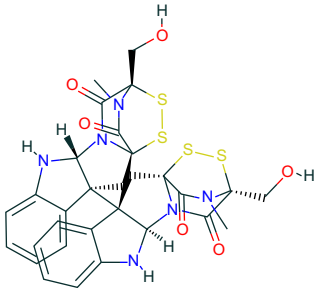
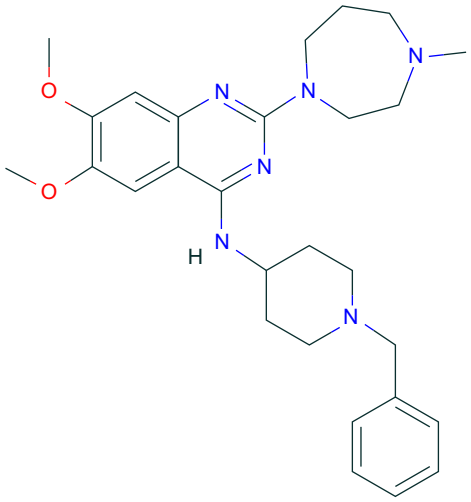
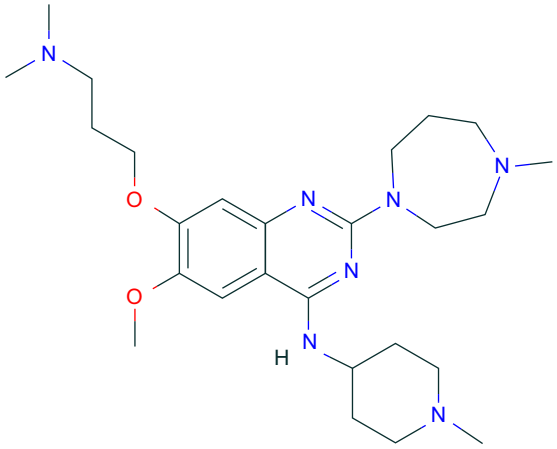
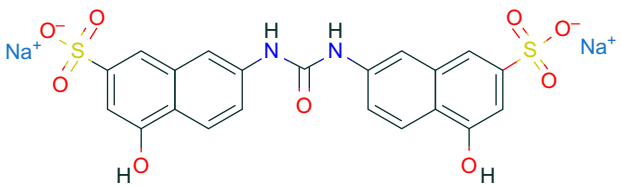
2D structure	Therapeutical agent
	**Name: *S*-Adenosylmethionine**; ademetionine; AdoMet; donamet; *S*-adenosyl-L-methionine; SAMe; methioninyladenylate; SAM-e; adenosylmethionine **Molecular formula:** $C_{15}H_{22}N_6O_5S$ **Molecular weight:** 398.44 g mol^{-1} **IUPAC name:** (2*S*)-2-Amino-4-[[(2*S*,3*S*,4*R*,5*R*)-5-(6-aminopurin-9-yl)-3,4-dihydroxyoxolan-2-yl]methyl-methylsulfonio]butanoate **Category:** Methyl radical donors **Mechanism:** HMT inhibitor **Targets:** HMT (KMT)

TABLE 6.9 Potential Epigenetic Drugs Modulating Histone Methylation—cont'd

2D structure	Therapeutical agent
	**Name: Chaetocin**; chaetocon from *Chaetomium minutum*; CHEMBL1089316; 28097-03-2; C30H28N6O6S4; (+)-chaetocin; SCHEMBL15791273 **Molecular formula:** $C_{30}H_{28}N_6O_6S_4$ **Molecular weight:** 696.83 g mol^{-1} **Category:** Mycotoxin antineoplastic agent **Mechanism:** HMT inhibitor **Targets:** HMT (KMT)
	**Name: BIX-01294**; BIX01294; 935693-62-2; BIX 01294; CHEMBL569864; *N*-(1-benzylpiperidin-4-yl)-6,7-dimethoxy-2-(4-methyl-1,4-diazepan-1-yl) quinazolin-4-amine **Molecular formula:** $C_{28}H_{38}N_6O_2$ **Molecular weight:** 490.65 g mol^{-1} **IUPAC name:** *N*-(1-Benzylpiperidin-4-yl)-6,7-dimethoxy-2-(4-methyl-1,4-diazepan-1-yl)quinazolin-4-amine **Category:** Heterocyclic compounds (quiazolines) **Mechanism:** HMT inhibitor **Targets:** H3K9
	**Name: UNC0224**; 1197196-48-7; CHEMBL576781; 7-[3-(dimethylamino)propoxy]-6-methoxy-2-(4-methyl-1,4-diazepan-1-yl)-*N*-(1-methylpiperidin-4-yl)quinazolin-4-amine **Molecular formula:** $C_{26}H_{43}N_7O_2$ **Molecular weight:** 485.68 g mol^{-1} **IUPAC name:** 7-[3-(Dimethylamino)propoxy]-6-methoxy-2-(4-methyl-1,4-diazepan-1-yl)-*N*-(1-methylpiperidin-4-yl)quinazolin-4-amine **Category:** Peptides **Mechanism:** HMT inhibitor **Targets:** G9a-HMT
	**Name: AMI-1**; 7,7′-(Carbonylbis(azanediyl))bis(4-oxidonaphthalene-2-sulfonate), sodium salt; arginine *N*-methylatransferase inhibitor-1 **Molecular formula:** $C_{12}H_{12}N_2Na_4O_9S_2$ **Molecular weight:** 592.41 g mol^{-1} **IUPAC name:** Disodium 4-hydroxy-7-[(5-hydroxy-7-sulfonatonaphthalen-2-yl)carbamoylamino]naphthalene-2-sulfonate **Category:** Small molecules **Mechanism:** HMT inhibitor **Targets:** HMT (KMT)

Continued

TABLE 6.9 Potential Epigenetic Drugs Modulating Histone Methylation—cont'd

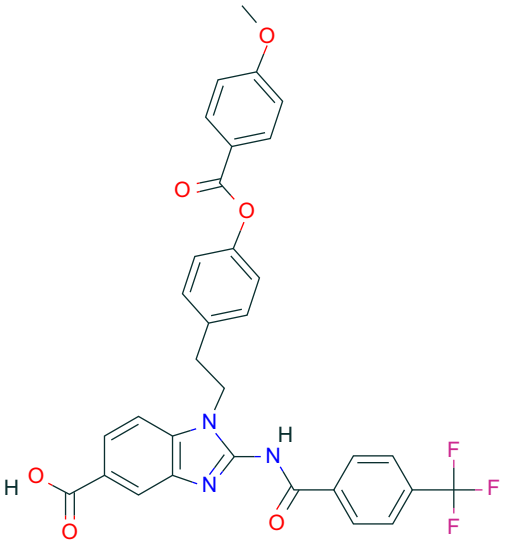
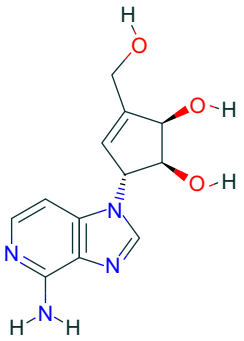
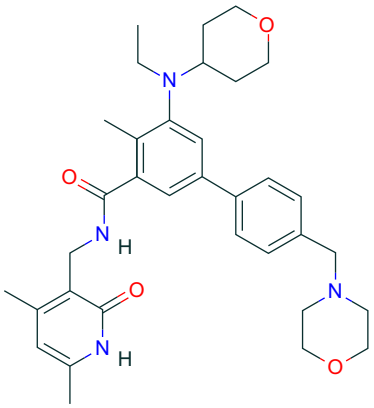
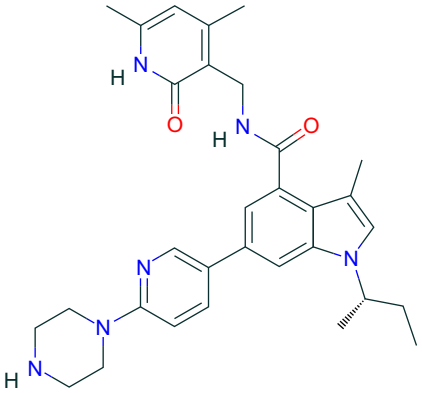
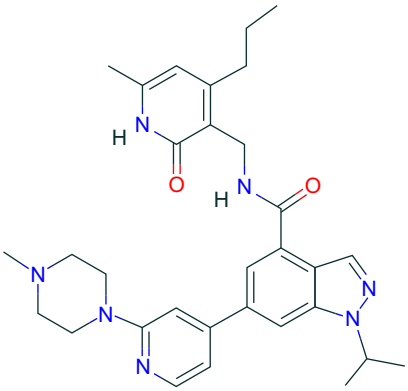
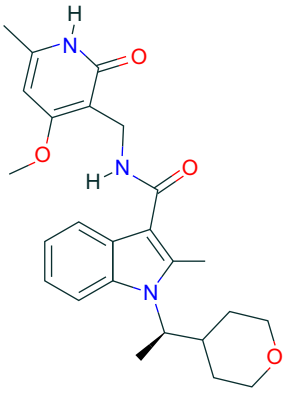
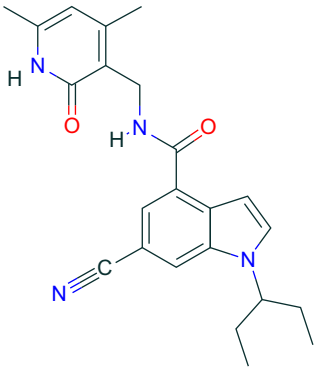
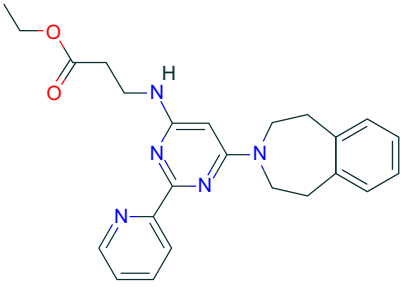
2D structure	Therapeutical agent
	**Name: BIX-01338**; ZINC72141366; 1-{2-[4-(4-Methoxybenzoyloxy)phenyl] ethyl}-2-(4-trifluoromethylbenzoylamino)-1*H*-benzoimidazole-5-carboxylic acid hydrate **Molecular formula:** $C_{32}H_{24}N_3F_3O_6$ **Molecular weight:** 603.55 g mol^{-1} **IUPAC name:** 1-[2-[4-(4-Methoxybenzoyl)oxyphenyl]ethyl]-2-[[4-(trifluoromethyl)benzoyl]amino]benzimidazole-5-carboxylic acid **Category:** Small molecules **Mechanism:** HMT inhibitor **Targets:** H3K9
	**Name: Deazaneplanocin A**; 7-deazanaplanocin A; 7-deaza NPA; BDBM50096907; (1*S*,2*R*,5*R*)-5-(4-aminopyrrolo[2,3-*d*]pyrimidin-7-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol **Molecular formula:** $C_{12}H_{14}N_4O_3$ **Molecular weight:** 262.27 g mol^{-1} **IUPAC name:** (1*S*,2*R*,5*R*)-5-(4-Aminoimidazo[4,5-*c*]pyridin-1-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol **Category:** EZH2 polycomb inhibitors **Mechanism:** HMT inhibitor **Targets:** HMT (KMT), SAH
	**Name: Tazemetostat**; EPZ-6438; 1403254-99-8; EPZ6438; UNII-Q40W93WPE1; EPZ 6438 **Molecular formula:** $C_{34}H_{44}N_4O_4$ **Molecular weight:** 572.74 g mol^{-1} **IUPAC name:** *N*-[(4,6-Dimethyl-2-oxo-1*H*-pyridin-3-yl)methyl]-3-[ethyl (oxan-4-yl)amino]-2-methyl-5-[4-(morpholin-4-ylmethyl)phenyl]benzamide **Category:** EZH2 polycomb inhibitors **Mechanism:** HMT inhibitor **Targets:** EZH2 (H3K27)
	**Name: GSK126**; 1346574-57-9; GSK-126; GSK 126; UNII-W4OGW9QZ97; W4OGW9QZ97 **Molecular formula:** $C_{31}H_{38}N_6O_2$ **Molecular weight:** 526.67 g mol^{-1} **IUPAC name:** 1-[(2*S*)-Butan-2-yl]-*N*-[(4,6-dimethyl-2-oxo-1*H*-pyridin-3-yl) methyl]-3-methyl-6-(6-piperazin-1-ylpyridin-3-yl)indole-4-carboxamide **Category:** EZH2 polycomb inhibitors **Mechanism:** HMT inhibitor **Targets:** HMT (KMT), SAH

TABLE 6.9 Potential Epigenetic Drugs Modulating Histone Methylation—cont'd

2D structure	Therapeutical agent
	**Name: GSK343**; 1346704-33-3; GSK 343; CHEMBL2204995; GSK-343; Compound 6 (PMID 24900432) **Molecular formula:** $C_{31}H_{39}N_7O_2$ **Molecular weight:** 541.69 g mol^{-1} **IUPAC name:** *N*-[(6-Methyl-2-oxo-4-propyl-1*H*-pyridin-3-yl)methyl]-6-[2-(4-methylpiperazin-1-yl)pyridin-4-yl]-1-propan-2-ylindazole-4-carboxamide **Category:** EZH2 polycomb inhibitors **Mechanism:** HMT inhibitor **Targets:** HMT (KMT), SAH
	**Name: CPI-360**; 1802175-06-9; SCHEMBL18463039; EX-A676; MolPort-039-193-848 **Molecular formula:** $C_{25}H_{31}N_3O_4$ **Molecular weight:** 437.53 g mol^{-1} **IUPAC name:** *N*-[(4-Methoxy-6-methyl-2-oxo-1*H*-pyridin-3-yl)methyl]-2-methyl-1-[(1*R*)-1-(oxan-4-yl)ethyl]indole-3-carboxamide **Category:** EZH2 polycomb inhibitors **Mechanism:** HMT inhibitor **Targets:** HMT (KMT), SAH
	**Name: EI1**; 1418308-27-6; KB-145943; 6-cyano-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-(pentan-3-yl)-1*H*-indole-4-carboxamide; D02CIT **Molecular formula:** $C_{23}H_{26}N_4O_2$ **Molecular weight:** 390.48 g mol^{-1} **IUPAC name:** 6-Cyano-*N*-[(4,6-dimethyl-2-oxo-1*H*-pyridin-3-yl)methyl]-1-pentan-3-ylindole-4-carboxamide **Category:** EZH2 polycomb inhibitors **Mechanism:** HMT inhibitor **Targets:** HMT (KMT), SAH
	**Name: GSK-J4**; GSK J4; 1373423-53-0; ethyl 3-((6-(4,5-dihydro-1*H*-benzo[*d*]azepin-3(2*H*)-yl)-2-(pyridin-2-yl)pyrimidin-4-yl)amino)propanoate **Molecular formula:** $C_{24}H_{27}N_5O_2$ **Molecular weight:** 417.50 g mol^{-1} **IUPAC name:** Ethyl 3-[[2-pyridin-2-yl-6-(1,2,4,5-tetrahydro-3-benzazepin-3-yl)pyrimidin-4-yl]amino]propanoate **Category:** Jumonji Fe^{2+}/α-ketoglutarate-dependent oxygenases **Mechanism:** HDM inhibitor **Targets:** H3K27

Continued

TABLE 6.9 Potential Epigenetic Drugs Modulating Histone Methylation—cont'd

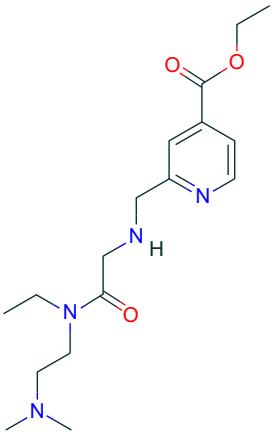
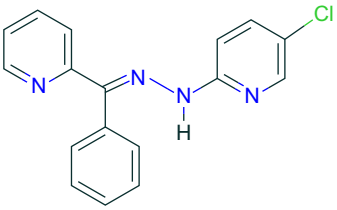
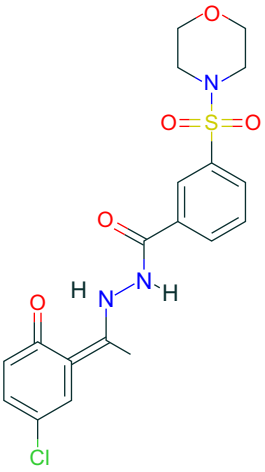
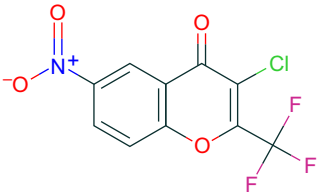
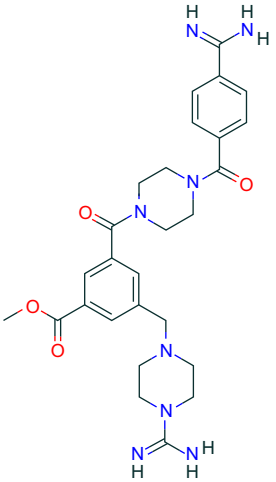
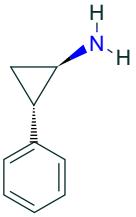
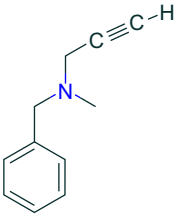
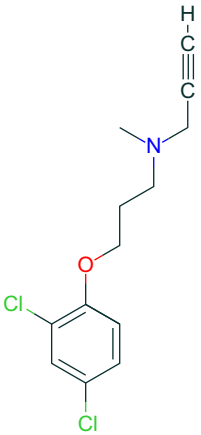
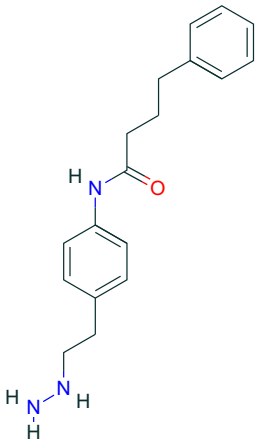
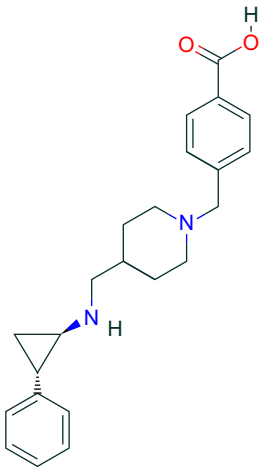
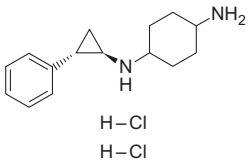
2D structure	Therapeutical agent
	**Name: KDM5-C70**; UNII-4288BE400F; 4288BE400F; 1596348-32-1; ethyl 2-[({2-[(2-(dimethylamino)ethyl)(ethyl)amino]-2-oxoethyl}amino)methyl] isonicotinate **Molecular formula:** $C_{17}H_{28}N_4O_3$ **Molecular weight:** 336.43 g mol^{-1} **IUPAC name:** Ethyl 2-[[[2-[2-(dimethylamino)ethyl-ethylamino]-2-oxoethyl] amino]methyl]pyridine-4-carboxylate **Category:** Jumonji Fe^{2+}/α-ketoglutarate-dependent oxygenases **Mechanism:** HDM inhibitor **Targets:** H3K4
	**Name: JIB-04**; 199596-05-9; NSC693627; (*E*)-5-chloro-2-(2-(phenyl(pyridin-2-yl)methylene)hydrazinyl)pyridine; JIB-04, NSC 693627 **Molecular formula:** $C_{17}H_{13}ClN_4$ **Molecular weight:** 308.76 g mol^{-1} **IUPAC name:** 5-Chloro-*N*-[(*E*)-[phenyl(pyridin-2-yl)methylidene]amino] pyridin-2-amine **Category:** Jumonji Fe^{2+}/α-ketoglutarate-dependent oxygenases **Mechanism:** HDM inhibitor **Targets:** Pan-Jumonji HDM inhibitor
	**Name: SP2509**; 1423715-09-6; C19H20ClN3O5S; (*E*)-*N*′-(1-(5-chloro-2-hydroxyphenyl)ethylidene)-3-(morpholinosulfonyl) benzohydrazide; SP-2509; SCHEMBL14696904 **Molecular formula:** $C_{19}H_{20}ClN_3O_5S$ **Molecular weight:** 337.90 g mol^{-1} **IUPAC name:** *N*′-[(1Z)-1-(3-chloro-6-oxocyclohexa-2,4-dien-1-ylidene)ethyl]-3-morpholin-4-ylsulfonylbenzohydrazide **Category:** Benzohydrazides **Mechanism:** HDM inhibitor **Targets:** LSD1
	**Name: Namoline**; 3-Chloro-6-nitro-2-(trifluoromethyl)chromen-4-one; namolin; AC1LEP3U; CHEMBL2426136; STOCK2S-44,578 **Molecular formula:** $C_{10}H_3ClF_3NO_4$ **Molecular weight:** 293.58 g mol^{-1} **IUPAC name:** 3-Chloro-6-nitro-2-(trifluoromethyl)chromen-4-one **Category:** Nonpeptide propargylamines **Mechanism:** HDM inhibitor **Targets:** LSD1
	Name: CBB1007; 1379573-92-8; SCHEMBL16273312; AKOS030526465; CBB 1007; CBB-1007; ZINC205876820 **Molecular formula:** $C_{27}H_{34}N_8O_4$ **Molecular weight:** 534.62 g mol^{-1} **IUPAC name:** Methyl 3-[4-(4-carbamimidoylbenzoyl)piperazine-1-carbonyl]-5-[(4-carbamimidoylpiperazin-1-yl)methyl]benzoate

TABLE 6.9 Potential Epigenetic Drugs Modulating Histone Methylation—cont'd

2D structure	Therapeutical agent
	**Category:** Nonpeptide chemical scaffolds **Mechanism:** HDM inhibitor **Targets:** LSD1
	**Name: Tranylcypromine**; transamine; parnate; PCPA; DL-tranylcypromine; tranylcypromine (INN); AC1L9ASR; (1*R*)-2-phenylcyclopropan-1-amine; DB00752; C07155 **Molecular formula:** $C_9H_{11}N$ **Molecular weight:** 133.19 g mol^{-1} **IUPAC name:** (1*R*,2*S*)-2-Phenylcyclopropan-1-amine **Category:** MAO inhibitors **Mechanism:** HDM inhibitor **Targets:** LSD1, H3K4, H3K9
	**Name: Pargyline**; 555-57-7; pargylamine; *N*-methyl-*N*-propargylbenzylamine; paragyline; eudatin **Molecular formula:** $C_{11}H_{13}N$ **Molecular weight:** 159.23 g mol^{-1} **IUPAC name:** *N*-Benzyl-*N*-methylprop-2-yn-1-amine **Category:** MAO inhibitors **Mechanism:** HDM inhibitor **Targets:** LSD1
	**Name: Clorgyline**; clorgiline; chlorgyline; clorgilina; clorgilinum; M and B 9302 **Molecular formula:** $C_{13}H_{15}Cl_2NO$ **Molecular weight:** 272.17 g mol^{-1} **IUPAC name:** 3-(2,4-Dichlorophenoxy)-*N*-methyl-*N*-prop-2-ynylpropan-1-amine **Category:** MAO inhibitors **Mechanism:** HDM inhibitor **Targets:** LSD1

Continued

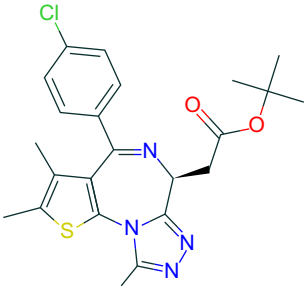
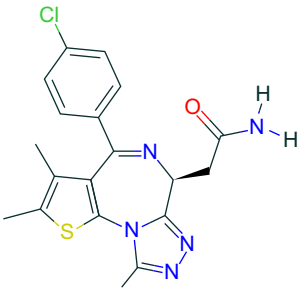
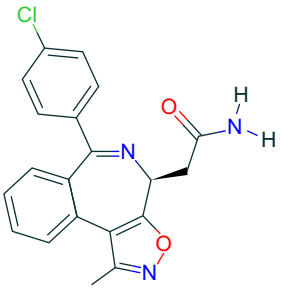
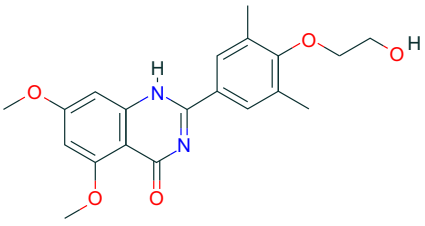
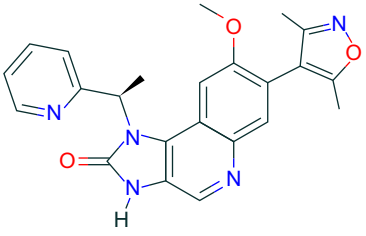
TABLE 6.9 Potential Epigenetic Drugs Modulating Histone Methylation—cont'd

2D structure	Therapeutical agent
	**Name: Bizine**; *N*-[4-(2-Hydrazinylethyl)phenyl]-4-phenylbutanamide; CHEMBL3785141; MolPort-044-561-629; BDBM113742; ZINC169621139 **Molecular formula:** $C_{18}H_2N_3O$ **Molecular weight:** 297.40 g mol^{-1} **IUPAC name:** *N*-[4-(2-Hydrazinylethyl)phenyl]-4-phenylbutanamide **Category:** MAO inhibitors **Mechanism:** HDM inhibitor **Targets:** LSD1
	**Name: GSK2879552**; 4-[[4-[[[(1*R*,2*S*)-2-Phenylcyclopropyl] amino]methyl] piperidin-1-yl]methyl]benzoic acid; 1401966-69-5; UNII-GT77Z6Y09Z; CHEMBL3786182 **Molecular formula:** $C_{23}H_{28}N_2O_2$ **Molecular weight:** 364.48 g mol^{-1} **IUPAC name:** 4-[[4-[[[(1*R*,2*S*)-2-Phenylcyclopropyl]amino]methyl] piperidin-1-yl]methyl]benzoic acid **Category:** MAO inhibitors **Mechanism:** HDM inhibitor **Targets:** LSD1
	**Name: ORY1001**; RG6016 **Molecular formula:** $C_{15}H_{22}N_2$ **Molecular weight:** 303.27 g mol^{-1} **IUPAC name:** 1,4-Cyclohexanediamine-*N*1-[(1*R*,2*S*)-2-phenylcyclopropyl]-hydrochloride (1:2), rel **Category:** MAO inhibitors **Mechanism:** HDM inhibitor **Targets:** LSD1

EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; *HDM*, histone demethylase; *HMT (KMT)*, histone methyltransferases; *LSD1*, lysine-specific histone demethylase-1; *MAO*, monoamine oxidases; *SAH*, *S*-adenosyl-L-homocysteine hydrolase; *SAMe*, *S*-adenosylmethionine.

benzamides, ketones, small molecules, quinoline-3-carboxamides, carbamates, hybrid compounds (pazopanib hybrids, dual indoleamine 2,3-dioxygenase 1 (IDO1) and histone deacetylase (HDAC) inhibitors, dual nicotinamide phosphoribosyltransferase (NAMPT) and histone deacetylase (HDAC) inhibitors, HDACi MS-275 + NO donors, polyamine-based HDACs-LSD1 dual binding inhibitors, dual G9a and HDAC inhibitors, triple inhibitors), sirtuin inhibitors and sirtuin activators (Table 6.7), and many other compounds (Tables 6.4 and 6.6); (iv) histone acetyltransferase (HAT) inhibitors (Table 6.8): these drugs may target many different proteins associated with the histone lysine acetyltransferase machinery (i.e., general control nonderepressible 5 (GCN5) KAT2A/GCN5, KAT2B/PCAF, KAT6–8, K (lysine) acetyltransferase 8 (KAT8, MOF), CREBBP/CBP, EP300-thymine DNA glycosylase (TDG), monocytic leukemia zinc finger protein-related factor (MORF), PHF20, ATP citrate lyase (ACL), super elongation complex (SEC), multiprotein histone acetyltransferase (HAT) complex: HBO1, inhibitor of growth family member (ING) 4/5, MYST/Esa1-associated factor (MEAF) 6, Jade family PHD finger (JADE) 1/2/3, bromodomain and PHD finger-containing protein (BRPF) 2/3; FATp300 inhibitor) (Tables 6.1, 6.4, and 6.8); (v) histone methyltransferase (HMT)

TABLE 6.10 Potential Bromodomain Inhibitors

2D structure	Therapeutical agent
	**Name: JQ-1**; (+)-JQ-1; JQ1 compound; UNII-1MRH0IMX0W **Molecular formula:** $C_{23}H_{25}ClN_4O_2S$ **Molecular weight:** 456.99 g mol^{-1} **Category:** Small molecules (PAHi) **Mechanism:** Bromodomain inhibitors **Targets:** Acetylated histones, BET
	**Name: CPI-203**; 1446144-04-2; CPI203; C19H18ClN5OS; (6*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine-6-acetamide **Molecular formula:** $C_{18}H_{18}ClN_5OS$ **Molecular weight:** 399.90 g mol^{-1} **IUPAC name:** (6*S*)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine-6-acetamide **Category:** Small molecules (PAHi) **Mechanism:** Bromodomain inhibitors **Targets:** Acetylated histones, BET
	**Name: CPI-0610**; 1380087-89-7; CPI-0610 anhydrous; UNII-U4017GUQ06; U4017GUQ06; GTPL9120 **Molecular formula:** $C_{20}H_{16}ClN_3O_2$ **Molecular weight:** 365.82 g mol^{-1} **IUPAC name:** 2-[(4*S*)-6-(4-Chlorophenyl)-1-methyl-4*H*-[1,2]oxazolo[5,4-*d*][2]benzazepin-4-yl]acetamide **Category:** Small molecules (PAHi) **Mechanism:** Bromodomain inhibitors **Targets:** Acetylated histones, BET
	**Name: RVX-208**; 1044870-39-4; apabetalone; 2-(4-(2-hydroxyethoxy)-3,5-dimethylphenyl)-5,7-dimethoxyquinazolin-4(3*H*)-one; RVX 208; RVX-000222 **Molecular formula:** $C_{20}H_{22}N_2O_5$ **Molecular weight:** 370.40 g mol^{-1} **IUPAC name:** 2-[4-(2-Hydroxyethoxy)-3,5-dimethylphenyl]-5,7-dimethoxy-1*H*-quinazolin-4-one **Category:** Small molecules (PAHi) **Mechanism:** Bromodomain inhibitors **Targets:** Acetylated histones, BET
	**Name: I-BET151 (GSK1210151A)**; 7-(3,5-dimethyl-4-isoxazolyl)-1,3-dihydro-8-methoxy-1-[(1*R*)-1-(2-pyridinyl)ethyl]-3*H*-imidazo[4,5-*c*]quinolin-2-one **Molecular formula:** $C_{23}H_{21}N_5O_3$ **Molecular weight:** 415.44 g mol^{-1} **IUPAC name:** 7-(3,5-Dimethyl-1,2-oxazol-4-yl)-8-methoxy-1-[(1*R*)-1-pyridin-2-ylethyl]-3*H*-imidazo[4,5-*c*]quinolin-2-one **Category:** Small molecules (PAHi) **Mechanism:** Bromodomain inhibitors **Targets:** Acetylated histones, BET

Continued

TABLE 6.10 Potential Bromodomain Inhibitors—cont'd

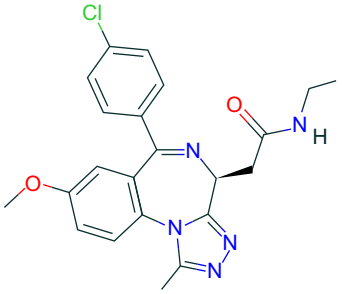
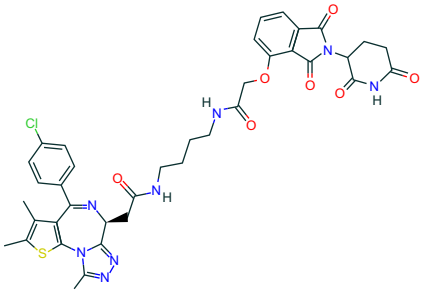
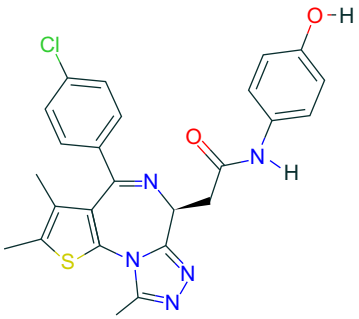
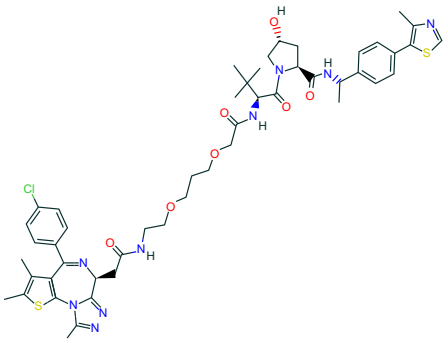
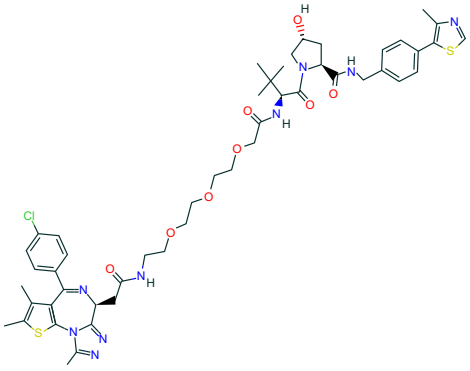
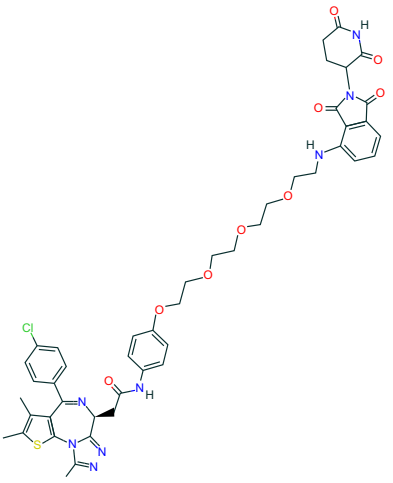
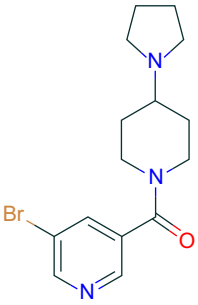
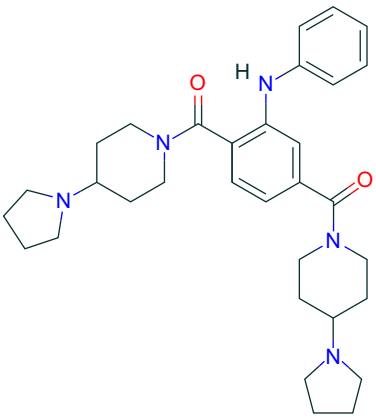
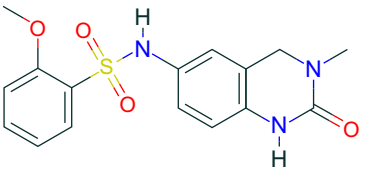
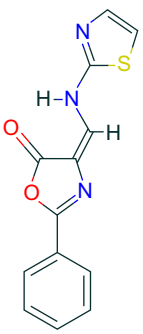
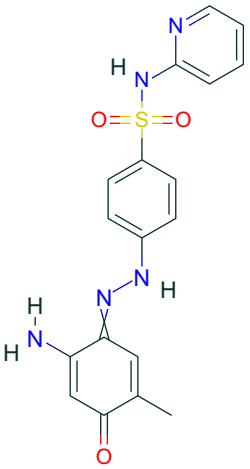
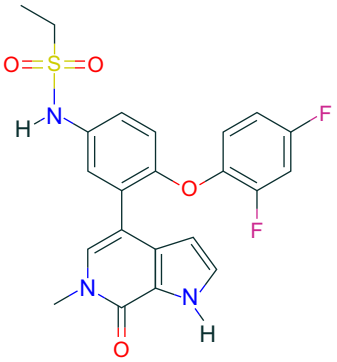
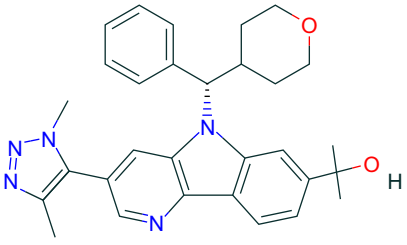
2D structure	Therapeutical agent
	**Name: IBET-762**; molibresib; GSK525762A; 1260907-17-2 **Molecular formula:** $C_{22}H_{22}ClN_5O_2$ **Molecular weight:** 426.90 g mol^{-1} **IUPAC name:** 2-[(4*S*)-6-(4-Chlorophenyl)-8-methoxy-1-methyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]-*N*-ethylacetamide **Category:** Small molecules (PAHi) **Mechanism:** Bromodomain inhibitors **Targets:** Acetylated histones, BET
	**Name: dBET1**; 1799711-21-9; SCHEMBL17553399; MolPort-044-561-471; AKOS032962867; HY-101838 **Molecular formula:** $C_{38}H_{37}ClN_8O_7S$ **Molecular weight:** 426.90 g mol^{-1} **Category:** Small molecules (PAHi) **Mechanism:** Bromodomain inhibitors **Targets:** Acetylated histones, BET
	**Name: OTX-015**; 202590-98-5; birabresib; OTX015; MK-8628; OTX 015 **Molecular formula:** $C_{25}H_{22}ClN_5O_2S$ **Molecular weight:** 491.99 g mol^{-1} **Category:** Small molecules (PAHi) **Mechanism:** Bromodomain inhibitors **Targets:** Acetylated histones, BRD2, BRD3, BRD4
	**Name: ARV-771**; 1949837-12-0; SCHEMBL18551355; ZINC616573431; CS-6389; HY-100972 **Molecular formula:** $C_{49}H_{60}ClN_9O_{72}$ **Molecular weight:** 986.65 g mol^{-1} **Category:** Small molecules (PAHi) **Mechanism:** Bromodomain inhibitors **Targets:** Acetylated histones, BRD4
	**Name: MZ 1**; HY-107425; 1797406-69-9; SCHEMBL18076362; AKOS032947179 **Molecular formula:** $C_{49}H_{60}ClN_9O_{82}$ **Molecular weight:** 1002.64 g mol^{-1} **Category:** Small molecules (PAHi) **Mechanism:** Bromodomain inhibitors **Targets:** Acetylated histones, BRD4

TABLE 6.10 Potential Bromodomain Inhibitors—cont'd

2D structure	Therapeutical agent
	**Name: ARV-825**; 1818885-28-7; SCHEMBL17162093; RWLOGRLTDKDANT-TYIYNAFKSA-N; MolPort-044-561-811; AKOS030526673 **Molecular formula:** $C_{46}H_{47}ClN_8O_9S$ **Molecular weight:** 923.44 g mol^{-1} **Category:** Small molecules (PAHi) **Mechanism:** Bromodomain inhibitors **Targets:** Acetylated histones, BRD4
	**Name: UNC-669**; CHEMBL1235119; (5-bromopyridin-3-yl)(4-(pyrrolidin-1-yl)piperidin-1-yl) methanone **Molecular formula:** $C_{15}H_{20}BrN_3O$ **Molecular weight:** 338.24 g mol^{-1} **IUPAC name:** (5-Bromopyridin-3-yl)(4-(pyrrolidin-1-yl)piperidin-1-yl)methanone **Category:** Small molecules (PAMi) **Mechanism:** Bromodomain inhibitors **Targets:** Methylated histones, BET
	**Name: UNC-1215**; 1415800-43-9; CHEMBL2426364; (2-(phenylamino)-1,4-phenylene)bis((4-(pyrrolidin-1-yl)piperidin-1-yl)methanone) **Molecular formula:** $C_{32}H_{43}N_5O_2$ **Molecular weight:** 529.72 g mol^{-1} **IUPAC name:** (2-(phenylamino)-1,4-phenylene)bis((4-(pyrrolidin-1-yl)piperidin-1-yl) methanone) **Category:** Small molecules (PAMi) **Mechanism:** Bromodomain inhibitors **Targets:** Methylated histones, BET
	**Name: PFI-1**; 1403764-72-6; PFI-1 (PF-6405761); QCR-192; 2-methoxy-*N*-(3-methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)benzenesulfonamide; PF-06405761 **Molecular formula:** $C_{16}H_{17}N_3O_4S$ **Molecular weight:** 347.39 g mol^{-1} **IUPAC name:** Methoxy-*N*-(3-methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl) benzenesulfonamide **Category:** Small molecules **Mechanism:** Bromodomain inhibitors **Targets:** BRD2, BRD4
	**Name: 9F-913**; AKOS005104753; ZINC100438770; 9F-913; (4*E*)-2-phenyl-4-[(1,3-thiazol-2-ylamino)methylidene]-1,3-oxazol-5-one; MolPort-002-882-077; 78666-78-1 **Molecular formula:** $C_{13}H_9N_3O_2S$ **Molecular weight:** 271.29 g mol^{-1} **IUPAC name:** (4*E*)-2-Phenyl-4-[(1,3-thiazol-2-ylamino) methylidene]-1,3-oxazol-5-one **Category:** Small molecules **Mechanism:** Bromodomain inhibitors **Targets:** BRD4

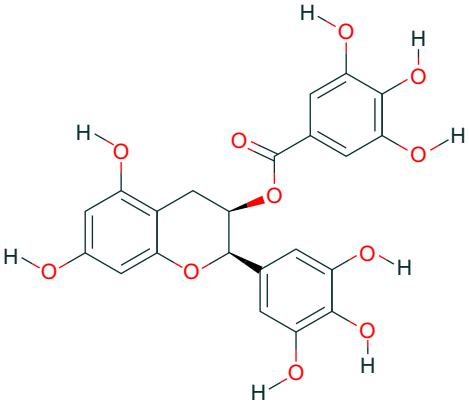
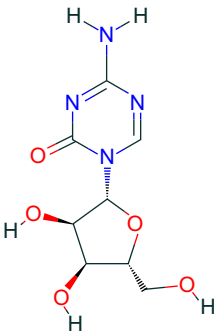
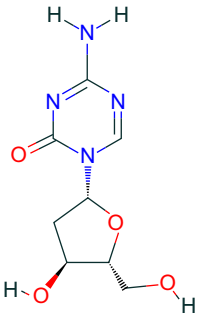
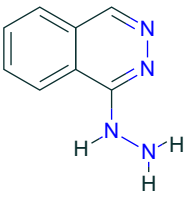
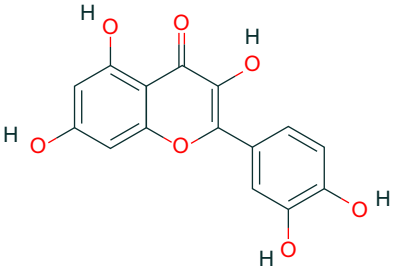
Continued

TABLE 6.10 Potential Bromodomain Inhibitors—cont'd

2D structure	Therapeutical agent
	**Name: MS436**; 1395084-25-9; MS 436; MS-436; 4-[2-(2-amino-5-methyl-4-oxocyclohexa-2,5-dien-1-ylidene)hydrazinyl]-*N*-pyridin-2-ylbenzenesulfonamide; GTPL7509 **Molecular formula:** $C_{18}H_{17}N_5O_3S$ **Molecular weight:** 383.43 g mol^{-1} **IUPAC name:** 4-[2-(2-Amino-5-methyl-4-oxocyclohexa-2,5-dien-1-ylidene)hydrazinyl]-*N*-pyridin-2-ylbenzenesulfonamide **Category:** Small molecules **Mechanism:** Bromodomain inhibitors **Targets:** BRD4
	**Name: Mivebresib**; 1445993-26-9; ABBV-075; UNII-VR86R11J7J; VR86R11J7J; *N*-(4-(2,4-difluorophenoxy)-3-(6-methyl-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)phenyl) ethanesulfonamide **Molecular formula:** $C_{22}H_{19}F_2N_3O_4S$ **Molecular weight:** 459.47 g mol^{-1} **IUPAC name:** *N*-(4-(2,4-Difluorophenoxy)-3-(6-methyl-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*] pyridin-4-yl)phenyl)ethanesulfonamide **Category:** Small molecules **Mechanism:** Bromodomain inhibitors **Targets:** BET
	**Name: BMS-986158**; 1800340-40-2; SCHEMBL16861831; KGERZPVQIRYWRK-GDLZYMKVSA-N; CS-7497; HY-101567 **Molecular formula:** $C_{30}H_{33}N_5O_2$ **Molecular weight:** 495.63 g mol^{-1} **IUPAC name:** 2-[3-(3,5-Dimethyltriazol-4-yl)-5-[(*S*)-oxan-4-yl(phenyl)methyl]pyrido[3,2-*b*] indol-7-yl]propan-2-ol **Category:** Small molecules **Mechanism:** Bromodomain inhibitors **Targets:** BET

BET, bromodomain and extraterminal proteins; *BRD*, bromodomain proteins; *PAHi*, inhibitors of protein binding to acetylated histones; *PAMi*, inhibitors of protein binding to methylated histones.

TABLE 6.11 Most Relevant Epidrugs Currently Submitted or Approved in Clinical Trials

Drug	Properties	Indication
	**Name: Epigallocatechin 3-gallate, EGCG**; (−)-epigallocatechin gallate, tea catechin, teavigo, catechin deriv., 989-51-5 **IUPAC name:** [(2*R*,3*R*)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-chromen-3-yl] 3,4,5-trihydroxybenzoate **Molecular formula:** $C_{22}H_{18}O_{11}$ **Molecular weight:** 458.37 g mol^{-1} **Mechanism:** DNA methyltransferase inhibitor **Targets:** DNMT1	• Alzheimer disease • Huntington disease • Diabetic nephropathy • Down syndrome • Fragile X • Influenza infection • Obesity • Light chain amyloidosis
	**Name: 5-Azacytidine**; azacitidine; azacytidine; ladakamycin; vidaza; mylosar; azacitidinum; 5-AZAC **IUPAC name:** 4-Amino-1-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3,5-triazin-2-one **Molecular formula:** $C_8H_{12}N_4O_5$ **Molecular weight:** 244.20 g mol^{-1} **Mechanism:** DNA methyltransferase inhibitor **Targets:** DNMT1	• Myelodysplastic syndrome • Various leukemias • Solid tumors
	**Name: Decitabine**; 5-Aza-2′-deoxycytidine; dacogen; dezocitidine; 2′-deoxy-5-azacytidine **IUPAC name:** 4-Amino-1-[(2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3, 5-triazin-2-one **Molecular formula:** $C_8H_{12}N_4O_4$ **Molecular weight:** 228.21 g mol^{-1} **Mechanism:** DNA methyltransferase inhibitor **Targets:** DNMT1	• Acute myeloid leukemia • Myelodysplastic syndrome • Other cancers
	**Name: Hydralazine**; 1-hydrazinophthlazine; hydrazinophthalazine; apresolin; apresoline; hydralazin; hypophthalin; apressin; aprezolin **IUPAC name:** Phthalazin-1-ylhydrazine **Molecular formula:** $C_8H_8N_4$ **Molecular weight:** 160.18 g mol^{-1} **Mechanism:** DNA methyltransferase inhibitor **Targets:** DNMT1	• Advanced cervical cancer • Lung cancer • Breast cancer • Congestive heart failure • Hypertension
	**Name: Quercetin**; sophoretin; quercetol; meletin; xanthaurine; quercitin; 3,3′,4′,5,7-pentahydroxyflavone **IUPAC name:** 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one **Molecular formula:** $C_{15}H_{10}O_7$ **Molecular weight:** 302.24 g mol^{-1} **Mechanism:** DNMT inhibitor **Targets:** DNMT1	• Chronic obstructive pulmonary disease • Stroke • Chronic hepatitis • Diabetes mellitus (type II) • Cystic fibrosis • Sarcoidosis • Autism spectrum disorders • Colon cancer

Continued

TABLE 6.11 Most Relevant Epidrugs Currently Submitted or Approved in Clinical Trials—cont'd

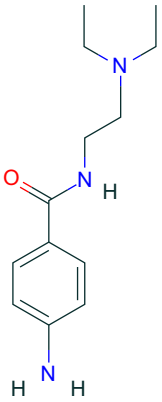
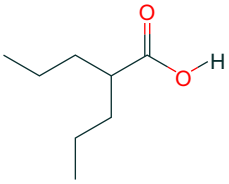
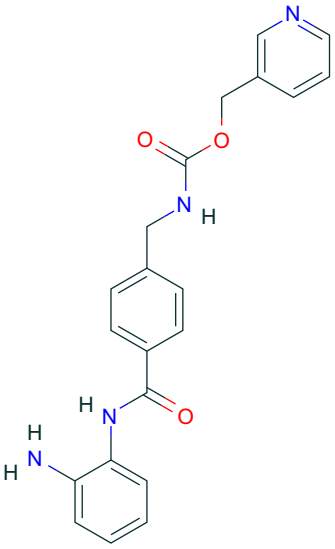
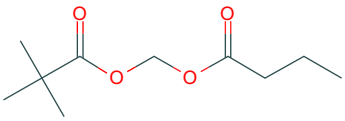
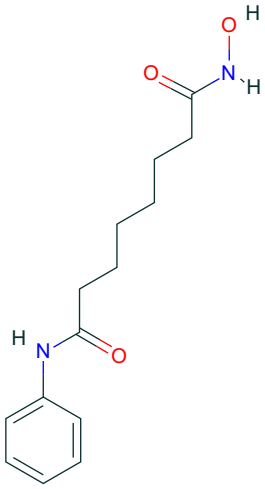
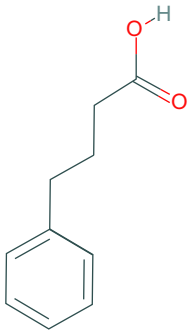
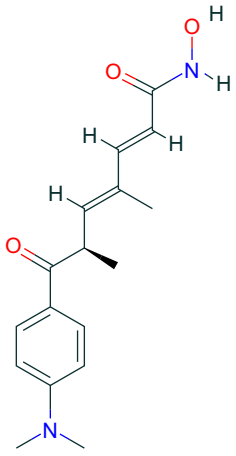
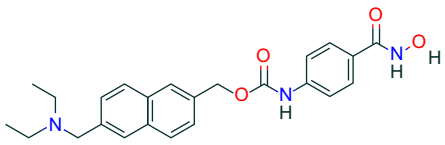
Drug	Properties	Indication
	**Name: Procainamide**; novocainamide; biocoryl; novocamid; procamide; procaine amide; pronestyl; novocainamid; novocaine amide; procan **IUPAC name: 4-amino-*N*-(2-diethylaminoethyl) benzamide** **Molecular formula:** $C_{13}H_{21}N_3O$ **Molecular weight:** 235.33 g mol^{-1} **Mechanism:** DNMT inhibitor **Targets:** DNMT1	• Arrhythmia • Cardiovascular diseases • Death, sudden, cardiac
	**Name: Valproic acid**; 2-propylpentanoic acid, depakene; depakine; ergenyl; dipropylacetic acid; mylproin; convulex; myproic acid **IUPAC name:** 2-Propylpentanoic acid **Molecular formula:** $C_8H_{16}O_2$ **Molecular weight:** 144.2 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDAC (HDAC1, 2, 3, 8)	• Advanced cancers • Autoimmune diseases • Renitis pigmentosa • Epilepsy • Bipolar disorder • Alzheimer disease • Amyotrophic lateral sclerosis
	**Name: Entinostat**; ms-275; 209783-80-2; SNDX-275; MS 275; MS-27-275; SNDX 275; histone deacetylase inhibitor I; S1053_Selleck; MS 27-275 **IUPAC name:** Pyridin-3-ylmethyl *N*-[[4-[(2-aminophenyl) carbamoyl]phenyl]methyl]carbamate **Molecular formula:** $C_{21}H_{20}N_4O_3$ **Molecular weight:** 376.41 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDAC (HDAC1, 2, 3)	• Hodgkin lymphoma • Renal cell carcinoma • Advanced breast cancer • Lung cancer
	**Name: Pivanex**; AN-9; pivalyloxymethyl butyrate; AN 9; 122110-53-6; BRN 4861411; ((2,2-dimethylpropanoyl)oxy) methyl butanoate **IUPAC name:** Butanoyloxymethyl 2,2-dimethylpropanoate **Molecular formula:** $C_{10}H_{18}O_4$ **Molecular weight:** 202.25 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDAC (HDAC1, 2, 3, 8)	• Melanoma • Nonsmall-cell lung cancer • Chronic lymphocytic leukemia • Small lymphocytic lymphoma • Heart failure • Insomnia • Depression • Multiple sclerosis
	Name: Vorinostat; suberoylanilide hydroxamic acid (SAHA); zolinza; suberanilohydroxamic acid; 149647-78-9; *N*-hydroxy-*N*′-phenyloctanediamide; SAHA cpd **IUPAC name:** *N*′-Hydroxy-*N*-phenyloctanediamide	• Acute myeloid leukemia • Melanoma • Pancreatic cancer

TABLE 6.11 Most Relevant Epidrugs Currently Submitted or Approved in Clinical Trials—cont'd

Drug	Properties	Indication
	**Molecular formula:** $C_{14}H_{20}N_2O_3$ **Molecular weight:** 264.32 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDAC (HDAC1, 2, 3, 8); Class IIb (HDAC6)	• Ovarian cancer • Multiple oncologic lesions
	**Name: Sodium phenylbutyrate**; buphenyl; 4-phenylbutyric acid; 4-phenylbutanoic acid; benzenebutanoic acid; benzenebutyric acid; butyric acid; 1821-12-1; γ-phenylbutyric acid, **IUPAC name:** 4-Phenylbutanoic acid **Molecular formula:** $C_{10}H_{12}O_2$ **Molecular weight:** 164.20 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3, 8); Class IIa HDACs (HDAC4,5,7,9); Class IIb HDACs (HDAC6,10)	• Parkinson disease • Huntington disease • Amyotrophic lateral sclerosis • Spinal muscular atrophy • Urea cycle disorders • Pulmonary tuberculosis • HIV infection • Diabetes (insulin resistance) • Lymphoma • Cystic fibrosis • Various cancers
	**Name: Trichostatin A**; 58880-19-6; trichostatin A (TSA); CHEBI:46024; TSA; 2,4-heptadienamide; 7-(4-(dimethylamino) phenyl)-*N*-hydroxy-4,6-dimethyl-7-oxo-7-(4-(dimethylamino) phenyl)-*N*-hydroxy-4,6-dimethyl-7-oxo-2,4-heptadienamide; [*R*-(*E*,*E*)]-7-[4-(dimethylamino)phenyl]-*N*-hydroxy-4,6-dimethyl-7-oxo-2,4-heptadienamide **IUPAC name:** (2*E*,4*E*,6*R*)-7-[4-(Dimethylamino)phenyl]-*N*-hydroxy-4,6-dimethyl-7-oxohepta-2,4-dienamide **Molecular formula:** $C_{17}H_{22}N_2O_3$ **Molecular weight:** 302.37 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3); Class IIa HDACs (HDAC4, 7, 9); Class IIb HDACs (HDAC6)	• Cardiovascular disorders • Obesity • Overactive bladder • HIV infection • Chronic myelogenous leukemia • Myelodysplastic syndrome • Hepatitis B infection • Stroke • Major depressive disorder
	**Name: Givinostat**; ITF2357; ITF-2357; UNII-5P60F84FBH; 497833-27-9; CHEMBL1213492; [6-(diethylaminomethyl)-2-naphthyl]methyl *N*-[4-(hydroxycarbamoyl)phenyl]carbamate **IUPAC name:** [6-(Diethylaminomethyl)naphthalen-2-yl]methyl *N*-[4-(hydroxycarbamoyl)phenyl]carbamate **Molecular formula:** $C_{24}H_{27}N_3O_4$ **Molecular weight:** 421.50 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3); Class IIa HDACs (HDAC4, 7, 9); Class IIb HDACs (HDAC6)	• Tymoma • Polycythemia vera • Duchenne muscular dystrophy (DMD) • Juvenile idiopathic arthritis • Crohn disease • Multiple myeloma • Myeloproliferative diseases • Hodgkin lymphoma

Continued

TABLE 6.11 Most Relevant Epidrugs Currently Submitted or Approved in Clinical Trials—cont'd

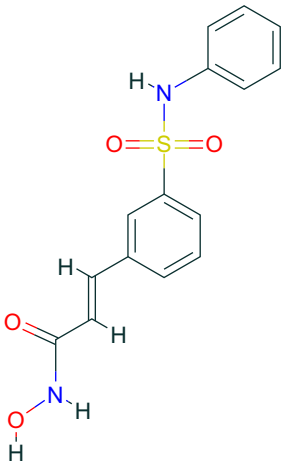
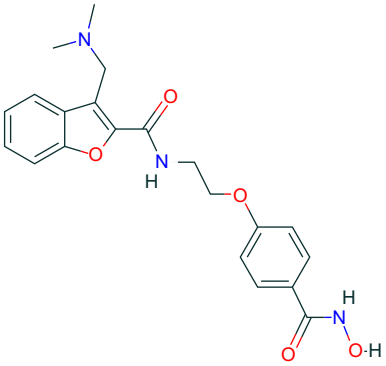
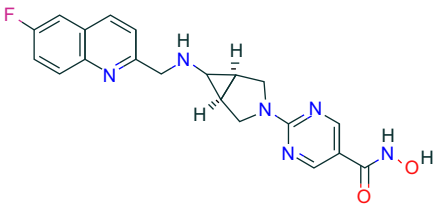
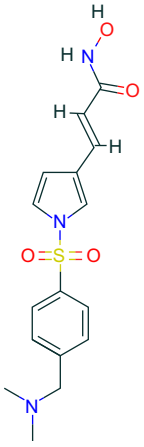
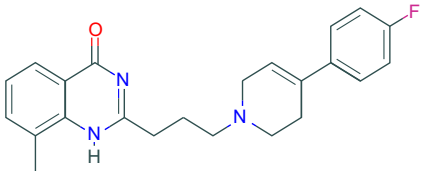
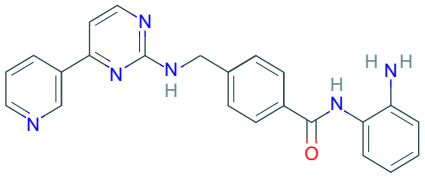
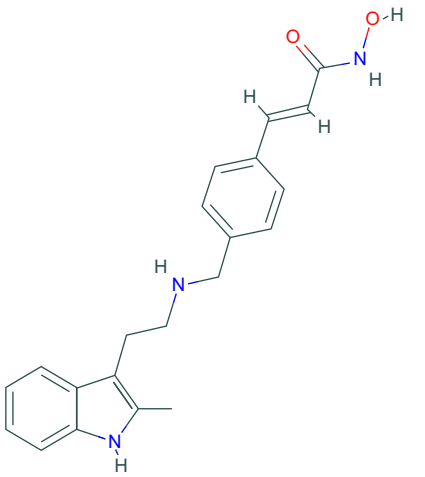
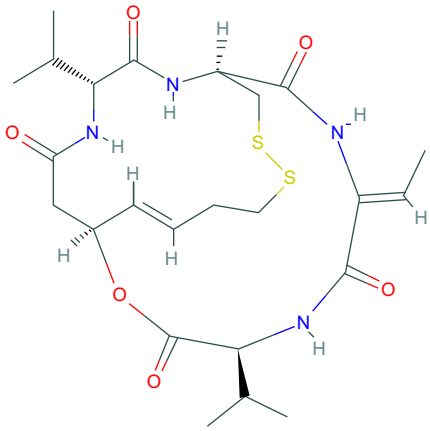
Drug	Properties	Indication
	**Name: Belinostat**; 414864-00-9; PXD101; PXD-101; Belinostat (PXD101); UNII-F4H96P17NZ; (*E*)-*N*-hydroxy-3-(3-(*N*-phenylsulfamoyl)phenyl)acrylamide **IUPAC name:** (*E*)-*N*-Hydroxy-3-[3-(phenylsulfamoyl)phenyl] prop-2-enamide **Molecular formula:** $C_{15}H_{14}N_2O_4S$ **Molecular weight:** 318.35 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3); Class IIa HDACs (HDAC4, 7, 9); Class IIb HDACs (HDAC6)	• Refractory T cell lymphomas • Non-Hodgkin lymphoma • Acute myeloid leukemia • Ovarian cancer • Soft-tissue sarcomas • Nonsmall-cell lung cancer • Solid tumors
	**Name: Abexinostat**; PCI-24781; 783355-60-2; CRA-024781; CRA 024781; 3-((dimethylamino)methyl)-*N*-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl)benzofuran-2-carboxamide **IUPAC name:** 3-[(Dimethylamino)methyl]-*N*-[2-[4-(hydroxycarbamoyl)phenoxy]ethyl]-1-benzofuran-2-carboxamide **Molecular formula:** $C_{21}H_{23}N_3O_5$ **Molecular weight:** 397.43 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3); Class IIa HDACs (HDAC4, 7, 9); Class IIb HDACs (HDAC6)	• Follicular lymphoma • Hematologic neoplasms • Hodgkin lymphoma • Non-Hodgkin lymphoma
	**Name: CHR-3996**; BDBM50347385; GTPL8391; 2-[(1*R*,5*S*,6*R*)-6-{[(6-fluoroquinolin-2-yl)methyl]amino}-3-azabicyclo[3.1.0] hexan-3-yl]-*N*-hydroxypyrimidine-5-carboxamide **IUPAC name:** 2-[(1*R*,5*S*)-6-[(6-Fluoroquinolin-2-yl) methylamino]-3-azabicyclo[3.1.0]hexan-3-yl]-*N*-hydroxypyrimidine-5-carboxamide **Molecular formula:** $C_{20}H_{19}FN_6O_2$ **Molecular weight:** 394.41 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3); Class IIa HDACs (HDAC4, 7, 9); Class IIb HDACs (HDAC6)	Advanced solid tumors
	**Name: Resminostat**; 864814-88-0; 4SC-201; resminostat (RAS2410); UNII-1578EUB98L; RAS2410; (E)-3-(1-((4-((dimethylamino)methyl)phenyl)sulfonyl)-1*H*-pyrrol-3-yl)-*N*-hydroxyacrylamide **IUPAC name:** (*E*)-3-[1-[4-[(Dimethylamino)methyl]phenyl] sulfonylpyrrol-3-yl]-*N*-hydroxyprop-2-enamide **Molecular formula:** $C_{16}H_{19}N_3O_4S$ **Molecular weight:** 349.40 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3); Class IIa HDACs (HDAC4, 7, 9); Class IIb HDACs (HDAC6)	• Advanced colorectal cancer • Hepatocellular carcinoma • Hodgkin lymphoma

TABLE 6.11 Most Relevant Epidrugs Currently Submitted or Approved in Clinical Trials—cont'd

Drug	Properties	Indication
	**Name: EVP-0334**; FRM-0334; 2-{3-[4-(4-fluorophenyl)-3,6-dihydro-1(2*H*)-pyridinyl]propyl}-8-methyl-4(3*H*)-quinazolinone; AC1L9MGN; quinazolinone analog 1e **IUPAC name:** 2-[3-[4-(4-Fluorophenyl)-3,6-dihydro-2*H*-pyridin-1-yl]propyl]-8-methyl-1*H*-quinazolin-4-one **Molecular formula:** $C_{23}H_{24}FN_3O$ **Molecular weight:** 377.46 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3); Class IIa HDACs (HDAC4, 7, 9); Class IIb HDACs (HDAC6)	• Frontotemporal dementia with granulin (*GRN*) mutation
	**Name: Mocetinostat**; MGCD0103; 726169-73-9; MGCD-0103; MGCD 0103; *N*-(2-aminophenyl)-4-([[4-(pyridin-3-yl) pyrimidin-2 yl]amino]methyl)benzamide **IUPAC name:** *N*-(2-Aminophenyl)-4-[[(4-pyridin-3-ylpyrimidin-2-yl)amino]methyl] benzamide **Molecular formula:** $C_{23}H_{20}N_6O$ **Molecular weight:** 396.44 g mol^{-1} **Mechanism:** HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3); Class IV HDACs (HDAC11)	• Advanced urothelial carcinoma • Nonsmall-cell lung cancer • Hodgkin lymphoma • Non-Hodgkin lymphoma • Metastatic solid tumors • Myelodysplastic syndrome • Breast cancer • Lung cancer
	**Name: Panobinostat**; LBH-589; 404950-80-7; LBH589; faridak; NVP-LBH589; LBH 589; S1030_Selleck; AC1OCFY8; panobinostat (LBH589) **IUPAC name:** (*E*)-*N*-Hydroxy-3-[4-[[2-(2-methyl-1*H*-indol-3-yl) ethylamino]methyl]phenyl] prop-2-enamide **Molecular formula:** $C_{21}H_{23}N_3O_2$ **Molecular weight:** 349.43 g mol^{-1} **Mechanism:** Pan-HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3, 8); Class IIa HDACs (HDAC4, 5, 7, 9); Class IIb HDACs (HDAC6, 10); Class IV HDACs (HDAC11)	• Multiple myeloma • Acute myeloid leukemia • Solid tumors • HIV infection • Various lymphomas • Sarcoma • Melanoma
	**Name: Romidepsin**; depsipeptide; chromadax; istodax; antibiotic FR 901228; FK228; FR 901228; FK-228; NSC 630176; NSC-630176 **IUPAC name:** (1*S*,4*S*,7*Z*,10*S*,16*E*,21*R*)-7-Ethylidene-4,21-di (propan-2-yl)-2-oxa-12,13-dithia-5,8,20,23-tetrazabicyclo[8.7.6] tricos-16-ene-3,6,9,19,22-pentone **Molecular formula:** $C_{24}H_{36}N_4O_6S_2$ **Molecular weight:** 540.70 g mol^{-1} **Mechanism:** Pan-HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3, 8); Class IIa HDACs (HDAC4, 5, 7, 9); Class IIb HDACs (HDAC6, 10); Class IV HDACs (HDAC11)	• Lymphoma • Myeloma • Solid tumors • Breast cancer • HIV infection • Anaplastic sarcocytoma • Anaplastic oligodendroglioma • Giant cell glioblastoma • Melanoma

Continued

TABLE 6.11 Most Relevant Epidrugs Currently Submitted or Approved in Clinical Trials—cont'd

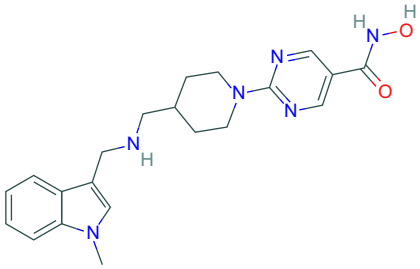
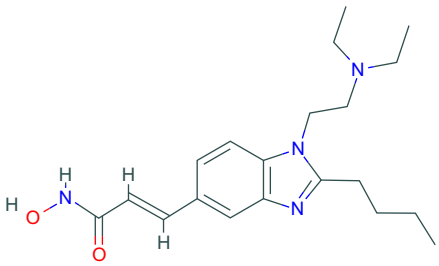
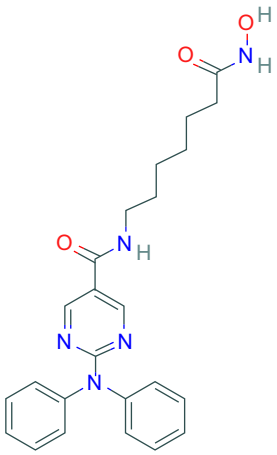
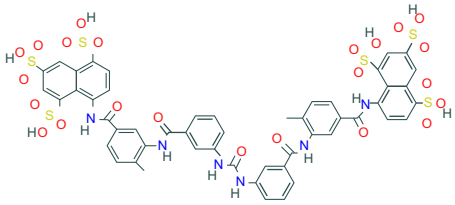
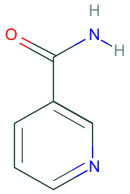
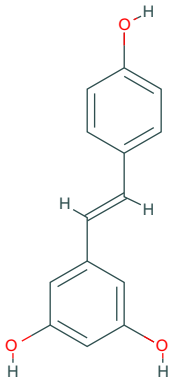
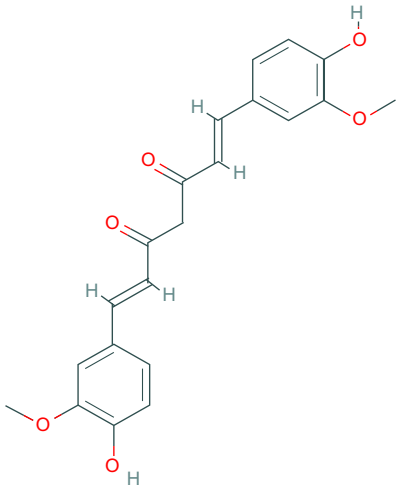
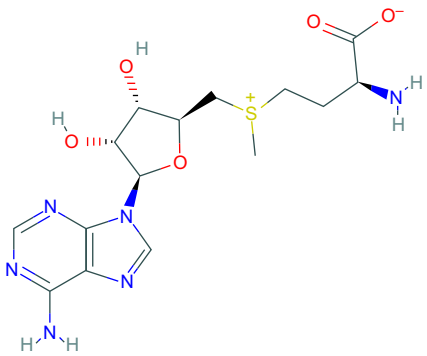
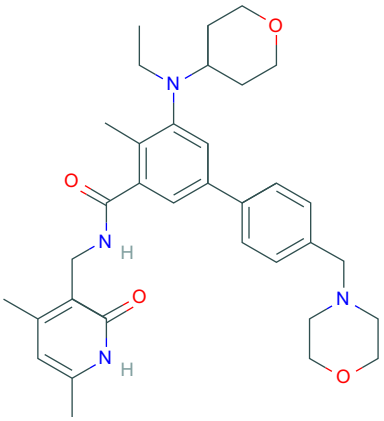
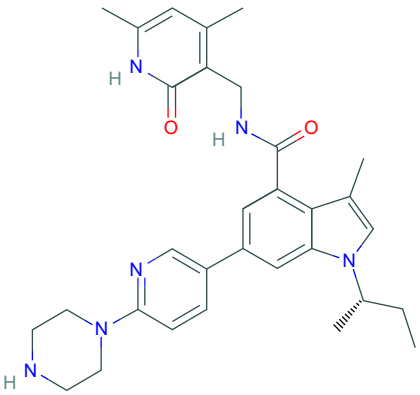
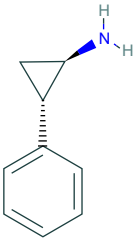
Drug	Properties	Indication
	**Name: Quisinostat**; 875320-29-9; JNJ-26481585; *N*-hydroxy-2-(4-((((1-methyl-1*H*-indol-3-yl)methyl)amino)methyl)piperidin-1-yl)pyrimidine-5-carboxamide; UNII-9BJ85K1J8S; JNJ26481585 **IUPAC name:** *N*-Hydroxy-2-[4-[[(1-methylindol-3-yl) methylamino]methyl]piperidin-1-yl]pyrimidine-5-carboxamide **Molecular formula:** $C_{21}H_{26}N_6O_2$ **Molecular weight:** 394.48 g mol^{-1} **Mechanism:** Pan-HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3, 8); Class IIa HDACs (HDAC4, 5, 7, 9); Class IIb HDACs (HDAC6, 10); Class IV HDACs (HDAC11)	• Ovarian cancer • T cell lymphoma • Multiple myeloma • Nonsmall-cell lung cancer • Advanced refractory leukemia
	**Name: Pracinostat**; 929016-96-6; SB 939; SB939; SB-939; pracinostat (SB939); UNII-GPO2JN4UON; (*E*)-3-(2-butyl-1-(2-(diethylamino)ethyl)-1*H*-benzo[*d*]imidazol-5-yl)-*N*-hydroxyacrylamide **IUPAC name:** (*E*)-3-[2-Butyl-1-[2-(diethylamino)ethyl] benzimidazol-5-yl]-*N*-hydroxyprop-2-enamide **Molecular formula:** $C_{20}H_{30}N_4O_2$ **Molecular weight:** 358.49 g mol^{-1} **Mechanism:** Pan-HDAC inhibitor **Targets:** Class I HDACs (HDAC1, 2, 3, 8); Class IIa HDACs (HDAC4, 5, 7, 9); Class IIb HDACs (HDAC6, 10); Class IV HDACs (HDAC11)	• Prostate cancer • Acute myeloid leukemia • Myelodysplastic syndrome • Myelofibrosis • Metastatic sarcoma • Solid tumors
	**Name: Ricolinostat**; ACY-1215; 1316214-52-4; rocilinostat; 2-(diphenylamino)-*N*-(7-(hydroxyamino)-7-oxoheptyl) pyrimidine-5-carboxamide; UNII-WKT909C62B **IUPAC name:** *N*-[7-(Hydroxyamino)-7-oxoheptyl]-2-(*N*-phenylanilino)pyrimidine-5-carboxamide **Molecular formula:** $C_{24}H_{27}N_5O_3$ **Molecular weight:** 433.51 g mol^{-1} **Mechanism:** Specific HDAC inhibitor **Targets:** HDAC6	• Leukemia • Multiple myeloma • Breast cancer • Cholangiocarcinoma
	**Name: Suramin**; naphuride; germanin; naganol; belganyl; fourneau; farma; antrypol; suramine; naganin **IUPAC name:** 8-[[4-Methyl-3-[[3-[[3-[[2-methyl-5-[(4,6,8-trisulfonaphthalen-1-yl)carbamoyl]phenyl]carbamoyl]phenyl] carbamoylamino]benzoyl]amino]benzoyl]amino]naphthalene-1,3,5-trisulfonic acid **Molecular formula:** $C_{51}H_{40}N_6O_{23}S_6$ **Molecular weight:** 1297.28 g mol^{-1} **Mechanism:** SIRT inhibitor **Targets:** SIRT1–3	• Autism spectrum disorders • Nonsmall-cell lung cancer • Breast cancer • Renal cell carcinoma • Prostate cancer • Central nervous system tumors • Bladder cancer • Multiple myeloma and plasma cell neoplasm
	Name: Nicotinamide; niacinamide, vitamin PP, aminicotin, nicotinic acid amide, amixicotyn, 3-pyridinecarboxamide, papulex, nicotylamide **IUPAC name:** Pyridine-3-carboxamide	• Alzheimer disease • HIV infection • Chronic renal failure

TABLE 6.11 Most Relevant Epidrugs Currently Submitted or Approved in Clinical Trials—cont'd

Drug	Properties	Indication
	**Molecular formula:** $C_6H_6N_2O$ **Molecular weight:** 122.12 g mol^{-1} **Mechanism:** SIRT inhibitor **Targets:** Class III HDAC (SIRT1–7)	• Cardiovascular disorders • Psoriasis • Schizophrenia • Ischemic stroke • Diabetes (type II) • Non-Hodgkin lymphoma
	**Name: Resveratrol**; trans-resveratrol; 501-36-0; 3,4′,5-trihydroxystilbene; 3,4′,5-stilbenetriol; 3,5,4′-trihydroxystilbene; resvida; (*E*)-resveratrol **IUPAC name:** 5-[(*E*)-2-(4-Hydroxyphenyl)ethenyl]benzene-1,3-diol **Molecular formula:** $C_{14}H_{12}O_3$ **Molecular weight:** 228.24 g mol^{-1} **Mechanism:** SIRT inducer/activator **Targets:** SIRT1	• Alzheimer disease • Huntington disease • Diabetes (type II) • Chronic obstructive pulmonary disease • Friedreich ataxia
	**Name: Curcumin**; Diferuloylmethane; natural yellow 3; turmeric yellow; turmeric; kacha haldi; Gelbwurz; Curcuma; haldar; souchet **IUPAC name:** (1*E*,6*E*)-1,7-bis(4-Hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione **Molecular formula:** $C_{21}H_{20}O_6$ **Molecular weight:** 368.38 g mol^{-1} **Mechanism:** HAT inhibitor **Targets:** p300/CBP	• Alzheimer disease • Mild cognitive impairment • Prostate cancer • Schizophrenia • Atopic asthma • Colorectal cancer • Urine cervical dysplasia • Advanced cancers
	**Name: *S*-Adenosylmethionine**; ademetionine; AdoMet; donamet; *S*-adenosyl-L-methionine; SAMe; methioninyladenylate; SAM-e; adenosylmethionine **IUPAC name:** (2*S*)-2-Amino-4-[[(2*S*,3*S*,4*R*,5*R*)-5-(6-aminopurin-9-yl)-3,4-dihydroxyoxolan-2-yl] methyl-methylsulfonio]butanoate **Molecular formula:** $C_{15}H_{22}N_6O_5S$ **Molecular weight:** 398.44 g mol^{-1} **Mechanism:** HMT inhibitor **Targets:** HMTs (KMTs)	• Alzheimer disease • Parkinson disease • Hyperhomocystinemia • Chronic hepatitis C • Hepatocellular carcinoma • Fibromyalgia syndrome • Depression • Major depressive disorder • Bipolar disorder • Chronic kidney disease
	Name: Tazemetostat; EPZ-6438; 1403254-99-8; EPZ6438; UNII-Q40W93WPE1; EPZ 6438 **IUPAC name:** *N*-[(4,6-Dimethyl-2-oxo-1*H*-pyridin-3-yl)methyl]-3-[ethyl(oxan-4-yl)amino]-2-methyl-5-[4-(morpholin-4-ylmethyl)phenyl]benzamide	• B cell lymphoma • Tumors with *EZH2* gain-of-function mutations

Continued

TABLE 6.11 Most Relevant Epidrugs Currently Submitted or Approved in Clinical Trials—cont'd

Drug	Properties	Indication
	**Molecular formula:** $C_{34}H_{44}N_4O_4$ **Molecular weight:** 572.74 g mol^{-1} **Mechanism:** HMT (EZH2) inhibitor **Targets:** EZH2 polycomb group	
	**Name: GSK126**; 1346574-57-9; GSK-126; GSK 126; UNII-W4OGW9QZ97; W4OGW9QZ97 **IUPAC name:** 1-[(2*S*)-Butan-2-yl]-*N*-[(4,6-dimethyl-2-oxo-1*H*-pyridin-3-yl)methyl]-3-methyl-6-(6-piperazin-1-ylpyridin-3-yl) indole-4-carboxamide **Molecular formula:** $C_{31}H_{38}N_6O_2$ **Molecular weight:** 526.67 g mol^{-1} **Mechanism:** HMT (EZH2) inhibitor **Targets:** EZH2 polycomb group	• Lymphoma • Solid tumors • Multiple myeloma
	**Name: Tranylcypromine**; transamine; parnate; PCPA; DL-tranylcypromine; tranylcypromine (INN); AC1L9ASR; (1*R*)-2-phenylcyclopropan-1-amine; DB00752; C07155 **IUPAC name:** (1*R*,2*S*)-2-phenylcyclopropan-1-amine **Molecular formula:** $C_9H_{11}N$ **Molecular weight:** 133.19 g mol^{-1} **Mechanism:** HDM (KDM) inhibitor **Targets:** LSD1, H3K4, H3K9	• ATRA-resistant myeloid cancers • Bipolar disorder • Major depression

ATRA, all-trans retinoic acid; *DNMT*, DNA methyltransferase; *EZH2*, enhancer of zeste 2 polycomb repressive complex 2 subunit; *HAT*, histone acetyltransferase; *HDM (KDM)*, histone demethylase; *HDAC*, histone deacetylase; *HMT (KMT)*, histone methyltransferase; *LSD1*, lysine-specific histone demethylase-1; *p300/CBP*, p300/cyclic AMP-responsive element binding protein p300/CBP-associated factor; *SIRT*, sirtuins.
Data from https://www.clinicaltrials.gov (Accessed 4 June 2018).

inhibitors (Table 6.9): HMT inhibitors target several components linked to histone methyltransferases (histone lysine methyltransferases (HKMT), G9a histone lysine methyltransferase (G9a) (KMT1C, EHMT2), SUV39 subfamily of KMTs (SUV39H1, SUV39H2, G9a, GLP, SETDB1, SETDB2), SET and MYND domain-containing protein (Smyd family) lysine methyltransferases, ESET protein (SETDB1), SETD8/SET8/Pr-SET7/KMT5A lysine methyltransferases, disruptor of telomeric silencing-1-like (DOT1L), KMT2A/MLL1 lysine methyltransferase complex, EZH2-polycomb repressive complex 2 (PRC2), histone arginine methyltransferases, protein arginine methyltransferases (PRMT1–9)) (Tables 6.1, 6.4, and 6.9); the most representative HMT inhibitors belong to the following categories: lysine methyltransferase inhibitors, G9a histone methyltransferase (G9a)(KMT1C, EHMT2) inhibitors, protein lysine methyltransferase (PKMT) SUV420H1 and SUV420H2 inhibitors, protein arginine methyltransferase (PRMT1–9) inhibitors (PRMT1 alkyl bis(oxy)dibenzimidamide derivatives, PRMT3 histone methyltransferase inhibitors, PRMT4 (CARM1) histone methyltranferase inhibitors), DOT1 histone methyltransferase inhibitors, EZH2 histone methyltransferase inhibitors, polycomb repressive complex 2 (PRC2) inhibitors, WD40 domain-containing protein EED, hybrid

HAT/EZH2 inhibitors, SET7/9 histone methyltransferase inhibitors, and other HMT inhibitors (Tables 6.4 and 6.9); (vi) histone demethylase inhibitors (Table 6.9): HDM inhibitors target components of the histone demethylating machinery (histone lysine demethylases, lysine-specific demethylase 1 (LSD1) (KDM1A), KDM1–8, Fe(II)/2-oxoglutarate-dependent dioxygenases, plant homeodomain (PHD), Jumonji C domain-containing histone lysine demethylases (JMJCs), JMJD1B, H3K9me2 lysine demethylase, JMJD-1.2-KDM7 family, JMJD2A-ETV2 complex, JMJD2D-ETV2 complex, prolyl hydroxylases, E26 transformation-specific (ETS) variant 2 (ETV2) protein), with the following most representative examples: lysine-specific demethylase 1 (LSD1) inhibitors, histone H3 peptide-based LSD1 inactivators, Fe(II)/2-oxoglutarate-dependent dioxygenase inhibitors, Jumonji C domain-containing histone lysine demethylase (JMJC) inhibitors, KDM1A inhibitors, KDM3 inhibitors, KDM4 inhibitors, KDM5A (KDM5A-PHD3) inhibitors, KDM6 histone lysine demethylase inhibitors, JMJD3 (KDM6B) inhibitors, and PHF8 inhibitors (Table 6.4); (vii) ATP-dependent chromatin remodelers would target ATP-dependent chromatin-remodeling complexes (SWI/SNF (switching defective/sucrose nonfermenting) family, ISWI (imitation SWI) family, CHD (chromodomain, helicase, DNA binding) family, INO (inositol requiring 80)); family); (viii) polycomb repressive complex 1 (PRC1) inhibitors (BMI-1 inhibitors); (ix) bromodomain inhibitors (Table 6.10); and (x) chromodomain inhibitors[67] (Tables 6.1, 6.4, and 6.10).

6.3.2 DNA Methyltransferase Inhibitors

6.3.2.1 Azacitidine

Azacitidine (AZA) (Tables 6.4 and 6.5) is a first-line treatment for patients with high-risk myelodysplastic syndromes (MDS). AZA induces a general increase in gene expression with 924 upregulated genes, with no correlation with changes in DNA methylation or H3K18ac, and only a weak association with changes in H3K9me3. AZA induces activation of transcripts containing 15 endogenous retroviruses (ERVs). DNA methylation decreases moderately in 99% of all genes, with pronounced effects in heterochromatin. AZA-induced hypomethylation correlates with changes in H3K9me3.[79] AZA is widely used in patients with high-risk MDS and is also useful in the treatment of acute myeloid leukemia (AML). In randomized clinical trials for MDS, major and overall response rates for AZA alone were 16%–33% and 38%–60%, respectively. In the AZA-001 trial the median overall survival (OS) for the AZA group was >24 months compared with 15 months for the control. However, other clinical trials have reported OS periods of 15–21 months, while population-based studies have reported OS rates much shorter than 20 months. Only a fraction of HR-MDS patients appears to benefit from AZA treatment. The risk may outweigh the benefit in a significant proportion of patients; therefore, the indication of AZA should be reconsidered if good biomarkers predicting its efficacy are discovered. The response rate to AZA was moderately higher in MDS patients with *TET2* mutations than in those without the mutations. Patients with *TP53* mutations tend to show a good response to higher doses of decitabine.[80] AZA has been shown to be effective in reducing human glioblastoma cell viability and increasing cellular apoptosis.[81]

During the past decade the human cytomegalovirus (HCMV) has been detected in several types of cancer, including medulloblastomas (MBs). Since DNA methylation occurs in the cell nucleus and this is considered a host defense response, Estekizadeh et al.[82] studied the impact of HCMV infection on DNA methyltransferase (DNMT1) in MB (D324) cells, human umbilical vein endothelial cells (HUVECs) as well as in MB tissue. DNMT1 localizes to the nucleus of uninfected and HCMV-IE-expressing D324 cells and HUVECs, but accumulates in the extra nuclear space in all HCMV-gB-positive cells. Inhibition of HCMV late protein expression by ganciclovir prevents the cytoplasmic localization of DNMT1. Treatment of HCMV-infected D324 cells and HUVECs with the methylation inhibitor AZA increases HCMV-IE and HCMV-gB gene transcription and protein expression. Increased viral protein synthesis in AZA-treated cells suggests that HCMV replication may benefit from a DNA methyltransferase-free cellular environment.

The ribonucleoside analog 5-azacytidine inhibits *Schistosoma mansoni* oviposition, egg maturation, and ovarian development. These antifecundity effects were associated with a loss of DNA methylation and other epigenetic changes.[83]

Stressor-induced memory enhancement involves DNA methylation in the mollusc *Lymnaea stagnalis*. Injection of the DNA methylation inhibitor 5-AZA 1 h before exposure to a memory-enhancing stressor obstructs memory augmentation.[84]

Subbanna et al.[85] studied the immediate and long-term effects of a single-day exposure to 5-azacytidine on neurobehavioral abnormalities in mice and found that AZA treatment inhibits DNA methylation, impairs extracellular signal-regulated kinase (ERK1/2) activation, and reduces expression of the activity-regulated cytoskeleton-associated protein (Arc). These events lead to the activation of caspase-3 in several brain regions, including the hippocampus and

cortex, two brain areas that are essential for memory formation and memory storage, respectively. AZA treatment of P7 mice induced significant deficits in spatial memory, social recognition, and object memory in adult mice and deficits in long-term potentiation (LTP) in adult hippocampal slices. The inhibition of DNA methylation by AZA in P7 mice causes neurodegeneration and impairs ERK1/2 activation and Arc protein expression in neonatal mice and induces behavioral abnormalities in adult mice. DNA methylation-mediated mechanisms appear to be necessary for the proper maturation of synaptic circuits during development, and disruption of this process by AZA can lead to abnormal cognitive function.

6.3.2.2 5-Aza-2′-Deoxycytidine (5-aza-dC) (Decitabine)

The most common DNA methyltransferase inhibitor, 5-aza-2′-deoxycytidine (5-aza-dC) (Tables 6.4 and 6.5), was approved for the treatment of acute myeloid leukemia and is under active investigation for the treatment of solid tumors. Early treatment with 5-aza-dC alters embryonic development, delays hatching, and increases teratology and mortality. Alterations induced by 5-aza-dC treatment can affect sexual development. The adult gonadal transcriptome of 5-aza-dC-exposed females shows changes in the expression of key reproduction-related genes (*cyp11a1*, *esr2b*, and *figla*), downregulates several profemale-related pathways such as the Fanconi anemia or the Wnt signaling pathways, and inhibits genes implicated in epigenetic regulatory mechanisms (*dnmt1, dicer, cbx4*).[86]

IFN-γ-induced PD-L1 expression represents the existence of tumor-specific T cells, which predicts a high-response rate to anti-PD-1/L1 therapy, but loss of function of *IFN* signals (*JAK* mutation) induces adaptive immune resistance in patients with a low-response rate. Interferon regulatory factors (IRF) are frequently epigenetic-silenced in carcinogenesis. Lai et al.[87] investigated the methylation status of *IFN*-γ-related genes *IRF1/8* and *IFN-α/β*-related genes *IRF3/7* in lung cancer tissues and found that only highly methylated IRF1 and 7 negatively correlated with cd274 (coding PD-L1) expression, similar to *JAK* mutation. Decitibine (DAC) can hypomethylate *IRF1/7* to restore the PD-L1 level. Additional treatment of DAC can rescue the ability of tumor cells to response to IFN in lung cancer patients with anti-PD-1/L1 therapy resistance.

Epithelial-mesenchymal transition (EMT) is a crucial driver of tumor progression. Tumor growth factor-beta 1 (TGF-β1) is an important factor in EMT induction in tumorigenesis. Decitabine reverses TGF-β1-induced EMT in PC9 cells, but not in A549 cells. This phenomenon is associated with epigenetic changes in the miR-200 family, which regulates EMT by altering the expression of ZEB1 and ZEB2. TGF-β1 induces aberrant methylation in *miR-200* promoters, leading to EMT in PC9 cells. Decitabine attenuates this effect and inhibits tumor cell migration. In A549 cells, however, neither TGF-β1 nor decitabine exhibited an effect on *miR-200* promoter methylation. Epigenetic regulation of the miR-200/ZEB axis is responsible for EMT induction by TGF-β1 in PC9 cells, and decitabine inhibits EMT in NSCLC cell PC9 through its epigenetic-based therapeutic activity.[88]

6.3.2.3 5-Aza-2′,2′-Difluorodeoxycytidine (NUC013) and 3′,5′-Di-Trimethylsilyl-2′,2′-Difluoro-5-Azadeoxycytidine (NUC041)

5-Aza-2′,2′-difluororodeoxycytidine (NUC013) is a novel DNA methyltransferase and ribonucleotide reductase inhibitor that is a more potent inhibitor of growth than decitabine in the NCI 60 cancer cell line panel. NUC013 is more active than decitabine against p53-null/mutant cancer cell lines but is even more so against p53 wild-type (WT) cell lines.[89] 5-Aza-2′,2′-difluorodeoxycytidine has been shown to be safer and more effective than decitabine in xenograft models of human leukemia and colon cancer. Its major problem is that its half-life is just as short as other DNA methyltransferase inhibitors with a 5-azacytosine base, which primarily target tumor cells in the S phase. 3′,5′-Di-trimethylsilyl-2′,2′-difluoro-5-azadeoxycytidine (NUC041) is a hydrophobic derivative of NUC013 with inhibitory activity on tumor growth. Tumor regression is likely mediated by derepression of the tumor suppressor gene *p53* and resultant activation of natural killer (NK) cells.[90]

6.3.2.4 Guadecitabine (SGI-110)

Guadecitabine (SGI-110) (Table 6.4) is a second-generation DNA methyltransferase inhibitor (DNMTi) currently in clinical trials for hepatocellular carcinoma (HCC). After SGI-110 treatment different cell lines have been shown to be sensitive to SGI-110 which has prolonged antiproliferation effects. The expression of upregulated genes, including tumor suppressors, are positively correlated with nucleosome accessibility and negatively correlated with gene promoter DNA methylation. Alternatively, the expression of downregulated genes, such as oncogenes, are negatively correlated with nucleosome accessibility and positively correlated with gene body DNA methylation. SGI-110 can also act as a dual inhibitor to downregulate *PRC2* complex genes by demethylating their gene bodies, resulting in reactivation of *PRC2*-repressed genes without involvement of DNA methylation. SGI-110 can also upregulate endogenous

retroviruses (ERVs) to reactivate immune pathways. About 48% of frequently altered genes in primary HCC tumors can be reversed by SGI-110 treatment.[91]

Guadecitabine (SGI-110) is formulated as a dinucleotide of decitabine and deoxyguanosine that is resistant to cytidine deaminase (CDA) degradation, resulting in prolonged in vivo exposure to decitabine following small-volume subcutaneous administration of guadecitabine. Guadecitabine is an effective demethylating agent and is able to prevent hepatocellular carcinoma (HCC) progression in preclinical models. Guadecitabine impedes tumor growth and inhibits angiogenesis, with no effect on liver fibrosis and inflammation in steatohepatitis. The demethylating efficacy of guadecitabine on LINE-1 elements is the highest 8 days post infusion in blood samples of mice. A signature of hypermethylated tumor suppressor genes (*CDKN1A*, *CDKN2A*, *DLEC1*, *E2F1*, *GSTP1*, *OPCML*, *E2F1*, *RASSF1*, *RUNX3*, and *SOCS1*) is modulated by this agent. A pronounced demethylating effect of guadecitabine is also obtained in the promoters of a subset of tumor suppressor genes (*CDKN2A*, *DLEC1*, and *RUNX3*) in HepG2 and Huh-7 HCC cells. The histone H2A variant MacroH2A1, an oncogene upregulated in human cirrhosis/HCC, synergizes with DNA methylation in suppressing tumor suppressor genes, and prevents the inhibition of cell growth triggered by decitabine in HCC cells. Guadecitabine, in contrast to decitabine, blocks growth in HCC cells that overexpress macroH2A1 histones and have high CDA levels, despite being unable to fully demethylate *CDKN2A*, *RUNX3*, and *DLEC1* promoters altered by macroH2A1.[92]

6.3.2.5 2-Amino-4-Halopyridine-C-Nucleosides (dXP) and Oligodeoxyribonucleotides (ODNs)

2-Amino-4-halopyridine-C-nucleosides (dXP) and oligodeoxyribonucleotides (ODNs) containing dXP are novel mechanism-based inhibitors of DNMTs. The designed ODN containing XPpG forms a complex with DNMTs via covalent bonding through a nucleophilic aromatic substitution (SNAr) reaction.[93]

6.3.2.6 Genipin

Genipin, the aglycon of geniposide, extracted from *Gardenia jasminoides* Ellis, has antidepressive effects. Genipin is capable of correcting depression-like behaviors induced by prenatal stress in offspring from prenatally stressed dams. Genipin inhibits DNA methyltransferase 1 (DNMT1), normalizing the expression of hippocampal BDNF.[94]

6.3.2.7 Genistein

Dietary polyphenols can be potential chemopreventive agents. Polyphenols reverse aberrant epigenetic patterns by targeting regulatory enzyme DNA methyltransferases (DNMTs) and histone deacetylases (HDACs). Genistein (Tables 6.4 and 6.5) reduces the expression and enzymatic activity of both DNMTs and HDACs in a time-dependent manner. Genistein can interact with various members of the DNMT and HDAC families, inhibiting their enzymatic activity.[95] At low doses this phytoestrogen nongenomically activates mitogen-activated protein kinase p44/42 (MAPKp44/42) via estrogen receptor alpha (ERα) leading to proliferation of human uterine leiomyoma cells. In hormone-responsive immortalized human uterine leiomyoma (ht-UtLM) cells, genistein activates MAPKp44/42 and MSK1 and increases phosphorylation of histone H3 at serine 10 (H3S10ph). Phosphorylation of both MSK1and H3S10ph is abrogated by PD98059 (PD), an MEK1 kinase inhibitor, thereby supporting genistein's activation of MSK1 and histone H3 downstream of MAPKp44/42. Genistein induces growth-related transcription factor genes (*EGR1*, *Elk1*, *ID1*, and *MYB* (cMyb)) downstream of MAPK in ht-UtLM cells. Genistein epigenetically modifies histone H3 by phosphorylation of serine 10, which is regulated by MSK1 and MAPK activation.[96]

6.3.2.8 Luteolin and Fisetin

Luteolin and fisetin display synergistic effects on proinflammatory cytokine secretion. Hyperglycemic conditions significantly induce histone acetylation, nuclear factor-kappa B (NF-κB) activation, interleukin 6, and tumor necrosis factor-α release from THP-1 cells; combination treatments with the phytochemicals fisetin and luteolin suppress NF-κB activity and inflammatory cytokine release. Fisetin, luteolin, and their combination treatments also decrease the activity of histone acetyltransferase, a known NF-κB coactivator, inhibit reactive oxygen species production, and activate sirtuin 1 (SIRT1) and forkhead box O3a (FOXO3a) expression.[97]

6.3.2.9 (−)-Epigallocatechin-3-Gallate (EGCG), Gallic acid, and Fermented Oolong Tea

(−)-Epigallocatechin-3-gallate (EGCG) (Tables 6.4 and 6.5) modulates gene expression by targeting DNA methyltransferases (DNMTs) through a proposed mechanism involving the gallate moiety. Gallic acid (GA) changes the methylome of lung cancer and premalignant oral cell lines and reduces both nuclear and cytoplasmic DNMT1 and DNMT3B within. GA exhibits stronger cytotoxicity against the lung cancer cell line H1299 than EGCG. GA reactivates the growth arrest and DNA damage-inducible 45 (GADD45) signaling pathway through the demethylation of CCNE2

and CCNB1 in H1299 cells. The fungus *Aspergillus sojae* increases the GA content in oolong tea via a fermentation process, enhancing the demethylation effects and reducing the nuclear levels of DNMT1, DNMT3A, and DNMT3B in lung cancer cell lines.[98]

Green tea (*Camellia sinensis*) catechin epigallocatechin-3-gallate (EGCG) has been shown to possess diverse anticancerous properties. EGCG inhibits acute promyelocytic leukemia (APL) cell proliferation, causes apoptosis, and elevates the expression of genes associated with cell cycle arrest and differentiation (*p27, PCAF, C/EBPα*, and *C/EBPε*). EGCG causes anticancerous epigenetic changes, including downregulation of epigenetic modifiers DNMT1, HDAC1, HDAC2, and G9a. Polycomb repressive complex 2 (PRC2) core components are also downregulated at the gene and protein level. EGCG treatment enhances hyperacetylated H4 and acetylated H3K14 histones binding to the promoter regions of *p27, PCAF, C/EBPα*, and *C/EBPε* and reduces the binding effect to PRC2 core component genes *EZH2*, *SUZ12*, and *EED*.[99]

6.3.2.10 Zebularine

Zebularine (Tables 6.4 and 6.5) acts as an inhibitor of DNA methylation and shows low toxicity and high efficacy, being a promising adjuvant agent for anticancer chemotherapy. Zebularine inhibits proliferation and clonogenicity, increases apoptosis and the number of cells in the S phase and the expression of *p53, p21*, and *Bax*, and decreases cyclin A, survivin, and Bcl-2 proteins. The combination of zebularine with vincristine and cisplatin results in synergism and antagonism, respectively. Zebularine also modulates activation of the SHH pathway, reducing SMO and GLI1 levels and one of its targets, PTCH1, without changing SUFU levels. Zebularine modulates several pathways, including the Toll-like receptor pathway and high levels of the *BATF2* gene.[100]

6.3.2.11 DNMT-G9a Dual Inhibitors

San José-Enériz et al.[101] designed and synthesized potent novel, selective, and reversible chemical probes that simultaneously inhibit G9a and DNMT methyltransferase activity. In in vitro treatment of hematological neoplasia (acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and diffuse large B cell lymphoma (DLBCL)), the lead compound CM-272 inhibits cell proliferation and promotes apoptosis, inducing interferon-stimulated genes and immunogenic cell death. CM-272 prolongs survival of AML, ALL, and DLBCL xenogeneic models.

6.3.2.12 Maleimide Derivatives of RG108

Nonnucleoside DNMT inhibitors are in development to address the high toxicity of nucleoside analogs. However, these compounds still have low activity in cancer cells and the mode of action of these compounds remains unclear. Maleimide derivatives of RG108 show cytotoxicity on mesothelioma cells and inhibitory potency against DNMTs.[102]

6.3.2.13 DNMT3A Inhibitors

DNA methyltransferase 3A (DNMT3A), acting as a de novo DNA methyltransferase, has gained widespread attention, especially in hematological diseases, and a large number of DNMT inhibitors have been discovered; however, small-molecular inhibitors targeting DNMT3A are still in a primitive situation. Shao et al.[103] reported the discovery of potent novel DNMT3A inhibitors. Compound 40 and 40_3 display comparable in vitro inhibitory activity against DNMT3A by binding to the *S*-adenosyl-L-methionine (SAM) pocket.

6.3.3 Histone Deacetylase (HDAC) Inhibitors

6.3.3.1 Hydroxamic Acids

A series of hydroxamic acids (Tables 6.4 and 6.6) linked by different lengths to a chiral imidazo-ketopiperazine scaffold have been synthesized as inhibitors of HDAC1, HDAC6, and HDAC8 isoforms.[104]

6.3.3.2 Suberoylanilide Hydroxamic Acid (SAHA) (Vorinostat)

Suberoylanilide hydroxamic acid (SAHA) (Tables 6.4 and 6.6) suppresses human nonsmall-cell lung cancer (NSCLC) cell invasiveness and sensitizes cancer cells to chemotherapeutic agents.[105]

Cyclooxygenase-2 (*COX-2*), with its main antifibrotic metabolite PGE2, is regarded as an antifibrotic gene. Repressed *COX-2* expression and deficient PGE2 contribute to the activation of lung fibroblasts and excessive deposition of collagen in pulmonary fibrosis. *COX-2* expression in lung fibroblasts from patients with idiopathic pulmonary fibrosis (IPF) is epigenetically silenced and can be restored by epigenetic inhibitors. *COX-2* downregulation induced by the profibrotic cytokine transforming growth factor-β1 (TGF-β1) in normal lung fibroblasts can be prevented by

epigenetic inhibitors. COX-2 protein expression and PGE2 production are markedly reduced by TGF-β1, and this can be prevented by the pan-histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) and to a lesser extent by the DNA demethylating agent decitabine (DAC), but not by the G9a histone methyltransferase (HMT) inhibitor BIX01294 or the EZH2 HMT inhibitor 3-deazaneplanocin A (DZNep). The effect of SAHA is unlikely mediated by histone modifications. Instead, 3′-untranslated region (3′-UTR) luciferase reporter assay indicated the involvement of posttranscriptional mechanisms. SAHA downregulates the 3′-UTR mRNA-binding protein *TIA-1* (T cell intracellular antigen-1), a negative regulator of *COX-2* translation. *TIA-1* knockdown by siRNA mimicked the effect of SAHA on *COX-2* expression. SAHA can prevent TGF-β1-induced *COX-2* repression in lung fibroblasts posttranscriptionally through a novel TIA-1-dependent mechanism.[106]

HDAC inhibition (HDACi) with the pan-HDAC inhibitor vorinostat attenuates prostaglandin (PG) E2 generation in the murine vasculature and in human vascular smooth muscle cells. The expression of microsomal PGE synthase-1 (*PTGES1*), a key enzyme for PGE2 synthesis, is reduced by HDACi. HDACs are involved in recruitment of the transcriptional activator p300 to the *PTGES1* gene, and HDACi prevents this effect. In line with the acetyltransferase activity of p300, H3K27 acetylation is reduced after HDACi and results in the formation of heterochromatin in the *PTGES1* gene.[107]

Systemic mastocytosis (SM) is a clonal bone marrow disorder with limited therapeutic options. Over 90% of patients carry the D816V point mutation in the *KIT* receptor that renders this receptor constitutively active. Primary mast cells (MCs) and mast cell lines HMC1.2 (D816V mutated), ROSA (KIT WT), and ROSA (KIT D816V) cells are sensitive to histone deacetylase inhibitor (HDACi) treatment. SAHA is the most effective at killing mutated MC. SAHA downregulates *KIT*, followed by major MC apoptosis. The level of the active chromatin mark H3K18ac/H3 decreases significantly in the *KIT* region. This epigenetic silencing is seen only in the *KIT* region and not in control genes upstream and downstream of *KIT*, indicating that the downregulation of *KIT* is exerted by specific epigenetic silencing. *KIT D816V* mutation sensitizes MC to HDACi-mediated killing, and SAHA may be a potential treatment for SM.[108]

Seven halogenated dihydroisocoumarins (palmaerones A–G), along with 11 known dihydroisocoumarins, have been isolated from *Lachnum palmae*, an endophytic fungus from *Przewalskia tangutica*, by exposure to SAHA. Palmaerones show antimicrobial, antiinflammatory, and cytotoxic activities. Palmaerones A–G showed antimicrobial activities against strains of *Cryptococcus neoformans*, *Penicillium* sp., *Candida albicans*, *Bacillus subtilis*, and *Staphyllococcus aureus*. Palmaerones A and E exhibited moderate inhibitory effects on NO production and palmaerone E showed weak cytotoxicity against HepG2.[109]

6.3.3.3 Sodium Butyrate

The potent HDAC inhibitor sodium butyrate (NaB) (Tables 6.4 and 6.6) can reprogram Ewing sarcoma (EWS) cells toward a more differentiated state and affect their growth and survival.[110]

Sodium butyrate and trichostatin A induce a rapid and transient increase in the oxygen consumption rate in the brain and enhance mitochondrial activity.[111]

Histone deacetylase inhibitors protect against noise-induced hearing loss (NIHL). The expression levels of acetylhistone H3 (Lys9) (H3-AcK9), histone deacetylase 1 (HDAC1), and 3-nitrotyrosine (3-NT), an oxidative stress marker, have been studied in a guinea pig model of NIHL. Sodium butyrate attenuates noise-induced permanent threshold shifts and outer hair cell (HC) loss. SB promotes H3-AcK9 expression, represses HDAC1 expression in the nuclei of HCs and Hensen's cells after noise exposure, and attenuates the noise-induced increase of 3-NT expression in HCs and Hensen's cells.[112]

Cerebral ischemia leads to neuroinflammation and activation of microglia which further contribute to stroke pathology. Sodium butyrate (SB) alters H3K9ac enrichment and transcription at the promoters of proinflammatory (*TNF-α, NOS2, STAT1, IL6*) and antiinflammatory (*IL10*) genes while inducing the expression of genes downstream of the IL10/STAT3 antiinflammatory pathway. SB mediates neuroprotection by epigenetically regulating the microglial inflammatory response, via downregulating the expression of proinflammatory mediators, TNF-α and NOS2, and upregulating the expression of antiinflammatory mediator IL10, in activated microglia.[113]

6.3.3.4 Trichostatin (TSA)

The effect of the HDAC inhibitor trichostatin A (TSA) (Tables 6.4 and 6.6) on MeCP2, a protein whose dysregulation plays an important role in brain disorders and cancer, has been studied.[114] MeCP2 binds to methylated DNA in a chromatin context. TSA decreases the phosphorylation state of MeCP2 resulting in a higher MeCP2 chromatin-binding affinity. HDAC inhibition also causes an overall decrease in MeCP2 levels of different cells and an increase in miR132 which is involved in downregulation of MeCP2.[114]

The use of histone deacetylase inhibitors for epigenetic transformation of mesenchymal stem cells (MSCs), whose nuclei are transferred into enucleated oocytes, is a novel approach in somatic cell cloning of mammalian species. TSA

has been used in cloning applications. TSA does not affect the expression of surface antigens related to MSC mesenchymal stemness origin and displays a stimulating effect on MSC transcription, affecting genes across the whole genome with some minor signs of site-specific genes acting in regions on SSC2 and SSC6. Genes with expression affected by TSA were related to development, differentiation, neurogenesis, or myogenesis. TSA interferes with Wnt signaling pathways by upregulation of several engaged genes. After TSA removal the expression levels of genes affected by TSA are restored to the initial levels. About 600 genes are altered by TSA.[115]

Staberg et al.[116] studied the sensitizing effect of trichostatin A on the alkylating agent lomustine (CCNU) for the treatment of glioblastoma (GBM). HDAC1, 3, and 6 expression levels are increased in GBM samples compared with nonneoplastic brain control samples. Pretreatment of GBM cells with TSA resulted in an enhancement of their sensitivity to CCNU, possibly via the accumulation of DSBs, decreased cell proliferation and viability rates, and an increased apoptotic rate.

6.3.3.5 Mocetinostat

Checkpoint inhibitor therapy has led to major treatment advances for several cancers including nonsmall-cell lung cancer (NSCLC), but a percentage of patients do not respond or develop resistance. Potential mechanisms of resistance include lack of expression of programmed death ligand 1 (PD-L1), decreased capacity to present tumor antigens, and the presence of an immunosuppressive tumor microenvironment. Mocetinostat (Tables 6.4 and 6.6) is a spectrum-selective inhibitor of class I/IV histone deacetylases (HDACs), a family of proteins implicated in epigenetic silencing of immune regulatory genes in tumor and immune cells. Mocetinostat upregulates PD-L1 and antigen presentation genes including class I and II human leukocyte antigen (HLA) family members in NSCLC cell lines. Mocetinostat target gene promoters are occupied by a class I HDAC and exhibit increased active histone marks after mocetinostat treatment. Mocetinostat synergizes with interferon γ (IFN-γ) in regulating class II transactivator (CIITA), a master regulator of class II HLA gene expression, decreases intratumoral T-regulatory cells (Tregs) and myeloid-derived suppressor cell (MDSC) populations, and increases intratumoral $CD8^+$ populations. Mocetinostat-treated Tregs also show downregulation of *FOXP3* and *HELIOS*. The combination of mocetinostat and a murine PD-L1 antibody antagonist increases antitumor activity compared with either therapy alone or syngeneic tumor models.[117]

6.3.3.6 Belinostat

The standard of care for advanced small-cell lung cancer (SCLC) is chemotherapy with cisplatin + etoposide (C + E). However, many patients do not respond to treatment. To overcome this problem a phase I trial combining belinostat (B) (Tables 6.4 and 6.6) with C + E has been designed. The combination was safe, although some patients were more susceptible to adverse events. Hematologic toxicities were most commonly observed. Objective responses were observed in 39% of 28 patients and 47% of 15 patients with neuroendocrine tumors. Patients carrying more than three copies of variant *UGT1A1* (*28 and *60) had higher serum levels of belinostat because of slower clearance. DNA damage peaked at 36 h after the initiation of belinostat, as did global lysine acetylation, but returned to baseline 12 h after the end of infusion.[118]

6.3.3.7 Panobinostat

The antineoplastic activity of histone deacetylase inhibitors (HDACi) in B cell lymphomas is lower than expected. The KDACi panobinostat (Tables 6.4 and 6.6) alters lipid metabolism and downstream survival signaling in diffuse large B cell lymphomas (DLBCL). Panobinostat induces metabolic adaptations resulting in newly acquired dependency on the choline pathway and activation of PI3K signaling. This metabolic reprogramming decreases the antineoplastic effect of panobinostat. Inhibition of these metabolic adaptations results in a superior antilymphoma effect as demonstrated by the combination of panobinostat with a choline pathway inhibitor.[119]

Dicer, a type III endoribonuclease, is a critical component in miRNA biogenesis and is required for mature miRNA production. Abnormal *Dicer* expression occurs in numerous cancer types and correlates with poor patient prognosis. Histone deacetylase inhibitors (HDACi) may regulate *Dicer* and miRNA expression. Panobinostat, a clinically approved HDACi, enhances *Dicer* expression via posttranscriptional mechanisms. Studies with proteasome inhibitors indicate that panobinostat regulates the proteasomal degradation of Dicer. Panobinostat, despite increasing Dicer protein expression, decreases Dicer activity, suggesting that Dicer protein levels do not necessarily correlate with Dicer activity and mature miRNA levels. Panobinostat posttranscriptionally regulates Dicer/miRNA biogenesis.[120]

Myelofibrosis is a chronic and progressive myeloproliferative neoplasm characterized by anemia, splenomegaly, debilitating symptoms, and leukemic transformation. Ruxolitinib, an oral JAK1/2 inhibitor, is highly effective in ameliorating systemic symptoms and reducing splenomegaly. Panobinostat is a pan-histone deacetylase inhibitor with clinical activity, as a single agent, in early phase trials of myelofibrosis.[121]

6.3.3.8 Valproic Acid

Mice injected with valproic acid (VPA) (Tables 6.4 and 6.6) exhibit neurobehavioral deficits typical of autism spectrum disorder (ASD) that are more prominent in males. Changes in the activity of SOD and CAT increase lipid peroxidation, and changes in the expression of antioxidant genes are observed in the prefrontal cortex of VPA-treated mice, more prominent in females, while ASD-like behavior is more prominent in males. The coadministration of VPA and the methyl donor *S*-adenosine methionine (SAM) alleviates most ASD-like neurobehavioral symptoms and normalizes redox potential in the prefrontal cortex.[122]

A retrospective cohort study evaluated the effects of exposure to VPA, an anticonvulsant and histone deacetylase inhibitor, on the risk for developing cancers. Cancer incidence in patients with bipolar disorder chronically treated with VPA was no different from patients treated with lithium or other anticonvulsants, except in the case of genitourinary cancer.[123]

VPA induces changes in chromatin structure making DNA more susceptible to damage induction and influences DNA repair efficiency. VPA is also a radiosensitizing agent. Human lymphocytes treated with VPA alone do not show any increase in the frequency of chromosomal aberrations. However, a moderate degree of sensitization is observed, through the increase of chromosomal aberrations, when 0.35 mM VPA is employed after γ-irradiation, whereas 0.70 mM VPA do not modify chromosomal aberration frequencies. The lower number of chromosomal aberrations obtained when VPA is employed at a higher dose after γ-irradiation might be related to the induction of cell cycle arrest.[124]

Valproic acid improves the efficacy of a second-line regimen (vindesine, doxorubicin, and cyclophosphamide) in small-cell lung carcinoma (SCLC) cells and in mouse models. Transcriptomic profiling integrating miRNA and mRNA data identifies key signaling pathways in the response of SCLC cells to valproic acid.[125]

6.3.3.9 JSL-1

JSL-1 is a novel HDAC inhibitor that effectively inhibits the proliferation of uveal melanoma cells. JSL-1 induces apoptosis with increased expression of proapoptotic BH3-only protein BIM, suppresses migration and invasion, blocks the canonical Wnt/β-catenin pathway, impairs self-renewal capacity, and decreases the percentage of $ALDH^+$ cells, thereby reflecting the elimination of UM cancer stem-like cells (CSCs) that seed metastasis.[126]

6.3.3.10 RGFP966

Histone deacetylases may regulate the specific sensory information that is captured for entry into long-term memory stores. The HDAC3-selective inhibitor RGFP966 modulates the expression of zenk (*zif268*, *egr-1*, *ngfi-a*, and *krox24*), which participates in memory processes and improves memory in birds.[127]

Epigenetic mechanisms are key to regulating long-term memory (LTM). One epigenetic mechanism is chromatin modification by histone acetylation. Blocking the action of histone deacetylases (HDACs), which normally negatively regulate LTM by repressing transcription, has been shown to enable memory formation. HDAC inhibition appears to facilitate memory by altering the dynamics of gene expression events important for memory consolidation. Systemic posttraining treatments with an HDAC3 inhibitor (RGPF966) in rats in the early phase of training facilitate auditory discriminative learning, change auditory cortical tuning, and increase the specificity for acoustic frequency formed in memory of both excitatory (S+) and inhibitory (S−) associations.[128]

HDAC3 regulates nuclear atrophy as an early response to axonal injury in retinal ganglion cells (RGCs) following optic nerve crush (ONC). Conditional knockout of *HDAC3* prevents nuclear atrophy post ONC. HDAC3-selective inhibition with RGFP966 is useful in acute and chronic models of optic nerve injury. A single intravitreal injection of RGFP966 prevents histone deacetylation, heterochromatin formation, apoptosis, and DNA damage post ONC.

Inhibition of HDAC3 activity with systemic dosing of RGFP966 prevents apoptosis-related histone deacetylation and attenuates RGC loss after acute optic nerve injury.[129]

6.3.3.11 Pazopanib Hybrids

A novel series of pazopanib hybrids with antitumor activity have been developed based on the crosstalk between histone deacetylases (HDACs) and the vascular endothelial growth factor (VEGF) pathway. Orthoaminoanilide 6d and hydroxamic acid 13f exhibit considerable total HDACs and VEGFR-2-inhibitory activities. Compounds 6d and 13f possess HDAC isoform selectivity profiles that are comparable with the clinical class I HDAC inhibitor MS-275 and the approved pan-HDAC inhibitor SAHA, respectively. Both compounds also exhibit multiple tyrosine kinase-inhibitory activities relative to pazopanib.[130]

6.3.3.12 m-*Carboxycinnamic Acid Bishydroxamide*

Agrawal et al.[131] studied the effect of histone deacetylase inhibitor *m*-carboxycinnamic acid bishydroxamide (CBHA) on in vitro development of buffalo embryos produced by hand-made cloning. Treatment of cloned embryos with CBHA improves the blastocyst rate, reduces the level of apoptosis, and alters the epigenetic status and gene expression pattern.

Epigenetic reprogramming is an indispensable process throughout the course of mammalian development. Study of the effect of donor cell treatment with the histone deacetylase (HDAC) inhibitor *m*-carboxycinnamic acid bishydroxymide (CBHA) on cloned embryo development has shown that CBHA treatment decreases the activity of HDACs and increases the level of gene activation mark H3K9ac and H3K4me3, with no changes in H3K27ac. Donor cell treatment with CBHA supports the reprogramming process and improves cloned preimplantation development.[132]

6.3.3.13 *Sirtuin Inhibitors*

Eurochevalierine (*Neosartorya pseudofischeri*). The fungal metabolite eurochevalierine from *Neosartorya pseudofischeri* inhibits sirtuin 1 and 2 activities without affecting sirtuin 3 activity. This sesquiterpene alkaloid induces histone H4 and α-tubulin acetylation in various cancer cell models, showing strong cytostatic effects.[133]

12-[18F]fluorododecanoic aminohexanoicanilide (12-[18F]DDAHA). Bonomi et al.[134] developed a SIRT2-specific substrate-type radiotracer for noninvasive PET imaging of epigenetic regulatory processes mediated by SIRT2. Radiosynthesis of 12-[18F]fluorododecanoic aminohexanoicanilide (12-[18F]DDAHA) was achieved by nucleophilic radiofluorination of 12-iododecanoic-AHA precursor.

8-Bromo-1,2-dihydro-3*H*-naphth[1,2-*e*][1,3]oxazine-3-thione N-alkylated derivatives. Nonpolar derivatives of heterocyclic aromatic screening hits such as the nonselective sirtuin inhibitor splitomicin tend to be poorly soluble in biological fluids. New SIRT2 inhibitors with improved aqueous solubility have been discovered. Derivatives of 8-bromo-1,2-dihydro-3*H*-naphth[1,2-*e*][1,3]oxazine-3-thione N-alkylated with a hydrophilic morpholino-alkyl chain at the thiocarbamate group intended for binding in the acetyl-lysine pocket of the enzyme appeared to be promising.[135]

2-((4,6-Dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide derivatives. 2-((4,6-Dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide derivatives are novel SIRT2 inhibitors. These compounds are potent inhibitors of breast cancer cells and increase the acetylation of α-tubulin in a dose-dependent manner.[136]

5-Methylmellein. Sirtuins are NAD^+-dependent histone deacetylases that are highly conserved among prokaryotes and eukaryotes. Sirtuins deacetylate histones and nonhistone proteins and are involved in fungal growth and secondary metabolite production. Shigemoto et al.[137] screened 579 fungal culture extracts that inhibited the histone deacetylase activity of sirtuin A (SirA), produced by the fungus *Aspergillus nidulans*. Eight fungal strains containing three Ascomycota, two Basidiomycota, and three Deuteromycetes produced SirA inhibitors. A SirA inhibitor from *Didymobotryum rigidum* JCM 8837, identified as the polyketide 5-methylmellein and its structurally related compound mellein, inhibit SirA activity. 5-Methylmellein modulates fungal secondary metabolism and is a potential tool for screening novel compounds derived from fungi.

6.3.3.14 *Spirohydantoins and 1,2,4-Triazole-3-Carboxamide Derivatives*

3′,4′-Dihydro-2′*H*-spiro[imidazolidine-4,1′-naphthalene]-2,5-dione and 1-(3-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamide are two structurally novel series of histone deacetylase inhibitors (HDACIs) with antiproliferative and HDAC-inhibitory activities; they have HDAC-inhibitory activity comparable with SAHA. 1-(3-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamide derivatives also behave as potential HDAC-tubulin dual inhibitors, with structural similarities to combretastatin A4.[138]

6.3.3.15 *α,β-Unsaturated Carboxylic Acid and Urea-Based Derivatives*

Some urea-containing compounds exhibit anticancer activity. These derivatives potently inhibit class I, II, and IV HDAC isoforms by hyperacetylation of lysine residues in A549 cells. These compounds (i) induce apoptosis, regulating tumor suppressor genes and proteins and facilitating the activation of the death receptor pathway by the TNF receptor; (ii) facilitate the induction of reactive oxygen species (ROS) generation leading to downregulation of Bcl2 and upregulation of Bax expression, with consequent dysregulation of mitochondrial membrane potential ($\Delta\Psi m$) to release cytochrome *c*; and (iii) downregulate the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) pathway to inhibit cell growth, proliferation, and metastasis through the matrix metalloproteinases (MMPs) MMP2 and MMP9 in A549 cells.[139]

6.3.3.16 Carbamates

Carbamates of the clinically approved HDAC (histone deacetylase) inhibitor vorinostat (suberoylanilide hydroxamic acid, SAHA) might serve as prodrugs for hydroxamic acid-containing HDAC inhibitors. Intact carbamates are inhibitors of histone deacetylases themselves, representing a new zinc-binding warhead in HDAC inhibitor design. A prototype of this series is the carbamate derivative bufexamac, an HDAC6-selective inhibitor.[140]

6.3.3.17 N-Substituted 7-Aminoheptanohydroxamic Acid-Based HDAC Inhibitors

A series of secondary and tertiary N-substituted 7-aminoheptanohydroxamic acid-based HDAC inhibitors have been developed. Secondary amines are more potent than the corresponding tertiary amines. Secondary amines with naphthalen-2-ylmethyl, 5-phenylthiophen-2-ylmethyl, and 1*H*-indol-2-ylmethyl (2j) substituents exhibit the highest potency against class I HDACs (HDAC1, HDAC2, HDAC3, HDAC8). The cytotoxicity of secondary and tertiary N-substituted 7-aminoheptanoic acid hydroxyamide-based inhibitors against HT-29, SH-SY5Y, and MCF-7 cancer cells correlate with their inhibition of HDAC1, 2, and 3, with a potency similar to that of suberoylanilide hydroxamic acid (SAHA). These compounds increased the acetylation of histones H3 and H4 in a time-dependent manner.[141]

6.3.3.18 Schistosoma mansoni Histone Deacetylase 8 (HDAC8) Inhibitors

A docking-based virtual-screening procedure using the crystal structure of histone deacetylase 8 from *Schistosoma mansoni* (smHDAC8) allowed the identification of eight novel *N*-(2,5-dioxopyrrolidin-3-yl)-*n*-alkylhydroxamate derivatives as smHDAC8 inhibitors. These newly identified inhibitors show activity against human histone deacetylases (hsHDAC1, 6, and 8).[141]

A predictive structure-based 3D QSAR model of the *S. mansoni* lysine deacetylase 8 enzyme (SmKDAC8) was developed, validated, and used to perform virtual screening of 1593 compounds. Two series characterized by 104 benzodiazepine derivatives and 60 simplified largazole analogs have been reported as human KDAC inhibitors. A nonhydroxamic acid benzothiadiazine dioxide derivative (NSC163639) showed interesting activity and selectivity against SmKDAC8.[142,143]

6.3.3.19 Abexinostat

Abexinostat (Tables 6.4 and 6.6), a novel pan-HDACi, combined with irradiation in normoxia and hypoxia enhances tumor radiosensitivity in a time-dependent manner. Abexinostat increases radio-induced caspase-dependent apoptosis and persistent DNA double-strand breaks associated with decreased DNA damage signaling and repair. Abexinostat potentiates tumor growth delay in combined modality treatments associating abexinostat, irradiation, and cisplatin.[144]

6.3.3.20 Polyoxometalates (PC-320)

Several polyoxometalate HDACi have antitumor activity. Dong et al.[145] investigated the antitumor mechanism of PAC-320, a polyoxometalate derivative. PAC-320 is a broad-spectrum HDACi that inhibits the growth of prostate cancer cells in vitro and in vivo. PAC-320 induces cell cycle arrest at the G2/M phase and apoptosis. PAC-320-induced cell cycle arrest is associated with an increase of p21 and decrease of cyclin A and cyclin B1, and PAC-320-induced apoptosis is mediated through the mitochondria-apoptotic pathway and is closely associated with an increase in BH3-only proteins Noxa and Hrk. The p38 MAPK pathway is involved in PAC-320-induced antiproliferative activities in prostate cancer.

6.3.3.21 Macrocyclic Nonribosomal Peptide HDAC Inhibitors

Over 30 macrocyclic nonribosomal peptide HDAC inhibitors obtained from natural sources have been evaluated. Some of them are highly potent class I and IIb HDAC inhibitors, with comparable effects to that of the approved drug istodax (romidepsin).[146]

6.3.3.22 Chidamide

Chidamide is a novel orally active benzamide-type histone deacetylase (HDAC) inhibitor that selectively targets HDACs 1, 2, 3, and 10. A low-dose chidamide enhances the cytotoxicity of DNA-damaging agents (daunorubicin, idarubicin, and cytarabine) in $CD34^+$ CD38-KG1α cells, $CD34^+$ CD38-Kasumi cells, and primary refractory or relapsed AML $CD34^+$ cells. These events are associated with DNA damage accumulation and repair defects. Cotreatment with chidamide and the DNA-damaging agent IDA gives rise to the production of γH2A.X and inhibits posttranslationally but not transcriptionally the repair of ATM, BRCA1, and checkpoint kinase 1 (CHK1) and 2 (CHK2) phosphorylation.

The combination of chidamide and IDA initiates caspase-3 and PARP cleavage and induces $CD34^{+}CD38$-KG1α cell apoptosis.[147]

6.3.3.23 Cd[L-Proline]2

Chidambaram et al.[148] synthesized cadmium-proline complexes using both the D- and L-isomers of proline and evaluated their biological activities by observing the efficiency of their inhibition of HDAC activity, their ability to reduce the expression of HDAC isoforms in A549 cells, and their effects on apoptosis. Both cadmium-proline complexes intensely inhibit HDAC activity at 2 μM concentration. Cd[L-proline]2 has been found to be a potent inhibitor for all HDAC isoforms, whereas Cd[D-proline]2 inhibited only HDAC1 and 2. These novel chemotherapeutic drugs induce hyperacetylation of histones H3 and H4, counteracting the aberrant repression of genes, such as insulin-like growth factor-binding protein 3 (*IGFBP-3*), *p53*, and *p21*. The ERK/MAPK signaling pathway results in downregulation of the expression of matrix metalloproteinases 2 and 9 (*MMP-2* and *MMP-9*), contributing to the inhibition of metastasis in A549 cells. Apoptosis induction is accompanied by the activation of death receptors and their ligands (which recruit initiator caspase 8), a decrease in mitochondrial membrane potential ($\Delta\Psi m$), an increase in the Bax/Bcl2 ratio, followed by activation of caspases 9 and 3.

6.3.3.24 Tetrahydroisoquinoline-Based HDAC Inhibitor

Structural modification of a previously reported tetrahydroisoquinoline-based HDAC inhibitor 1 has been carried out to improve its plasma stability for more feasible drug delivery. Of three newly synthesized analogs the in vitro rat plasma stability of compound 2 was more than fivefold better than its parent 1. These compounds exhibited similar HDAC-inhibitory activity and antitumor activity in a human breast carcinoma (MDA-MB-231) xenograft model.[149]

6.3.3.25 Dithienylethenes and Fulgimides

Dithienylethenes (DTEs) and fulgimides were functionalized with hydroxamic acid, which is a known moiety binding to zinc-dependent HDACs, to gain photoswitchable HDAC inhibitors. The new DTE-based inhibitors showed moderate photochromic properties in polar solvents, and inhibitory activity improved by a factor >4.[150]

6.3.3.26 Isatin/o-Phenylenediamine-Based HDAC Inhibitors

A novel series of HDAC inhibitors with isatin-based caps and o-phenylenediamine-based zinc-binding groups have been designed and synthesized using the scaffold-hopping strategy. The most potent compound 9n exhibits similar HDAC-inhibitory and antiproliferative activities against multiple tumor cell lines to the positive control entinostat (MS-275).[151]

6.3.3.27 Benzodiazepine Derivatives

New benzodiazepine (BZD) derivatives are potent and selective human lysine deacetylase inhibitors (hKDACi). A total of 108 BZD compounds have been designed, synthesized, and biologically evaluated against human lysine deacetylases (hKDACs) 1, 3, and 8 (class I) and 6 (class IIb). The most active compounds showed mid-nanomolar potencies against hKDACs 1, 3, and 6 and micromolar activity against hKDAC8, while a promising compound (6q) showed selectivity toward hKDAC3.[152]

6.3.3.28 7-Ureido-N-Hydroxyheptanamide Derivative (CKD5)

7-Ureido-*N*-hydroxyheptanamide derivative (CKD5) is a pan-HDACi that is comparable with suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA) in vitro and in vivo. CKD5 has improved cytotoxic effects, apoptosis, antiproliferative activity, and cell cycle arrest at the G2/M phase. CKD5 is a promising therapeutic candidate for glioblastoma.[153]

6.3.3.29 Phenylpyrrole-Based Derivatives

Brindisi et al.[154] have reported the development of a series of novel phenylpyrrole-based derivatives stemmed from combined computational and medicinal chemistry efforts to modulate HDAC1/6 isoform selectivity. These compounds show in vitro activity on HDAC1 and HDAC6 isoforms and on histone H3 and α-tubulin acetylation.

6.3.3.30 Resveratrol

Sirtuin-1 (SIRT-1) downregulation in type 2 diabetes mellitus (T2DM) has been associated with epigenetic markers of oxidative stress. Bo et al.[155] evaluated whether an increase in SIRT-1 expression affects histone 3 acetylation at the 56 lysine residue (H3K56ac) in T2DM patients treated with resveratrol. SIRT-1 levels were increased by resveratrol treatment, and boosting SIRT-1 expression/activation influenced redox homeostasis in diabetics.

Resveratrol possesses antithyroid cancer (TC) activity. It diminishes serum carcinoembryonic antigen and thyroglobulin levels, downregulates expression of IL-6 and cyclooxygenase-2 (COX-2), reduces NF-κB/p65 nuclear translocation, and elevates IkBα expression.[156] Anaplastic thyroid cancer (ATC) is a highly lethal undifferentiated malignancy without reliable therapies. Retinoic acid (RA) has been employed to promote redifferentiation of thyroid cancers by increasing their I^{131} uptake and radiosensitivity. Resveratrol induces cancer redifferentiation. RA exerts a small inhibitory effect on different cell lines. The total cell number in resveratrol-treated THJ-16T and THJ-21T cultures decreased, and this effect was accompanied by reduced cyclin D1 immunolabeling, increased apoptotic fractions, and distinct caspase-3 activation. Resveratrol failed to inhibit growth but enhanced RA sensitivity of THJ-11T cells, suppressed peroxisome proliferator-activated receptor-β/δ (PPAR-β/δ), and upregulated cellular retinoic acid-binding protein 2 (CRABP2) and retinoic acid receptor beta (RARβ) expression. Increased thyroglobulin and E-cadherin levels and the appearance of membranous E-cadherin were evidenced in resveratrol-treated THJ-11T cells.[157]

Resveratrol shows some effects in ARV7-positive prostate cancer. Resveratrol is capable of inhibiting ARV7 transcriptional activity by downregulating ARV7 protein levels. It downregulates ARV7 by enhancing ARV7 polyubiquitination and subsequent proteasome-mediated degradation.[158]

Activated STAT3 signaling is critical for human medulloblastoma cells. SHP2, SOCS3, and PIAS3 are negative regulators of STAT3 signaling. SHP2, SOCS3, and PIAS3 levels are reduced in medulloblastomas. In resveratrol-suppressed medulloblastoma cells in which STAT3 is downregulated and there is a decreased incidence of STAT3 nuclear translocation, PIAS3 is upregulated, the SHP2 level remains unchanged, and SOCS3 is downregulated. SOCS3 proteins accumulate in the distal ends of axon-like processes of resveratrol-differentiated medulloblastoma cells. Knockdown of *SOCS3* expression by siRNA does not influence cell proliferation, STAT3 activation, or resveratrol sensitivity, but inhibits resveratrol-induced axon-like process formation.[159]

Autophagic activity reflects the cellular response to drug treatment and can be regulated by STAT3 signaling. Resveratrol inhibits STAT3 activation and causes remarkable growth arrest and cell death of ovarian cancer (OC) cells. Resveratrol efficiently suppresses growth, induces apoptosis, and inactivates STAT3 signaling of the two OC cell lines. Autophagic activity accompanied by Beclin-1 upregulation and LC3 enzymatic cleavage has been found in resveratrol-treated OC cells, as has an increase in autophagosomes and mitochondrial spheroids in both resveratrol- or AG490-treated OC cells.[160]

6.3.3.31 2-[18F]Fluoroethyltriazolesuberohydroxamine Acid

Kim et al.[161] have reported the radiochemical synthesis of 2-[^{18}F]fluoroethyltriazolesuberohydroxamine acid ([^{18}F]FETSAHA) as an HDAC-targeted radiolabel probe for positron imaging tomography/computed tomography. [^{18}F]FETSAHA shows radioactivity accumulation in tumors with rapid blood clearance and both gastrointestinal track and renal excretion. [^{18}F]FETSAHA has favorable in vivo tumor-imaging properties and may be useful for noninvasive evaluation of the correlation between cancer and HDACs.

6.3.3.32 Dual/Hybrid Inhibitors

The first generation of dual indoleamine 2,3-dioxygenase 1 (IDO1) and histone deacetylase (HDAC) inhibitors have been designed. Compound 10 shows acceptable pharmacokinetic profiles as an orally active antitumor agent and as a valuable probe to clarify the relationships and mechanisms between cancer immunotherapy and epigenetics.[162]

Novel dual nicotinamide phosphoribosyltransferase (NAMPT) and histone deacetylase (HDAC) inhibitors have also been designed using a pharmacophore fusion approach. Thiazolocarboxamide inhibitors were highly active for both targets, with compound 7f showing potent in vivo antitumor efficacy in the HCT116 xenograft model.[163] The first small molecules to simultaneously inhibit nicotinamide phosphoribosyltransferase (NAMPT) and histone deacetylase (HDAC) have been reported by Dong et al.[164] Compound 35 has excellent and balanced activities against both NAMPT and HDAC1, effectively inducing cell apoptosis and autophagy.

HDAC inhibitors and NO donors have been combined in hybrid molecules. Nitrooxy groups or substituted furoxan derivatives were joined to the α position of the pyridine ring of the selective class I HDAC inhibitor MS-275. Their association with the dinitrooxy compound 31 or the furoxan derivative 16 gives hybrid compounds the ability to preserve single-moiety activities. These compounds may represent new therapeutic tools for cardiovascular, neuromuscular, and inflammatory diseases.[165]

Both HDAC1/2 and LSD1 are found in association with the repressor protein CoREST in a transcriptional corepressor complex that is responsible for gene silencing. Combined modulation of both targets results in synergistic antiproliferative activity. A new series of polyamine-based HDACs-LSD1 dual-binding inhibitors obtained by coupling vorinostat and tranylcypromine have been developed.[166]

Histone 3 lysine 9 methylation (H3K9Me2) and global deacetylation on histone proteins are associated with multiple cancer phenotypes including leukemia, prostatic carcinoma, hepatocellular carcinoma, and pulmonary

carcinoma. Zang et al.[167] have reported the first small molecule capable of acting as a dual inhibitor targeting both G9a and HDAC.

6.3.3.33 Triple Inhibitors

Some authors postulate that inhibition of multiple signaling pathways in cancer with a single molecule might result in more effective treatments than monotherapy. Yao et al.[168] designed triple-inhibiting ligands to block three completely different target types: a kinase (JAK2), an epigenetic target (HDAC), and a chaperone (HSP90). Although these enzymes have totally different functions they are related through interdependent pathways in the developing cancer cell. A lead compound (lead compound 47) has been discovered that has low micromolar activity for the three targets.

6.3.4 Histone Acetyltransferase (HAT) Inhibitors

Histone acetyltransferases (HATs) are epigenetic drivers that catalyze the acetyl transfer from acetyl-CoA to lysines of both histone and nonhistone substrates and thereby induce transcription either by chromatin remodeling or direct transcription factor activation. Histone deacetylases (HDACs) conduct the reverse reaction to counter HAT activity. HDACs have been extensively characterized and targeted by small molecules, including four FDA-approved HDAC inhibitors; in contrast, HATs have not been active targets for therapeutic development.[169]

Very few HAT inhibitors (HATi) have been identified to date (Tables 6.4 and 6.6): Lys-CoA for p300, H3-CoA-20 for PCAF, anacardic acid (from cashew nut shell liquid) and garcinol (from *Garcinia indica*) for p300/CBP and PCAF. The only known p300-specific activator is *N*-(4-chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl benzamide (CTPB). CTPB is cell-impermeable, but it appears to cross the blood-brain barrier after i.p. administration, inducing hyperacetylation of histone 3 in the mouse brain.[170] Another histone acetylase activator is pentadecylidenemalonate 1b (SPV-106).[171] None of these compounds is in clinical trials.[9] Curcumin (diferuloylmethane) is a phytochemical compound with HTAi properties, extracted from the rhizome of *Curcuma longa*, a constituent of the ancient herbal medicine Jiawei-Xiaoyaosan.[6,7,74,172] Curcumin (Tables 6.4 and 6.8) is a component of turmeric and has been shown to be effective in the treatment of several diseases modulating epigenetic effects. The expression levels of histone deacetylase 1 (HDAC1), matrix metalloproteinase-2 (MMP-2), and transforming growth factor β (TGF-β) are decreased under curcumin treatment. Curcumin is capable of promoting the transcription activation of *TIMP1* through suppressing HDAC1 expression and increasing histone H3 acetylation at the *TIMP1* promoter region in hypertensive rats. Curcumin might relieve extracellular matrix degradation and interstitial fibrosis induced by hypertension, lowering blood pressure, and might also improve vascular structure through inhibiting the expression of HDAC1, promoting *TIMP1* transcription activation, and suppressing the expression of *MMP-2* and *TGFβ*.[173]

The pathogenesis of breast cancer is paralleled by distinct alterations in the expression profile of several miRNAs. The putative antitumor properties of curcumin are mediated by diverse mechanisms including inhibition of cell proliferation, metastasis, migration, invasion and angiogenesis, and induction of G2/M cell cycle arrest, apoptosis, and paraptosis. Curcumin can interact with several oncogenic and tumor suppressor miRNAs involved in different stages of breast cancer. Upregulation of miR181b, miR-34a, miR-16, miR-15a, and miR-146b-5p and downregulation of miR-19a and miR-19b have been shown following the treatment of several breast cancer cell lines with curcumin. These effects lead to the suppression of tumorigenesis and metastasis, and the induction of apoptosis.[174] Curcumin inhibits the growth of prostate cancer, promotes apoptosis, inhibits the JNK pathway, and represses H3K4me3 in LNCaP cells. The combination of curcumin and JQ-1 inhibits prostate cancer efficiently.[175] Curcumin and its analogs exhibit antileukemic activity either as a single agent or in combination therapy. Dimethoxycurcumin (DMC) is a more metabolically stable curcumin analog that has been shown to induce the expression of promoter-methylated genes without reversing DNA methylation. Cotreatment with DMC and DNA methyltransferase (DNMT) inhibitors might enhance the reexpression of promoter-methylated tumor suppressor genes. The combination of DMC and the DNMT inhibitor decitabine (DAC) in primary leukemia samples and cell lines shows antagonistic cytotoxic effects and is not cytotoxic to primary leukemia cells. The combination increases H3K27 acetylation (H3K27Ac) near the promoter region of promoter-methylated genes. The enhanced induction of promoter-methylated genes by this combination compared with DAC alone is mediated by a mechanism that involves increased histone acetylation and not through potentiation of the DNA-hypomethylating activity of DAC.[176]

FATp300 (p300 with intrinsic factor acetyltransferase activity) is an essential epigenetic regulator of fibrogenesis, which is increased in several fibrotic tissues. A novel FATp300 inhibitor (L002) has been studied in a murine model of hypertensive cardiorenal fibrosis. L002 blunts FATp300-mediated acetylation of specific histones. L002 suppresses

several profibrogenic processes including cellular proliferation, migration, myofibroblast differentiation, and collagen synthesis. This histone acetyltransferase p300 inhibitor reduces hypertension-associated pathological hypertrophy, cardiac fibrosis, and renal fibrosis.[177]

Clear cell renal carcinoma (ccRCC) is often metastasized at diagnosis, and surgery remains the main treatment. The KAT inhibitor CPTH2 lowers histone H3 acetylation, induces apoptosis in colon cancer and cultured cerebellar granule neurons, and decreases cell viability, adhesion, and invasiveness in the ccRCC cell line 786-O. There is preferential inhibition for KAT3B-p300 with hypoacetylating effects on histone H3 at specific H3K18 histones.[178]

Histone-modifying enzymes have been identified as potential targets for development of antimalarials. PfGCN5, a HAT family member of *Plasmodium falciparum*, is predominantly involved in H3K9 acetylation. Kumar et al.[179] have elucidated differences in the catalytic pocket of PfGCN5 that can be exploited to design selective inhibitors. They have reported 20 potential inhibitors of PfGCN5 and experimentally validated one molecule (C14) that has antimalarial activity in the low nanomolar range.

p300/CBP-associated factor (PCAF) and related GCN5 bromodomain-containing lysine acetyl transferases are members of subfamily I of the bromodomain phylogenetic tree. Iterative cycles of rational inhibitor design and biophysical characterization have led to the discovery of the triazolopthalazine-based L-45 (dubbed L-Moses) as the first potent, selective, and cell-active PCAF bromodomain (Brd) inhibitor. Synthesis from readily available (1*R*,2*S*)-(−)-norephedrine furnished L-45 in enantiopure form. L-45 disrupts the PCAF-Brd histone H3.3 interaction, with high selectivity for PCAF and GCN5 bromodomains.[180]

6.3.5 Histone Methyltransferase Inhibitors

6.3.5.1 S-Adenosylmethionine (SAM)

S-Adenosylmethionine (SAM) (Tables 6.4 and 6.9) is the methyl donor for biological methylation modifications that regulate protein and nucleic acid functions. Methylation of the phospholipid phosphatidylethanolamine (PE), essential for the synthesis of phosphatidylcholine (PC), consumes SAM. The induction of phospholipid biosynthetic genes is accompanied by induction of the enzyme that hydrolyzes *S*-adenosylhomocysteine (SAH), a product and inhibitor of methyltransferases. PE facilitates the turnover of SAM for the synthesis of cysteine and glutathione through trans-sulfuration. Cells that lack PE methylation accumulate SAM, which leads to hypermethylation of histones and the major phosphatase PP2A.[181]

The universal methyl group donor *S*-adenosylmethionine (SAM) has been shown to potentially block breast cancer development, growth, and metastasis in in vitro and in vivo studies. SAM treatment induces a dose-dependent decrease in cell proliferation, invasion, migration, anchorage-independent growth, and increased apoptosis in vitro. In vivo, the oral administration of SAM reduces tumor volume and metastasis in green fluorescent protein (GFP)-tagged MDA-MB-231 xenograft models.[182]

6.3.5.2 SMYD2 Inhibitors

SMYD2 is a lysine methyltransferase that catalyzes the monomethylation of several protein substrates including p53. SMYD2 is overexpressed in a significant percentage of esophageal squamous primary carcinomas, and its overexpression correlates with poor patient survival. LLY-507 is a cell-active, potent small-molecule inhibitor of SMYD2.[183]

6.3.5.3 SET7/9 Inhibitors

SET domain-containing (lysine methyltransferase) 7 (SETD7) is implicated in multiple signaling and disease-related pathways and has a broad diversity of reported substrates. (*R*)-PFI-2 is a potent, selective, and cell-active inhibitor of the methyltransferase activity of human SETD7. (*R*)-PFI-2 exhibits an unusual cofactor-dependent and substrate-competitive inhibitory mechanism by occupying the substrate peptide-binding groove of SETD7, including the catalytic lysine-binding channel, and by making direct contact with the donor methyl group of the cofactor, *S*-adenosylmethionine.[184]

SET7 is the only histone methyltransferase that monomethylates “Lys-4” of histone H3. Based on DC-S239, Ding et al.[185] identified DC-S285 as a new hit compound targeting SET7. DC-S303 also shows selectivity against other epigenetic targets, including SETD1B, SETD8, G9a, SMYD2, and EZH2. A few selective small-molecule inhibitors have been reported that target SETD7, the most potent being (*R*)-PFI-2.[186]

The histone methyltransferase SET7/9 methylates histone and nonhistone protein substrates, and some SET7/9 inhibitors have been developed. Cyproheptadine, a serotonin receptor antagonist and histamine receptor (H1)

antagonist, is a novel scaffold of the SET7/9 inhibitor. Dibenzosuberene is a substructure of cyproheptadine and, together with 2-hydroxycyproheptadine, may display SET7/9-inhibitory activity.[187]

6.3.5.4 Polycomb Repressive Complex 2 (PRC2) Inhibitors

Polycomb repressive complex 2 (PRC2) is a histone H3 lysine 27 methyltransferase and epigenetic drug target for cancer therapy. The WD40 domain-containing protein EED is the regulatory subunit of PRC2. It binds to trimethylated lysine 27 of histone H3 (H3K27me3), through which it stimulates the activity of PRC2 allosterically. Li et al.[188] reported data on a novel PRC2 inhibitor (EED226) that binds to the K27me3-pocket on EED and has strong antitumor activity, as well as four other EED binders along with EED162, the parental compound of EED226. The five compounds interact with Arg367, displaying unique features in its interaction with EED.

6.3.5.5 EZH2 Inhibitors

Dysregulation of the histone methyltransferase EZH2 plays a critical role in the development of a variety of malignancies including B cell lymphomas. Three EZH2 inhibitors (tazemetostat (EPZ-6438), GSK2816126, and CPI-1205) are in phase I/phase II clinical trials for the treatment of non-Hodgkin lymphoma and genetically defined solid tumors. Early data from the tazemetostat trials indicate an acceptable safety profile and early signs of activity in diffuse large B cell lymphomas and follicular lymphomas, including patients with EZH2 wild-type and mutant tumors.[189]

Enhancer of zeste homolog 2 (EZH2) is the catalytic unit of polycomb repressive complex 2 (PRC2) that epigenetically silences many genes involved in tumor suppressor mechanisms via the trimethylation of lysine 27 of histone H3 (H3K27me3). Overexpression of EZH2 is associated with poor outcome of glioblastoma (GBM). Yu et al.[190] studied the antitumor effects of the EZH2 inhibitor GSK343 on glioma cells. GSK343 reduces proliferation, attenuates cell motility, and reverses epithelial-mesenchymal transition in U87 and LN229 glioma cells. GSK343 also suppresses the stemness of cell lines and patient-derived glioma stem cells. GSK343 inhibits histone H3K27 methylation and upregulates the expression of EZH2 target genes, thereby regulating the levels of markers involved in epithelial-mesenchymal transition and stemness.

Novel pyrazole-based EZH2 inhibitors have been prepared through molecular pruning of known inhibitors bearing a bicyclic moiety as a central scaffold. The hit compound 1o (*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide) has shown low micromolar EZH2/PRC2 inhibition and high selectivity toward a panel of other methyltransferases, with effects on cell growth arrest in breast MDA-MB231, leukemia K562, and neuroblastoma SK-N-BE cancer cells.[191]

Petraglia et al.[192] identified a novel hybrid epidrug MC2884, a HAT/EZH2 inhibitor, able to induce cancer-selective cell death. Anticancer action is the result of epigenome modulation by H3K27me3, H3K27ac, H3K9/14ac decrease and caspase-dependent apoptosis induction. MC2884 triggers mitochondrial pathway apoptosis by upregulation of cleaved-BID and strong downregulation of BCL2. MC2884 induces massive apoptosis in human primary leukemia blasts by targeting *BCL2* expression and reduces acetylation of the *BCL2* promoter.

EZH2 enzymatic inhibitors show antitumor effects in EZH2-mutated lymphoma and *ARID1A*-mutated ovarian cancer; however, many cancers do not respond because EZH2 can promote cancer independently of its histone methyltransferase activity. ZRANB1 is the EZH2 deubiquitinase that binds, deubiquitinates, and stabilizes EZH2. Depletion of ZRANB1 in breast cancer cells results in EZH2 destabilization and growth inhibition. Systemic delivery of *ZRANB1* small interfering RNA (siRNA) leads to marked antitumor and antimetastatic effects in preclinical models of triple-negative breast cancer (TNBC). In patients with breast cancer, ZRANB1 levels correlate with EZH2 levels and poor survival. EZH2 deubiquitinase ZRANB1 is a novel therapeutic target to be explored in cancer.[193]

Enhancer of zeste homolog 2 (EZH2) inhibitors affect epigenetic programming in sperm and oocytes. Inhibition of EZH1/2 with tazemetostat severely depletes H3K27me3 in growing oocytes of adult female mice.[194]

Loss-of-function mutations in genes encoding contractile proteins are present in cases of thoracic aortic aneurysms (TAAs). Expression of *SM22α* is inversely correlated with aneurysm size. *SM22α*-deficient mice show pregnancy-induced aortic dissection, and SM22α deficiency worsens aortic aneurysm in *Fbn1C1039G/+* (Marfan) mice. Repression of SM22α is enforced by increased activity of the methyltransferase EZH2. Treatment with the EZH2 inhibitor GSK343 improves cytoskeletal architecture and restores *SM22α* expression.[195]

Head and neck squamous cell carcinoma (HNSCC) is the sixth most prevalent cancer worldwide with rates of HPV-positive oropharyngeal squamous cell carcinoma (OPSCC) dramatically increasing. The overexpression of enhancer of zeste homolog 2 (EZH2) is associated with poor clinical prognosis and aggressive HPV-positive phenotypes. Lindsay et al.[196] studied three EZH2 pathway inhibitors (GSK-343, DZNeP, EPZ-5687) in HPV-positive and HPV-negative OPSCC cell lines. Treatment with GSK-343 decreased H3K27me3 in all cell lines, and treatment with DZNeP decreased

H3K27me3 in only HPV-negative cell lines. Cells treated with EPZ-5687 displayed no appreciable change in H3K27me3. Cells treated with DZNeP showed the most dramatic expressional changes, with decreased EGFR in HPV-positive cell lines and an overall increase in proliferation markers in HPV-negative cell lines. GSK-343-treated cells displayed moderate expressional changes, with CCND1 increased in HPV-positive cell lines and decreased TP53 in HPV-negative SCC-1. EPZ-5687-treated cell lines displayed few expressional changes overall. Only DZNeP-treated cells displayed antiproliferative characteristics shown in wound-healing assays.

Infectious pathogens such as herpesviruses are regulated by the cellular epigenetic machinery, and epigenetic therapeutics represents a novel approach used to control infection, persistence, and the resulting recurrent disease. The histone H3K27 methyltransferases EZH2 and EZH1 (EZH2/1) are epigenetic repressors that suppress gene transcription via the propagation of repressive H3K27me3-enriched chromatin domains. EZH2/1 are implicated in the repression of herpesviral gene expression, and EZH2/1 inhibitors suppress primary herpes simplex virus (HSV) infection. These compounds have been shown to block lytic viral replication following induction of HSV reactivation in latently infected sensory ganglia. Suppression correlates with the induction of multiple inflammatory, stress, and antipathogen pathways, as well as enhanced recruitment of immune cells to in vivo infection sites. EZH2/1 inhibitors induce a cellular antiviral state that also suppresses infection with DNA (human cytomegalovirus, adenovirus) and RNA (Zika virus) viruses.[197]

EZH2 and EZH1, the catalytic components of polycomb repressive complex 2 (PRC2), trigger trimethylation of H3K27 (H3K27me3) to repress the transcription of target genes. UNC1999 is a dual inhibitor of EZH2 and EZH1 that, in combination with proteasome inhibitors, is effective in multiple myeloma and prostate cancer. Proteasome inhibitors repress EZH2 transcription via abrogation of the RB/E2F pathway, thereby sensitizing EZH2-dependent multiple myeloma cells to EZH1 inhibition by UNC1999. A combination of proteasome inhibitors with UNC1999, but not with an EZH2-specific inhibitor, induces synergistic antimyeloma activity. Bortezomib combined with UNC1999 inhibits the growth of myeloma cells in vivo. The tumor suppressor gene *NR4A1* is a direct target of UNC1999. Derepression of *NR4A1* by UNC1999 results in suppression of *MYC*, which is enhanced by combination with bortezomib, suggesting a cooperative blockade of PRC2 function.[198]

6.3.5.6 PKMT SUV420H1 and SUV420H2 Inhibitors

Protein lysine methyltransferases (PKMTs) regulate diverse physiological processes including the transcription and maintenance of genomic integrity. PKMTs SUV420H1 and SUV420H2 facilitate proficient nonhomologous end-joining (NHEJ)-directed DNA repair by catalyzing the dimethylation and trimethylation (me2 and me3, respectively) of lysine 20 on histone 4 (H4K20). Bromberg et al.[199] identified A-196, a potent and selective inhibitor of SUV420H1 and SUV420H2, which is a substrate-competitive inhibitor of both SUV420 enzymes. A-196 induces a global decrease in H4K20me2 and H4K20me3 and a concomitant increase in H4K20me1. A-196 inhibits 53BP1 foci formation on ionizing radiation and reduces NHEJ-mediated DNA break repair but did not affect homology-directed repair. A-196 represents a first-in-class chemical probe of SUV420 to investigate the role of histone methyltransferases in genomic integrity.

6.3.5.7 PRMT Inhibitors

Protein arginine methylation, a posttranslational modification critical for a variety of biological processes, is catalyzed by protein arginine *N*-methyltransferases (PRMTs). The protein arginine methyltransferase (PRMT) family of proteins participates in multiple disease states. Some new specific inhibitors have been discovered that have the potential to treat both benign and malignant conditions.[200] PRMT1 is responsible for over 85% of arginine methylation in mammalian cells. Dysregulation of PRMT1 is involved in diverse pathological diseases including cancers. A series of alkyl bis(oxy)dibenzimidamide derivatives have been identified as selective PRMT1 inhibitors. The most potent compound corresponds to hexamidine, which is an antimicrobial agent. Hexamidine has been shown to effectively block cell proliferation in cancer cell lines related to PRMT1 overexpression.[201]

Halby et al.[202] developed a convergent synthetic pathway starting from a protected bromomethylcytosine derivative to synthesize transition state analogs of DNA methyltransferases. 5-Methylcytosine-adenosine compounds were inactive against hDNMT1, hDNMT3Acat, TRDMT1, and other RNA human and viral methyltransferases; however, two compounds showed inhibitory activity against PRMT4, opening new routes for the conception of new potent PRMT4 inhibitors based on the 5-methylcytosine-adenosine scaffold.

Protein arginine methyltransferase-5 (PRMT5) is reported to have a role in diverse cellular processes, including tumorigenesis, and its overexpression is observed in cell lines and primary patient samples derived from lymphomas, particularly mantle cell lymphoma (MCL). Chan-Penebre et al.[203] characterized a potent and selective inhibitor of PRMT5 with antiproliferative effects in both in vitro and in vivo models of MCL. EPZ015666 (GSK3235025) is an orally

available inhibitor of PRMT5 enzymatic activity in biochemical assays with a half-maximal inhibitory concentration (IC50) of 22 nM and broad selectivity against a panel of other histone methyltransferases.

Protein arginine methyltransferase-5 (PRMT5) regulates gene expression either transcriptionally by symmetric dimethylation of arginine residues on histones H4R3, H3R8, and H2AR3, or at the posttranslational level by methylation of nonhistone target proteins. PRMT5 functions as an oncogene. A high-fat diet upregulates PRMT5 levels in the liver, but not in other metabolically relevant tissues such as skeletal muscle or white and brown adipose tissue. This has been associated with repression of master transcription regulators involved in mitochondrial biogenesis. Lentiviral shRNA-mediated reduction of *PRMT5* decreases PI3K/AKT signaling in mouse AML12 liver cells. *PRMT5* knockdown or knockout decreases basal AKT phosphorylation and boosts the expression of *PPARα* and *PGC-1α* with a concomitant increase of mitochondrial biogenesis. The enzymatic activity of PRMT5 is required for regulation of *PPARα* and *PGC-1α* expression and mitochondrial biogenesis, suggesting that targeting PRMT5 may have therapeutic potential for treatment of fatty liver.[204]

6.3.5.8 CARM1 Inhibitors

CARM1 is an arginine methyltransferase with diverse histone and nonhistone substrates implicated in the regulation of cellular processes including transcriptional coactivation and RNA processing. CARM1 overexpression has been reported in multiple cancer types and has been shown to modulate oncogenic pathways. Drew et al.[205] reported the first potent and selective inhibitor of CARM1 with antiproliferative effects in multiple myeloma (MM). EZM2302 (GSK3359088) is an inhibitor of CARM1 enzymatic activity with broad selectivity against other histone methyltransferases. EZM2302 inhibits *PABP1* and *SMB* methylation.

6.3.5.9 DOT1L Inhibitors

Disruptor of telomeric silencing 1-like (DOT1L) lysine methyltransferase plays a key role in MLL-rearranged acute leukemia. Selective inhibition of DOT1L is an attractive strategy to break down aberrant H3K79 methylation and overexpression of leukemogenic genes. The chemical structure of several DOT1L inhibitors and novel candidate drugs able to inhibit DOT1L at the micromolar level have been reported.[206]

6.3.5.10 Histone Lysine Methyltransferases G9a Inhibitors

Histone H3 lysine 9 dimethylation (H3K9me2) is mainly regulated by histone lysine methyltransferase *G9a* and is associated with the repression of transcription. Knockdown of *G9a* reduces H3K9me2 levels and impairs both HCC cell growth and sphere formation. Transforming growth factor β1-induced epithelial mesenchymal transition (EMT) is not suppressed by *G9a* knockdown. Yokoyama et al.[207] identified 96 candidate epigenetic targets of *G9a*. Pharmacological inhibition of G9a by BIX-01294 resulted in both cell growth inhibition and induction of apoptosis in HCC cells. Intraperitoneal administration of BIX-01294 suppressed the growth of xenograft tumors generated by implantation of HCC cells in nonobese diabetic/severe combined immunodeficient mice. *G9a* expression levels positively correlate with H3K9me2 levels in tumor tissues. Pharmacological interference of *G9a* might be a novel epigenetic approach for the treatment of hepatocellular carcinoma.

Histone lysine methyltransferases G9a and GLP are validated targets for the development of new epigenetic drugs. Inhibitors of G9a and GLP target the histone substrate binding site and/or the *S*-adenosylmethionine cosubstrate binding site. Lenstra et al.[208] reported an alternative approach for inhibiting the methyltransferase activity of G9a and GLP. For proper folding and enzymatic activity, G9a and GLP contain structural zinc fingers, one of them being adjacent to the *S*-adenosylmethionine binding site. Targeting these labile zinc fingers with electrophilic small molecules results in ejection of structural zinc ions, and consequently inhibition of methyltransferase activity. Effective Zn(II) ejection and inhibition of G9a and GLP was observed with ebselen, disulfiram, and cisplatin.[208]

6.3.5.11 Trimethyllysine Analogs

Histone *Nε*-lysine methylation is a widespread posttranslational modification that is specifically recognized by a diverse class of *Nε*-methyllysine binding reader proteins. Reader proteins efficiently bind trimethylornithine and trimethylhomolysine, the simplest *Nε*-trimethyllysine analogs that differ in the length of the side chain.[209]

6.3.5.12 Verticillin A

The highly resistant nature of pancreas ductal adenocarcinoma (PDAC) to all therapies suggests that intrinsic tumor cell factors, likely on the deregulated apoptosis pathway, are key mechanisms underlying PDAC nonresponse to these therapies, in addition to the restricted pharmacological properties of drugs. Inhibition of histone methyltransferase (HMTase) by a selective HMTase inhibitor, verticillin A, increases human PDAC cell sensitivity

to gemcitabine-induced growth suppression. Verticillin A treatment decreases FLIP, Mcl-1, Bcl-x and increases the Bak, Bax, and Bim protein level in tumor cells, resulting in activation of caspases, elevated cytochrome C release, and increased apoptosis as determined by upregulated PARP cleavage in tumor cells. The expression levels of the antiapoptotic mediators Bcl-x, Mcl-1, and FLIP are higher, whereas the expression levels of the proapoptotic mediators Bim, Bak, and Bax are dramatically lower in human PDAC tissues than in the normal pancreas. Verticillin A downregulates H3K4me3 levels at the *BCL2L1, CFLAR*, and *MCL-1* promoter to decrease the Bcl-x, FLIP, and Mcl-1 expression level, and inhibits H3K9me3 levels at the *BAK1*, *BAX*, and *BCL2L11* promoter to upregulate the Bak, Bax, and Bim expression level. PDAC cells use H3K4me3 to activate Bcl-x, FLIP, and Mcl-1, and use H3K9me3 to silence Bak, Bax, and Bim to acquire an apoptosis-resistant phenotype. Selective inhibition of H3K4me3 and H3K9me3 is potentially an effective approach to overcome PDAC cell resistance to gemcitabine.[210]

6.3.5.13 Difluorinated Propanediones

Difluorinated propanediones show histone methyltransferase inhibitory potential in leukemic cell lines. A small-molecule inhibitor PR-4 alters the methylation levels in leukemic cell lines, histiocytic lymphoma, and acute T-cell leukemia.[211]

6.3.6 Histone Demethylase Inhibitors

6.3.6.1 Lysine-Specific Demethylase 1 (LSD1) Inhibitors

Lysine-specific demethylase 1 (LSD1) (Tables 6.4 and 6.9) is a flavin-dependent amine oxidase that selectively removes one or two methyl groups from H3 at Lys4 and is recognized as a promising therapeutic target for cancer and other diseases. LSD1 was the first histone demethylase identified as catalyzing the removal of monomethylation and dimethylation marks on histone H3-K4. Despite the potential broad action of LSD1 in transcription regulation, LSD1 may also coordinate multiple epigenetic regulatory complexes including the CoREST/HDAC complex, NuRD complex, SIRT1, and PRC2. LSD1 is an integral component of the SIN3A/HDAC complex. The LSD1/SIN3A/HDAC complex targets several cellular signaling pathways that are critically involved in cell proliferation, survival, metastasis, and apoptosis, especially the p53 signaling pathway. LSD1 in the SIN3A/HDAC complex inhibits a series of genes such as *CASP7, TGFB2, CDKN1A(p21), HIF1A, TERT*, and *MDM2*, some of which are oncogenic. LSD1 and SIN3A are required for optimal survival and growth of breast cancer cells, while also essential for the maintenance of epithelial homoeostasis and chemosensitivity. The LSD1/SIN3A/HDAC complex might be a target for breast cancer therapeutic strategies.[212]

Ota et al.[213] identified novel peptide-based LSD1 inactivators, focusing on the X-ray structure of LSD1 complexed with an H3 peptide-based suicide substrate. These synthetic peptides incorporate two 1-aminocyclohexanecarboxylic acids at both sides of a lysine residue bearing a *trans*-2-phenylcyclopropylamine (PCPA) moiety, which is a pharmacophore for LSD1 inactivation and further development of histone H3 peptide-based LSD1 inactivators.

A series of 4-(4-benzyloxy)phenoxypiperidines with potent and reversible inhibitory activity against LSD1 have been synthesized and evaluated. These compounds inhibit the migration of HCT-116 colon cancer cells and A549 lung cancer cells.[214]

Mould et al.[215] developed reversible inhibitors of LSD1 and identified a series of 4-(pyrrolidin-3-yl)benzonitrile derivatives that act as successful scaffold hops of the inhibitor GSK-690. The most active compound (21 g) improved selectivity over the hERG ion channel compared with GSK-690, and had no activity against the related enzymes MAO-A and B. In human THP-1 acute myeloid leukemia cells 21g was found to increase the expression of the surrogate cellular biomarker CD86.

The expression and function of LSD1 are tightly regulated in human-induced pluripotent stem cells (hiPSCs), and their deregulation underlies the development of teratomas. S2157, an LSD1 inhibitor, prevents teratoma formation from hiPSCs transplanted into immunodeficient mice.[216]

The overexpression of LSD1 decreases methylation at histone 3 lysine 4 and aberrantly silences tumor suppressor genes. Cyclic peptide 9 is a potent and reversible LSD1 inhibitor. Some derivative peptidomimetics have been reported. Cyclic mutant peptides 11 and 16 produce the greatest LSD1 inhibition, and 11, 16, 27, and 28 increase global H3K4me2 in K562 cells. Mutant peptides 16, 27, and 28 promote significant increases in H3K4me2 levels at the promoter sites of genes *IGFBP2* and *FEZ1*.[217]

A series of reversible inhibitors of LSD1 with a 5-hydroxypyrazole scaffold has been developed. These compounds upregulate the expression of the surrogate cellular biomarker CD86.[218] Another series of 3-oxoaminobenzenesulfonamides has been synthesized and evaluated for their inhibitory activity against LSD1. The LSD1

inhibition of compounds 7b and 7h are reversible and selective.[219] Speranzini et al.[220] reported the discovery of two classes of noncovalent inhibitors displaying unique structural features: antibiotic polymyxins and quinazoline-based compounds. A modified H3 peptide with substitution of Lys4 to Met (H3K4M) is a potent competitive inhibitor of LSD1. Amano et al.[221] developed a series of H3K4M peptide derivatives and evaluated their LSD1-inhibitory activities in vitro. Substitution of Ala1 to Ser resulted in more potent inhibitory activity toward LSD1. H3K4M derivatives bind to the LSD1/CoREST complex.

6.3.6.2 KDM1 Inhibitors

The lysine-specific demethylase KDM1A is a key regulator of stem cell potential in acute myeloid leukemia (AML). ORY-1001 is a highly potent and selective KDM1A inhibitor that induces H3K4me2 accumulation on KDM1A target genes, blast differentiation, and reduction of leukemic stem cell capacity in AML. ORY-1001 exhibits potent synergy with standard-of-care drugs and selective epigenetic inhibitors, reduces growth of an AML xenograft model, and extends survival in a mouse PDX model of T cell acute leukemia.[222]

6.3.6.3 KDM3 Inhibitors (JMJD3 Histone Demethylase Inhibitors)

Jumonji-type histone H3K27 demethylases are key regulators of cytokine production in human NK cell subsets. The prototypic JMJD3/UTX (Jumonji domain-containing protein 3) H3K27 demethylase inhibitor GSK-J4 increases global levels of the repressive H3K27me3 mark around transcription start sites of effector cytokine genes. GSK-J4 reduces IFN-γ, TNF-α, granulocyte macrophage colony-stimulating factor (GM-CSF), and interleukin-10 levels in cytokine-stimulated NK cells, while sparing their cytotoxic killing activity against cancer cells. The antiinflammatory effect of GSK-J4 in NK cell subsets suggests that histone demethylase inhibition has broad utility for modulating immune and inflammatory responses. H3K27me3 is a dynamic and important epigenetic modification during NK cell activation, and JMJD3/UTX-driven H3K27 demethylation is critical for NK cell function.[223]

Potential inhibitors from various other scaffolds such as hydroxyquinolines, hydroxamic acids, and triazolopyridines have been identified and reported. Myricetin is the most promising flavonol inhibitor for JMJD3, and myricetin analogs have been identified as potential inhibitors of JMJD3 (KDM6B).[224]

Nonsmall-cell lung cancer (NSCLC) patients may benefit from standard taxane–platin chemotherapy, but many develop drug resistance. Dalvi et al.[225] established preclinical taxane-platin chemoresistance models and identified a 35-gene resistance signature, which was associated with poor recurrence-free survival in neoadjuvant-treated NSCLC patients and included upregulation of the Jumonji C lysine demethylase KDM3B. Multidrug-resistant cells progressively increased the expression of many Jumonji C demethylases, had altered histone methylation, and showed hypersensitivity to Jumonji C inhibitors. Increasing taxane-platin resistance in progressive cell line series was accompanied by progressive sensitization to JIB-04 and GSK-J4. These Jumonji C inhibitors partly reversed deregulated transcriptional programs, prevented the emergence of drug-tolerant colonies from chemonaive cells, and synergized with standard chemotherapy.

6.3.6.4 KDM4 Inhibitors

By interacting with and coactivating the androgen receptor the KDM4 subfamily (KDM4A-E) promotes aggressive phenotypes of prostate cancer (PCa). Knockdown of *KDM4* expression or inhibition of KDM4 enzyme activity reduces the proliferation of PCa cell lines and highlights the inhibition of lysine demethylation as a possible therapeutic method for PCa treatment. Carter et al.[226] identified several compounds (hydroxyquinoline scaffold, benzimidazole pyrazolone scaffold) with inhibitory activity on the human KDM4E isoform, with antiproliferative effects in cellular models of PCa.

The Jumonji C (JmjC) family of 2-oxoglutarate (2OG)-dependent oxygenases regulates transcription via the catalysis of demethylation of *N*ε-methylated lysine residues in histone tails, especially the N-terminal tail of histone H3. Most human JmjC *N*ε-methyl lysine demethylases (KDMs) are complex enzymes, with "reader domains" in addition to their catalytic domains. Some JmjC KDMs also have *N*ω-methyl arginyl demethylase (RDM) activity. JmjC KDM activity has been linked to multiple cancers, and some JmjC proteins are therapeutic targets. Bonnici et al.[227] reported biochemical studies on the potential dual inhibition of JmjC KDM and RDM activities using a model JmjC demethylase, KDM4E (JMJD2E).

KDM4B (lysine demethylase 4B) plays a critical role in energy balance, oxidation, lipolysis, and thermogenesis in adipose tissues. Loss of *KDM4B* in mice results in obesity associated with reduced energy expenditure and impaired adaptive thermogenesis. Adipocyte-specific deletion of *Kdm4b* revealed that adipose tissues were the main sites for KDM4B antiobesity effects. KDM4B controls the expression of multiple metabolic genes, including *Ppargc1a* and *Ppara*, and has been postulated as a potential therapeutic target for the treatment of obesity.[228]

The potent KDM4 inhibitor compound 6 (QC6352) is effective in breast and colon cancer PDX models.[229] Fang et al.[230] reported the discovery of a series of new small-molecule inhibitors of histone lysine demethylase 4D (KDM4D) based on pyrazolo[1,5-*a*]pyrimidine-3-carbonitrile derivatives.

PKF118-310 is an antagonist of transcription factor 4 (TCF4)/β-catenin signaling and inhibitor of KDM4A. PKF118-310 inhibitor activity was discovered by virtual screening of the crystal structure of KDM4A. PKF118-310 anticancer activity has been observed in both liquid and solid tumor cells.[231]

6.3.6.5 KDM5 Inhibitors

Lysine demethylase 5A (KDM5A/RBP2/JARID1A) is a histone lysine demethylase that is overexpressed in several human cancers including lung, gastric, breast, and liver cancers. Gale et al.[232] characterized KDM5A and, after conducting a screen of about 9000 small molecules for inhibitors, found several 3-thio-1,2,4-triazole compounds that inhibited KDM5A. These compounds showed great specificity and did not inhibit its close homolog KDM5B (PLU1/JARID1B) or the related H3K27 demethylases KDM6A (UTX) and KDM6B (JMJD3). One compound (YUKA1) was able to increase H3K4me3 levels in human cells and selectively inhibited the proliferation of cancer cells whose growth depends on KDM5A. YUKA1 prevents drug tolerance in EGFR-mutant lung cancer cells treated with gefitinib and $HER2^+$ breast cancer cells treated with trastuzumab.

Loss of the male-specific histone demethylase lysine-specific demethylase 5D (KDM5D) encoded on the Y chromosome epigenetically modifies histone methylation marks and alters gene expression, resulting in aggressive prostate cancer. Segmental or total deletion of the Y chromosome in prostate cancer cells is one of the causes of decreased *KDM5D* mRNA expression. Loss of *KDM5D* expression with dysregulated H3K4me3 transcriptional marks has been associated with acceleration of the cell cycle and mitotic entry, leading to increased DNA replication stress. Loss of expression of *KDM5D* confers a poorer prognosis. Stress-induced DNA damage is present in the serine/threonine protein kinase ATR with loss of *KDM5D*. In *KDM5D*-deficient cells, blocking ATR activity with an ATR inhibitor enhances DNA damage, thus leading to subsequent apoptosis and opening new windows for therapeutic intervention.[233]

The H3K4 demethylase KDM5B is amplified and overexpressed in luminal breast cancer, suggesting that it might constitute a potential cancer therapy target. Leadem et al.[234] studied, in breast cancer cells, the molecular effects of a recently developed small-molecule inhibitor of the KDM5 family of proteins (KDM5i), either alone or in combination with the DNA demethylating agent 5-aza-2′-deoxycytidine (DAC). KDM5i treatment alone increased the expression of a small number of genes, whereas combined treatment with DAC enhanced the effects of the latter on increasing the expression of hundreds of DAC-responsive genes. Cells treated with the drug combination exhibited increased promoter and gene body H3K4me3 occupancy at DAC-responsive genes compared with DAC alone. Treatment with either DAC or DAC + KDM5i induced a dramatic increase in H3K27ac at enhancers with an associated significant increase in target gene expression, suggesting an effect of DAC on transcriptional regulation. KDM5i synergized with DAC to reduce the viability of luminal breast cancer cells in in vitro assays.

Pyrazole analog 35 is a novel KDM5A inhibitor with improved biochemical, cell potency, reduced MW, and lower lipophilicity.[235]

In glioblastomas several histone demethylase genes (KDM) are overexpressed compared with normal brain tissue, and the development of temozolomide (TMZ) resistance is accompanied by the transient further increased expression of *KDM5A* and other KDMs following epigenetic resilience. Banelli et al.[236] hypothesized that targeting KDMs may kill the cells that survive the cytotoxic therapy, and determined the effect of JIB 04 and CPI-455, two KDM inhibitors, on glioblastoma cells and found that both molecules are more effective against TMZ-resistant rather than native cells. JIB 04 activates the autophagic and apoptotic pathways, interferes with cell cycle progression, inhibits cell clonogenicity, and dephosphorylates Akt, thus inactivating a potent prosurvival pathway. The combination of temozolomide and JIB 04 shows a strong synergistic effect.

Members of the KDM5 (JARID1) subfamily are 2-oxoglutarate (2-OG) and Fe^{2+}-dependent oxygenases acting as histone 3 lysine 4 trimethyl (H3K4me3) demethylases, regulating proliferation, stem cell self-renewal, and differentiation. KDOAM-25 is a new inhibitor of KDM5 enzymes. KDOAM-25 has shown biochemical half-maximal inhibitory concentration values of <100 nM for KDM5A-D, high selectivity toward other 2-OG oxygenase subfamilies, and no off-target activity on a panel of 55 receptors and enzymes. KDOAM-25 has good selectivity toward other demethylases. *KDM5B* is overexpressed in multiple myelomas and negatively correlates with overall survival. Multiple myeloma MM1S cells treated with KDOAM-25 have demonstrated increased global H3K4 methylation at transcriptional start sites as well as impaired proliferation.[237]

6.3.6.6 KDM6 Inhibitors

Peptides derived from substrates of the histone H3 C-terminus have been used as potential inhibitors of the KDM6 subfamily of histone lysine demethylases, with high sequence similarity to the catalytic domain of Jumonji C histone demethylases. A K18I variant of a histone H3-derived peptide increases the affinity toward KDM6 enzymes. Peptide R17 residue has increased hydrophilic interactions. These interactions of the optimized peptide are likely to be responsible for the increased affinity to KDM6 enzymes.[238]

6.3.6.7 Tranylcypromine Derivatives

FAD-dependent lysine-specific demethylase 1 (LSD1) is overexpressed or deregulated in many cancers. Drug candidate inhibitors are N-substituted derivatives of the dual LSD1/monoamine oxidase inhibitor tranylcypromine (2-PCPA) that have a basic amine function in the N-substituent. These derivatives are selective over MAO-A and MAO-B enzymes. N-substituted 2-PCPA derivatives without a basic function or even a polar group are still potent inhibitors of LSD1 and effectively inhibit colony formation of leukemic cells in culture.[239] A new series of tranylcypromine analogs containing a fluorine in the cyclopropyl ring have been reported as inhibitors of the LSD1 enzyme, with effects in acute myeloid leukemia cell lines.[240]

6.3.6.8 Plant Homeodomain (PDH) Inhibitors

Plant homeodomain (PHD)-containing proteins are important epigenetic regulators and potential drug targets. Amiodarone derivatives inhibit PHD finger 3 of KDM5A (KDM5A (PHD3)). Amiodarone and its derivatives disrupt the interactions of a histone H3K4me3 peptide with KDM5A (PHD3). Selected amiodarone derivatives inhibit catalysis of KDM5A, but in a PHD finger-independent manner. Amiodarone derivatives also bind to H3K4me3-binding PHD fingers from the KDM7 subfamily.[241]

6.3.6.9 PHF8 Inhibitors

The histone demethylase PHF8 catalyzes the demethylation of monomethylated and dimethylated Lys9 on histone H3 (H3K9me1/2), and is a transcriptional activator involved in the development of cancer. The affinity and specificity of PHF8 toward H3K9me2 are affected by interacting with both the catalytic domain and a PHD reader domain. The latter specifically recognizes trimethylated Ly4 on histone H3. A fragment of the histone H3 tail with trimethylated Lys4 has been used as a template for the structure-based design of a cyclic, cell-penetrating peptide that exhibits micromolar binding affinity to PHF8. This PHF8 cyclic peptide inhibitor has lower affinity toward KDM2 enzymes and to the KDM3 and KDM6 subfamilies. Selectivity is marginal toward an enzyme from the KDM4 family, which shares histone tail specificity with PHF8. It is a substrate of KDM5B.[242]

6.3.7 Bromodomain Inhibitors

Bromodomains (Tables 6.4 and 6.10) and chromodomains have emerged as attractive candidates for the development of inhibitors targeting gene transcription. Posttranslational modifications of nucleosomal histone proteins orchestrate chromatin organization and gene expression in normal and cancer cells. The acetylation of N-terminal histone tails represents the fundamental epigenetic mark of open-structure chromatin and active gene transcription. Bromodomain and extraterminal (BET) proteins are epigenetic readers that utilize tandem bromodomain (BRD) modules to recognize and dock themselves on to acetylated lysine tails. BET proteins act as scaffolds for the recruitment of transcription factors and chromatin organizers required in transcription initiation and elongation. Some small molecules are capable of blocking their lysine-binding pocket. Inhibitors of the bromo and extraterminal family have shown promising activity in disease models. JQ1 is a prototype benzodiazepine molecule and a specific BET inhibitor with antineoplastic activity both in solid tumors and hematological malignancies. The quinolone I-BET151 and the I-BET762 benzodiazepine have also shown potent antitumor activity in preclinical studies. I-BET762 is currently being tested in early-phase clinical trials[243] and has shown antiviral effects in lesions caused by DNA papillomaviruses.[244] The pleiotropic nature of BET proteins regulating tissue-specific transcription has raised safety concerns. RVX-208, a compound currently in phase II clinical trials, is a BET bromodomain inhibitor specific for second bromodomains (BD2s). RVX-208 displaces BET proteins from chromatin, and BD2 inhibition only modestly affects BET-dependent gene transcription.[245]

Apabetalone, a small-molecule inhibitor, targets the epigenetic readers of BET proteins. Apabetalone has antiinflammatory and antiatherosclerotic properties, and in phase II clinical trials reduced the incidence of major adverse cardiac events in patients with cardiovascular disease. Apabetalone induces changes in the plasma proteome of patients with impaired kidney function. At the trial baseline 169 plasma proteins (cystatin C and β2 microglobulin)

were differentially enriched in patients with renal disease. In the plasma proteome, apabetalone activates 42 pathways that control immunity and inflammation, oxidative stress, endothelial dysfunction, vascular calcification, and coagulation.[246]

Crawford et al.[247] reported the discovery of a potent and selective inhibitor of the bromodomain of cat eye syndrome chromosome region candidate 2 (*CECR2*). They identified a pyrrolopyridine chemical lead, and subsequent structure-based drug design resulted in a potent and selective CECR2 bromodomain inhibitor (GNE-886) suitable for use as an in vitro tool compound.

3,5-Dimethylisoxazole-based BET bromodomain ligand (OXFBD02) inhibits interactions between BRD4(1) and the RelA subunit of NF-κB, in addition to histone H4. This ligand has demonstrated a promising profile in a screen of the NCI-60 panel but is rapidly metabolized. The 3-pyridyl-derived OXFBD04 shows improved BRD4(1) affinity, optimized physicochemical properties, and greater metabolic stability.[248]

Childhood MLL-rearranged acute lymphoblastic leukemia is a very aggressive leukemia, with a dismal prognosis. Small-molecule inhibitors targeting the epigenetic regulators of the MLL complex have emerged as a promising strategy for the development of a targeted therapy. Studies on the effects of BET function abrogation in a preclinical mouse model of MLL-AF4+ infant ALL using the BET inhibitor I-BET151 revealed that I-BET151 is capable of arresting the growth of MLL-AF4+ leukemic cells in vitro, by blocking cell division and rapidly inducing apoptosis. Treatment with I-BET151 in vivo impairs the leukemic engraftment of patient-derived primary samples and lowers the disease burden in mice. I-BET151 affects the transcriptional profile of MLL-rearranged ALL by deregulating the *BRD4, HOXA7/HOXA9*, and *RUNX1* gene networks. I-BET151 treatment sensitizes glucocorticoid-resistant MLL-rearranged cells to prednisolone in vitro and is more efficient when used in combination with HDAC inhibitors.[249]

Bromodomain and extraterminal (BET) protein inhibition has displayed antitumor activity in a wide range of cancers, including *KRAS*-driven malignancies. Jauset et al.[250] evaluated the effects of a new BET inhibitor, BAY 1238097, against pancreatic ductal adenocarcinoma (PDAC) and nonsmall-cell lung cancer (NSCLC) models harboring *RAS* mutations. BET inhibition has displayed a significant therapeutic impact in genetic mouse models of *KRAS*-driven PDAC and NSCLC, reducing both tumor area and tumor grade. BET inhibition by BAY 1238097 decreases *MYC* expression in some cell lines; in PDAC cells its antitumorigenic effect is independent of *MYC* regulation.

2-Methylisoquinoline-1-one is a novel BET bromodomain-binding motif. Structure-guided SAR exploration resulted in a >10,000-fold potency improvement for the BRD4-BD1 bromodomain. Lead compounds have exhibited excellent potencies in both biochemical and cellular assays in MYC-dependent cell lines. Compound 36 demonstrated good physicochemical properties and promising exposure levels in exploratory PK studies.[251]

eIF4E regulates gene translation and plays an important role in the progression of lung cancer. BET inhibitors JQ1 and I-BET151 suppress the growth of NSCLCs, in parallel with downregulation of *eIF4E* expression. Knockdown of *BRD4* expression using siRNAs inhibits the growth of NSCLCs and decreases eIF4E protein levels. Overexpression of *eIF4E* partially abrogates the growth-inhibitory effect of JQ1, while knockdown of *eIF4E* enhances the inhibitory effect of JQ1. JQ1 treatment or knockdown of *BRD4* expression decreases *eIF4E* mRNA levels and inhibits its promoter activity. JQ1 treatment decreases the binding of *eIF4E* promoter with BRD4. Inhibition of BET by JQ1, I-BET151, or *BRD4* silencing suppresses the growth of nonsmall-cell lung carcinoma through decreasing *eIF4E* transcription and subsequent mRNA and protein expression.[252] Most small-cell lung cancer (SCLCs) cell lines are sensitive to JQ1. Expression of *MYCL* as well as *MYCN*, *ASCL1*, and other driver oncogenes including *CDK6* is reduced by JQ1 treatment, in particular in cell lines with high expression of the respective genes. The levels of *CDK6* expression and its reduction rates by JQ1 are associated with JQ1 sensitivity, indicating that *CDK6* is a novel target of JQ1 and predictive marker for JQ1 sensitivity in SCLC cells.[253]

Malignant pleural mesothelioma (MPM) is an asbestos-associated tumor with poor prognosis and few therapeutic options. JQ1, a selective antagonist of BRD4, modulates transcription of oncogenes, including MPM chemoresistance-associated *c-myc* and *Fra-1*. JQ1 can enhance the efficacy of cisplatin against MPM. JQ1 in combination with cisplatin elicits additive or synergistic (superadditive) antiproliferative effects on MPM cells as a result of increased senescence and apoptosis, along with *c-myc* repression.[254]

Ozer et al.[255] studied BRD4 profiling to identify critical pathways involved in the pathogenesis of chronic lymphocytic leukemia (CLL). *BRD4* is overexpressed in CLL and is enriched proximal to genes upregulated or de novo expressed in CLL with known functions in disease pathogenesis and progression. These genes, including key members of the B cell receptor (BCR) signaling pathway, provide a rationale for this therapeutic approach to identify new targets in alternative types of cancer. PLX51107 is a structurally distinct BET inhibitor with novel pharmacologic properties that emulates or exceeds the efficacy of BCR-signaling agents in preclinical models of CLL. BRD4 is involved in the core CLL transcriptional program and justifies the rationale for clinical investigation of PLX51107 as an epigenetic therapy in CLL.

Bernasconi et al.[256] characterized the antitumor activity of the novel bromodomain and extraterminal (BET) inhibitor BAY 1238097 in preclinical lymphoma models. BAY 1238097 shows antiproliferative activity in a large panel of

lymphoma-derived cell lines. BAY 1238097 targets the *NFKB/TLR/JAK/STAT* signaling pathways, *MYC* and *E2F1*-regulated genes, cell cycle regulation, and chromatin structure. The gene expression profiling signatures highly overlap with the signatures of other BET bromodomain inhibitors and partially overlap with HDAC inhibitors, mTOR inhibitors, and demethylating agents. BAY 1238097 shows synergism with EZH2, mTOR, and BTK inhibitors.

Resistance to the antiandrogen enzalutamide (Enz) in prostate cancer can occur through bypass of androgen receptor (AR) blockade by the glucocorticoid receptor (GR). GR-mediated antiandrogen resistance is adaptive and reversible as a result of regulation of *GR* expression by a tissue-specific enhancer. *GR* expression is silenced in prostate cancer by a combination of AR binding and EZH2-mediated repression at the *GR* locus, but it is restored in advanced prostate cancers upon reversion of both repressive signals. BET bromodomain inhibition resensitizes drug-resistant tumors to Enz by selectively impairing the GR signaling axis via this enhancer.[257]

Huang et al.[258] studied the therapeutic potential of I-BET-762, an inhibitor of the bromodomain and extraterminal (BET) protein family, in experimental acute pancreatitis (AP). The infusion of taurolithocholic acid sulfate into the biliopancreatic duct (TLCS-AP), intraperitoneal injections of ethanol and palmitoleic acid (FAEE-AP), and injections of caerulein (CER-AP) resulted in characteristic elevations in serum amylase and cytokine levels, increased pancreatic trypsin and myeloperoxidase activity, typical pancreatic histopathological changes, and lung injury. Treatment with I-BET-762 significantly reduced biochemical, cytokine, and histopathological responses in TLCS-AP and FAEE-AP, but not CER-AP.

Bromodomain proteins are "readers" of acetylated lysine of histones. As a member of bromodomain proteins, bromodomain-containing protein 9 (BRD9) is a subunit of mammalian SWI/SNF chromatin-remodeling complexes. A series of chemical ligands against BRD9 have been developed in recent years, with still limited clinical application.[259]

Crawford et al.[260] identified a small-molecule inhibitor of the bromodomain-containing protein 9 (BRD9). Starting from a pyrrolopyridine lead, they characterized a highly selective compound 11 (GNE-375) with remarkable potency in preventing the emergence of a drug-tolerant population in *EGFR*-mutant PC9 cells treated with EGFR inhibitors. Such tolerance has been linked to an altered epigenetic state, and this was associated with decreased expression of *ALDH1A1*, a gene previously shown to be important in drug tolerance. BRD9 inhibitors may therefore show utility in preventing epigenetically defined drug resistance.

Tripartite motif-containing protein 24 (TRIM24) is related to multiple cancers, and the bromodomain of TRIM24 is essential for the proliferation of lethal castration-resistant prostate cancer. Liu et al.[261] identified three new inhibitors (5H1T, 5H1U, and 5H1V) of the TRIM24 bromodomain with some features similar to benzoimidazolone inhibitors.

The BET protein BRD4 has been shown to be involved in the papillomavirus life cycle, as a cofactor for viral E2 and mediating viral partitioning in some virus types. Several BET inhibitors reduced *HPV11* mRNA expression in vitro, and topical therapeutic administration of I-BET762 abrogates CRPV cutaneous wart growth in rabbits, demonstrating the translation of antiviral effects to efficacy in vivo. I-BET762 reduces the viability of HPV16-infected W12 cells compared with noninfected C33A cells.[262]

Inhibition of BET bromodomain proteins may suppress chondrocyte differentiation and restrain bone growth. BET bromodomain inhibitors reduce Col2a1, an elementary collagen of the chondrocyte. BET inhibitors I-BET151 and (+)-JQ1 may affect *EGFP* expression and chondrocyte differentiation. BET inhibitors affect the depletion of RNA polymerase II from the *Col2a1* promoter. Consequently, BET bromodomain inhibition may have side effects on skeletal bone structures.[263]

Tumor cells with high aldehyde dehydrogenase (ALDH) activity as a result of *ALDH1A1* expression are prone to chemotherapy resistance and tumor relapse. Bromodomain and extraterminal (BET) inhibitors suppress ALDH activity by abrogating BRD4-mediated *ALDH1A1* expression through a superenhancer element and its associated enhancer RNA. The small-molecule BET inhibitor JQ1 suppresses the outgrowth of cisplatin-treated ovarian cancer cells. Combining JQ1 with cisplatin has been shown to improve the survival of ovarian cancer-bearing mice in an orthotopic model. These phenotypes correlate with inhibition of *ALDH1A1* expression through a superenhancer element and other stem-related genes in promoter regions bound by BRD4.[207]

BET bromodomain proteins, Brd2, Brd3, and Brd4 are downregulated in neural stem cells and progenitor cells (NPCs) upon differentiation, while their levels remain unaltered in proliferating NPCs. Treatment with the bromodomain-selective inhibitor (+)-JQ-1 or knockdown of each BET protein results in an increase in the number of neurons with simultaneous reduction in both astrocytes and oligodendrocytes. BET bromodomain inhibition induces a broad transcription program enhancing differentiation of NPCs into neurons, while suppressing cell cycle progression and gliogenesis.[264]

Histone acetylation is essential for memory formation and its deregulation contributes to the pathogenesis of neurodegenerative disorders. The histone acetylation landscape is shaped by chromatin writer and eraser proteins, while readers link the chromatin state to cellular function. Chromatin readers are emerging as novel drug targets for the treatment of brain disorders. JQ1 is a small-molecule inhibitor of the chromatin readers BRD2, BRD3, BRD4, and BRDT. JQ1

enhances cognitive performance and long-term potentiation in wild-type animals and Alzheimer disease animal models. JQ1 regulates hippocampal gene expression affecting ion channel activity, transcription, and DNA repair.[265]

6.4 OTHER PHARMACOEPIGENETICS-RELATED PRODUCTS

6.4.1 Polycomb Group Protein BMI-1 Inhibitor PTC-209

Polycomb group (PcG) proteins are potential targets for therapy in multiple myeloma (MM), a tumor of plasmablasts/plasma cells (PCs) characterized by the expansion of malignant PCs with complex genetic aberrations in the bone marrow (BM). The PcG protein BMI-1 of the polycomb repressive complex 1 (PRC1) is overexpressed in MM and displays oncogenic functions in MM. The BMI-1 inhibitor PTC-209 is a potent antimyeloma agent. PTC-209 reduces the viability of MM cells via induction of apoptosis, and its anti-MM actions are mediated by on-target effects (downregulation of BMI-1 protein and the associated repressive histone mark H2AK119ub), leaving other PRC1 subunits such as CBX-7 and the catalytic subunit RING1B unaffected. PTC-209 has been shown to exhibit synergistic and additive antimyeloma activity when combined with other epigenetic inhibitors targeting EZH2 and BET bromodomains.[266]

6.4.2 All-Trans Retinoic Acid (ATRA)

Retinoic acid (RA) is an active metabolite of vitamin A that improves the clinical symptoms of patients with systemic sclerosis (SSc). The addition of all-trans retinoic acid (ATRA) to human naive $CD4^+$ cells can promote the maturation of Tregs and increase the stable expression of *Foxp3*. ATRA acts as an inducer of Treg response in SSc $CD4^+$ T cells via demethylation of the *FOXP3* promoter and activation of *FOXP3* expression.[267]

6.4.3 Engrailed 1 Interference Peptides (EN1-iPeps)

The neural-specific transcription factor Engrailed 1 is overexpressed in basal-like breast tumors. Synthetic interference peptides comprising a cell-penetrating peptide/nuclear localization sequence and the Engrailed 1-specific sequence from the N-terminus have been engineered to produce a strong apoptotic response in tumor cells overexpressing *EN1*, with no toxicity to normal or non-Engrailed 1-expressing cells.[268]

6.4.4 TET2 Disruptors

Cancer immunotherapy based on genetically redirecting T cells has been used successfully to treat B cell malignancies. The T cell genome can be modified by integration of viral vectors or transposons encoding chimeric antigen receptors (CARs) that direct tumor cell killing. In a patient with chronic lymphocytic leukemia treated with CAR T cells targeting the CD19 protein, Fraietta et al.[269] demonstrated that, following infusion of CAR T cells, antitumor activity was evident in peripheral blood, lymph nodes, and bone marrow, accompanied by complete remission. It was shown that 94% of CAR T cells originated from a single clone in which lentiviral vector-mediated insertion of the CAR transgene disrupted the methylcytosine dioxygenase *TET2* gene. *TET2*-disrupted CAR T cells exhibited an epigenetic profile consistent with altered T cell differentiation. The progeny of a single CAR T cell induces leukemia remission, and TET2 modification may be useful for improving immunotherapies.

6.4.5 GCN5 Inhibitors

The general control nonrepressed protein 5 (GCN5) plays a crucial role in cellular processes, and its dysregulation may cause human diseases, especially cancers. Xiong et al.[270] discovered a novel GCN5 inhibitor DC_G16 with a 1,8-acridinedione scaffold. A highly potent inhibitor (DC_G16-11) inhibits proliferation and induces cell cycle arrest and apoptosis in cancer cells.

6.4.6 RRx-001

The hypoxic bone marrow (BM) microenvironment confers growth/survival and drug resistance in multiple myeloma (MM) cells. RRx-001 is a novel molecule with hypoxia-selective epigenetic and nitric oxide-donating properties.

RRx-001 decreases the viability of MM cell lines and overcomes drug resistance. RRx-001 inhibits MM cell growth in the presence of BM stromal cells. RRx-001-induced apoptosis is associated with the activation of caspases, release of ROS and nitrogen species, induction of DNA damage via ATM/γ-H2AX, and decrease in DNA methyltransferase (DNMT) and global methylation. There is a predominant role of DNMT1 in MM cell survival vs DNMT3a or DNMT3b. The deubiquitylating enzyme USP7 stimulates DNMT1 activity, and *USP7*-siRNA reduces DNMT1 activity and decreases MM cell viability. RRx-001 plus USP7 inhibitor P5091 triggers synergistic anti-MM activity. Combining RRx-001 with pomalidomide, bortezomib, or SAHA induces synergistic anti-MM activity.[271]

6.4.7 Crambescidin 800

Crambescidin 800 (C800) is a potent cytotoxic compound isolated from the marine sponge *Monanchora viridis*. C800 exhibits cytotoxic potency in a panel of breast cancer cells. C800 induces cell cycle arrest at the G2/M phase, resulting in a decline in the expression of *cyclin D1*, *CDK4*, and *CDK6* in TNBC cells. This effect is associated with the inhibition of phosphorylation of the Akt, NF-κB, and MAPK pathways, resulting in apoptosis in triple-negative breast cancer (TNBC) cells.[272]

6.4.8 Ginkgolic Acid

Ginkgolic acid (GA) is a potent sumoylation inhibitor that also acts as an inhibitor of histone acetyltransferase. Sumoylation is a posttranslational modification process that influences mesenchymal stem cell differentiation. GA promotes the differentiation of mouse bone marrow stromal cells into adipocytes and enhances preadipogenic gene expression.[273]

6.4.9 Corosolic Acid

Corosolic acid (CRA) is found in plants and has been used as a health food supplement worldwide. CRA exhibits anticancer activity. Yang et al.[274] investigated the effect of CRA on cellular transformation and the reactivation of nuclear factor erythroid 2-related factor 2 (Nrf2) through epigenetic regulation in TRAMP-C1 prostate cells. CRA inhibits anchorage-independent growth of prostate cancer TRAMP-C1 cells but not *Nrf2* knockout prostate cancer TRAMP-C1 cells. CRA induces mRNA and protein expression of *Nrf2*, heme oxygenase-1 (*HO-1*), and NAD(P)H quinone oxidoreductase 1 (*NQO1*). CRA treatment decreases methylation of the first five CpG sites of the *Nrf2* promoter, increases the acetylation of histone H3 lysine 27 (H3K27ac), while decreasing the trimethylation of histone H3 lysine 27 (H3K27me3) in the promoter region of *Nrf2*, and attenuates the protein expression of DNA methyltransferases (DNMTs) and histone deacetylases (HDACs).

6.4.10 Puerarin

Puerarin is an active ingredient of pueraria, used in injections for the treatment of cardiovascular diseases including arrhythmia, myocardial ischemia, and hypertension. Puerarin reduces the myocardial infarct area and increases left ventricular pressure in diabetic rats with myocardial I/R. Oxidative stress, inflammation, and nuclear factor-κB protein expression are reduced by puerarin. Puerarin activates the protein expression levels of *VEGFA* and *Ang-I*, and increases nitric oxide production, phosphorylated-endothelial nitric oxide synthase protein expression, and caspase-3 activity. The myocardial-protective effect of puerarin serves to reduce myocardial I/R injury, via upregulation of VEGFA/Ang-1 and suppression of apoptosis, in diabetic rats with myocardial I/R.[275]

6.4.11 Terrein

Terrein is a bioactive fungal metabolite isolated from *Aspergillus terreus*, with antimelanogenic activity and antiproliferative effects on a number of types of cancer. Terrein inhibits the proliferation of Eca109 esophageal cancer cells in a dose- and time-dependent manner. Terrein treatment leads to the G2/M phase arrest of Eca109 cells by indirectly regulating cyclin B1 and phosphorylating cell division cycle protein 2 genes. Terrein exhibits a synergistic effect with cisplatin.[276]

6.4.12 Naringenin

Naringenin is a flavanone present in citrus fruit as a mixture of chiral isomers. The expression levels of *miR-17-3p* and *miR-25-5p* are decreased in response to naringenin. miR-17-3p behavior is in agreement with increased levels of target mRNAs, coding for two antioxidant enzymes, manganese-dependent superoxide dismutase (MnSOD) and glutathione peroxidase 2 (GPx2), while expression levels of *miR-25-5p* are not in agreement with its target mRNAs, coding for two pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). Naringenin may exert its antioxidant activity through epigenetic regulation operated by miRNAs, while antiinflammatory activity is regulated by other mechanisms.[277]

6.4.13 Eriocitrin

Eriocitrin is a flavonoid isolated from lemons, with strong antioxidant properties. Eriocitrin can inhibit the proliferation of hepatocellular carcinoma cell lines by arresting the cell cycle in the S phase through the upregulation of *p53*, cyclin A, cyclin D3, and *CDK6*. Eriocitrin triggers apoptosis by activating the mitochondria-involved intrinsic signaling pathway.[278]

6.4.14 Berberine

The plant isoquinoline alkaloid berberine (BBR) may act as an HDAC inhibitor in the human lung cancer A549 cell line. BBR represses total HDAC and class I, II, and IV HDAC activity through the hyperacetylation of histones. BBR triggers positive regulation of the sub-G0/G1 cell cycle progression phase in A549 cells. BBR downregulates oncogene (*TNF-α, COX-2, MMP-2*, and *MMP-9*) and upregulates tumor suppressor gene (*p21* and *p53*) mRNA and protein expressions. BBR regulates Bcl-2/Bax family proteins and triggers the caspase cascade apoptotic pathway in A549 cells. BBR mediates epigenetic reprogramming by HDAC inhibition, which may be the key mechanism for its antineoplastic activity.[279]

6.4.15 Thymoquinone

The natural product thymoquinone (TQ) targets a vast number of signaling pathways in carcinogenesis in different cancers. TQ shows time-dependent and dose-dependent cytotoxic effects and inhibits the migration and invasion processes in different cervical cancer cells. TQ inhibits the migration and invasion of cervical cancer cells by targeting the epithelial-mesenchymal transition-associated transcription factors Twist1 and Zeb1.[280]

6.4.16 Vitamin E Phosphate Nucleoside Prodrugs

Vitamin E phosphate (VEP) nucleoside prodrugs have been designed to bypass two mechanisms of tumor resistance to therapeutic nucleosides: nucleoside transport and kinase downregulation. Some isoforms of vitamin E show activity against solid and hematologic tumors, contributing to chemosensitization. δ-Tocopherol-monophosphate (MP) gemcitabine (NUC050) and δ-tocotrienol-MP gemcitabine (NUC052) are two constructs of vitamin E isoforms conjugated with gemcitabine at the 5′ position. NUC050 delivers gemcitabine-MP intracellularly by a nucleoside transport-independent mechanism and is effective against nonsmall-cell lung cancer (NSCLC) cells. NUC052 also inhibits tumor growth. NUC050 and NUC052 appear to be safe and effective in a mouse xenograft of NSCLC.[281]

6.4.17 Matrine

Matrine is a naturally occurring alkaloid extracted from the Chinese herb *Sophora flavescens*. This alkaloid exhibits antiproliferative properties, promotes apoptosis, and inhibits cell invasion in a number of cancer cell lines by modulating the NF-κB pathway to downregulate the expression of MMP2 and MM9. It also improves the efficacy of chemotherapy when it is combined with other chemotherapeutic drugs. Differential gene expression in KEGG pathways between matrine-treated and untreated prostate cancer cell lines revealed that *GADD45B* is a major target of matrine. Matrine promotes the expression of *GADD45B*, a tumor suppressor gene that is involved in regulation of the cell cycle, DNA damage repair, cell survival, aging, apoptosis, and other cellular processes through the p38/JNK, ROS-GADD45B-p38, and other signaling pathways. Although GADD45B is elevated in prostate cancer tissues, its levels

in prostate tumor tissues are reduced at late stages of tumor invasion, and higher levels of GADD45B predict better survival for prostate cancer patients.[282]

6.4.18 Taxifolin

Taxifolin (TAX) is a potent cancer-chemopreventive agent in skin cancer. Nuclear factor erythroid-2 related factor 2 (Nrf2) is a vital transcription factor that regulates the antioxidative defense system. TAX inhibits the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced colony formation of JB6 P^+ cells and induces antioxidant response element (ARE)-luciferase activity in HepG2-C8 cells, together with upregulation of mRNA and protein levels of Nrf2 and its downstream genes heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase 1 (NQO1). TAX treatment reduces the methylation level of the first 15 CpG sites in the *Nrf2* promoter and the expression levels of DNA methyltransferase (DNMT) and histone deacetylase (HDAC) proteins.[283]

6.4.19 3,3′-Diindolylmethane

Androgen receptor (AR) is a transcription factor involved in normal prostate physiology and prostate cancer (PCa) development. 3,3′-Diindolylmethane (DIM) is a promising phytochemical agent against PCa that affects AR activity and epigenetic regulators in PCa cells. DIM treatment leads to epigenetic suppression of the AR target genes involved in DNA repair (*PARP1, MRE11, DNA-PK*). Decreased expression of these genes is accompanied by an increase in repressive chromatin marks, loss of AR occupancy, and EZH2 recruitment to their regulatory regions. Decreased DNA repair gene expression is associated with an increase in DNA damage (γH2Ax) and upregulation of genomic repeat elements LINE1 and α-satellite.[284]

6.4.20 Hydrogen Sulfide

Hydrogen sulfide (H_2S) has a protective role in the pathogenesis of atherosclerosis, and does so through multiple pathways, and the histone deacetylase sirtuin-1 (SIRT1) displays antiatherogenic effects by regulating the acetylation of functional proteins. In *ApoE* knockout atherosclerosis mice, treatment with an H_2S donor (NaHS or GYY4137) has been shown to reduce the atherosclerotic plaque area, macrophage infiltration, aortic inflammation, and plasma lipid level. H_2S treatment increased aorta and liver *SIRT1* mRNA expression. Overexpression of cystathionine gamma lyase (*CSE*) also changed intracellular *SIRT1* expression. CSE/H_2S treatment increased *SIRT1* deacetylation in endothelium, induced the deacetylation of target proteins (p53, p65, and sterol response element-binding protein), and reduced endothelial and macrophage inflammation. CSE/H_2S induced SIRT1 sulfhydration in its two zinc finger domains, increased its zinc ion-binding activity to stabilize the alpha-helix structure, lowered its ubiquitination, and reduced its degradation. According to these data reported by Du et al.[285] it appears that H_2S is a novel SIRT1 activator by direct sulfhydration.

Hydrogen sulfide mitigates renal damage by reducing blood pressure and ROS. miRNAs are dysregulated in response to angiotensin II (ANG II)-induced hypertension in the kidney, and an H_2S donor (GYY4137) can reverse miRNA alteration and kidney function.[286]

6.4.21 *N*-Acetylcysteine

Intrauterine growth restriction (IUGR) is associated with vascular dysfunction, oxidative stress, and signs of endothelial epigenetic programming of umbilical vessels. Maternal treatment with *N*-acetylcysteine (NAC) during the second half of gestation restores fetal growth by increasing placental efficiency and reversing the functional and epigenetic programming of eNOS in the arterial endothelium of IUGR guinea pigs. NAC treatment restores eNOS-dependent relaxation in aorta and umbilical arteries, normalizing *eNOS* mRNA levels in EC fetal and umbilical arteries. The DNA methylation of IUGR-derived ECs is decreased at CpG-170, and this epigenetic signature is absent in NAC-treated fetuses. IUGR-ECs have common molecular markers of eNOS programming in umbilical and systemic arteries, and this effect is prevented by maternal treatment with antioxidants.[287]

6.4.22 CPUK02 (15-Oxosteviol Benzyl Ester)

CPUK02 (15-oxosteviol benzyl ester) is a new ent-kaurenoid derivative of stevioside with strong anticancer activity. CPUK02 decreases *DNMT3b* mRNA levels and the methylated allele of *MGMT* and *SFRP2* genes. A positive correlation was found between mRNA expression of *DNMT3b* and gene promoter hypermethylation after treatment with CPUK02 and 5-AZA, acting as demethylating agents.[288]

6.4.23 Silibinin

Silibinin, extracted from milk thistle (*Silybum marianum* L.), shows preclinical activity against prostate carcinoma. Its antitumor and chemopreventive activities have been associated with diverse effects on cell cycle, apoptosis, and receptor-dependent mitogenic signaling pathways. Silibinin's pleiotropic effects may reflect its interference with epigenetic mechanisms in human prostate cancer cells. Silibinin reduces the gene expression levels of polycomb repressive complex 2 (*PRC2*) members of enhancer of zeste homolog 2 (*EZH2*), suppressor of zeste homolog 12 (*SUZ12*), and embryonic ectoderm development (*EED*) components in DU145 and PC3 human prostate cancer cells. Silibinin-mediated reduction of EZH2 levels is accompanied by an increase in trimethylation of histone H3 on lysine (K)-27 residue (H3K27me3) levels, and this response is dependent on decreased expression levels of phosphorylated Akt (ser473) (pAkt) and phosphorylated EZH2 (ser21) (pEZH2). Silibinin displays other epigenetic effects, such as an increase in total DNA methyltransferase (DNMT) activity and a decrease in histone deacetylases 1–2 (HDACs1–2) expression levels.[289]

6.4.24 Dihydroartemisinin

Dihydroartemisinin (DHA) is a promising anticancer agent. DHA induces the downregulation of *UHRF1* and *DNMT1*, accompanied by upregulation of *p16* and decreased *p16* promoter methylation levels in PC-3 cells. DHA induces apoptosis and G1/S cell cycle arrest in PC-3 cells. Downregulation of *UHRF1/DNMT1* is upstream of many cellular events, including G1 cell arrest, demethylation of *p16*, and apoptosis of prostate cancer cells.[290]

6.4.25 miRNA/UHRF1 Pathway Modulators

Ubiquitin-like containing plant homeodomain and RING finger domain 1 (UHRF1) is an antiapoptotic protein involved in the silencing of several tumor suppressor genes (TSGs) through epigenetic modifications including DNA methylation and histone posttranslational alterations, as well as epigenetic-independent mechanisms. *UHRF1* overexpression is observed in a number of solid tumors and hematological malignancies and is considered a primary mechanism in inhibiting apoptosis. UHRF1 exerts its inhibitory activity on TSGs by binding to functional domains, and therefore influences several epigenetic actors including DNA methyltransferase, histone deacetylase 1, histone acetyltransferase Tat-interacting protein 60, and histone methyltransferases G9a and Suv39H1. UHRF1 controls a large macromolecular protein complex termed the epigenetic code replication machinery to maintain epigenetic silencing of TSGs during cell division, thus enabling cancer cells to escape apoptosis. miRNAs are able to regulate the expression of their target genes by functioning as either an oncogene or a tumor suppressor. Choudhry et al.[291] elegantly dissected the role of tumor suppressor miRNAs in the regulation of UHRF1 and highlighted the importance of targeting the microRNA/UHRF1 pathways to reactivate silenced TSGs and subsequent apoptosis.

6.4.26 1-Trifluoromethoxyphenyl-3-(1-Propionylpiperidine-4-yl) Urea (TPPU)

1-Trifluoromethoxyphenyl-3-(1-propionylpiperidine-4-yl) urea (TPPU), a novel soluble epoxide hydrolase inhibitor (sEHI), has been used to increase epoxyeicosatrienoic acid (EET) levels in an animal model before myocardial infarction (MI) surgery. TPPU enhances exercise-induced cardiac recovery in mice after MI by increasing EET levels and promoting angiogenesis around the ischemic area.[292]

6.4.27 Suxiao Jiuxin

Suxiao Jiuxin Pill (SJP) is a traditional medicine for the treatment of acute coronary syndrome in China. SJP contains two principal components: tetramethylpyrazine and borneol. Treatment with SJP increases the protein levels of histone

3 lysine 27 trimethylation (H3K27me3), a key epigenetic chromatin marker for cardiac transcriptional suppression, in HL-1 cells. The mRNA expression levels of key histone methylases (*EZH1*, *EZH2*, and *EED*) and demethylases (*JMJD3* and *UTX*) are also modified by SJP in exosome-treated HL-1 cells. SJP increases cardiomyocyte proliferation and modulates C-MSC-derived exosomes to cause epigenetic chromatin remodeling in recipient cardiomyocytes.[293]

6.4.28 *trans*-2-Phenylcyclopropylamine (2-PCPA)

Epigenetic modifications extensively occur in mammalian embryonic development and cell differentiation. They play an essential role in the reprogramming of nuclei during somatic cell nuclear transfer (SCNT) and subsequent in vitro embryonic development. SCNT embryos contain a subnormal level of histone H3K4 dimethylation (H3K4me2) in contrast to in vitro fertilized embryos. Increasing H3K4me2 levels may ameliorate the aberrant development of cloned embryos. Mao et al.[294] studied the effects of *trans*-2-phenylcyclopropylamine (2-PCPA), a specific inhibitor of lysine-specific demethylase 1 (LSD1), on embryogenesis, H3K4me2 level, and gene expression in cloned goat embryos. H3K4me2 levels in treated GFFs increased gradually as the 2-PCPA concentration increased and had no obvious influence on cell viability. The 2-PCPA-induced upregulation of H3K4me2 levels led to G0/G1 cell cycle arrest. The development rate of goat SCNT embryos in vitro was improved and aberrant H3K4me2 levels were effectively corrected in 2-PCPA-treated SCNT embryos in contrast to that in SCNT control embryos. 2-PCPA treatment promoted the mRNA expression of developmental genes (*Oct4*, *Sox2*) without affecting the expression levels of imprinted genes (*IGF2R*, *H19*) in goat SCNT embryos. An abnormal H3K4me2 status can be corrected, and SCNT embryo development can be promoted by treating donor cells with 2-PCPA.[294]

6.4.29 Ivermectin

Ivermectin belongs to the group of avermectins, a series of 16-member macrocyclic lactone compounds discovered in 1967 and FDA-approved for human use in 1987. Ivermectin exerts antitumor effects in different types of cancer. Ivermectin interacts with several targets including the multidrug resistance protein (MDR), Akt/mTOR, and WNT-TCF pathways, purinergic receptors, PAK-1 protein, certain cancer-related epigenetic deregulators such as SIN3A and SIN3B, RNA helicase, chloride channel receptors, and the preferentially targeted cancer stem cell-like population.[295]

6.4.30 Senataxin

Deletions and chromosome rearrangements are common features of cancer cells. Brustel et al.[296] established a new two-component system reporting on epigenetic silencing or deletion of an actively transcribed gene adjacent to a double-strand break (DSB). A targeted DSB results in a misrepair event of kilobase deletions encompassing the DSB site and transcribed gene. Deletions are reduced upon *RNaseH1* overexpression and increased after knockdown of the DNA/RNA helicase Senataxin, implicating a role for DNA/RNA hybrids. The majority of these large deletions are dependent on the 3′ flap of endonuclease XPF. These hybrids were reduced by *RNaseH1* overexpression and increased by Senataxin knockdown, consistent with a role in deletions.

6.4.31 Nanaomycin A

Pluripotent stem cell-derived hepatocyte-like cells (HLCs) are candidates for use in drug screening. However, the hepatocyte functions of HLCs are still lower than those of human hepatocytes. The maintenance of DNA methyltransferase (DNMT) 1 and the de novo DNMTs DNMT3A and DNMT3B are essential for mammalian development. The expression levels of DNMT3B are decreased during hepatoblast differentiation. To accelerate hepatoblast differentiation a DNMT3B-selective inhibitor (nanaomycin A) has been used. The gene expression levels of hepatoblast markers (alpha-fetoprotein, hepatocyte nuclear factor 4 alpha) are increased by nanaomycin A treatment and decreased by DNMT3B overexpression, indicating that it might be possible to promote hepatoblast differentiation by DNMT3B inhibition using nanaomycin A.[297]

6.4.32 Strigolactone Analogs

Strigolactones (SLs) are a new class of phytohormones, which play a crucial role in the development of plant roots and shoots. Synthetic analogs of SLs show proapoptotic effects on different cancer cell lines. The SL analogs TIT3 and TIT7 reduce HepG2 cell viability in a dose- and time-dependent manner and induce apoptosis. The migration of cancer cells is suppressed upon treatment with TIT3 and TIT7, which might exert their selective inhibitory effects on cancer cells by targeting microtubules.[298]

6.4.33 Betaine

Betaine is a feed additive widely used in livestock production because of its ability to promote growth. Maternal betaine supplementation alters hepatic metabolism in offspring. Betaine-fed female chicks show lower body weight and lower level of biologically active thyroid hormone in plasma, which is associated with decrease in the expression of type 1 iodothyronine deiodinase (*Dio1*). Betaine also changes hepatic expression of betaine-homocysteine-*S*-methyltransferase (*BHMT*) and DNA methyltransferase 1 (*DNMT1*), which may contribute to hypermethylation of the *Dio1* promoter. Betaine treatment of hens causes none of these effects in male chicks except *Dio1* expression. Maternal betaine administration affects the growth of offspring through differential modification of *Dio1* gene methylation.[299]

6.4.34 D-2-Hydroxyglutarate

Isocitrate dehydrogenases 1 and 2 (IDH1,2), key Krebs cycle enzymes that generate NADPH-reducing equivalents, undergo heterozygous mutations in over 70% of low- to mid-grade gliomas and in approximately 20% of acute myeloid leukemias (AMLs), and gain an unusual new activity of reducing α-ketoglutarate (α-KG) to D-2 hydroxyglutarate (D-2HG) in a NADPH-consuming reaction. The oncometabolite D-2HG is widely accepted to drive progressive oncogenesis in addition to exacerbating already increased oxidative stress in these cancers. D-2HG competes with α-KG and inhibits a large number of α-KG-dependent dioxygenases such as TET (ten-eleven translocation), JmjC domain-containing KDMs (histone lysine demethylases), and the ALKBH DNA repair proteins that ultimately lead to hypermethylation of CpG islands in the genome. The resulting CpG island methylator phenotype (CIMP) accounts for major gene expression changes including the silencing of the *MGMT* (O^6-methylguanine DNA methyltransferase) repair protein in gliomas. Glioma patients with *IDH1* mutations also show better therapeutic responses and longer survival.[300]

6.4.35 β-Hydroxybutyrate

Histone 3-lysine 9-β-hydroxybutyrylation (H3k9bhb), a novel histone modification mark induced by β-hydroxybutyrate, may participate in the development of depression. H3k9bhb is reduced in the brain of depressive mice. Exogenous β-hydroxybutyrate ameliorates depressive behaviors and reverses the reduction of H3K9bhb and BDNF.[301]

6.4.36 Astaxanthin and Fucoxanthin Carotenoids

Nuclear factor erythroid-2-related factor-2 (Nrf2 or NFE2L2) is a master regulator of antioxidative stress response, which is involved in the defense against many oxidative stress/inflammation-mediated diseases. Epigenetic modification of the *Nrf2* gene plays a key role in restoring the expression of *Nrf2*. Astaxanthin (AST) and fucoxanthin (FX) are carotenoids obtained from microalgae and seaweed that have effects on *Nrf2* expression. FX induces antioxidant response element (ARE)-luciferase and upregulates the mRNA and protein levels of *Nrf2* and *Nrf2* downstream genes in HepG2-C8 cells that overexpress the ARE-luciferase reporter. Both FX and AST decrease colony formation in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced transformation of JB6 P+ cells. FX decreases methylation of the *Nrf2* promoter region in JB6 P+ cells. Both FX and AST have been shown to reduce DNA methyltransferase (DNMT) activity, but did not affect histone deacetylase (HDAC) activity in JB6 P+ cells. FX activates the Nrf2 signaling pathway, induces the epigenetic demethylation of CpG sites in *Nrf2*, and blocks TPA-induced transformation of JB6 P+ cells, indicating the potential health-promoting effects of FX in skin cancer prevention.[302]

Astaxanthin (AST) has synergistic antioxidant effects on polyunsaturated fatty acids at low concentrations via nuclear factor (erythroid-derived 2)-like 2 (NFE2L2 or Nrf2)/antioxidant response element (ARE) signaling. Chromatin remodeling and DNA methylation-based gene silencing represent common mechanisms in prostate carcinogenesis and tumor progression from normal cells to preinitiated cells and ultimately to invasive carcinoma. The control of

epigenetic modification and the transcriptional/translational control of the activation of Nrf2 and Nrf2 target genes, including glutathione *S*-transferases (GSTs), appear to be an important mechanism for protecting cells against injuries from oxidative stress and cancer development. AST in human LNCaP cells reduces the methylation of 21 CpG sites of the *GSTP1* CpG island but does not affect the three CpG sites of the *Nrf2* promoter region. AST induces the mRNA expression and protein expression of both *Nrf2* and *GSTP1* and increases the mRNA expression of *NQO1* in sh-mock LNCaP cells but not in sh-SETD7 LNCaP cells. AST reduces the protein expression of DNMT3b and inhibits DNMT and HDAC activities in vitro. AST decreases the methylation status of *GSTP1*.[303]

6.4.37 Plant Alkaloids

Some plant-derived alkaloids have been shown to be effective against neurodegenerative disorders. Alkaloids include among their number isoquinoline, indole, pyrroloindole, oxindole, piperidine, pyridine, aporphine, vinca, β-carboline, methylxanthene, lycopodium, and erythrine byproducts. Alkaloids may act as muscarinic and adenosine receptor agonists, antioxidant, antiamyloid, and MAO inhibitors, acetylcholinestrase and butyrylcholinesterase inhibitors, inhibitors of α-synuclein aggregation, dopaminergic and nicotine agonists, and NMDA antagonists.[304]

6.4.38 Spindlin1 Inhibitors

The discovery of inhibitors of the methyl- and acetyl-binding domains has provided evidence for the "druggability" of epigenetic effector molecules. The small-molecule probe UNC1215 prevents methyl-dependent protein-protein interactions by engaging the aromatic cage of MBT domains and, with lower affinity, Tudor domains. Using a library of tagged UNC1215 analogs, Bae et al.[305] identified a compound (EML405) that acquired a novel interaction with the Tudor domain-containing protein Spindlin1 (SPIN1). Structural studies facilitated the rational synthesis of SPIN1 inhibitors with increased selectivity (EML631-633), which engage SPIN1 in cells, block its ability to "read" H3K4me3 marks, and inhibit its transcriptional-coactivator activity.

6.4.39 Organoruthenium Compounds

Ruthenium complexes are potential replacements for platinum compounds in oncotherapy. Three histones (H3.1, H2A, H2B) are possible targets for an anticancer redox organoruthenium compound (RDC11). A comparative study of the ruthenium complex vs cisplatin showed differential epigenetic modifications on histone H3 that correlated with differential expression of histone deacetylase (*HDAC*) genes. Cisplatin preferentially triggered p53 and folate biosynthesis, while the ruthenium complex induced the endoplasmic reticulum stress and trans-sulfuration pathways. Suberoylanilide hydroxamic acid (SAHA) synergizes with cisplatin cytotoxicity while antagonizing ruthenium complex activity.[306]

6.5 lncRNAs, miRNAs, AND DERIVED TECHNOLOGIES

MicroRNAs (miRNAs) are key regulators of gene expression, representing largely unexplored layers of DMET (metabolizing enzymes and transporters) gene regulation.[307] They have been shown to have strong therapeutic potential for different modalities of intervention, including long-term transcriptional gene silencing.[308]

Patent applications have been lodged for a series of reagents and kits that can be used for the early diagnosis of cancer. They do so by detecting specific lncRNAs, which have the advantages of high sensitivity, specificity, and stability. In addition, lncRNAs have been used as effective targets for developing targeted cancer drugs. The role played by lncRNAs in cancer involves the regulation of target genes, by medicating cancer physiological and pathological processes via diverse mechanisms at the epigenetic, transcriptional, and posttranscriptional level.[309]

miRNAs play an important role in cancer cell proliferation, survival, and apoptosis. Epigenetic modifiers regulate miRNA expression. Histone deacetylases (HDACs) function as key regulators of miRNA expression. miRNA-based treatments are gaining importance for use in anticancer therapy. Epigenetic regulation of miR-200 is a potential strategy for therapy against triple-negative breast cancer.[310]

Arteriogenesis is an important shear stress-induced adaptation to bypass arterial occlusion with implications for treating peripheral arterial disease. miR-146a is a candidate regulator of vascular remodeling that regulates in vitro

angiogenic endothelial cell behavior, as well as perfusion recovery, arteriogenesis, and angiogenesis in response to arterial occlusion.[311]

A cationic branched tea polysaccharide (CTPSA) derivative, bearing *N*-acylurea and 3-(dimethylamino)-1-propylamine residues, has been synthesized and characterized. A nonspecific siRNA (NsiRNA) has been used as a model molecule of functional siRNA that could downregulate overexpressed glycometabolism enzymes in liver. CTPSA and NsiRNA could form stable complexes when their weight ratio is larger than 18. The CTPSA/NsiRNA complex has been observed to comprise spherical nanoparticles 100 nm in size. CTPSA/NsiRNA complexes exhibited lower cytotoxicity in HL-7702 cells when compared with branched PEI (bPEI) and bPEI/NsiRNA complexes. The CTPSA derivative might be useful as a nonviral vector for targeted delivery of functional siRNA to hepatocytes.[312]

Persistent activation of the phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin (mTOR) pathway is an important mechanism in the resistance of breast cancer to endocrine therapy. Everolimus has potent inhibitory effects on the mTOR pathway, with modest clinical activity as a single agent. lncRNAs are involved in everolimus resistance.

Long noncoding RNA N-acylsphingosine amidohydrolase 2B-2 (*Inc-ASAH2B-2*) knockdown in BT474 and MCF7 breast cancer cells has shown that *Inc-ASAH2B-2* is upregulated by everolimus in cells with and without serum. Moreover, reduced *Inc-ASAH2B-2* expression has been shown to inhibit the proliferation of BT474 and MCF7 cells. *Inc-ASAH2B-2* might be a new therapeutic target for breast cancer.[313]

Metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) represents one of the best characterized lncRNAs. The aberrant expression and dysregulated activity of *MALAT1* in human malignancies makes *MALAT1* a potential target for novel treatment strategies against cancer.[314]

A hominid-specific long noncoding RNA, *MORT* (*ZNF667-AS1*), is expressed in all normal cell types and is epigenetically silenced during cancer-associated immortalization of human mammary epithelial cells. The 10 most common cancers in humans display DNA methylation-associated *MORT* silencing in a large fraction of their tumors. Of the 16 common cancer types 7 show DNA methylation linked to *MORT* silencing. *MORT* expression is silenced by aberrant DNA methylation in 22 of the 33 TCGA cancer types. These 22 cancers include most carcinoma types, blood-derived cancers, and sarcomas.[315] Since epigenetic aberrations in the *MORT* gene are the most common changes seen in human cancer, it is very likely that MORT acts as a tumor suppressor, representing an attractive pharmacoepigenetic target.

miRNAs are key mediators of the host response to infection, predominantly by regulating proteins involved in innate and adaptive immune pathways. miRNAs can govern the cellular tropism of some viruses, are implicated in the resistance of some individuals to infections like HI, and are associated with impaired vaccine response in older people. RG-101 and miravirsen hepatitis C treatments target host miRNAs, and miRNAs are also being used to design attenuated vaccines.[316]

H19 is an lncRNA regulated by genomic imprinting through methylation at the locus between H19 and IGF2. H19 is important in normal liver development, controlling proliferation and impacting genes involved in an important network that manages fetal development. H19 also plays a major role in disease progression, particularly in hepatocellular carcinoma. H19 participates in the epigenetic regulation of many processes impacting diseases, such as activating the miR-200 pathway by histone acetylation to inhibit the epithelial-mesenchymal transition to suppress tumor metastasis. H19 has been proposed as a potential therapeutic target in liver disease.[317]

Dysregulation of miRNAs contributes to the pathogenesis of all types of cancer. The diminished expression of tumor suppressor miRNAs, such as members of the Let-7 and miR-34 family, promotes tumor progression, invasion, and metastasis. Some advances have been documented in miRNA replacement therapy in cancer. This approach aims to restore tumor suppressor miRNA function in tumor cells using synthetic miRNA mimics or miRNA expression plasmids.[318]

TNF-related apoptosis-inducing ligand (TRAIL) possesses the capacity to induce apoptosis in a wide variety of tumor cells without affecting most normal cells. However, many primary cancer cells are resistant to TRAIL monotherapy. The miR-221/222 cluster is upregulated in TRAIL-resistant liver cancer cells. Specific inhibitors of miR-221 and/or miR-222 (sponge, TuD, miR-Zip) have been constructed and evaluated to overcome TRAIL resistance. AAV-mediated gene therapy, by means of coexpression of TRAIL with miR-221-Zip, showed the greatest synergistic activity in the induction of apoptosis in vitro. In vivo treatment of nude mice bearing human TRAIL-resistant liver cancer xenografts with AAV-TRAIL-miR-221-Zip also led to growth inhibition. This sensitizing effect of miR-221-Zip has been associated with increased expression of *PTEN*, the miR-221 target, as well as with decreasing levels of survivin. miR-221 expression has been found to be concomitant with promotion of survivin expression and suppression of *PTEN* expression. TRAIL sensitivity of cancer cells isolated from liver cancer tissues correlated with *miR-221* expression, and miR-221 blood expression levels in liver cancer patients correlated with TRAIL sensitivity, showing potential as a predictor of TRAIL sensitivity in liver cancer.[319]

lncRNA *MIAT* expression is associated with tumor size, lymph node metastasis, distant metastasis, and TNM stage. The expression of *MIAT* in lung cancer tissues is upregulated. lncRNA *MIAT* is an independent factor for predicating the prognosis of lung cancer patients. Patients with low lncRNA *MIAT* have longer overall survival time and progression-free survival time than patients with high lncRNA *MIAT* expression. The knockdown of *MIAT* sensitizes PC9 and gefitinib-resistant PC9 cells to gefitinib and increases the expression of miR-34a and inactivated PI3K/Akt signaling. MIAT interacts with miR-34a and epigenetically controls miR-34a expression by hypermethylating its promotor. The knockdown of *MIAT* by siRNA enhances lung cancer cells to gefitinib through the PI3K/Akt signaling pathway by epigenetically regulating miR-34a.[320]

miR-149 has been implicated in tumor progression by regulating cellular proliferation, migration, invasion, epithelial-mesenchymaltransition, and chemoresistance. miR-149 can function both as an oncogene or tumor suppressor, depending on the type of cancer, thereby giving rise to the possibility of utilizing *miR-149* mimics and inhibitors for cancer therapy. *miR-149* is known to be epigenetically silenced via DNA methylation, which can be reversed with the use of demethylating agents. miR-149-based therapy appears to be an attractive anticancer strategy.[321]

Chemokine CXC receptor 4 (CXCR4) in spinal glial cells has been implicated in neuropathic pain. *CXCR4* expression increases in spinal glial cells of mice that have pSNL-induced neuropathic pain. Blocking CXCR4 alleviates pain behavior, and overexpressing *CXCR4* induces pain hypersensitivity. MicroRNA-23a-3p (miR-23a) directly binds to

TABLE 6.12 Pharmacological Profile and Pharmacogenetics of Selected Epigenetic Drugs

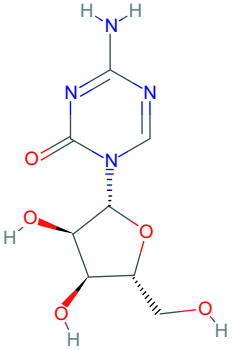
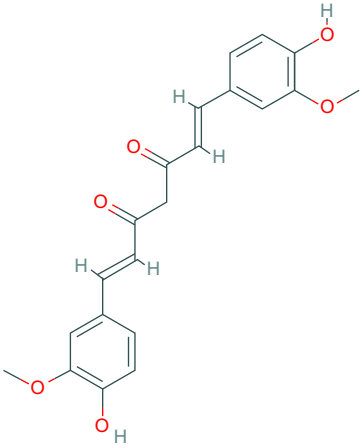
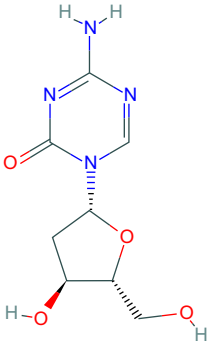
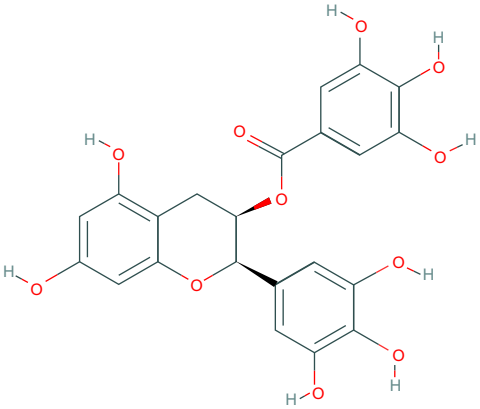
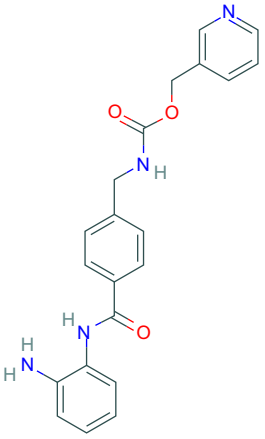
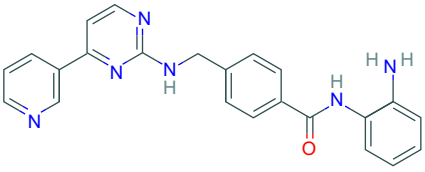
Drug	Properties	Pharmacogenetics
	**Name: 5-Azacytidine**; azacitidine; azacytidine; ladakamycin; vidaza; mylosar; azacitidinum; 5-AZAC **IUPAC name:** 4-Amino-1-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3,5-triazin-2-one **Molecular formula:** $C_8H_{12}N_4O_5$ **Molecular weight:** 244.20 g mol^{-1} **Category:** Pyrimidine nucleoside cytidine analog **Mechanism:** DNA methyltransferase inhibitor; Telomerase inhibitor **Target:** DNA (cytosine-5)-methyltransferase 1 (DNMT1) **Interactions:** Cytidine deaminase **Effect:** Antineoplastic; antimetabolite; methylates CpG residues; methylates hemimethylated DNA; mediates transcriptional repression by direct binding to HDAC2	**Pathogenic genes:** *ALDH3A1, CDKN2A, MGMT, PLA2R1, RRM1, TNFRSF1B* **Mechanistic genes:** *ALDH1A1, DAPK1, DNMT1, DPYD, CDKN2A, MGMT, PLCB1* **Metabolic genes:** **Substrate:** *CDA, DCK, SLC28A1, SLC29A1, RRM1, RRM2, UCK1, UCK2* **Inhibitor:** *CYP1A2 (weak), CYP2E1 (weak), DNMT1* **Inducer:** *SULT1C2* **Transporter genes:** *SLC5A5, SLC28A1, SLC29A1* **Pleiotropic genes:** *BLK*
	**Name: Curcumin**; diferuloylmethane; natural yellow 3; turmeric yellow; turmeric; kacha haldi; Gelbwurz; Curcuma; haldar; souchet **IUPAC name:** (1*E*,6*E*)-1,7-bis(4-Hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione **Molecular formula:** $C_{21}H_{20}O_6$ **Molecular weight:** 368.38 g mol^{-1} **Category:** Natural product (*Curcuma longa*) **Mechanism:** Histone acetyltransferase (HAT) inhibitor **Effect:** Nonsteroidal antiinflammatory agent; antineoplastic; antioxidant; cognitive enhancer; coloring agent; enzyme inhibitor	**Pathogenic genes:** *BACE1, CCND1, CDH1, GSK3B, IL1A, IL6, JUN, MSR1, PSEN1, PTGS2, SNCA, SREBF1, TNF* **Mechanistic genes:** *AKT1, PRKAs, BACE1, CCND1, CDH1, CDKs, CRM1, CTNNB1, EGF, GSK3B, HDACs, HIF1A, IL1A, IL6, JUN, MMPs, MSR1, NFKB1, NOS2, PDGFRs, PSEN1, PTGS2, SNCA, SOCS1, SOCS3, SREBF1, STAT3, TNF, VEGFA* **Metabolic genes:** **Inhibitor:** *CYP2C8, CYP2C9, EP300* **Inducer:** *CYP2C8, CYP2C9, CYP2D6, CYP3A4* **Transporter genes:** *ABCA1, SNCA* **Pleiotropic genes:** *CTNNB1, MSR1*

TABLE 6.12 Pharmacological Profile and Pharmacogenetics of Selected Epigenetic Drugs—cont'd

Drug	Properties	Pharmacogenetics
	**Name: Decitabine**; 5-aza-2′-deoxycytidine; dacogen; dezocitidine; 2′-deoxy-5-azacytidine **IUPAC name:** 4-Amino-1-[(2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3, 5-triazin-2-one **Molecular formula:** $C_8H_{12}N_4O_4$ **Molecular weight:** 228.21 g mol^{-1} **Category:** Nucleoside **Mechanism:** DNA methyltransferase inhibitor **Target:** DNA (cytosine-5)-methyltransferase 1 (DNMT1) **Interactions:** Deoxycytidine kinase **Effect:** Antineoplastic; antimetabolite; enzyme inhibitor; teratogen	**Pathogenic genes:** *BRCA1, CDKN2B, DNMT3A, EGFR, FOS, MGMT, MLH1, MMP9, MYC, NOS3, NQO1, TP53, VHL* **Mechanistic genes:** *APAF1, BRCA1, CDKN2B, EGFR, ICAM1, MAGED1, MGMT, MLH1, MMP2, MMP9, MYC, NOS3, TIMP3, TP53, VHL, ZNF350.* **Metabolic genes:** **Substrate:** *DCK, DNMT1, CDA, SLC29A1* **Inhibitor:** *DNMT1, DNMT3B* **Inducer:** *DPYD* **Transporter genes:** *ABCs, SLC15s, SLC22s, SLC28A1, SLC29As* **Pleiotropic genes:** *HBG1, NQO1, NTRK2, MMP2, MSH2*
	**Name: Epigallocatechin 3-gallate, EGCG**; (−)-epigallocatechin gallate; tea catechin, teavigo, catechin deriv., 989-51-5 **IUPAC name:** [(2*R*,3*R*)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-chromen-3-yl] 3,4,5-trihydroxybenzoate **Molecular formula:** $C_{22}H_{18}O_{11}$ **Molecular weight:** 458.37 g mol^{-1} **Category:** DNMT inhibitors **Targets:** DNMT1	**Pathogenic genes:** *APP, BACE1, CDX2, EGFR, FAS, PIK3CA, ROS1* **Mechanistic genes:** *APP, BACE1, BMP2, CDX2, CHRNA7, ECEs, EGFR, IRS1, PIK3CA, ROS1* **Metabolic genes:** **Inhibitor:** *SOD* **Transporter genes:** *CD36, SLC5A1, SLC27A4, SLCO1B1, SLCO1B3* **Pleiotropic genes:** *ACACA, CHRNA7, SCD*
	**Name: Entinostat**; ms-275; 209783-80-2; SNDX-275; MS 275; MS-27-275; SNDX 275; histone deacetylase inhibitor I; S1053_Selleck; MS 27-275 **IUPAC name:** Pyridin-3-ylmethyl *N*-[[4-[(2-aminophenyl)carbamoyl]phenyl]methyl] carbamate **Molecular formula:** $C_{21}H_{20}N_4O_3$ **Molecular weight:** 376.41 g mol^{-1} **Category:** Benzamide **Mechanism:** Class I HDAC inhibitor (HDAC1, 2, 3) **Effect:** Antineoplastic agent; histone deacetylase inhibitor; memory enhancer	**Pathogenic genes:** *CDH1* **Mechanistic genes:** *CDH1, HDAC1, HDAC2, HDAC3, KLRK1* **Metabolic genes:** **Inhibitor:** *HDAC1, HDAC2, HDAC3* **Inducer:** *CYP19A1*
	**Name: Mocetinostat**; MGCD0103; 726169-73-9; MGCD-0103; MGCD 0103; *N*-(2-aminophenyl)-4-([[4-(pyridin-3-yl) pyrimidin-2 yl]amino]methyl)benzamide **IUPAC name:** *N*-(2-Aminophenyl)-4-[[(4-pyridin-3-ylpyrimidin-2-yl)amino]methyl] benzamide **Molecular formula:** $C_{23}H_{20}N_6O$ **Molecular weight:** 396.44 g mol^{-1} **Category:** Benzamide **Mechanism:** Class I HDAC inhibitor	**Pathogenic genes:** *CDKN1A, CDKN2B, TNF* **Mechanistic genes:** *CDKN1A, CDKN2B, HDAC1, HDAC2, HDAC3, HDAC11, NFKB2, TNF* **Metabolic genes:** **Inhibitor:** *HDAC1, HDAC2, HDAC3, HDAC11*

Continued

TABLE 6.12 Pharmacological Profile and Pharmacogenetics of Selected Epigenetic Drugs—cont'd

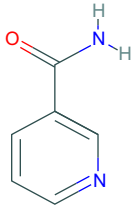
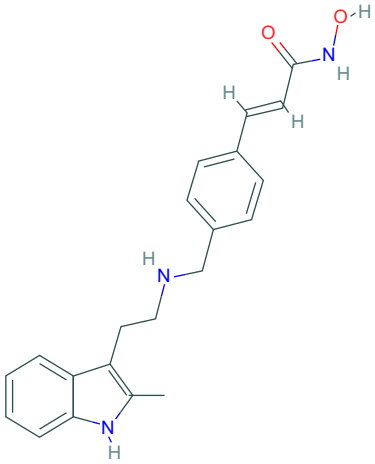
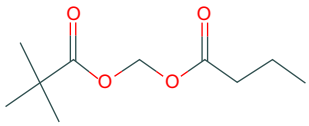
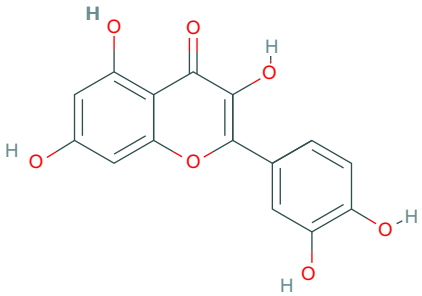
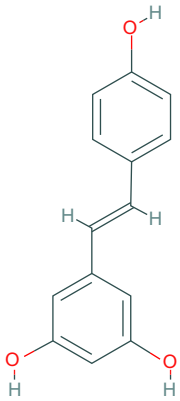
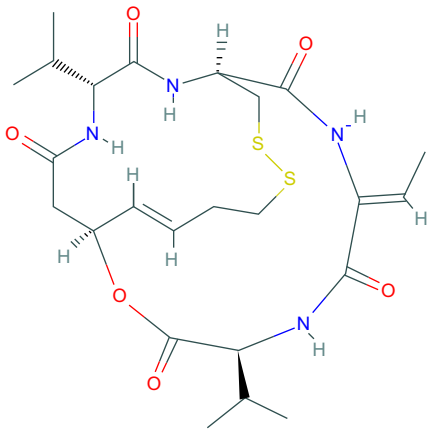
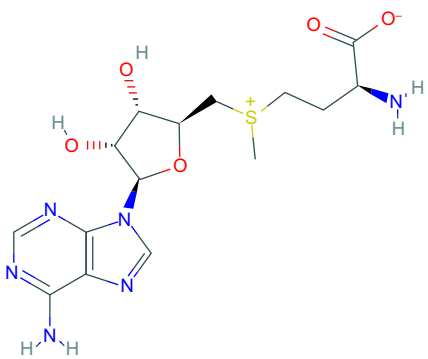
Drug	Properties	Pharmacogenetics
	(HDAC1, 2, 3); Class IV HDAC inhibitor (HDAC11) **Effect:** Antineoplastic agent; histone deacetylase inhibitor	
	**Name: Nicotinamide**; niacinamide, vitamin PP, aminicotin, nicotinic acid amide, amixicotyn, 3-pyridinecarboxamide, papulex, nicotylamide **IUPAC name:** Pyridine-3-carboxamide **Molecular formula:** $C_6H_6N_2O$ **Molecular weight:** 122.12 g mol^{-1} **Category:** SIRT inhibitors **Targets:** Class III HDACs (SIRT1–7)	**Pathogenic genes:** *IL6, IL8, PTGS2, TNF* **Mechanistic genes:** *ARTs, CAT, CLOCK, FOXO3, GPXs, IL6, IL8, PARP1, PTGS2, SIRT1, SOD1, TNF* **Metabolic genes:** **Inhibitor:** *CYP2D6, CYP3A4, CYP2E1, SIRT1-7* **Pleiotropic genes:** *CAT, PARP1*
	**Name: Panobinostat**; LBH-589; 404950-80-7; LBH589; faridak; NVP-LBH589; LBH 589; S1030_Selleck; AC1OCFY8; panobinostat (LBH589) **IUPAC name:** (*E*)-*N*-hydroxy-3-[4-[[2-(2-methyl-1*H*-indol-3-yl)ethylamino]methyl] phenyl]prop-2-enamide **Molecular formula:** $C_{21}H_{23}N_3O_2$ **Molecular weight:** 349.43 g mol^{-1} **Category:** Hydroxamic acid **Mechanism:** Class I HDAC inhibitor (HDAC1, 2, 3, 8); Class IIa HDAC inhibitor (HDAC4, 5, 7, 9); Class IIb HDAC inhibitor (HDAC6, 10); Class IV HDAC inhibitor (HDAC11); Pan-histone deacetylase inhibitor **Effect:** Antineoplastic agent; histone deacetylase inhibitor	**Pathogenic genes:** *CDKN1A, EGFR, IL6, RASSF1* **Mechanistic genes:** *AKT1, CDKN1A, DAPK1, DNMT1, EGFR, HDACs, HIST3H3, HIST4H4, HSP90As, IL6, IL10, IL12, IL23A, NFKB2, RASSF1, TLR3* **Metabolic genes:** **Substrate:** *CYP2C19, CYP2D6, CYP3A4* **Inhibitor:** *AKT1, CYP19A1 (strong), HDACs* **Pleiotropic genes:** *IL10*
	**Name: Pivanex**; AN-9; pivalyloxymethyl butyrate; AN 9; 122110-53-6; BRN 4861411; ((2,2-dimethylpropanoyl)oxy)methyl butanoate **IUPAC name:** Butanoyloxymethyl 2,2-dimethylpropanoate **Molecular formula:** $C_{10}H_{18}O_4$ **Molecular weight:** 202.25 g mol^{-1} **Category:** Short chain fatty acid **Mechanism:** Class I HDAC inhibitor (HDAC1, 2, 3, 8) **Effect:** Antineoplastic agent; histone deacetylase inhibitor	**Pathogenic genes:** *BCL2, TP53* **Mechanistic genes:** *BAX, BCL2, BCR-ABL, HDACs, TP53* **Metabolic genes:** **Inhibitor:** *ABCB1, HDACs* **Transporter genes:** *ABCB1*
	**Name: Quercetin**; sophoretin; quercetol; meletin; xanthaurine; quercitin; 3,3′,4′,5,7-pentahydroxyflavone **IUPAC name:** 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one **Molecular formula:** $C_{15}H_{10}O_7$ **Molecular weight:** 302.24 g mol^{-1} **Category:** DNMT inhibitors **Targets:** DNMT1	**Pathogenic genes:** *IL1R, NFkB, Ccl8, IKK, STAT3, CD4, CDK2, IL2* **Mechanistic genes:** *MTND4, CDKN2A, PRDX4, DIO2, HSD17B1, MSH2, GSS, COMT, FOS, CRP, NR1I3, PON1* **Metabolic genes:**

TABLE 6.12 Pharmacological Profile and Pharmacogenetics of Selected Epigenetic Drugs—cont'd

Drug	Properties	Pharmacogenetics
		Substrate: *UGT1A1, UGT1A3, GSTT1, CYP2J2, GSTK1, CYP2C8, CYP1A1, CYP1A2, CYP1B1, GSTA1, CYP19A1* **Inhibitor:** *SULT1E1* **Transporter genes:** *ABCB1, ABCG2*
	**Name: Resveratrol**; trans-resveratrol; 501-36-0; 3,4′,5-trihydroxystilbene; 3,4′,5-stilbenetriol; 3,5,4′-trihydroxystilbene; resvida; (*E*)-resveratrol **IUPAC name:** 5-[(*E*)-2-(4-Hydroxyphenyl) ethenyl]benzene-1,3-diol **Molecular formula:** $C_{14}H_{12}O_3$ **Molecular weight:** 228.24 g mol^{-1} **Category:** Natural polyphenol **Mechanism:** SIRT1 inducer/activator **Effect:** Nonsteroidal antiinflammatory agent; anticarcinogenic; antimutagenic; antineoplastic; antioxidant; platelet aggregation inhibitor; enzyme inhibitor; lifespan extension; memory improvement; Aβ decrease; reduction of plaque formation	**Pathogenic genes:** *BCL2, CAV1, ESR1, ESR2, GRIN2B, NOS3, PTGS2, TNFRSF10A, TNFRSF10B* **Mechanistic genes:** *APP, ATF3, BAX, BAK1, BBC3, BCL2, BCL2L1, BCL2L11, BIRC5, CASP3, CAV1, CFTR, ESR1, ESR2, GRIN1, GRIN2B, HTR3A, NFKB1, NOS3, PMAIP1, PTGS1, PTGS2, SIRT1, SIRT3, SIRT5, SRC, TNFRSF10A, TNFRSF10B, TRPs* **Metabolic genes:** **Substrate:** *CYP1A1, CYP1A2, CYP1B1, CYP2E1, GSTP1, PTGS1, PTGS2* **Inhibitor:** *CYP1A1, CYP1B1, CYP2C9, CYP2D6, CYP3A4, NQO2* **Inducer:** *CYP1A2, SIRT1* **Transporter genes:** *ABCC1, ABCC2, ABCC3, ABCC4, ABCC8, ABCG1, ABCG2, CFTR, TRPs*
	**Name: Romidepsin**; depsipeptide; chromadax; istodax; antibiotic FR 901228; FK228; FR 901228; FK-228; NSC 630176; NSC-630176 **IUPAC name:** (1*S*,4*S*,7*Z*,10*S*,16*E*,21*R*)-7-ethylidene-4,21-di(propan-2-yl)-2-oxa-12,13-dithia-5,8,20,23-tetrazabicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-pentone **Molecular formula:** $C_{24}H_{36}N_4O_6S_2$ **Molecular weight:** 540.70 g mol^{-1} **Category:** Cyclic peptide **Mechanism:** Class I HDAC inhibitor (HDAC1, 2, 3, 8); Class IIa HDAC inhibitor (HDAC4,5,7,9); Class IIb HDAC inhibitor (HDAC6, 10); Class IV HDAC inhibitor (HDAC11) **Effect:** Antibiotic; antineoplastic agent; histone deacetylase inhibitor	**Pathogenic genes:** *BCL2, CCDN1, CDKN1A, MYC, NF2, RB1, ROS1, TNFSF10, VHL* **Mechanistic genes:** *BCL2, CCDN1, CDKN1A, FLT1, HDAC1, HDAC2, HDAC3, HDAC4, HSP90As, KDR, MYC, NF2, TNFSF10, VEGFs, VHL* **Metabolic genes:** **Substrate:** *ABCB1, ABCG2, CYP1A1 (minor), CYP2B6 (minor), CYP2C19 (minor), CYP3A4 (major), CYP3A5 (minor), NR1I3, SLCO1B3* **Inhibitor:** *ABCB1, HDACs* **Inducer:** *ABCG2* **Transporter genes:** *ABCB1, ABCC1, ABCG2, SLCO1B3* **Pleiotropic genes:** *CDH1, CDKN1A*
	**Name: *S*-Adenosylmethionine**; ademetionine; AdoMet; donamet; *S*-adenosyl-L-methionine; SAMe; methioninyladenylate; SAM-e; adenosylmethionine **IUPAC name:** (2*S*)-2-Amino-4-[[(2*S*,3*S*,4*R*,5*R*)-5-(6-aminopurin-9-yl)-3,4-dihydroxyoxolan-2-yl] methyl-methylsulfonio]butanoate **Molecular formula:** $C_{15}H_{22}N_6O_5S$ **Molecular weight:** 398.44 g mol^{-1} **Category:** Methyl radical donor **Mechanism:** Histone methyltransferase inhibitor	**Pathogenic genes:** *AKT1, ERK, GNMT, MAT1A, PSEN1* **Mechanistic genes:** *AMD1, CAT, CBS, GCLC, GNMT, GSS, NOS2, ROS1, STAT1, TNF* **Metabolic genes:** **Substrate:** *COMT, GNMT, TPMT, SRM* **Inhibitor:** *ABCB1, CYP2E1, NOS2* **Transporter genes:** *SLC25A26* **Pleiotropic genes:** *CAT, TNF*

Continued

TABLE 6.12 Pharmacological Profile and Pharmacogenetics of Selected Epigenetic Drugs—cont'd

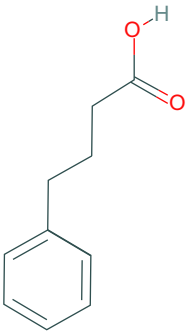
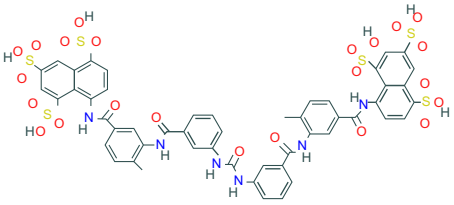
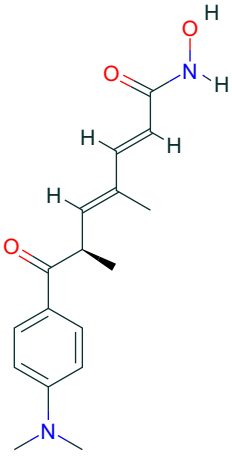
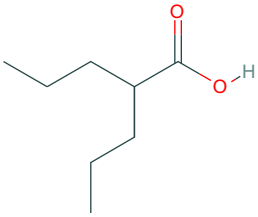
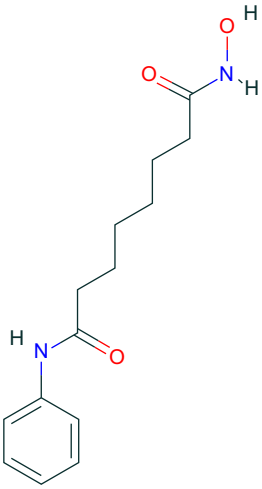
Drug	Properties	Pharmacogenetics
	Effect: Antineoplastic; antiinflammatory; memory enhancer; PSEN1 repressor	
	**Name: Sodium phenylbutyrate**; buphenyl; 4-phenylbutyric acid; 4-phenylbutanoic acid; benzenebutanoic acid; benzenebutyric acid; butyric acid; 1821-12-1; γ-phenylbutyric acid **IUPAC name:** 4-Phenylbutanoic acid **Molecular formula:** $C_{10}H_{12}O_2$ **Molecular weight:** 164.20 g mol^{-1} **Category:** Short chain fatty acid **Mechanism:** Class I HDAC inhibitor (HDAC1, 2, 3, 8); Class IIa inhibitor (HDAC4, 5, 7, 9); Class IIb inhibitor (HDAC6, 10) **Effect:** Antineoplastic agent; histone deacetylase inhibitor; memory improvement; pTau decrease via GSK3β inactivation; C99 and Aβ decrease; amyloid burden reduction	**Pathogenic genes:** *ARG1*, *ASS1*, *BCL2*, *CPS1*, *NAGS*, *OTC* **Mechanistic genes:** *BCL2*, *BDNF*, *EDN1*, *HDACs*, *HSPA8*, *ICAM1*, *NFKB2*, *NT3*, *VCAM1* **Metabolic genes:** **Inhibitor:** *HDACs* **Inducer:** *ARG1*, *CFTR*, *CYP2B6*, *NFKB2* **Transporter genes:** *CFTR* **Pleiotropic genes:** *ASL*, *BDNF*, *VCAM1*
	**Name: Suramin**; naphuride; germanin naganol; belganyl; fourneau; farma; antrypol; suramine; naganin **IUPAC name:** 8-[[4-Methyl-3-[[3-[[3-[[2-methyl-5-[(4,6,8-trisulfonaphthalen-1-yl) carbamoyl]phenyl]carbamoyl]phenyl] carbamoylamino]benzoyl]amino]benzoyl] amino]naphthalene-1,3,5-trisulfonic acid **Molecular formula:** $C_{51}H_{40}N_6O_{23}S_6$ **Molecular weight:** 1297.28 g mol^{-1} **Category:** Polyanionic compound **Mechanism:** Class III HDAC/sirtuin inhibitor (SIRT1–3) **Effect:** Antineoplastic agent; trypanocidal agent; antiparasitic; antinematodal (African trypanosomiasis, onchocerca); sirtuin inhibitor	**Mechanistic genes:** *FSHR*, *IL10*, *P2RY2*, *PDGFRB*, *RYR1*, *SIRT1*,*SIRT2*, *SIRT3*, *SIRT5* **Metabolic genes:** **Inhibitor:** *SIRT1*, *SIRT2*, *SIRT3*
	**Name: Trichostatin A**; 58880-19-6; TSA; trichostatin A (TSA); CHEBI:46024; TSA; 2,4-heptadienamide; 7-(4-(dimethylamino) phenyl)-*N*-hydroxy-4,6-dimethyl-7-oxo-7-(4-(dimethylamino)phenyl)-*N*-hydroxy-4,6-dimethyl-7-oxo-2,4-heptadienamide; [*R*-(*E*, *E*)]-7-[4-(dimethylamino)phenyl]-*N*-hydroxy-4,6-dimethyl-7-oxo-2,4-heptadienamide **IUPAC name:** (2*E*,4*E*,6*R*)-7-[4-(dimethylamino)phenyl]-*N*-hydroxy-4,6-dimethyl-7-oxohepta-2,4-dienamide **Molecular formula:** $C_{17}H_{22}N_2O_3$ **Molecular weight:** 302.37 g mol^{-1} **Category:** Hydroxamic acid **Mechanism:** Class I HDAC inhibitor (HDAC1, 2, 3); Class IIa HDAC inhibitor (HDAC4, 7, 9); Class IIb inhibitor (HDAC6) **Effect:** Antifungal agent; antibacterial agent; histone deacetylase inhibitor; protein	**Pathogenic genes:** *BCL2* **Mechanistic genes:** *BCL2*, *HDACs*, *IL8*, *IL12A*,*IL12B*, *NFKB2*, *RARB* **Metabolic genes:** **Substrate:** *CYP3A4 (mayor)* **Inhibitor:** *HDACs* **Inducer:** *CYP1A1*, *CYP1B1*, *CYP2B6*, *CYP2E1*, *CYP7A1*, *SLC19A3* **Transporter genes:** *SLC19A3*

TABLE 6.12 Pharmacological Profile and Pharmacogenetics of Selected Epigenetic Drugs—cont'd

Drug	Properties	Pharmacogenetics
	synthesis inhibitor; antineoplastic; memory improvement; rescue of CA3-CA1 LTP in APP/PS1 transgenic models	
	**Name: Valproic acid**; 2-propylpentanoic acid; depakene; depakine; ergenyl; dipropylacetic acid; mylproin; convulex; myproic acid **IUPAC name:** 2-Propylpentanoic acid **Molecular formula:** $C_8H_{16}O_2$ **Molecular weight:** 144.21 g mol^{-1} **Category:** Short chain fatty acid **Mechanism:** Class I HDAC inhibitor (HDAC1, 2, 3, 8) **Effect:** Anticonvulsant; mood stabilizer; antimanic agent; enzyme inhibitor; histone deacetylase inhibitor; GABA modulator; memory improvement; Aβ and pTau decrease; CDK5 inactivation	**Pathogenic genes:** *CREB1, IL6, LEP, SCN2A, TGFB1, TNF, TRNK* **Mechanistic genes:** *ABAT, CDK5, GSK3B, HDAC1, HDAC2, HDAC3, HDAC8, HDAC9, LEP, LEPR, SCNs, SMN2* **Metabolic genes:** **Substrate:** *ABCB1, CYP1A1 (minor), CYP2A6 (major), CYP2B6 (minor), CYP2C9 (major), CYP2C19 (minor), CYP2E1 (minor), CYP3A4 (minor), CYP4B1 (major), CYP4F2 (minor), UGT1A4, UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B7* **Inhibitor:** *ABCB1, ACADSB, AKR1A1, CYP2A6 (moderate), CYP2C9 (strong), CYP2C19 (moderate), CYP2D6 (weak), CYP3A4 (moderate), HDAC1, HDAC2, HDAC3, HDAC8, HDAC9, UGT1A9, UGT2B1, UGT2B7* **Inducer:** *ABCB1, AKR1C4, CASR, CYP2A6, CYP2B6, CYP3A4, CYP7A1, MAOA, NR1I2, SLC5A5, SLC6A2, SLC12A3, SLC22A16* **Transporter genes:** *ABCB1, ABCC2, ABCG1, ABCG2, SCNs, SLC5A5, SLC6A2, SLC12A3, SLC22A16* **Pleiotropic genes:** *ABL2, AGPAT2, ASL, ASS1, CDK4, CHRNA1, COL1A1, CPS1, CPT1A, DRD4, FMR1, FOS, HBB, HFE, HLA-A, HLA-B, ICAM1, IFNG, IL6, IL10, LEPR, NAGS, NR3C1, OTC, PTGES, STAT3, TGFB1, TNF, TP53.*
	**Name: Vorinostat**; suberoylanilide hydroxamic acid (SAHA); zolinza; suberanilohydroxamic acid; 149647-78-9; *N*-hydroxy-*N*′-phenyloctanediamide; SAHA cpd **IUPAC name:** *N*′-Hydroxy-*N*-phenyloctanediamide **Molecular formula:** $C_{14}H_{20}N_2O_3$ **Molecular weight:** 264.32 g mol^{-1} **Category:** Hydroxamic acid **Mechanism:** Class I HDAC inhibitor (HDAC1, 2, 3, 8); Class IIb inhibitor (HDAC6) **Effect:** Antineoplastic; memory improvement	**Pathogenic genes:** *BIRC3, CCND1, CDKN1A, CFLAR, CYP19A1, ERBB2, ERBB3, EGFR, RB1, TP53, TNF* **Mechanistic genes:** *CDKN1A, EGFR, ERBB2, ERBB3, STATs, TYMS, VEGFs* **Metabolic genes:** **Substrate:** *CYP2A6 (minor), CYP2C9 (minor), CYP2C19 (major), CYP2D6 (minor), CYP3A4 (major)* **Inhibitor:** *HDAC1, HDAC2, HDAC3, HDAC6* **Inducer:** *CYP1A1, CYP1A2, CYP1B1* **Pleiotropic genes:** *ALPs, TNF, TYMS*

ABAT, 4-aminobutyrate aminotransferase; *ABCs*, ATP-binding cassette family; *ABCA1*, ATP-binding cassette, subfamily A (ABC1), member 1; *ABCB1*, ATP-binding cassette, subfamily B (MDR/TAP), member 1; *ABCC1*, ATP-binding cassette, subfamily C (CFTR/MRP), member 1; *ABCC2*, ATP-binding cassette, subfamily C (CFTR/MRP), member 2; *ABCC3*, ATP-binding cassette, subfamily C (CFTR/MRP), member 3; *ABCC4*, ATP-binding cassette, subfamily C (CFTR/MRP), member 4; *ABCC8*, ATP-binding cassette, subfamily C (CFTR/MRP), member 8; *ABCG1*, ATP-binding cassette, subfamily G (WHITE), member 1; *ABCG2*, ATP-binding cassette, subfamily G (WHITE), member 2 (Junior blood group); *ABL2*, ABL protooncogene 2, nonreceptor tyrosine kinase; *ACACA*, acetyl-CoA carboxylase alpha; *ACADSB*, acyl-CoA dehydrogenase, short/branched chain; *AGPAT2*, 1-acylglycerol-3-phosphate *O*-acyltransferase 2; *AKR1A1*, aldo-keto reductase family 1, member A1 (aldehyde reductase); *AKR1C4*, aldo-keto reductase family 1, member C4; *AKT1*, v-akt murine thymoma viral oncogene homolog 1; *ALDH1A1*, aldehyde dehydrogenase 1 family,

member A1; *ALDH3A1*, aldehyde dehydrogenase 3 family, member A1; *ALPs*, alkaline phosphatases; *AMD1*, adenosylmethionine decarboxylase 1; *APAF1*, apoptotic peptidase activating factor 1; *APP*, amyloid beta (A4) precursor protein; *ARG1*, arginase 1; *ARTs*, ADP ribosyltransferases; *ASL*, argininosuccinate lyase; *ASS1*, argininosuccinate synthase 1; *ATF3*, activating transcription factor 3; *BACE1*, beta-site APP-cleaving enzyme 1; *BAK1*, BCL2-antagonist/killer 1; *BAX*, BCL2-associated X protein; *BBC3*, BCL2 binding component 3; *BCL2*, B cell CLL/lymphoma 2; *BCL2L1*, BCL2-like 1; *BCL2L11*, BCL2-like 11 (apoptosis facilitator); *BCR-ABL*, *BCR-ABL* tyrosine kinase fusion; *BDNF*, brain-derived neurotrophic factor; *BIRC3*, baculoviral IAP repeat containing 3; *BIRC5*, baculoviral IAP repeat containing 5; *BLK*, BLK protooncogene, Src family tyrosine kinase; *BMP2*, bone morphogenetic protein 2; *BRCA1*, breast cancer 1, early onset; *CASP3*, caspase 3, apoptosis-related cysteine peptidase; *CASR*, calcium-sensing receptor; *CAT*, catalase; *CAV1*, caveolin 1, caveolae protein, 22 kDa; *CBS*, cystathionine-beta-synthase; *CCDN1*, cyclin D1; *CCL8*, C-C motif chemokine ligand 8; *CD36*, CD36 molecule; *CDA*, cytidine deaminase; *CDH1*, cadherin 1, type 1; *CDK2*, cyclin-dependent kinase 2; *CDK4*, cyclin-dependent kinase 4; *CDK5*, cyclin-dependent kinase 5; *CDKN1A*, cyclin-dependent kinase inhibitor 1A (p21, Cip1); *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *CDKN2B*, cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4); *CDKs*, cyclin-dependent kinases; *CDX2*, caudal type homeobox 2; *CFLAR*, CASP8 and FADD-like apoptosis regulator; *CFTR*, cystic fibrosis transmembrane conductance regulator (ATP-binding cassette subfamily C, member 7); *CHRNA1*, cholinergic receptor, nicotinic, alpha 1 (muscle); *CHRNA7*, cholinergic receptor, nicotinic, alpha 7 subunit; *CLOCK*, circadian locomotor output cycles kaput; *COL1A1*, collagen, type I, alpha 1; *COMT*, catechol-*O*-methyltransferase; *CPS1*, carbamoyl-phosphate synthase 1, mitochondrial; *CPT1A*, carnitine palmitoyltransferase 1A (liver); *CREB1*, cAMP-responsive element binding protein 1; *CRP*, C-reactive protein; *CTNNB1*, catenin (cadherin-associated protein), beta 1, 88 kDa; *CYP1A1*, cytochrome P450, family 1, subfamily A, polypeptide 1; *CYP19A1*, cytochrome P450, family 19, subfamily A, polypeptide 1; *CYP1A2*, cytochrome P450, family 1, subfamily A, polypeptide 2; *CYP1B1*, cytochrome P450, family 1, subfamily B, polypeptide 1; *CYP2A6*, cytochrome P450, family 2, subfamily A, polypeptide 6; *CYP2C8*, cytochrome P450, family 2, subfamily C, polypeptide 8; *CYP2C9*, cytochrome P450, family 2, subfamily C, polypeptide 9; *CYP2C19*, cytochrome P450, family 2, subfamily C, polypeptide 19; *CYP2D6*, cytochrome P450, family 2, subfamily D, polypeptide 6; *CYP2E1*, cytochrome P450, family 2, subfamily E, polypeptide 1; *CYP2J2*, cytochrome P450, family 2, subfamily J, member 2; *CYP3A4*, cytochrome P450, family 3, subfamily A, polypeptide 4; *CYP3A5*, cytochrome P450, family 3, subfamily A, polypeptide 5; *CYP4B1*, cytochrome P450, family 4, subfamily B, polypeptide 1; *CYP4F2*, cytochrome P450, family 4, subfamily F, polypeptide 2; *CYP7A1*, cytochrome P450, family 7, subfamily A, polypeptide 1; *DAPK1*, death-associated protein kinase 1; *DCK*, deoxycytidine kinase; *DIO2*, iodothyronine deiodinase 2; *DNMT1*, DNA (cytosine-5-)-methyltransferase 1; *DNMT3A*, DNA (cytosine-5-)-methyltransferase 3 alpha; *DNMT3B*, DNA (cytosine-5-)-methyltransferase 3 beta; *DPYD*, dihydropyrimidine dehydrogenase; *DRD4*, dopamine receptor D4; *ECEs*, endothelin-converting enzymes; *EDN1*, endothelin 1; *EGF*, epidermal growth factor; *EGFR*, epidermal growth factor receptor; *EP300*, E1A-binding protein p300; *ERBB2*, erb-b2 receptor tyrosine kinase 2; *ERBB3*, erb-b2 receptor tyrosine kinase 3; *ERK*, elk-related tyrosine kinase; *ESR1*, estrogen receptor 1; *ESR2*, estrogen receptor 2 (ER beta); *FAS*, Fas (TNF receptor superfamily, member 6); *FLT1*, fms-related tyrosine kinase 1; *FMR1*, fragile X mental retardation 1; *FOS*, FBJ osteosarcoma oncogene; *FOXO3*, forkhead box O3; *FSHR*, follicle-stimulating hormone receptor; *GCLC*, glutamate-cysteine ligase, catalytic subunit; *GNMT*, glycine *N*-methyltransferase; *GPXs*, phage tail proteins; *GRIN1*, glutamate receptor, ionotropic, *N*-methyl D-aspartate 1; *GRIN2B*, glutamate receptor, ionotropic, *N*-methyl D-aspartate 2B; *GSK3B*, glycogen synthase kinase 3 beta; *GSS*, glutathione synthetase; *GSTA1*, glutathione *S*-transferase alpha 1; *GSTK1*, glutathione *S*-transferase kappa 1; *GSTP1*, glutathione *S*-transferase pi 1; *GSTT1*, glutathione *S*-transferase theta 1; *HBB*, hemoglobin, beta; *HBG1*, hemoglobin, gamma A; *HDAC1*, histone deacetylase 1; *HDAC11*, histone deacetylase 11; *HDAC2*, histone deacetylase 2; *HDAC3*, histone deacetylase 3; *HDAC4*, histone deacetylase 4; *HDAC6*, histone deacetylase 6; *HDAC8*, histone deacetylase 8; *HDAC9*, histone deacetylase 9; *HDACs*, histone deacetylases; *HFE*, hemochromatosis; *HIF1A*, hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor); *HIST3H3*, histone cluster 3, H3; *HIST4H4*, histone cluster 4, H4; *HLA-A*, major histocompatibility complex, class I, A; *HLA-B*, major histocompatibility complex, class I, B; *HSD17B1*, hydroxysteroid 17-beta dehydrogenase 1; *HSP90As*, heat shock protein 90 kDa alpha (cytosolic), class A; *HSPA8*, heat shock 70 kDa protein 8; *HTR3A*, 5-hydroxytryptamine (serotonin) receptor 3A, ionotropic; *ICAM1*, intercellular adhesion molecule 1; *IFNG*, interferon, gamma; *IKK*, I-kappaB kinase beta; *IL2*, interleukin 2; *IL6*, interleukin 6; *IL8*, interleukin 8; *IL10*, interleukin 10; *IL12*, interleukin 12; *IL1A*, interleukin 1, alpha; *IL1R*, interleukin receptor; *IL12A*, interleukin 12A; *IL23A*, interleukin 23, alpha subunit p19; *IL12B*, interleukin 12B; *IRS1*, insulin receptor substrate 1; *JUN*, jun protooncogene; *KDR*, kinase insert domain receptor; *KLRK1*, killer cell lectin-like receptor subfamily K, member 1; *LEP*, leptin; *LEPR*, leptin receptor; *MAGED1*, melanoma antigen family D1; *MAOA*, monoamine oxidase A; *MAT1A*, methionine adenosyltransferase I, alpha; *MGMT*, *O*-6-methylguanine-DNA methyltransferase; *MLH1*, mutL homolog 1; *MMP2*, matrix metallopeptidase 2; *MMP9*, matrix metallopeptidase 9; *MMPs*, matrix metallopeptidases; *MSH2*, mutS homolog 2; *MSR1*, macrophage scavenger receptor 1; *MTND4*, mitochondrially encoded NADH dehydrogenase 4; *MYC*, v-myc avian myelocytomatosis viral oncogene homolog; *NAGS*, *N*-acetylglutamate synthase; *NF2*, neurofibromin 2 (merlin); *NFKB1*, nuclear factor of kappa light polypeptide gene enhancer in B cells 1; *NFKB2*, nuclear factor of kappa light polypeptide gene enhancer in B cells 2 (p49/p100); *NOS2*, nitric oxide synthase 2, inducible; *NOS3*, nitric oxide synthase 3 (endothelial cell); *NQO1*, NAD(P)H dehydrogenase, quinone 1; *NQO2*, NAD(P)H dehydrogenase, quinone 2; *NR1I2*, nuclear receptor subfamily 1, group I, member 2; *NR1I3*, nuclear receptor subfamily 1, group I, member 3; *NR3C1*, nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor); *NT3*, 3′-nucleotidase; *NTRK2*, neurotrophic tyrosine kinase, receptor, type 2; *OTC*, ornithine carbamoyltransferase; *P2RY2*, purinergic receptor P2Y, G-protein coupled, 2; *PARP1*, poly(ADP-ribose) polymerase 1; *PDGFRB*, platelet-derived growth factor receptor, beta polypeptide; *PDGFRs*, platelet-derived growth factor receptors; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *PLA2R1*, phospholipase A2 receptor 1, 180 kDa; *PLCB1*, phospholipase C, beta 1 (phosphoinositide specific); *PMAIP1*, phorbol-12-myristate-13-acetate-induced protein 1; *PON1*, paraoxonase 1; *PRDX4*, peroxiredoxin 4; *PRKAs*, protein kinase family, AMP-activated; *PSEN1*, presenilin 1; *PTGES*, prostaglandin E synthase; *PTGS1*, prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase); *PTGS2*, prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase); *RARB*, retinoic acid receptor, beta; *RASSF1*, Ras association (RalGDS/AF-6) domain family, member 1; *RB1*, retinoblastoma 1; *RRM1*, ribonucleotide reductase M1; *ROS1*, ROS protooncogene 1, receptor tyrosine kinase; *RRM1*, ribonucleotide reductase M1; *RRM2*, ribonucleotide reductase M2; *RYR1*, ryanodine receptor 1 (skeletal); *SCD*, stearoyl-CoA desaturase; *SCN2A*, sodium channel, voltage gated, type II alpha subunit; *SCNs*, sodium channel family; *SIRT1*, sirtuin 1; *SIRT2*, sirtuin 2; *SIRT3*, sirtuin 3; *SIRT5*, sirtuin 5; *SLC5A1*, solute carrier family 5, member 1; *SLC5A5*, solute carrier family 5 (sodium/iodide cotransporter), member 5; *SLC6A2*, solute carrier family 6 (neurotransmitter transporter), member 2; *SLC12A3*, solute carrier family 12 (sodium/chloride transporter), member 3; *SLC15s*, solute carrier family 15; *SLC19A3*, solute carrier family 19 (thiamine transporter), member 3; *SLC22s*, solute carrier family 22; *SLC22A16*, solute carrier family 22 (organic cation/carnitine transporter), member 16; *SLC25A26*, solute carrier family 25 (*S*-adenosylmethionine carrier), member 26; *SLC27A4*, solute carrier family 27, member 4; *SLC28A1*, solute carrier family 28 (concentrative nucleoside transporter), member 1; *SLC29As*, solute carrier family 29; *SLC29A1*, solute carrier family 29 (equilibrative nucleoside transporter), member 1; *SLCO1B1*, solute carrier organic anion transporter family member 1B1; *SLCO1B3*, solute carrier organic anion transporter family, member 1B3; *SMN2*, survival of motor neuron 2, centromeric; *SNCA*, synuclein, alpha (non-A4 component of amyloid precursor); *SOCS1*, suppressor of cytokine signaling 1; *SOCS3*, suppressor of cytokine signaling 3; *SOD*, superoxide dismutase; *SRC*, SRC protooncogene, nonreceptor tyrosine kinase; *SREBF1*, sterol regulatory element-binding transcription factor 1; *SRM*, spermidine synthase; *STATs*, signal transducer and activator of transcription family; *STAT1*, signal transducer and activator of transcription 1, 91 kDa; *STAT3*, signal transducer and activator of transcription 3 (acute phase response factor); *SULT1C2*, sulfotransferase family, cytosolic 1C, member 2; *SULT1E1*, sulfotransferase family 1E, member 1; *TGFB1*, transforming growth factor, beta 1; *TIMP3*, TIMP metallopeptidase inhibitor 3; *TLR3*, Toll-like receptor 3; *TNF*, tumor necrosis factor; *TNFRSF10A*, tumor necrosis factor receptor superfamily, member 10a; *TNFRSF10B*, tumor necrosis factor receptor superfamily, member 10b; *TNFRSF1B*, tumor necrosis factor receptor superfamily, member 1B; *TNFSF10*, tumor necrosis factor (ligand) superfamily, member 10; *TP53*, tumor protein p53; *TPMT*, thiopurine *S*-methyltransferase; *TRNK*, mitochondrially encoded tRNA lysine; *TRPs*, transient receptor potential cation channels; *TYMS*, thymidylate synthetase; *UCK1*, uridine-cytidine kinase 1; *UCK2*, uridine-cytidine kinase 2; *UGT1A1*, UDP glucuronosyltransferase family 1, member A1; *UGT1A3*, UDP glucuronosyltransferase family 1, member A3; *UGT1A4*, UDP glucuronosyltransferase 1 family, polypeptide A4; *UGT1A6*, UDP glucuronosyltransferase 1 family, polypeptide A6; *UGT1A8*, UDP glucuronosyltransferase 1 family, polypeptide A8; *UGT1A9*, UDP glucuronosyltransferase 1 family, polypeptide A9; *UGT1A10*, UDP glucuronosyltransferase 1 family, polypeptide A10; *UGT2B1*, UDP glucuronosyltransferase 1 family, polypeptide B1; *UGT2B7*, UDP glucuronosyltransferase 2 family, polypeptide B7; *VCAM1*, vascular cell adhesion molecule 1; *VEGFA*, vascular endothelial growth factor A; *VEGFs*, vascular endothelial growth factor family; *VHL*, von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase; *ZNF350*, zinc finger protein 350.

the 3′-UTR of *CXCR4* mRNA. pSNL-induced neuropathic pain reduces the mRNA expression of miR-23a. Overexpression of *miR-23a* by intrathecal injection of miR-23a mimics (or lentivirus) reduces spinal CXCR4 and prevents pSNL-induced neuropathic pain. The knockdown of *miR-23a* by intrathecal injection of the miR-23a inhibitor (or lentivirus) induces pain-like behavior, which is reduced by CXCR4 inhibition. *miR-23a* knockdown or *CXCR4* overexpression in naive mice can increase thioredoxin-interacting protein (TXNIP), which is associated with induction of the NOD-like receptor protein 3 (NLRP3) inflammasome. CXCR4 and TXNIP are coexpressed, and there is a direct interaction between CXCR4 and TXNIP, which is increased in the spinal cord of pSNL mice. Inhibition of TXNIP reverses the pain behavior elicited by pSNL, *miR-23a* knockdown, or *CXCR4* overexpression. *miR-23a* overexpression or *CXCR4* knockdown inhibits the increase of the TXNIP and NLRP3 inflammasome in pSNL mice. These data reported by Pan et al.[322] suggest that miR-23a, by directly targeting CXCR4, regulates neuropathic pain via the TXNIP/NLRP3 inflammasome axis in spinal glial cells and that epigenetic interventions against miR-23a, CXCR4, or TXNIP may potentially serve as novel therapeutic avenues in treating peripheral nerve injury-induced nociceptive hypersensitivity.

A nutrient starvation-responsive lncRNA, JHDM1D antisense 1 (JHDM1D-AS1), promotes tumorigenesis by regulating angiogenesis in response to nutrient starvation. The expression of *JHDM1D-AS1* is increased in cancer cells and in clinical tumor samples compared with that in normal tissue. Stable expression of *JHDM1D-AS1* in human pancreatic cancer (PANC-1 and AsPC-1) cells promotes cell growth. The expression of genes for tumor-derived angiogenic factors (*hHGF and hFGF1*) concomitant with host-derived inflammation-responsive genes (*mMmp3, mMmp9, mS100a8*, and *mS100a9*) is increased in tumor xenografts of *JHDM1D-AS1*-expressing pancreatic cancer cells, leading to poor prognosis. Increased *JHDM1D-AS1* expression under nutrient starvation accelerates tumor growth by upregulating angiogenesis.[323]

6.6 PHARMACOGENETICS OF EPIGENETIC DRUGS

Like any other xenobiotic agent, epigenetic drugs are processed via the pharmacogenetic apparatus (Table 6.12).[4,5,6,7] Epidrugs can act mechanistically on different components of the epigenetic machinery, hence influencing the expression of pathogenic, metabolic, transporter, and pleiotropic genes. Likewise, by-products of the gene clusters integrating the pharmacogenetic apparatus may bidirectionally interact with epidrugs modulating their effects. The integrity of the pharmacogenetic network and the reciprocal interaction of epidrugs with pharmacoepigenetic effectors are responsible for drug efficacy and safety. Structural anomalies, dysfunctional interactions, and specific pathogenic conditions, together with mutations in the genes encoding components of the epigenetic machinery as well as polymorphic variants in pathogenic, mechanistic, metabolic, transporter, and pleiotropic genes, may lead to drug resistance and, consequently, ineffective therapeutic interventions in different human pathologies.[15,16]

6.7 PHARMACOEPIGENETIC EFFECTS OF SELECTED THERAPEUTIC INTERVENTIONS

6.7.1 Anticancer Strategies

6.7.1.1 Aurora Kinase A Oncogene-H3K9 Combined Inhibition

Pancreatic cancer (PDAC) may develop and progress in response to the interaction between known oncogenes and downstream epigenomic regulators. Mathison et al.[324] tested a new combinatorial therapy based on the inhibition of the Aurora kinase A (*AURKA*) oncogene and one of its targets, the H3K9 methylation-based epigenetic pathway. This therapeutic combination is effective at inhibiting the in vitro growth of PDAC cells both in monolayer culture systems and in three-dimensional spheroids and organoids. The combination also reduces the growth of PDAC xenografts in vivo. Inhibiting methyltransferases of the H3K9 pathway in cells, which are arrested in G2-M after targeting *AURKA*, decrease H3K9 methylation at centromeres, induce mitotic aberrations, trigger an aberrant mitotic checkpoint response, and lead to mitotic catastrophe.[324]

Dietrich et al.[325] measured the ex vivo sensitivity of 246 blood cancers to 63 drugs alongside genome, transcriptome, and DNA methylome analysis to understand the determinants of drug response. In chronic lymphocytic leukemia (CLL), responses to 62% of drugs were associated with two or more mutations and linked the B cell receptor (BCR) pathway to trisomy 12, an important driver of CLL. According to drug responses, CLL may be differentiated into clusters of signaling pathways (BCR, mTOR, MEK), mutations, gene expression, and DNA methylation. mTOR

signaling drives 14% of CLLs, and immunoglobulin heavy chain variable gene (*IGHV*) mutation status and trisomy 12 are the most important modulators of response to kinase inhibitors.[325]

6.7.1.2 p38α Inhibitors and Taxanes

Cánovas et al.[326] reported a role for the protein kinase p38α in coordinating the DNA damage response and limiting chromosome instability during breast tumor progression and identified the DNA repair regulator CtIP as a p38α substrate. Decreased p38α signaling results in impaired ATR activation and homologous recombination repair, with concomitant increases in replication stress, DNA damage, and chromosome instability, leading to cancer cell death and tumor regression. Pharmacological inhibition of p38α potentiates the effects of taxanes by boosting chromosome instability, suggesting potential interest in combining p38α inhibitors with chemotherapeutic drugs.

6.7.1.3 Bortezomib

Painful neuropathy is a severe side effect of bortezomib and a common reason for treatment discontinuation. Bortezomib increases the expression of NOD-like receptor family pyrin domain-containing 3 (*NLRP3*) and phosphorylates the signal transducer and activator of transcription-3 (*STAT3*) in dorsal root ganglion (DRG). Intrathecal injection of *NLRP3* siRNA prevents mechanical allodynia induced by bortezomib, and intrathecal injection of recombinant adeno-associated virus vector encoding *NLRP3* markedly decreases the paw withdrawal threshold of naive rats. Bortezomib increases the recruitment of STAT3 and the acetylation of histone H3 and H4 in the *NLRP3* promoter region in DRG neurons. The inhibition of STAT3 activity by S3I-201 or DRG local deficiency of STAT3 prevent upregulated H3 and H4 acetylation in the *NLRP3* promoter region following bortezomib treatment. The upregulation of *NLRP3* in DRG via STAT3-dependent histone acetylation is critically involved in bortezomib-induced mechanical allodynia.[327]

Bortezomib treatment induces the upregulation of methylglyoxal in the spinal dorsal horn of rats. Spinal local application of methylglyoxal also induces mechanical allodynia and central sensitization in normal rats. Bortezomib upregulates the expression of receptors for advanced glycation end products (RAGE) and phosphorylated STAT3 (p-STAT3) in dorsal horn. Intrathecal injection of metformin, a known scavenger of methylglyoxal, attenuates the upregulation of methylglyoxal and RAGE in dorsal horn, central sensitization, and mechanical allodynia induced by bortezomib treatment. Blockage of RAGE also prevents the upregulation of p-STAT3, central sensitization, and mechanical allodynia induced by bortezomib treatment. Inhibition of STAT3 activity by S3I-201 attenuates bortezomib-induced mechanical allodynia and central sensitization. Local knockdown of *STAT3* ameliorates the mechanical allodynia induced by bortezomib. The accumulation of methylglyoxal may activate the RAGE/STAT3 signaling pathway in dorsal horn, contributing to the spinal central sensitization and persistent pain induced by bortezomib.[328]

6.7.1.4 Sorafenib

Patients with acute myeloid leukemia (AML) harboring an internal tandem duplication (ITD) in the gene encoding Fms-related tyrosine kinase 3 (*FLT3*) who relapse after allogeneic hematopoietic cell transplantation (allo-HCT) have a 1-year survival rate below 20%. Sorafenib, a multitargeted tyrosine kinase inhibitor, increases IL-15 production by FLT3-ITD+ leukemia cells. This synergizes with the allogeneic $CD8^+$ T cell response, leading to long-term survival in mouse models of FLT3-ITD+ AML. Sorafenib-related IL-15 production causes an increase in $CD8^+CD107a^+IFN\text{-}\gamma^+$ T cells that display features of longevity, which eradicates leukemia in secondary recipients. Sorafenib has been found to reduce expression of the transcription factor *ATF4*, thereby blocking negative regulation of interferon regulatory factor 7 (IRF7) activation, which enhances *IL-15* transcription. Both *IRF7* knockdown and *ATF4* overexpression in leukemia cells antagonize sorafenib-induced IL-15 production in vitro. Human FLT3-ITD+ AML cells obtained from sorafenib responders following sorafenib therapy show increased levels of IL-15, phosphorylated IRF7, and a transcriptionally active IRF7 chromatin state. The mitochondrial spare respiratory capacity and glycolytic capacity of $CD8^+$ T cells increase upon sorafenib treatment in sorafenib responders but not in nonresponders. The synergism of T cells and sorafenib is mediated via reduced *ATF4* expression, causing activation of the IRF7-IL-15 axis in leukemia cells and thereby leading to metabolic reprogramming of leukemia-reactive T cells in humans.[329]

6.7.1.5 Imatinib

Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasm whose pathogenesis is linked to presence of the Philadelphia chromosome that generates the BCR-ABL1 fusion oncogene. Tyrosine kinase inhibitors (TKI) (imatinib mesylate, IM) improve the treatment efficiency and survival of CML patients by targeting BCR-ABL tyrosine kinase. Patients in the chronic phase respond well to treatment; however, patients in the accelerated phase or blast crisis usually show therapy resistance and CML relapse. Dysregulations in epigenetic modulators such as histone

methyltransferases have been described for some hematologic malignancies. Lysine methyltransferase MLL2/KMT2D and MLL3/KMT2C are important players.

Both methyltransferases are either upregulated or have basal expression levels unchanged during the chronic phase. MLL3/KMT2C and especially MLL2/KMT2D levels decrease during disease progression correlating with distinct clinical stages. MLL2/KMT2D is decreased in patients resistant to IM treatment. The expression of both MLL genes was observed to be restored in KCL22S, a CML cell line sensitive to IM, after treatment with dasatinib or nilotinib, which was associated with a higher rate of apoptosis, an enhanced expression of p21 (*CDKN1A*), and a concomitant decrease in the expression of *CDK2, CDK4*, and cyclin B1 (*CCNB1*) in comparison with the untreated KCL22S control or IM-resistant KCL22R cell line, which suggests involvement of the p53-regulated pathway.[330]

6.7.1.6 Ibrutinib

B cell receptor (BCR) signaling is key to the survival of chronic lymphocytic leukemia (CLL) cells, and BCR signaling inhibitors have been shown to be clinically active. However, relapse and resistance to treatment are very frequent. To detect novel candidate therapeutic targets, Wolf et al.[331] performed a genome-wide DNA methylation screen and identified aberrant promoter DNA methylation in 2192 genes. The transcription factor *NFATC1*, which is a downstream effector of BCR signaling, was among the top hypomethylated genes and was concomitantly transcriptionally upregulated in CLL. *NFATC1* promoter DNA hypomethylation levels correlated with Binet disease staging and thymidine kinase levels, strongly suggesting a central role of *NFATC1* in CLL development. DNA hypomethylation at the *NFATC1* promoter inversely correlated with RNA levels of *NFATC1*, and dysregulation correlated with expression of target genes *BCL-2, CCND1*, and *CCR7*. Inhibition of the NFAT regulator calcineurin using tacrolimus, cyclosporin A, and the BCR-signaling inhibitor ibrutinib significantly reduced NFAT activity in leukemic cell lines, and NFAT inhibition resulted in increased apoptosis of primary CLL cells.

6.7.1.7 Doxorubicin

Doxorubicin (DOX) is a widely used treatment for human cancers. This drug increases the risk of life-threatening congestive heart failure (CHF). DOX-induced mitochondrial damage is cumulative and persistent. Chronic DOX therapy is associated with epigenetic modifications of DNA methylation status. DOX exposure alters DNA methylation landscapes, with altered methylation in several genes (*Rbm20, Nmnat2, Klhl29, Cacna1c, Scn5a*). The gene expression of *Rbm20, Klhl29*, and *Nmnat2* is altered in DOX-treated animals. Klhl29 and Nmnat2 protein expression is also altered in response to DOX.[332]

The stromal cell-derived factor-1/C-X-C chemokine receptor type 4 (SDF1/CXCR4) axis exerts a cardioprotective effect. In a mouse model of DOX-induced cardiomyopathy, $CXCR4^+$ cells are increased in response to DOX, mainly in human cardiac mesenchymal progenitor cells (CmPCs), a subpopulation with regenerative potential. CXCR4 is induced after 24 h of DOX exposure in CmPC. SDF1 administration protects against DOX-induced cell death and promotes CmPC migration. *CXCR4* promoter analysis revealed zinc finger E box-binding homeobox 1 (ZEB1) binding sites. Upon DOX treatment, ZEB1 binding decreases and RNA-polymerase-II increases, suggesting a DOX-mediated transcriptional increase in *CXCR4*. DOX induces the upregulation of *miR-200c*, which directly targets *ZEB1*. SDF1 administration in DOX-treated mice partially reverses adverse remodeling, decreasing left ventricular (LV) end diastolic volume, LV ejection fraction, and LV anterior wall thickness in diastole and recovering LV and systolic pressure. In vivo administration of SDF1 partially reverses DOX-induced miR-200c and p53 protein upregulation in mouse hearts. Downmodulation of *ZEB1* mRNA and protein by DOX is increased by SDF1.[333]

The DNA methylation status is altered in the male germline during testicular toxicity induced by doxorubicin. Reduced testicular expression levels of DNA methyltransferases DNMT3a and DNMT3b are present in DOX-treated animals. Hypomethylation is the most frequent change induced by DOX.[334]

6.7.1.8 Methotrexate

Methotrexate (MTX) is administered to treat childhood acute lymphoblastic leukemia (ALL). It acts by inhibiting dihydrofolate reductase, which reduces methyltetrahydrofolate, a key component in 1-carbon metabolism, thus reducing cell proliferation. Further perturbations to 1-carbon metabolism, such as reduced vitamin B12 levels via the use of nitrous oxide for sedation during childhood ALL treatment, may increase neurotoxicity risk. With vitamin B12 as an enzymatic cofactor, methyltetrahydrofolate is essential for the production of methionine, which is critical for DNA methylation. MTX treatment increases *LINE-1* methylation in neuronal cell lines (SH-SY5Y, DAOY) and increases *FKBP5* methylation in MO3.13 cells. Altered DNA methylation of brain cells might be one mechanism involved in MTX treatment-related neurotoxicities and neurocognitive late effects in ALL survivors.[335]

6.7.1.9 Polo-Like Kinase 4 (PLK4) Inhibitors

Polo-like kinase 4 (PLK4) is a critical regulator of centriole duplication and mitotic progression. *PLK4* is overexpressed in rhabdoid tumors (RT) and medulloblastomas (MB). Inhibiting PLK4 with a small-molecule inhibitor impairs the proliferation, survival, migration, and invasion of RT cells. PLK4 inhibition induces apoptosis, senescence, and polyploidy in RT and MB cells, thereby increasing the susceptibility of cancer cells to DNA-damaging agents. Targeting PLK4 with small-molecule inhibitors alone or in combination with other cytostatic agents might be a novel strategy for the treatment of RT and MB.[336]

6.7.1.10 Baicalin

Baicalin, a flavonoid compound isolated from the roots of *Scutellaria lateriflora* Georgi (Huang Qin), is an effective agent for the treatment of a variety of cancers. Baicalin has the potential to suppress the migration and invasion of highly aggressive breast cancer cells in a dose-dependent manner, but has no effect on the viability of these cancer cells. Baicalin reverses the epithelial-mesenchymal transition (EMT) process and downregulates the expression of β-catenin mRNA and protein. Baicalin reduces liver and lung metastasis of breast cancer, inhibits the expression of β-catenin, and degrades the EMT molecules vimentin and slug in orthotopic tumor tissues.[337]

Baicalin hydrate (BH) inhibits NPC cell growth by inducing apoptosis and cell cycle arrest. BH epigenetically regulates genome instability by upregulating the expression of satellite 2 (Sat2), alpha satellite (α-Sat) and major satellite (Major-Sat). BH also increases the level of IKKα, Suv39H1, and H3K9me3 and decreases LSH expression. BH promotes the splicing of Suv39H1 via the enhancement of m6A RNA methylation, rather than DNA methylation.[338]

Cisplatin is a primary anticancer drug against ovarian cancer, but recurrent tumors after treatment frequently show acquired chemoresistance. Extract of *Scutellaria baicalensis* (SbE), which contains baicalin, in combination with cisplatin reduces cell viability in CSC and in CRC. Cisplatin-induced cell death in CSC is mediated by p53-induced apoptosis accompanied by expression of the damage-regulated autophagy modulator (DRAM). In CRC, decreased *DRAM* expression hinders p21-mediated cell death and contributes to cisplatin resistance. Treatment of SbE also induces cell death in CSC by p53-dependent apoptosis. Cell death is mediated by autophagy with increased expression of *Atg5* and *Atg12*, rather than the p53-dependent pathway, with repressed expression of *p21* through HDAC1 activation. SbE treatment in combination with cisplatin has shown potential as a chemotherapeutic agent in cisplatin-resistant ovarian cancer.[339]

6.7.1.11 Sulforaphane

Sulforaphane (SFN) is a natural compound obtained from cruciferous vegetables that has potent anticancer activities. SFN may exert its chemopreventive effects partly through epigenetic demethylation and restoration of miR-9-3. CpG methylation is reduced in the *miR-9-3* promoter, and *miR-9-3* expression is increased after treatment with SFN. SFN treatment increases H3K4me1 enrichment at the *miR-9-3* promoter and attenuates enzymatic DNMT activity and DNMT3a, HDAC1, HDAC3, HDAC6, and CDH1 protein expression.[340]

The natural compound withaferin A (WA), from the Indian winter cherry, also has anticancer effects. The combinatorial effects of low concentrations of WA and SFN on breast cancer cell proliferation, histone deacetylase1 (HDAC1), and DNA methyltransferases (DNMTs) indicate a synergistic inhibition of cellular viability and induction of apoptosis. HDAC expression is downregulated at multiple levels. This combination decreases in BCL-2 and increases in BAX.[341]

Sulforaphane is one of the most potent histone deacetylase inhibitors (HDACis), with effects in the regulation of miRNAs and human telomerase reverse transcriptase (hTERT). SFN treatment decreases cell density, inhibits cell viability, induces apoptosis, and downregulates oncogenic *miR-21*, *HDAC* and *hTERT* mRNA, protein, and enzyme levels in CRC cells.[342]

Sulforaphane attenuates the expression of cancer-associated lncRNAs. SFN alters the expression of approximately 100 lncRNAs in different cell types and normalizes the expression of some lncRNAs that were differentially expressed in cancer cells. SFN-mediated alterations in lncRNA expression correlate with genes that regulate the cell cycle, signal transduction, and metabolism. *LINC01116* is overexpressed in several cancers and transcriptionally repressed after SFN treatment. Knockdown of *LINC01116* with siRNA decreases the proliferation of prostate cancer cells and upregulates several genes including *GAPDH* (regulates glycolysis), *MAP1LC3B2* (autophagy), and *H2AFY* (chromatin structure). A fourfold decrease in the ability of cancer cells to form colonies is found when the *LINC01116* gene is disrupted through a CRISPR/CAS9 method, further supporting an oncogenic function for LINC01116 in PC-3 cells.[343]

Sulforaphane acts via multiple mechanisms to modulate gene expression, including the induction of nuclear factor (erythroid-derived 2)-like 2 (Nrf2)-dependent signaling and the inhibition of histone deacetylase activity. In addition

to NAD(P)H quinone dehydrogenase 1 (NQO1) and other well-known Nrf2-dependent targets, SFN strongly induces the expression of *Loc344887*. This noncoding RNA is a novel functional pseudogene for NmrA-like redox sensor 1, under the name NmrA-like redox sensor 2 pseudogene (*NMRAL2P*). NMRAL2P also serves as a coregulator of NQO1 in human colon cancer cells. The silencing of *NMRAL2P* via CRISPR/Cas9 genome-editing protects against SFN-mediated inhibition of cancer cell growth, colony formation, and migration. *NMRAL2P* is the first functional pseudogene to be identified both as a direct transcriptional target of Nrf2 and as a downstream regulator of Nrf2-dependent NQO1 induction.[344]

6.7.1.12 Withaferin A

Withaferin A (WA) is a plant-derived steroidal lactone that holds promise as a therapeutic agent for the treatment of breast cancer (BC). Triple-negative breast cancer (TNBC) is characterized by poor prognosis and a DNA hypomethylation profile. In contrast to the DNA demethylating agent 5-aza-2′-deoxycytidine (DAC), WA treatment of MDA-MB-231 cells instead tackles an epigenetic cancer network through gene-specific DNA hypermethylation of tumor-promoting genes including ADAM metallopeptidase domain 8 (*ADAM8*), urokinase-type plasminogen activator (*PLAU*), tumor necrosis factor (ligand) superfamily, member 12 (*TNFSF12*), and genes related to detoxification (glutathione *S*-transferase mu 1, *GSTM1*) or mitochondrial metabolism (malic enzyme 3, *ME3*). Withaferin A induces epigenetic suppression of multiple cancer hallmarks associated with cell cycle regulation, cell death, cancer cell metabolism, cell motility, and metastasis. DNA hypermethylation of corresponding CpG sites in *PLAU, ADAM8, TNSF12, GSTM1*, and *ME3* genes correlates with receptor tyrosine–protein kinase erbB-2 amplification (HER2)/estrogen receptor (ESR)/progesterone receptor (PR) status in primary BC tumors. WA silences *HER2/PR/ESR*-dependent gene expression programs to suppress aggressive TNBC characteristics in favor of luminal BC hallmarks, with an improved therapeutic sensitivity.[345]

6.7.1.13 α-Lipoic Acid

α-Lipoic acid is a pleiotropic molecule with antioxidant and antiinflammatory properties mediated through modulation of NF-κB which regulates cytokines. *IL-1B* and *IL-6* undergo DNA methylation-dependent modulation in neuroblastoma cells in response to α-lipoic acid.[346]

6.7.1.14 PLK1 Inhibitors

The inhibition of PLK1 has proven potent antiproliferative activity in vitro, but the effectiveness of most synthetic targeted drugs has not translated into clinics. Brassesco et al.[347] studied the in vitro effects of two second-generation PLK1 inhibitors (BI 6727 and GSK461364) in breast cancer cell lines as a monotherapy or in combination with other drugs or ionizing radiation.

Mild effects on the viability of cell lines (MCF-7 and Hs578T) have been observed irrespective of the PLK1 inhibitor used. Alternately, the abrogation of PLK1 reduced clonogenicity while effectively sensitizing cells to ionizing radiation. Drug interactions showed dissimilar results, with antagonistic effects with any drug combination in MCF-7 and clear synergistic interactions between both PLK1 inhibitors and cisplatin, temozolomide, or doxorubicin in Hs578T, which is *TP53* mutated. The disparate responses of cell lines to drug combinations might denote a partial reflection of the substantial differences in the vast spectrum of genetic, biological, and epigenetic burden observed in breast cancer.

6.7.1.15 Proteasome Inhibitors

Mucinous type of epithelial ovarian cancer (MuOC) is a unique subtype with a poor survival outcome in recurrent and advanced stages. Liew et al.[348] analyzed the methylomic profiles of 6 benign mucinous adenomas, 24 MuOCs, 103 serous-type epithelial ovarian cancers (SeOCs), and 337 nonepithelial ovarian cancers. MuOC and SeOC exhibited distinct DNA methylation profiles comprising 101 genes, 81 of which exhibited low methylation in MuOC and were associated with the response to glucocorticoid, ATP hydrolysis-coupled proton transport, proteolysis involved in the cellular protein catabolic process, and ion transmembrane transport. The profiles of MuOC were similar to colorectal adenocarcinoma and stomach adenocarcinoma. Genetic interaction network analysis of differentially methylated genes in MuOC showed that a dominant network module is the proteasome subunit beta (PSMB) family. PSMB8 is a candidate marker for MuOC. PSMB8 is commonly expressed in MuOC and gastrointestinal cancer, but not in SeOC. Carfilzomib, a second-generation proteasome inhibitor, suppresses MuOC cell growth in vitro.

Posttranslational modification of the p53 signaling pathway plays an important role in cell cycle progression and stress-induced apoptosis. Dysregulation of *p53* and its E3 ligase *MDM2* by the ubiquitin-proteasome system (UPS) promotes carcinogenesis and malignant transformation. Drug discovery efforts have focused on the restoration of wild-type p53 activity or inactivation of oncogenic mutant *p53* by targeted inhibition of UPS components, particularly

key deubiquitinases (DUBs) of the ubiquitin-specific protease (USP) class. Zhang et al.[349] used a phage-displayed ubiquitin variant (UbV) library to develop inhibitors targeting the DUBs USP7 and USP10, which are involved in regulating levels of p53 and MDM2.

6.7.1.16 Selenium Compounds

Selenium compounds are promising chemotherapeutic agents with proposed epigenetic effects. Khalkar et al.[350] have assessed the effects of the inorganic selenium compound selenite and the organic form methylseleninic acid (MSA) in a leukemic cell line K562 on active (histone H3 lysine 9 acetylation (H3K9ac) and histone H3 lysine 4 trimethylation (H3K4me3)) and repressive (histone H3 lysine 9 trimethylation (H3K9me3)) histone marks. Both selenite and MSA had major effects on histone marks, but the effects of MSA were more pronounced. Selenite affected genes involved in the response to oxygen and hypoxia, whereas MSA affected distinct gene sets associated with cell adhesion and glucocorticoid receptors.

6.7.1.17 Epigenetic Silencing of O-6-Methylguanine-DNA Methyltransferase

Epigenetic silencing of *O*-6-methylguanine-DNA methyltransferase (*MGMT*) promoter via methylation in glioblastoma (GBM) has been correlated with a more favorable response to alkylating chemotherapeutic agents such as temozolomide. Selective targeting of altered epigenomes in recurrent GBMs may facilitate the future development of both prognostic biomarkers and enhanced therapeutic strategies.[351]

6.7.1.18 Cancer Immunotherapy

Cancer immunotherapy has become increasingly important compared with traditional cancer treatments, including surgery, chemotherapy, and radiotherapy. The clinical successes of immune checkpoint blockade, such as PD-1 and CTLA-4, represent a landmark event in cancer immunotherapy development.[352] Immune checkpoints can be regulated by stimulatory and inhibitory checkpoint molecules. Inhibitory checkpoints (cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein-1 (PD-1), and programmed cell death ligand 1 (PD-L1)) have been identified as suppressing antitumor immune responses in solid tumors. PD-1 blockers have been approved for treatment of melanoma and for treatment of nonsmall-cell lung cancer in 2015. The combination of anti-PD-1/PD-L1 with anti-CTLA-4 antibodies is under evaluation in clinical trials. Epigenetic modulators of checkpoints also contribute to improving the tumor microenvironment and restoring immune recognition and immunogenicity.[353]

BRAF and MEK inhibitor combinations or immune checkpoint inhibitors are current first-line treatments for metastatic melanoma, with unsatisfactory results. The molecular principles and drug classes that may hold promise for improved tumor therapy combination regimens include kinase inhibition, induction of apoptosis, DNA-damage response inhibition, epigenetic reprogramming, telomerase inhibition, redox modulation, metabolic reprogramming, proteasome inhibition, cancer stem cell trans-differentiation, immune cell signaling modulation, and others.[354]

ARID1A (the AT-rich interaction domain 1A) (BAF250a) is one of the most commonly mutated genes in cancer. *ARID1A* mutations are inactivating mutations that lead to loss of *ARID1A* expression, which makes ARID1A a poor therapeutic target. ARID1A interacts with mismatch repair (MMR) protein MSH2. ARID1A recruits MSH2 to chromatin during DNA replication and promotes MMR. ARID1A inactivation compromises MMR and increases mutagenesis. ARID1A deficiency correlates with a microsatellite instability genomic signature and a predominant C > T mutation pattern as well as increased mutation load across multiple human cancer types. Tumors formed by an ARID1A-deficient ovarian cancer cell line in syngeneic mice display increased mutation load, elevated numbers of tumor-infiltrating lymphocytes, and *PD-L1* expression. Treatment with the anti-PD-L1 antibody reduces tumor burden and prolongs survival of mice bearing ARID1A-deficient but not ARID1A wild-type ovarian tumors.[355]

Several epigenetic modifiers, such as histone deacetylase inhibitors, DNA methyl transferase inhibitors, bromodomain inhibitors, lysine-specific histone demethylase 1 inhibitors, and enhancer of zeste homolog 2 inhibitors, display intrinsic immunomodulatory properties. Several promising combinations, notably with immune checkpoint blockers or adoptive T cell therapy, can be explored.[356]

6.7.1.19 Synergistic and Multimodal Strategies

Combination strategies with two or more drugs to synergize their therapeutic effects are common in the treatment of cancer. These combinations can be made with epigenetic drugs, antitumor drugs plus epigenetic drugs, or common drugs able to display pharmacoepigenetic effects.

6.7.1.19.1 Liver Cancer

The combination of the DNA methyltransferase inhibitor 5-Aza-2′-deoxycytidine (5aza-dC) and the pan-deacetylase inhibitor trichostatin A (TSA) at low cytotoxic concentrations modulates the canonical Wnt/β-catenin pathway in liver cancer cells. 5Aza-dC and TSA treatments are enough to induce the expression of pathway antagonists, decrease β-catenin protein levels, relocalize protein to the plasma membrane, and reduce pathway transcriptional activity, accompanied by an antitumoral outcome with reduction of cell migration and clonogenic capability. Epigenetic drugs have been shown to modulate the Wnt/β-catenin pathway through E-cadherin upregulation under an activation pathway background, such as *CTNNB1* and *TP53* mutations, in liver cancer.[357]

6.7.1.19.2 Pancreatic Cancer

Pancreatic adenocarcinoma (PDAC) is classified into four molecular subtypes: squamous, immunogenic, pancreatic progenitor, and aberrantly differentiated endocrine-exocrine. Of all the subtypes the squamous subtype has the worst prognosis. Er et al.[358] identified 26 small molecules that may target the squamous subtype of PDAC, including inhibitors targeting the SRC protooncogene (*SRC*) and the mitogen-activated protein kinase kinase 1/2 (*MEK1/2*). SRC inhibitors (dasatinib and PP2) and MEK1/2 inhibitors (pimasertib) synergize gemcitabine sensitivity specifically in the squamous subtype of PDAC cells (SW1990 and BxPC3), but not in PDAC progenitor cells (AsPC1). Synergistic effects are dependent on SRC or MEK1/2 activities, as overexpression of *SRC* or *MEK1/2* completely abrogates the synergistic effects of SRC and MEK1/2 inhibitors. Combinations of SRC or MEK inhibitors with gemcitabine have synergistic effects on the squamous subtype of PDAC cells.[358]

Somatostatin receptors are a pivotal target for treatment of pancreatic neuroendocrine tumors (pNETs), either with somatostatin analogs (SSAs) or radiolabeled SSA. The highest affinity target for the most commonly used SSA is somatostatin receptor type 2 (sst2). Treatment with the epidrugs 5-aza-2′-deoxycytidine (5-Aza-dC) and valproic acid (VPA) induces upregulation of *sst2* mRNA, increased uptake of radiolabeled octreotide, and increased sensitivity to the SSA octreotide in functional cAMP inhibition. At the epigenetic level low methylation levels of the *sst2* gene promoter region are seen, irrespective of expression. The activation of histone mark H3K9Ac can be regulated with epidrug treatment. Repressive histone mark H3K27me3 is not regulated by either 5-Aza-dC or VPA. Combined 5-Aza-dC and VPA treatment might represent a promising strategy for future treatment of patients with pNETs.[359]

6.7.1.19.3 Myeloproliferative Neoplasms and Myeloid Leukemia

Patients with myeloproliferative neoplasms (MPNs) frequently progress to bone marrow failure or acute myeloid leukemia (AML), and mutations in epigenetic regulators such as the metabolic enzyme isocitrate dehydrogenase (*IDH*) are associated with poor outcomes. Combined expression of *Jak2V617F* and mutant *IDH1R132H* or *Idh2R140Q* induces MPN progression, alters stem/progenitor cell function, and impairs differentiation in mice. *Jak2V617F Idh2R140Q*-mutant MPNs are sensitive to small-molecule inhibition of IDH. Combined inhibition of JAK2 and IDH2 normalizes the stem and progenitor cell compartments in the murine model and reduces disease burden to a greater extent than was seen with JAK inhibition alone. Combined *JAK2* and *IDH2* inhibitor treatment also reverses aberrant gene expression in MPN stem cells and metabolite perturbations induced by concurrent *JAK2* and *IDH2* mutations.[360]

A major obstacle to curing chronic myeloid leukemia (CML) is residual disease maintained by tyrosine kinase inhibitor (TKI)-persistent leukemic stem cells (LSC) that are BCR-ABL1 kinase independent, refractory to apoptosis, and serve as a reservoir to drive relapse or TKI resistance. Polycomb repressive complex 2 is misregulated in chronic phase CML LSCs. This is associated with extensive reprogramming of H3K27me3 targets in LSCs, thus sensitizing them to apoptosis upon treatment with an EZH2-specific inhibitor (EZH2i). Treatment of primary CML cells with either EZH2i or TKI alone causes upregulation of H3K27me3 targets, and combined treatment further potentiates these effects and results in significant loss of LSCs compared with TKI alone.[361]

6.7.1.19.4 Thyroid Cancer

The prognosis of anaplastic (ATC) and poorly differentiated thyroid cancer (PDTC) is poor, as a result of their radioiodine refractoriness (RAI-R), high metastatic potential, and current lack of effective treatment strategies. Wächter et al.[362] studied the efficacy of the tyrosine kinase inhibitors (TKIs) sorafenib and selumetinib and the histone deacetylase inhibitor (HDACI) panobinostat in patient-derived tumor tissue (PDTT) of ATCs/PDTCs, as well as the expression of sodium iodide symporter (*NIS*) and radioiodine uptake (*RAI-U*). Panobinostat showed the strongest cytotoxic effect in all PDTTs and HF and caused a significant overexpression of the *NIS* transcript. *RAI-U* was upregulated after 24 h of treatment with TKIs and panobinostat. Selumetinib caused a significant suppression of *HMGA2* in PDTT and

HF, whereas sorafenib caused no change in *HMGA2* expression. Panobinostat suppressed *HMGA2* in PDTT and HF. The expression of miRNAs *hsa-let-7f-5p*, *has-let-7b-5p*, *hsa-miR-146b-5p*, and *hsa-miR-146b-3p* was modulated heterogeneously. PDTT might be a useful tool to test the efficacy of compounds and to develop new and individualized multimodal treatment options for PDTCs and ATCs.

6.7.1.19.5 Lymphoma

Peripheral T cell lymphoma is a rare heterogeneous group of diseases that are characterized by poor outcomes to treatment and short overall survival. Several new therapies targeting T cell biology have been approved, including pralatrexate, romidepsin, belinostat, and brentuximab vedotin, with no major impact in terms of efficacy. Early-phase clinical studies have demonstrated that combination therapy with romidepsin plus other agents known to have activity in T cell lymphoma have enhanced clinical benefit. The antibody drug conjugate brentuximab vedotin has shown potent activity in T cell lymphomas expressing CD30.[363]

6.7.1.19.6 Acute Lymphocytic Leukemia

The HDACi vorinostat and entinostat have been tested in combination with adaphostin in human acute lymphocytic leukemia (ALL) cell lines. Both combinations synergistically induced apoptotic DNA fragmentation, preceded by an increase in superoxide levels, a reduction in mitochondrial membrane potential, and an increase in caspase-9 activation. The antioxidant *N*-acetylcysteine (NAC) blocked superoxide generation and prevented reduction of mitochondrial membrane potential. NAC decreased DNA fragmentation and caspase activity in cells treated with adaphostin and vorinostat, but not in those treated with adaphostin and entinostat. Gene expression arrays revealed differential regulation of several redox genes prior to cell death induction. The redox modulatory agent, adaphostin, may enhance the efficacy of HDACi, vorinostat, or entinostat.[364]

6.7.1.19.7 Urothelial Carcinoma

The class I histone deacetylase (HDAC) inhibitor romidepsin efficiently kills urothelial carcinoma (UC) cells, but does not elicit canonical apoptosis; it affects benign urothelial cells indiscriminately. Combinations of HDAC inhibitors with JQ1, an inhibitor of bromodomain-containing acetylation reader proteins like BRD4, have shown efficacy in several tumor types.

Romidepsin and JQ1 act in a synergistic manner across all UC cell lines, efficiently inhibiting cell cycle progression, suppressing clonogenic growth, and inducing caspase-dependent apoptosis. The antiapoptotic and oncogenic factors *survivin*, *BCL-2*, *BCL-XL*, *c-myc*, *EZH2*, and *SKP2* are consistently downregulated by drug combination, and AKT phosphorylation is diminished. Around the transcriptional start sites of these genes the drug combination enhances H3K27 acetylation and decreases H3K4 trimethylation. The cell cycle inhibitor CDKN1C/p57KIP2 is induced at the mRNA and protein level.[365]

6.7.1.19.8 Sarcoma

Addition of an antiangiogenic agent (bevacizumab) to standard chemotherapy in the treatment of sarcoma has been studied in clinical trials, but most of the findings have not supported its use. Monga et al.[366] proposed that the addition of valproic acid (VPA), a weak histone deacetylase inhibitor, and bevacizumab, a monoclonal antibody against vascular endothelial growth factor, together with the cytotoxic effects of gemcitabine and docetaxel, may enhance responses and alter chemoresistance. According to the authors the combination of VPA, bevacizumab, gemcitabine, and docetaxel appears to be moderately safe and well tolerated.

6.7.1.19.9 Prostate Cancer

The expression of estrogen receptor (ER)-β appears to be lost during prostate cancer progression through the hypermethylation mechanism. Epigenetic drugs such as 5-aza-2′-deoxycytidine (5-Aza-dC) and trichostatin A (TSA) show efficacy in restoring ERβ expression in prostate cancer cells. Motawi et al.[367] designed a study to explore the potential anticarcinogenic effects resulting from reexpressing *ERβ1* using 5-Aza-dC and/or TSA, followed by its stimulation with diarylpropionitrile (DPN), a selective ERβ1 agonist, in prostate cancer cell line PC-3. Treatment with these drugs exhibited an increase in *ERβ1* expression to different extents as well as active caspase-3 levels, with a reduction in cyclin D1, VEGF, and β-catenin levels. The triple-combination regimen led to the most prominent antitumor responses in terms of increased apoptosis, reduced proliferation, and angiogenesis.

6.7.1.19.10 Breast Cancer

Yu et al.[368] investigated the efficacy and safety of double- vs single-agent chemotherapy (CT) plus trastuzumab (H) as a first-line therapy for human epidermal growth factor 2 receptor (HER2)-positive metastatic breast cancer (MBC). Compared with single-agent CT the combination of double CT with trastuzumab as a first-line therapy for HER2-positive MBC is associated with longer progression-free survival and overall survival, but more treatment-related grade 3 or 4 toxicities.

Targeting cancer using small-molecule prodrugs should help overcome problems associated with conventional cancer-targeting methods. Ota et al.[369] focused on lysine-specific demethylase 1 (LSD1) to trigger the controlled release of anticancer drugs in cancer cells, where *LSD1* is highly expressed. Conjugates of the LSD1 inhibitor *trans*-2-phenylcyclopropylamine (PCPA) were used as novel prodrugs to selectively release anticancer drugs by LSD1 inhibition. Like PCPA-drug conjugate (PDC) prototypes, PCPA-tamoxifen conjugates have been designed to release 4-hydroxytamoxifen in the presence of LSD1. These compounds inhibit the growth of breast cancer cells by the simultaneous inhibition of LSD1 and the estrogen receptor without exhibiting cytotoxicity toward normal cells.

6.7.1.19.11 Melanoma

Epigenetic modifications play an important role in the progression and development of resistance in *V600EBRAF*-positive metastatic melanoma. Zakharia et al.[370] designed a phase lb study to determine the dose-limiting toxicity and maximum tolerated dose of a combination of subcutaneous decitabine with oral vemurafenib in patients with *V600EBRAF*-positive metastatic melanoma with or without any prior treatment. The combination of oral vemurafenib with subcutaneous decitabine is safe and showed activity in *V600EBRAF*-positive metastatic melanoma.

6.7.1.19.12 Glioma

Diffuse intrinsic pontine glioma (DIPG) is an infiltrative, often high-grade glioma of the brainstem that is not amenable to surgical resection. Radiation therapy is effective, but the tumor tends to recur rapidly. EZH2 is a potential therapeutic target for *H3K27M*-mutant pediatric gliomas, and the BET family protein is an attractive target in many different types of cancers, including DIPG. The combination of these two inhibitors exhibits better inhibition of tumor growth by blocking proliferation and promoting cell apoptosis.[371]

6.7.1.19.13 Colorectal Cancer

Only a subset of colorectal cancer (CRC) cells respond to bromodomain inhibitors (BETi). Wu et al.[372] investigated additional agents that could be combined with BETi to overcome this obstacle. In BETi-sensitive CRC cells, JQ1 also impairs tumor angiogenesis through the c-myc/miR-17-92/CTGF + THBS1 axis. *CTGF* knockdown moderately counteracts the antiangiogenic effect of JQ1 and leads to partially attenuated tumor regression. JQ1 decreases *c-myc* expression and NF-κB activity in BETi-sensitive CRC cells but not in resistant cells. Bortezomib synergistically sensitizes BETi-resistant cells to JQ1 treatment, and JQ1 + bortezomib induce G2/M arrest in CRC cells. The inhibition of NF-κB by bortezomib, by an NF-κB inhibitor, or by an *IKK1/2* siRNA all render BETi-resistant cells more sensitive to BETi by synergistic repression of *c-myc*, which in turn induces *GADD45* expression, and by synergistic repression of *FOXM1*, which in turn inhibits G2/M checkpoint gene expression. The activation of NF-κB by *IκBα* siRNA induces resistance to JQ1 in BETi-sensitive CRC cells. JQ1 + bortezomib inhibit tumor growth and angiogenesis. The antiangiogenic effect of JQ1 plays a vital role in the therapeutic effect of JQ1 in CRC and provides a rationale for combined inhibition of BET proteins and NF-κB as a potential therapy for CRC.

Therapy with the demethylating agent 5-azacitidine and histone deacetylase inhibitor entinostat has shown synergistic reexpression of tumor suppressor genes and growth inhibition in colorectal (CRC) cell lines and in in vivo studies.[373]

6.7.1.19.14 Renal Carcinoma

Xi et al.[374] have studied the antitumor activity of valproic acid (VPA), a histone deacetylase inhibitor (HDACi), and 5-aza-2′-deoxycytidine (5-Aza-dC), an inhibitor of DNA methyltransferases, on renal cell carcinoma (RCC) cell lines 786-O and 769-P. VPA and 5-Aza-dC can individually induce decreased viability and have an inhibitory effect on the proliferation of 786-O and 769-P cells. This antigrowth effect is more pronounced when the cells are treated with both VPA and 5-Aza-dC. The combination of VPA and 5-Aza-dC also elicits greater apoptosis and produces greater cell cycle arrest in the G1 phase for both cell lines.

6.7.1.19.15 Other Neoplasms

Studies on the epigenetic response of a set of colon, breast, and leukemia cancer cell lines to small-molecule inhibitors against DNA methyltransferases (DAC), histone deacetylases (Depsi), histone demethylases (KDM1A inhibitor S2101), and histone methylases (EHMT2 inhibitor UNC0638 and EZH2 inhibitor GSK343) have revealed that (i) DAC preferentially regulates genes that are silenced in cancer and that are methylated at their promoters; (ii) Depsi affects the expression of 30.4% of the transcriptome, but shows little selectivity for gene upregulation or silenced genes; (iii) S2101, UNC0638, and GSK343 affect only 2% of the transcriptome, with UNC0638 and GSK343 preferentially targeting genes marked with H3K9me2 or H3K27me3, respectively; (iv) when combined with histone methylase inhibitors the extent of gene upregulation by DAC is extended while still maintaining selectivity for DNA-methylated genes and silenced genes; and (v) genes upregulated by combination treatment exhibit limited overlap, indicating the possibility of targeting distinct sets of genes based on different epigenetic therapy combinations. DNA methyltransferase inhibitors preferentially target cancer-relevant genes and can be combined with inhibitors targeting histone methylation for synergistic effects while still maintaining selectivity.[375]

Epigenetic drugs, such as DNA methylation inhibitors (DNMTi) or histone deacetylase inhibitors (HDACi), are approved in monotherapy for cancer treatment. These drugs reprogram gene expression profiles and reactivate tumor suppressor genes (TSG), producing cancer cell differentiation and apoptosis. Epigenetic drugs have been shown to synergize with other epigenetic drugs or various anticancer drugs. To discover new molecular entities that enhance epigenetic therapy, Raynal et al.[376] performed high-throughput screening using FDA-approved libraries in combination with DNMTi or HDACi and discovered that 45 FDA-approved drugs in FDA-approved libraries enhanced DNMTi and HDACi activity, mainly belonging to anticancer and antiarrhythmic drug classes. Transcriptome analysis revealed that the combination of decitabine (DNMTi) with the antiarrhythmic proscillaridin A produced profound gene expression reprogramming, which was associated with downregulation of 153 epigenetic regulators, including two known oncogenes in colon cancer (*SYMD3* and *KDM8*). About 85 FDA-approved drugs antagonized DNMTi and HDACi activity through cytotoxic mechanisms, suggesting detrimental drug interactions for patients undergoing epigenetic therapy.

Matei et al.[377] performed a phase 1 study of guadecitabine and carboplatin in patients with recurrent, platinum-resistant, high-grade serous ovarian cancer, primary peritoneal carcinoma, or fallopian tube cancer with promising, unconclusive results.

6.7.2 Immunosuppressants

6.7.2.1 Rapamycin

Rapamycin is an immunosuppressant used in patients who have had a kidney transplant. Some studies revealed deleterious effects of rapamycin, predominantly when administered for $\geq$24 h, and a few studies have focused on the short-term effects of rapamycin administered only during the initial reperfusion phase. Rapamycin maintains renal function and attenuates ischemia reperfusion (I/R)-induced apoptosis during the initial reperfusion phase, especially at 8 h after reperfusion. Simultaneously, rapamycin activates autophagy and inhibits endoplasmic reticulum (ER) stress and three pathways of unfolding protein response: ATF6, PERK, and IRE1α. The protective effects of rapamycin are suppressed when autophagy is inhibited by chloroquine and 3-methyladenine or when ER stress is induced by thapsigargin.[378]

6.7.2.2 Tacrolimus and Mycophenolic Acid

Immunosuppressive drug therapy is required to treat patients with autoimmune disease and patients who have undergone organ transplantation. The main targets of the immunosuppressive drugs tacrolimus and mycophenolic acid (MPA, the active metabolite of mycophenolate mofetil) are T cells. These immunosuppressive drugs have effects on DNA methylation of the gene promoter region of interferon gamma (*IFN-γ*), a proinflammatory cytokine. MPA induces changes in *IFN-γ* DNA methylation of naive T cells and counteracts the decrease in methylation after stimulation. Tacrolimus does not affect *IFN-γ* DNA methylation of naive T cells. In memory T cells both immunosuppressive drugs do not affect *IFN-γ* DNA methylation. Differentiation of naive T cells into a central memory-like phenotype ($CD45RO^+$) is inhibited by both immunosuppressive drugs, while differentiation of memory T cells remains unaffected by both MPA and tacrolimus. IFN-γ protein production is suppressed by tacrolimus.[379]

6.7.3 β2-Adrenoreceptor Agonists (Formoterol) and Glucocorticoids

The combination of inhaled long-acting β2-adrenoreceptor (LABA) and inhaled glucocorticoid (ICS) is a common therapy for asthma, with risk for infection as a major concern. Plasmacytoid dendritic cells (pDCs) are the predominant cells against infection. Glucocorticoids (GCS) suppress Toll-like receptor (TLR)-induced interferon (IFN)-α expression, and LABAs enhance the suppressive effect. LABAs alone also suppress TLR-induced *IFN-α/β* expression, and the effect is reversed by the β2-adrenoreceptor antagonist ICI118551. Dibutyryl-cAMP, a cAMP analog, confers a similar suppressive effect, and this effect is abrogated by the exchange protein directly activated by the cAMP (Epac) inhibitor HJC0197 or the intracellular-free Ca^{2+} chelator BAPTA-AM. Formoterol suppresses TLR-induced phosphorylation of mitogen-activated protein kinase *(MAPK)-p38/ERK*, interferon regulatory factor *(IRF)-3/IRF-7* expression, CpG-induced translocation of H3K4-specific methyltransferase *WDR5*, and H3K4 trimethylation in the *IFNA* and *IFNB* gene promoter region. LABAs suppress *TLR7/9*-induced type 1 IFN production via the β2-adrenoreceptor/cAMP-Epac-Ca^{2+}, IRF-3/IRF-7, and MAPK-p38/ERK pathways, and epigenetic regulation by suppressing histone H3K4 trimethylation through inhibiting the translocation of WDR5 from cytoplasm to the nucleus.[380]

6.7.4 Nonsteroidal Antiinflammatory Drugs (NSAIDs)

Nonsteroidal antiinflammatory drugs (NSAIDs) are well known for their effects on inflammatory gene expression. The NSAID salicylate can disrupt histone acetylation, in part through direct inhibition of the lysine acetyltransferase (KAT) p300/CBP. Salicylate is a relatively weak KAT inhibitor, but its CoA-linked metabolite is more potent. Many carboxylate-containing NSAIDs, including ibuprofen, are able to function as weak inhibitors. The linkage of ibuprofen to CoA increases its biochemical potency toward p300 and other KAT enzymes. Carboxylate-containing NSAIDs inhibit histone acetylation.[381]

NSAIDs have also shown chemoprevention and chemosuppression activities. Complexing the drugs with bioactive metal obliterates their negative charge and allows them to bind to DNA, thereby opening the possibility of genome level interaction. The interaction of piroxicam and its copper complex with histone/chromatin results in structural alterations with different biological manifestations. The complex shows alterations in the key epigenetic signatures implicated in transcription in the global context, although piroxicam causes no significant changes.[382]

6.7.5 Paracetamol

Long-term exposure to paracetamol during pregnancy is associated with attention-deficit/hyperactivity disorder (ADHD). There are significant differences in DNA methylation (6211 CpGs) associated with prenatal exposure to paracetamol for more than 20 days in children diagnosed with ADHD compared with controls (193 CpGs). Gene ontology analysis has revealed the enrichment of pathways involved in oxidative stress, neurological processes, and the olfactory sensory system, which have previously been implicated in the etiology of ADHD.[383]

6.7.6 Aspirin

Aspirin has positive effects on bone marrow mesenchymal stem cell (BMSC) osteogenic differentiation. Aspirin inhibits BMSCs adipogenesis. The level of HDAC activity, global histone H3 acetylation, and H3k9 acetylation are all downregulated during adipogenic differentiation, and aspirin can reverse these decreases. The expression of *HDAC9* is increased in a dose-dependent manner when aspirin is introduced during BMSC adipogenic differentiation. HDAC9 may play an important role in the process of aspirin-induced suppression of adipogenesis.[384]

6.7.7 Psychotropic Drugs

Antipsychotic drugs can modulate the host epigenome, and drug-induced epigenetic modulation can explain in part the heterogeneity in drug response. Antipsychotic drugs induce epigenetic changes by downregulating miRNA that target DNA methyltransferases, resulting in global hypermethylation.[385]

Changes in DNA methylation of *ANKK1* (ankyrin repeat and kinase domain containing 1) have been observed in response to aripiprazole in antipsychotic-free acute schizophrenia patients. DNA methylation levels at CpG site 387 of *ANKK1* were higher in responders to treatment with aripiprazole and correlated with changes in Positive and Negative Syndrome Scale scores. In responders, methylation at all CpG sites correlated with the baseline plasma levels of

homovanillic acid and 3-methoxy-4-hydroxyphenylglycol. Methylation levels at CpG site 387 of *ANKK1* may be associated with treatment response to aripiprazole.[386]

Studies on neuroblastoma cells have revealed that perospirone exposure alters DNA methylation at 4098 of 470,000 probes. These probes are enriched with genes for neural development. Probes showing hypermethylation are mainly found in the gene body and intergenic regions, whereas those that show hypomethylation are located near the promoter regions. DNA methylation changes are found in the probes for dopamine receptor 2 and serotonin receptor (*HTR*) 2A and *HTR1A*, which are the pharmacological targets of atypical antipsychotics.[387]

Atypical antipsychotics cause insulin resistance that leads to an increased risk for diabetes mellitus and cardiovascular disease. Skeletal muscle is the primary tissue for uptake of glucose, and its dysfunction is considered one of the primary defects in the development of insulin resistance. Protein kinase B (AKT) plays an important role in overall skeletal muscle health and glucose uptake into the muscle. Burghardt et al.[388] have measured AKT isoform-specific gene methylation differences in the skeletal muscle of patients with bipolar disorder treated with atypical antipsychotic or mood stabilizer maintenance therapy. In patients treated with atypical antipsychotics, *AKT1* and *AKT2* methylation was found to be increased compared with patients treated with mood stabilizers, and there was a positive trend for *AKT2* hypermethylation with increasing insulin resistance, whereas for patients receiving mood stabilizers a trend for decreased *AKT2* methylation with increasing insulin resistance was observed.

In vitro evaluation of the pharmacoepigenetic response of haloperidol, clozapine, and olanzapine shows increased expression of *ABCB1*, *CYP1A2*, and *CYP3A4* regulated by miR-27a and miR-128a. Antipsychotic drugs can influence miRNA-mediated epigenetic response in pharmacogenes that probably modulate drug efficacy and safety.[389]

Older patients can be especially susceptible to antipsychotic-induced side effects. Age-related epigenetic alterations may lead to decreased expression and functionality of the dopamine D2 receptor (D2R), contributing to this susceptibility. The motor side effects of haloperidol are more exaggerated in aged mice than young mice, and HDAC inhibitors are able to reverse the severity of these deficits. Haloperidol-induced motor deficits in aged mice are associated with age- and drug-dependent decrease in striatal D2R protein levels and functionality. Histone acetylation is reduced while histone trimethylation is increased at specific lysine residues of H3 and H4 within the *Drd2* promoter in the striatum of aged mice. HDAC inhibitors, particularly valproic acid, restore striatal D2R protein levels and functionality and reverse age- and drug-related histone modifications at the *Drd2* promoter.[390]

Antidepressant treatment can modulate DNA methylation in the promoter region of genes related to neuroplasticity and mood regulation. Systemic administration of DNA methyltransferase (DNMT) inhibitors induces antidepressant-like effects in rodents. DNA methylation is conveyed by DNMT1, 3a and 3b isoforms, which are differentially expressed in the brain. Stress increases DNA methylation as well as *DNMT3a* and *DNMT3b* expression in the dorsal hippocampus and prefrontal cortex. Chronic imipramine administration attenuates stress effects only in the prefrontal cortex.[391] Epigenetic modifications of *SLC6A4*, *BDNF*, and *IL11* genes have shown promising results as biomarkers for prediction of antidepressant response.[392]

6.7.8 Acetylcholinesterase Inhibitors

Donepezil is the acetylcholinesterase inhibitor most prescribed worldwide for the treatment of Alzheimer disease.[3,4] Adolescent intermittent ethanol (AIE) exposure produces persistent impairments in cholinergic and epigenetic signaling and alters the markers of synapses in hippocampal formation, effects that are thought to drive hippocampal dysfunction in adult rodents. Donepezil prevents some neuropathological impairments in preclinical models of neuropsychiatric disorders and partially reverses epigenetic changes in the hippocampus of adult rats exposed to AIE. AIE exposure reduces dendritic spine density, alters the morphological characteristics of subclasses of dendritic spines, and increases mRNA levels and *H3-K27* acetylation occupancy of the fragile X mental retardation 1 (*Fmr1*) gene in the hippocampus. This phenotype can be potentially reversed by donepezil.[393]

6.7.9 Morphine

During embryo-fetal development, morphine increases GFP in nestin/GFP embryos and overexpresses the NSC marker nestin. Morphine induces hyperacetylation of H3K27 and decreases DNA methylation within a region located 18 Kb upstream of the nestin transcription starting site. Morphine upregulates the transcription factor complex Sox2/Oct4/Nanog and increases the histone acetyl transferase p300. The inhibition of p300 activity decreases nestin.

Morphine facilitates nestin increase by several mechanisms, which include hyperacetylation of H3K27, decreased DNA methylation, and the overexpression of the transcription factors *sox2*, *oct4*, and *nanog*. Nestin expression delays the normal differentiation of neural stem cells.[394]

Diacetylmorphine (DAM) reduces the activation of the hypothalamic-pituitary-adrenal (HPA) axis in opioid-maintained patients. DAM injection increases methylation, blunts stress hormone levels, and modifies *POMC* promoter methylation of heroin-dependent patients.[395]

6.7.10 5-HT2CR Antagonist SB243213/5-HT2CR Inverse Agonist SB206553

Social isolation enhances aggressive behavior, and epigenetics-related Htr2c RNA editing is related to aggressive behavior. Social isolation affects adenosine deaminase acting on the RNA-editing enzyme RNA 1 (*ADAR1*) and on *Htr2c* RNA editing, leading to aggressive behavior.

The 5-HT2CR antagonist SB243213/5-HT2CR inverse agonist SB206553 recovers the increased aggressive behavior of isolated BALB/c mice mediated by *ADAR1 (p110)* expression and *Htr2c* RNA editing. *ADAR1 (p110)* expression in the amygdala decreases in accordance with moderate increase in *Htr2c* RNA editing at the A and B sites in the amygdala of aggressive isolated BALB/c mice. Treatment with the 5-HT2CR antagonist SB243213/5-HT2CR inverse agonist SB206553 recovers the enhanced aggressive behavior of isolated mice and returns the protein expression of ADAR1 (p110) to normal levels, and the animals exhibit a lower percentage of *Htr2c* RNA editing.[396]

6.7.11 Statins

Atorvastatin represses *miR-29a-3p, miR-29b-3p, miR-300, miR-33a-5p, miR-33b-5p*, and *miR-454-3p* in patients with hypercholesterolemia, and simvastatin does not affect miRNA expression. Atorvastatin-modulated miRNAs regulate key cholesterol genes (*ABCA1, HMGCR, INSIG1, LDLR, LPL, SCAP*, and *SREBF1*). miR-106b-5p, miR-17-3p, and miR-590-5p are repressed in hypercholesterolemic patients.[397]

Statins alone, or in combination with vismodegib (an FDA-approved smoothened antagonist), have been utilized to inhibit medulloblastoma growth in vivo. Cholesterol biosynthesis was markedly enhanced in Hh-MB from both humans and mice. The inhibition of cholesterol biosynthesis dramatically decreased Hh pathway activity and reduced the proliferation of medulloblastoma cells. Statins effectively inhibited medulloblastoma growth in vivo and functioned synergistically in combination with vismodegib. Cholesterol biosynthesis is required for smoothened activity in the hedgehog pathway, and it is indispensable for the growth of Hh-MB. Targeting cholesterol biosynthesis has been proposed as a potential strategy for treatment of Hh-MB.[398]

Lovastatin alters the stem-like state of cells to a more differentiated condition and reduces stemness. Lovastatin treatment may influence the expression and methylation patterns of genes regulating the differentiation of endometrial-mesenchymal stem cells (eMSCs), such as *BMP2, GATA2*, and *RUNX2*, as well as *eMSC* markers. Treatment with lovastatin increases the expression of *BMP2* and *RUNX2* and induces *BMP2* promoter demethylation. Lovastatin downregulates *GATA2* expression by inducing methylation.[399]

6.7.12 Telmisartan and Esculetin

An esculetin and telmisartan combination reverses histone posttranslational modifications in diabetic cardiomyopathy. This combination alleviates the pathological features of diabetic cardiomyopathy including metabolic perturbations, morphometric alterations, altered vascular reactivity, and increased *Keap1* and fibronectin expression. Telmisartan alone or in combination with esculetin attenuates increased levels of histone PTMs such as H3K9me2, H3K9Ac, H2AK119Ub, and H2BK120Ub in the heart of diabetic rats.[400]

6.7.13 Metformin

Metformin influences the activity of numerous epigenetic-modifying enzymes, mostly via modulating the activation of AMP-activated protein kinase (AMPK). Activated AMPK can phosphorylate numerous substrates, including epigenetic enzymes, such as histone acetyltransferases, class II histone deacetylases, and DNA methyltransferases, generally resulting in their inhibition, although HAT1 activity may be increased. Metformin decreases the expression of multiple histone methyltransferases, increases the activity of class III HDAC *SIRT1*, and decreases the influence of DNMT inhibitors. These alterations influence the epigenome and gene expression and may contribute to the

antidiabetic properties of metformin. The expression levels of numerous miRNAs are also reportedly influenced by metformin treatment and may confer antidiabetic and anticancer activities. According to Bridgeman et al.[401], as the reported effects of metformin on epigenetic enzymes act to both increase and decrease histone acetylation, histone and DNA methylation, and gene expression, a significant degree of uncertainty exists on the overall effect of metformin on the epigenome, gene expression, and subsequent effect on the health of metformin users.

Hepatic metformin transporters are responsible for the pharmacologic action of metformin. DNA methylation in liver OCT1 (*SLC22A1*), OCT3 (*SLC22A3*), and MATE1 (*SLC47A1*) is affected by metformin, with a clear tendency to hypomethylation.[402]

GWA analyses have identified 198 mRNA expression probe sets, 12 SNP loci, and 5 DNA methylation loci associated with metformin. At least 14 genes are downregulated by metformin. The E3 ubiquitin ligase STUB1 can influence the metformin response by facilitating proteasome-mediated degradation of cyclin A.[403]

Metformin treatment decreases the cell growth of prostate cancer (PCa) cell line 22Rv1 and stalls cells at the G1/S checkpoint in a time- and dose-dependent manner, resulting in increased cells in G1 and decreased cells in the S phase. Metformin activates the AMPK/mTOR signaling pathway, which results in increased p-AMPK and decreased p-p70S6K. Metformin treatment induces changes in 136 chromatin-modifying genes. The multiple myeloma SET domain (*MMSET*) shows increased expression in PCa cell lines, and its expression is decreased upon metformin treatment. siRNA-mediated knockdown of *MMSET* shows decreased cellular migration and invasion in DU-145 cells. *MMSET* knockdown in combination with metformin treatment results in further reduction in the capacity of PCa cells to migrate and invade. MMSET may play a role in the inhibitory effect of metformin on PCa and might serve as a potential novel therapeutic target for PCa.[404]

6.7.14 Allopurinol

Allopurinol-induced severe cutaneous adverse reactions (SCARs), including drug rash with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome (SJS), and toxic epidermal necrosis (TEN), are life-threatening autoimmune reactions. Epigenetic variation, particularly DNA methylation, is associated with autoimmune diseases. In a genome-scale DNA methylation profiling study, Sun et al.[405] identified 41 differentially methylated CpG loci annotated to 26 genes showing altered DNA methylation between allopurinol-SCARs and allopurinol-tolerants. Among these genes, significant hypomethylation of *PSORS1C1 (cg24926791)* was validated in a larger sample cohort, suggesting that *PSORS1C1* hypomethylation is associated with allopurinol-SCARs.

6.7.15 Antiprogeroid Treatments

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic condition associated with mutations in the *LMNA* gene. The typical *LMNA* mutation results in the production of a truncated prelamin A protein, progerin, that remains permanently farnesylated and abnormally associated with the nuclear envelope. Farnesyltransferase inhibitors (FTIs) reverse nuclear structure abnormalities that are characteristic of HGPS cells. Treatment with FTIs (Ionafarnib) shows some improvements in HGPS children. Sulforaphane efficiently stimulates autophagy and enhances progerin clearance in HGPS fibroblasts. The coadministration of lonafarnib and sulforaphane in HGPS fibroblast cultures shows synergistic and additive effects on autophagy activity but is cytotoxic to HGPS cells. In contrast, intermittent treatment with lonafarnib followed by sulforaphane separately and in repeated cycles rescued the HGPS cellular phenotype.[406]

6.7.16 Granulocyte Colony-Stimulating Factor

Bacterial meningitis during the perinatal period may cause long-term neurological deficits. Yang et al.[407] investigated whether bacterial lipopolysaccharide (LPS) derived from *Escherichia coli* led to neuronal apoptosis and impaired performance of long-term cognitive function, involving the activation of histone modification in the *TNF-α* gene promoter, and looked into the therapeutic efficacy of granulocyte colony-stimulating factor (G-CSF) in a neonatal brain suffering from perinatal bacterial meningitis. LPS injection increases the expression of NF-κB phosphorylation and trimethylated H3K4 in the *TNFA* gene promoter locus, and alters caspase-3, neuronal apoptosis expression, and cognitive functions. These deleterious outcomes can be alleviated by G-CSF therapy. Selective therapeutic action sites of G-CSF through epigenetic regulation in the *TNFA* gene promoter locus may exert a potentially beneficial role for the neonatal brain suffering from perinatal bacterial-induced meningitis.

6.7.17 α-Oxoglutarate

Urinary α-oxoglutarate (α-OG) is elevated in interstitial cystitis. α-OG, a tricarboxylic acid (TCA) cycle intermediate, suppresses the proliferation of immortalized normal human bladder epithelial cells. AT-rich interactive domain 1A (ARID1A) is a chromatin remodeler that is hypomethylated and upregulated by α-OG treatment. α-OG suppresses ten-eleven translocation (TET) activity, but does not affect DNA methyltransferase (DNMT) activity.[408]

6.7.18 Melatonin

Melatonin may exert neuroprotective effects in several models of brain injury. Melatonin has been found to be able to prevent lipopolysaccharide (LPS)-induced fetal brain damage in a model of LPS-induced preterm labor. In the absence of melatonin, fetuses from LPS-treated mothers show histological signs of brain damage, microglial/macrophage activation, and higher levels of *IL-1β*, inducible nitric oxide synthase (*NOS*), and neuronal *NOS* mRNAs as well as increased histone acetyltransferase activity and histone H3 hyperacetylation. In contrast, antenatal administration of melatonin prevented LPS-induced fetal brain damage.[409]

Fang et al.[410] studied the effects of melatonin treatment of donors on methylation modification of prepubertal cumulus cells, which are dysfunctional in domestic animals, perhaps as a result of age-specific epigenetic events. Melatonin upregulates expression of *MT1, Bcl2, DNMT1, DNMT3a*, and *DNMT3b* and downregulates expression of *p53*, caspase 3, and *Bax*. Melatonin methylates two CpG sites of *DNMT1* and hypermethylates two CpG sites of *DNMT3a*, with no major differences from the *DNMT1* and *DNMT3a* promoter regions.

6.7.19 Acyl-CoA Synthetase Short Chain Family Member 2 (ACSS2)-Driven Histone Crotonylation

The eradication of HIV-1 (HIV) is hindered by stable viral reservoirs. Viral latency is epigenetically regulated. The effects of histone acetylation and methylation on HIV long-terminal repeats (LTRs) have been described, and HIV LTR histone crotonylation is a regulator of HIV latency. Reactivation of latent HIV is achieved following the induction of histone crotonylation by increased expression of the crotonyl-CoA-producing enzyme acyl-CoA synthetase short chain family member 2 (ACSS2). This reprograms local chromatin at HIV LTRs by increased histone acetylation and reduces histone methylation. Pharmacologic inhibition or siRNA knockdown of *ACSS2* diminishes histone crotonylation-induced HIV replication and reactivation. ACSS2 induction is highly synergistic in combination with either a protein kinase C agonist (PEP005) or a histone deacetylase inhibitor (vorinostat) in reactivating latent HIV.[411]

6.7.20 Antisense Oligonucleotides

Using the principle of antibody-drug conjugates that deliver highly potent cytotoxic agents to cancer cells for cancer therapy, Cao et al.[412] have reported on the possibility of synthesizing antisense-oligonucleotides (ASO) and thyroid hormone T3 conjugates for obesity treatment. ASOs primarily target fat and liver with poor penetrance to other organs. Pharmacological T3 treatment increases energy expenditure and causes weight loss; however, T3 is contraindicated for obesity treatment as a result of its systemic effects on multiple organs. ASO-T3 conjugates may knock down target genes and enrich T3 action in fat and liver. Nicotinamide *N*-methyltransferase (NNMT)-ASO prevents diet-induced obesity in mice. Apolipoprotein B (ApoB)-ASO is an FDA-approved drug for treating familial hypercholesterolemia. NNMT-ASO and ApoB-ASO are chemically conjugated with T3 using a noncleavable sulfo-SMCC linker. NNMT-ASO-T3 (NAT3) and ApoB-ASO-T3 (AAT3) enhance thyroid hormone receptor activity. The treatment of obese mice with NAT3 or AAT3 decreases adiposity and increases lean mass. ASO-T3 enhances white fat browning, decreases genes for fatty acid synthesis in liver, and shows limited effects on T3 target genes in heart and muscle.[412]

In plants, treatment by short oligonucleotides homologous to a different sequence of chalcone synthase (*CHS*) gene isoforms, which encode an essential enzyme in the phenylpropanoid biosynthesis pathway, revealed that those directed to regulatory gene regions (5′- and 3′-UTR) activated gene expression, those directed to noncoding region (introns) caused gene activity reduction, while those homologous to a coding region may have variable influence on its activity. Gene expression changes were accompanied by changes in the methylation status and rearrangement of the nucleosome location.[413]

6.7.21 Plasmids

Plasmid DNA (pDNA) is an attractive therapeutic biomolecule in several diseases. Plasmid DNA for transgene expression or vaccine applications needs novel approaches to bioprocessing. Lin et al.[414] described the synthesis, characterization, and evaluation of aminoglycoside-derived hydrogel microbeads ("Amikabeads") for pDNA binding, as well as a novel chemotherapeutic drug-conjugated microbead for application in pDNA binding and recovery. Chemotherapeutic drug-conjugated Amikabeads demonstrated higher binding of methylated pDNA compared with unmethylated pDNA in the presence of high salt concentrations. Desorption of plasmids from drug-conjugated microbeads was facilitated by the use of organic modifiers.

6.7.22 Programmable Epigenetic Editors

Targeted epigenome editing is an emerging technology designed to specifically regulate cellular gene expression to modulate cell phenotypes or dissect the epigenetic mechanisms involved in their control. Stepper et al.[415] employed a DNA methyltransferase Dnmt3a-Dnmt3L construct fused to the nuclease-inactivated dCas9 programmable targeting domain to introduce DNA methylation into the human genome specifically at the *EpCAM, CXCR4*, and *TFRC* gene promoters. Targeting these loci using single gRNAs leads to efficient and widespread methylation of the promoters. The peaks of targeted methylation have been observed around 25 bp upstream and 40 bp downstream of the PAM site, while 20–30 bp of the binding site itself are protected against methylation. Potent methylation is dependent on the multimerization of Dnmt3a/Dnmt3L complexes on DNA. Such methylation causes transcriptional repression of targeted genes.

Gene silencing is instrumental to interrogating gene function and holds promise for therapeutic applications. Amabile et al.[416] modified the endogenous retrovirus-silencing machinery of embryonic stem cells to stably silence three highly expressed genes in somatic cells by epigenetics. This was achieved by transiently expressing combinations of engineered transcriptional repressors that bind to and synergize at the target locus to instruct repressive histone marks and de novo DNA methylation, thus ensuring long-term memory of the repressive epigenetic state. Silencing was found to be highly specific, as shown by genome-wide analyses, sharply confined to the targeted locus without spreading to nearby genes, resistant to activation induced by cytokine stimulation, and relieved only by targeted DNA demethylation.[416]

6.7.23 Nanoparticles

Oxygen-encapsulated nanosize carboxymethyl cellulosic nanobubbles have been developed to mitigate the hypoxic regions of tumors to weaken hypoxia-driven pathways and inhibit tumor growth. 5-Methylcytosine (5mC) hypomethylation in hypoxic regions of a tumor can be reversed to enhance cancer treatment by epigenetic regulation using oxygen nanobubbles. Oxygen nanobubbles (ONB) are effective at delaying tumor progression and improving survival rates in experimental models. ONB treatment induces *BRCA1* hypermethylation and is able to reprogram tumor suppressor genes (*MAT2A, PDK-1*).[417]

Nanoformulations of EGFR T790M targeted inhibitor AZD9291 and paclitaxel (PTX) have been developed for combination therapy of lung cancer.[418]

Nanocomposites for integrating imaging and therapy have attracted attention in biomedicine. Fe@Bi2S3 nanocomposites modified with polyethylene glycol (PEG) molecules have been fabricated for synergistic thermoradiotherapy.[419]

Li et al.[420] developed a combined therapy consisting of miRNA-21 antisense oligonucleotides (ASO-miR-21) and gemcitabine (Gem) using a targeted codelivery nanoparticle (NP) carrier, and investigated the synergistic inhibitory effects on pancreatic cancer cell metastasis and growth. Polyethylene glycol-polyethylenimine-magnetic iron oxide NPs were used to codeliver ASO-miR-21 and Gem. An anti-CD44v6 single-chain variable fragment (scFvCD44v6) was used to coat the particles to obtain active and targeted delivery. The downregulation of oncogenic miR-21 by ASO results in upregulation of the tumor suppressor genes *PDCD4* and *PTEN* and suppression of epithelial-mesenchymal transition, which inhibits proliferation and induces clonal formation, migration, and invasion of pancreatic cancer cells in vitro. Codelivery of ASO-miR-21 and Gem induces more cell apoptosis and inhibits the growth of pancreatic cancer cells to a greater extent than single ASO-miR-21 or Gem treatment. In animals, more scFvCD44v6-PEG-polyethylenimine/ASO-magnetic iron oxide NP/Gem accumulates at the tumor site than nontargeted NPs, inducing potent inhibition of tumor proliferation and metastasis. The combination of *miR-21* gene silencing and Gem therapy using an scFv-functionalized NP carrier exerts synergistic antitumor effects on pancreatic cancer cells.[420]

The clinical success of cancer radiotherapy is usually limited by insufficient DNA damage and rapid DNA repair after treatment. Jiang et al.[421] reported a DNA dual-targeting approach for enhanced cancer radiotherapy using a hierarchical multiplexing nanodroplet, which can simultaneously promote DNA lesion formation and prevent subsequent DNA damage repair.

The synthesis of zinc oxide nanoparticles (ZnO NPs) can be achieved using *Glycyrrhiza glabra* seed aqueous extract. The ZnO NPs synthesized are green, around 35 nm in size, and have an irregular spherical shape. *G. glabra* seed aqueous extract-mediated synthesized ZnO NPs were used to treat human glioblastoma cells with the help of temozolomide.[422]

Choudhury et al.[423] evaluated the cytotoxic effects of zinc oxide nanoparticles (ZnO NPs) and found severe actin depolymerization, increased release of mitochondrial cytochrome C, nuclear enlargement, global reduction of 5-methylcytosine, and increased 5-hydroxymethylcytosine content. The authors also observed an increase in the expression of ten-eleven translocation (TET)-methylcytosine dioxygenase genes, but not in the expression of DNA methyltransferases. ZnO NPs induce an abundant increase in ROS to promote multimodal structural and functional anomalies in cells. ZnO NP-induced ROS may promote global hypomethylation in cells by triggering the expression of TET enzymes, thus avoiding DNMT interference.

Hypermethylation of the transcription factor AP-2 epsilon (*TFAP2E*) gene affects 5-fluorouridine (5-FU) resistance in gastric cancer (GC) patients. The epigenetic inhibitor 5-Aza-2′-deoxycytidine (5-Aza-dC), which reverses DNA methylation by targeting DNA methyltransferases (DNMTs), has shown potential to sensitize GC to 5-FU. DNA demethylation by 5-Aza-dC transiently occurs since 5-Aza-dC is unstable in aqueous solutions, which limits its potential. Hong et al.[424] developed intelligent nanoparticles (NPs) comprising gelatinase with polyethylene glycol (PEG) and poly-ε-caprolactone (PCL) to specifically deliver 5-Aza-dC (DAC-TNPs) to tumors. The combination treatment of DAC-TNPs and 5-FU greatly improves tumor suppression in GC cells and mouse xenograft models by hypermethylation of *TFAP2E* (MKN45 cells).

Many products, including cosmetics, pharmaceuticals, electronics, and food, have nanomaterials (NMs) incorporated in them. Nanotechnology has yielded many promising benefits, yet there remains much uncertainty about the hazards posed by NMs to humans. Different types of commonly used NMs (containing silicon dioxide, titanium dioxide, silver, or zinc oxide) in food products can display potential toxic effects, inducing epigenetic toxicity mediated via altered miRNA expression.[425] Constant exposure to nanoparticles has raised concerns regarding their adverse side effects on human health. Nanoparticles can display potential cytotoxicity and genotoxicity and induce epigenetic changes.[426]

6.7.24 Stem Cell Therapy

Pluripotent stem cells (PSCs) are essential tools in modern regenerative medicine and in personalized cell-based therapies as a result of their properties, which include unlimited self-renewal and the ability to differentiate into cell types representative of the three embryonic germ layers: mesoderm, ectoderm, and endoderm.[427]

Stem cells have been shown to hold much promise in the treatment of several brain disorders and pathologies including brain tumors. Bhere et al.[428] explored their potential for creating induced pluripotent stem cell (iPSC)-derived therapeutic NSCs (ipNSCs) by using either unmodified or gene-modified somatic cells, and tested their fate and therapeutic efficacies in vitro and in vivo. Cells engineered in a somatic state lose transgene expression during the neural induction process, which is partially restored by HDAC inhibitor treatment, whereas cells engineered in an ipNSC state have sustained expression of transgenes. Bimodal mouse and human ipNSCs engineered to express tumor-specific death-receptor ligand and suicide-inducing proteins have profound antitumor efficacy when encapsulated in a synthetic extracellular matrix (sECM) and transplanted in mouse models of resected GBMs.

Mesenchymal stromal cells (MSCs) can differentiate into multiple tissues. Preclinical studies have shown that MSC-based therapy has great potential as a new way of treating ischemic stroke.[429]

The transplantation of human hematopoietic stem cells into immunodeficient mice provides a powerful in vivo means of gaining functional insights into hematopoietic differentiation. Mice have a much shorter life expectancy than humans, and the xenogeneic environment might greatly accelerate the epigenetic clock. Genome-wide DNA methylation patterns of normal human hematopoietic development are indeed recapitulated upon engraftment in mice, particularly those of normal early B cell progenitor cells. Epigenetic changes in human hematopoietic development are recapitulated in the murine transplantation model, whereas epigenetic aging is not accelerated by the shorter life expectancy of mice faster aging environment and seems to occur in the cell intrinsically.[430]

6.7.25 CRISPR-Related Personalized Therapy

Drug efficacy and safety are unsatisfactory in complex disorders in which fewer than 40% of patients can be catalogued as good responders.[3,4,431] In addition to necessary improvements in the search for more specific targets and the characterization of better drugs, emerging new technologies and the incorporation of pharmacoepigenetic procedures may help in this endeavor. Genome-editing technology clustered regularly interspaced short palindromic repeats (CRISPRs) in combination with the CRISPR-associated (Cas) 9 system may provide some new solutions. However, these promising technologies are not devoid of technical limitations and additional pathogenic risks when dealing with polygenic/multifactorial disorders.[432]

Gao and Liang[433] developed a new inducible method to integrate dCas9-based genome targeting with abscisic acid (ABA)-based chemically induced proximity (CIP) technologies to modify histone tail modifications at specific genome loci in living cells. ABA leads to rapid heterodimerization of PYL and ABI proteins, which can be individually fused to dCas9 and a histone-modifying enzyme core domain. In the presence of ABA and locus-specific sgRNAs this histone-modifying activity can be recruited to a specific genome locus to achieve histone editing with perfect temporal control. This technique has been used to control recruitment of the p300 acetyltransferase core domain to the human *IL1RN* locus to ectopically increase the acetylation of H3K27 and induce expression of the *IL1RN* gene.

6.8 DRUG RESISTANCE

Drug resistance is the result of several anomalies in pharmacological treatment, including (i) pharmacological features of particular drugs, (ii) mutations in different genes involved in the pharmacogenomic network (pathogenic, mechanistic, metabolic, transporter, and pleiotropic genes), and (iii) aberrations in the epigenetic machinery leading to deficiencies in the pharmacoepigenetic processor.[5,7] Some other potential mechanisms cannot be excluded in the phenomenon of drug resistance in complex disorders. According to recent studies reported by Wang et al.,[434] contrary to prevailing dogma, cells exhibit an immediate to early response to changes in histone proteoforms, recovering basal-like conditions upon removal of epigenetic inhibitors. For instance, inhibition of SUV4–20 results in decreased H4K20me2; however, no effects on H4K20me3 have been observed, implying that another enzyme mediates H4K20me3. Strikingly, SUV4–20 inhibition results in an increase in histone H4 acetylation attributable to proteoforms containing K20me2. This response suggests that hyperacetylated proteoforms protect K20me2 from demethylation as an evolved compensatory mechanism. Pretreatment with an HDACi diminishes the effects of SUV4–20 inhibition in prone cells and HATi-facilitating SUV4–20 inhibition decreases discrete H4K20me2 in resistant cells.[434]

Some authors suggest that the Warburg effect might contribute to drug resistance. Cancer cells have both a conventional oxidative metabolism and a glycolytic anaerobic metabolism, and their proliferation is marked by a shift toward increasing glycolytic metabolism even in the presence of O_2 (Warburg effect). The Warburg effect also favors an intracellular alkaline pH, which is a driving force in many aspects of cancer cell proliferation (enhancement of glycolysis and cell cycle progression) and of cancer aggressiveness (resistance to various processes including hypoxia, apoptosis, cytotoxic drugs, and immune response).[435]

Transporter associated with antigen processing 2 (TAP2) is involved in the development of multidrug resistance and the etiology of immunological diseases. *TAP2* expression can be perturbed by SNPs located in the 3′-untranslated region (3′-UTR) of the gene via interactions with miRNAs. The SNP rs241456, located in the 3′-UTR of *TAP2*, resides in a potential binding site for hsa-miR-1270 and hsa-miR-620. hsa-miR-1270 suppresses the production of *TAP2* by binding to this SNP in the 3′-UTR of this gene.[436]

Transcriptional changes result from immediate therapeutic response or resistance, whereas epigenetic alterations only occur with resistance. Genes with epigenetic alterations associated with resistance stabilize the resistant phenotype. These genes include *FGFR1*, which was associated with EGFR inhibitor resistance.[437]

Wyce et al.[438] investigated the biomarkers of activity of the clinical BET inhibitor GSK525762 (I-BET, I-BET762) across cancer cell lines and demonstrated that *KRAS* mutations are novel resistance biomarkers. The authors combined BET with RAS pathway inhibition using MEK inhibitors to overcome resistance, which resulted in synergistic effects on growth and survival in *RAS* pathway mutant models as well as a subset of cell lines lacking *RAS* pathway mutations. GSK525762 treatment upregulated p-ERK1/2 levels in both RAS pathway wild-type and mutant cell lines, suggesting that MEK/ERK pathway activation may also be a mechanism of adaptive BET inhibitor resistance. The BET/MEK combination uniquely sustains the downregulation of genes associated with mitosis, leading to prolonged growth arrest that is not observed with either single-agent therapy.

Bioinformatic tools can help to approach complex multiomic data sets containing transcriptomics, proteomics, and epigenomics data. With one of these procedures Kel et al.[439] identified the following potential drug targets against induced resistance of cancer cells toward chemotherapy by methotrexate (MTX): TGFalpha, IGFBP7, and alpha9-integrin. They also identified the following chemical compounds: zardaverine and divalproex as well as human metabolites such as nicotinamide N-oxide.

Carcinoma-associated fibroblasts (CAFs) are abundant and heterogeneous stromal cells in the tumor microenvironment and are critically involved in cancer progression. Two cell surface molecules, CD10 and GPR77, specifically define a CAF subset correlated with chemoresistance and poor survival in multiple cohorts of breast and lung cancer patients. $CD10^+GPR77^+$ CAFs promote tumor formation and chemoresistance by providing a survival niche for cancer stem cells (CSCs). $CD10^+GPR77^+$ CAFs are driven by persistent NF-κB activation via p65 phosphorylation and acetylation, which is maintained by complement signaling via GPR77, a C5a receptor. $CD10^+GPR77^+$ CAFs promote successful engraftment of patient-derived xenografts (PDXs), and targeting these CAFs with a neutralizing anti-GPR77 antibody abolishes tumor formation and restores tumor chemosensitivity.[440]

SET domain-containing epigenetic factors govern drug efficacy to the medically relevant azole class of antifungal drugs. Set4 is induced when *Saccharomyces cerevisiae* yeasts are treated with azole drugs or azole drugs grown under hypoxic conditions, two conditions that deplete cellular ergosterol and increase sterol precursors. Set4 induction is controlled by the sterol-sensing transcription factors, Upc2 and Ecm22. Set4 is required for global changes in gene expression. Loss of Set4 leads to the upregulation of nearly all ergosterol genes including *ERG11* and *ERG3*, suggesting that Set4 has a role in gene repression. Set4 interacts with the hypoxic-specific transcriptional repressor Hap1 when this interaction is necessary for Set4 recruitment of ergosterol gene promoters under hypoxia. Sterol precursors are needed for Set4 to be induced via an Upc2-mediated mechanism. This new sterol-signaling pathway governs azole antifungal drug resistance and mediates the repression of sterol genes under hypoxic conditions.[441]

The potential role of lncRNAs and their epigenetic regulation in response to platinum treatment have been studied. Differential expression in response to therapy has been observed more frequently in cis-acting than in overlapping lncRNAs, while significantly altered methylation profiles were more commonly associated with overlapping. Five lncRNAs under epigenetic regulation appear to be involved in cisplatin resistance (*AC091814.2, AC141928.1, RP11-65J3.1-002, BX641110*, and *AF198444*).[442]

Piulats et al.[443] investigated the genetic basis of cisplatin resistance and identified recurrent chromosomal rearrangements across cisplatin-resistant tumors, showing gains at the 9q32-q33.1 region. There was a clinical correlation between the presence of 9q32-q33.1 gains in cisplatin-refractory patients and poorer overall survival in metastatic tumors. *POLE3* and *AKNA* were the only two genes deregulated in resistant tumors harboring a 9q32-q33.1 gain. Another four genes (*GCS, ZNF883, CTR1*, and *FLJ31713*) were deregulated in all five resistant tumors independently of 9q32-q33.1 amplification. The influence of 9q32-q33.1 genes on cisplatin resistance can be driven by either upregulation or downregulation. The glucosylceramide synthase (GCS) inhibitor DL-threo-PDMP resensitizes cisplatin-resistant germline-derived orthoxenografts to cisplatin.

Loss of TET1 may play a role in the formation of tumors. There is potential involvement of ten-eleven translocation 1 (TET1) in the DNA damage response (DDR). In response to clinically relevant doses of ionizing radiation (IR), human glial cells made TET1 deficient by using lentiviral vectors display greater numbers of colony-forming units and lower levels of apoptotic markers compared with glial cells transduced using control vectors. The G2/M checkpoint and expression of cyclin B1 are greatly diminished in TET1-deficient cells, and TET1-deficient cells display lower levels of γH2A.x following exposure to IR. The levels of DNA-PKcs, which are DNA-PK complex members, are lower in TET1-deficient cells compared with control cell lines. Cyclin B1, DNA-PKcs, and γH2A.x levels are each rescued by reintroduction of the TET1-catalytic domain. Cytosine methylation within intron 1 of *PRKDC*, the gene encoding DNA-PKcs, is significantly higher upon depletion of TET1.[444]

6.8.1 Glioma and Glioblastoma

Histone H3 mutations are frequently found in diffuse midline gliomas (DMGs), which include diffuse intrinsic pontine gliomas and thalamic gliomas, with dismal prognoses. One reason for the poor prognoses is that *O6*-methylguanine-DNA methyltransferase (MGMT) promoter frequently lacks methylation in DMGs. Isocitrate dehydrogenase-mutant gliomas frequently have methylated MGMT promoters and are sensitive to temozolomide.[445]

The chromatin regulator JmjC domain histone H3K36me2/me1 demethylase KDM2B is highly expressed in glioblastoma surgical specimens compared with normal brain. Targeting KDM2B function genetically or pharmacologically impairs the survival of patient-derived primary glioblastoma cells by inducing DNA damage and apoptosis,

sensitizing them to chemotherapy. KDM2B loss decreases the GSC pool, which is potentiated by the coadministration of chemotherapy. KDM2B is crucial for glioblastoma maintenance, with inhibition causing loss of GSC cell survival, genomic stability, and chemoresistance.[446]

Glioma stem cells (GSCs) express low levels of MKP1, a dual-specificity phosphatase that acts as a negative inhibitor of JNK, ERK1/2, and p38 MAPK, while the induction of high levels of MKP1 expression are associated with the differentiation of GSCs. High levels of MKP1 correlate with better prognosis and overall increased survival. Elevated MKP1 impairs self-renewal and induces the differentiation of GSCs while reducing tumorigenesis. *MKP1* is epigenetically regulated and mediates the antitumor activity of histone deacetylase inhibitors (HDACIs) alone or in combination with temozolomide. The activation of MKP1 through epigenetic regulation might be a novel therapeutic strategy to overcome therapy resistance in glioblastoma.[447]

6.8.2 Lung Cancer

Patients with lung adenocarcinoma can benefit from antiangiogenic therapies to a degree; in contrast, patients with squamous cell lung carcinoma (SQLC) do not respond to this therapeutic intervention. One potential reason for this discrepancy might be the role of soluble VEGF receptor-1 (sVEGFR1-i13), a truncated splice variant of the cell membrane spanning VEGFR1 that has no transmembrane or tyrosine kinase domain. sVEGFR1-i13 is an antiangiogenic factor that counteracts VEGF-A/VEGFR signaling in endothelial cells.

Antiangiogenic therapies specifically increase the levels of sVEGFR1-i13 in SQLC cell lines and chemically induced SQLC murine tumor grafts. An sVEGFR1-i13/β1 integrin/VEGFR autocrine loop determines whether SQLC cells proliferate or go into apoptosis in response to antiangiogenic therapies. High levels of sVEGFR1-i13 and β1 integrin mRNAs and proteins are associated with advanced stages in SQLC patients and with a poor clinical outcome in patients with early-stage SQLC.[448]

Several homeobox-related gene (HOX) transcription factors, such as mesenchyme HOX-2 *(MEOX2)*, are associated with cancer drug resistance, malignant progression, and/or clinical prognostic responses in lung cancer patients. Hedgehog-*GLI1* gene promoter sequences from −2192 to −109 are occupied by *MEOX2*, accompanied by transcriptionally active RNA pol II and epigenetically linked to the active histones H3K27Ac and H3K4me3. The MEOX2-GLI1 axis is involved in cellular cytotoxic resistance to cis-platinum in a dose-dependent manner. MEOX2-dependent GLI-1 protein expression is associated with clinical progression and poorer overall survival in NSCLC patients undergoing platinum-based oncological therapy with both epidermal growth factor receptor (EGFR)-nonmutated and EGFR-mutated status.[449]

Claudin-2 is highly expressed in lung adenocarcinoma tissues and increases proliferation in adenocarcinoma cells. Azacitidine (AZA), a DNA methylation inhibitor, and trichostatin A (TSA) and sodium butyrate (NaB), both of which are histone deacetylase (HDAC) inhibitors, decrease claudin-2 levels. The effect of AZA is mediated by the inhibition of phosphorylated Akt and NF-κB. LY-294002, an inhibitor of phosphatidylinositol 3-kinase (PI3K), and BAY 11-7082, an NF-κB inhibitor, decrease claudin-2 levels. The reporter activity of claudin-2 is decreased by AZA and LY-294002, which is blocked by a mutation in a putative NF-κB-binding site. NF-κB binds to the promoter region of claudin-2, which is inhibited by AZA and LY-294002. AZA may decrease the claudin-2 mRNA level mediated by inhibition of the PI3K/Akt/NF-κB pathway. TSA and NaB do not change phosphorylated Akt and NF-κB levels. These inhibitors do not change the reporter activity of claudin-2, but decrease the stability of claudin-2 mRNA mediated by the elevation of *miR-497* miRNA. The binding of histone H3 to the promoter region of *miR-497* is inhibited by TSA and NaB, whereas that of claudin-2 is not. HDAC inhibitors decrease claudin-2 levels mediated by the elevation of *miR-497* expression. Cell proliferation is additively decreased by AZA, TSA, and NaB, and is partially rescued by ectopic expression of claudin-2.[450]

6.8.3 Breast Cancer

About 75% of breast cancers are estrogen receptor alpha (ERα) positive and are treatable with endocrine therapies; however, many patients develop lethal resistant disease. Frequent mutations (10%–40%) in ligand-binding domain (LBD) codons in the gene encoding ERα (*ESR1*) have been identified, resulting in ligand-independent, constitutively active receptors. *ESR1* chromosomal translocations can occur, resulting in fusion proteins that lack the LBD and are entirely unresponsive to all endocrine treatments. Identifying coactivators that bind to these mutant ERα proteins may offer new therapeutic targets for endocrine-resistant cancer.[451]

The oncogenic histone methyltransferase EZH2 confers tamoxifen resistance by silencing the expression of the estrogen receptor α (ERα) cofactor *GREB1*. The induction of DNA methylation of a particular CpG-enriched region at the *GREB1* promoter negatively correlates with GREB1 levels and cell sensitivity to endocrine agents. GREB1 also ensures

proper cellular reactions to different ligands by recruiting distinct sets of ERα cofactors to cis-regulatory elements, which explains the contradictory biological effects of GREB1 on breast cancer cell growth in response to estrogen or antiestrogen. In refractory cells EZH2-dependent repression of *GREB1* triggers chromatin reallocation of ERα coregulators, converting the antiestrogen into an agonist.[452]

Tamoxifen resistance is accountable for relapse in many ER-positive breast cancer patients. Tamoxifen-resistant breast cancer cells overexpress *BARD1* and *BRCA1*, leading to resistance to DNA-damaging chemotherapy including cisplatin and adriamycin, but not to paclitaxel. Silencing *BARD1* or *BRCA1* expression or inhibiting BRCA1 phosphorylation by dinaciclib restores the sensitivity to cisplatin in tamoxifen-resistant cells. An activated PI3K/AKT pathway is responsible for the upregulation of *BARD1* and *BRCA1*. PI3K inhibitors decrease the expression of *BARD1* and *BRCA1* in tamoxifen-resistant cells and resensitize them to cisplatin. Higher *BARD1* and *BRCA1* expression is associated with worse prognosis of early breast cancer patients, especially those receiving radiotherapy, indicating the potential use of PI3K inhibitors to reverse chemoresistance and radioresistance in ER-positive breast cancer patients.[453]

GALNT14 is a member of the *N*-acetylgalactosaminyltransferase enzyme family and mediates breast cancer cell development. GALNT14 regulates multidrug resistance (MDR) in breast cancer. The expression of *GALNT14* is associated with MDR in breast cancer. A higher level of GALNT14 facilitates MCF-7 cells to resist adriamycin, whereas knockdown of *GALNT14* sensitizes cells to adriamycin. The expression of *GALNT14* also associates with the expression of P-gp, the efflux pump localized on the cell membrane, which might be the underlying mechanism by which GALNT14 induces MDR. GALNT14 regulates the stability of P-gp and associates with a higher level of P-gp in chemotherapy-resistant human breast cancer tissues.[454]

Yang et al.[455] studied the role of neuregulin 1 (NRG1)-dependent human epidermal growth factor receptor 3 (HER3) activation in trastuzumab primary resistance. NRG1-dependent activation of HER3 induces primary resistance to trastuzumab in HER2-overexpressing breast cancer cells. HER3 monoclonal antibodies combined with trastuzumab may serve as a treatment choice for patients with primary resistance to trastuzumab.

miR-34a is downregulated in breast cancer tissues and cell lines, correlating with breast cancer multidrug resistance (MDR). The expression of *miR-34a* is downregulated in multidrug-resistant MDR-MCF-7 cells. Patients with a low expression of *miR-34a* have poorer overall survival (OS) and disease-free survival (DFS) than those with high expression. Transfecting miR-34a mimics into MDR-MCF-7 breast cancer cells leads to partial MDR reversal. miR-34a reduces both the mRNA and protein expressions of BCL-2, CCND1, and NOTCH1, with no changes in p53 or TOP-2a expression. In breast cancer tissue samples the expression of *miR-34a* is related to BCL-2, CCND1, and NOTCH1, but not to HER-2, p53, and TOP-2a. miR-34a is an MDR and prognosis indicator of breast cancer that may participate in the regulation of drug-resistant breast cancer by targeting *BCL-2, CCND1,* and *NOTCH1*.[456]

miRNAs regulate chemotherapy-induced epithelial–mesenchymal transition (EMT) and drug resistance. Overexpression of *miR-200b* in chemoresistant cells reverses the EMT phenotype and increases sensitivity to doxorubicin. Inhibition of miR-200b in parental cells induces EMT and resistance to doxorubicin. Overexpression of *miR-200b* downregulates *FN1* expression, and knockdown of *FN1* reverses mesenchymal morphology, inhibited cell migration and invasion, and sensitized cells to doxorubicin. miR-200b regulates the EMT of chemoresistant breast cancer cells by targeting *FN1*.[457]

The expression levels of chemoresistance-associated long noncoding RNA (*CRALA*), a newly discovered long noncoding RNA, have been measured in biopsied primary breast cancer samples. Nonresponding tumors had a fourfold higher CRALA expression than responding tumors. CRALA is upregulated in chemoresistant breast cancer cell lines compared with their parental lines. Silencing of *CRALA* in chemoresistant breast cancer cells resensitizes the cells to chemotherapy in vitro. Higher *CRALA* expression is associated with poor prognosis. *CRALA* expression may be an important biomarker for predicting the clinical response to chemotherapy and prognosis in breast cancer patients, and it might be possible to target *CRALA* to reverse chemoresistance in breast cancer patients.[458]

Circular RNAs (circRNAs) are associated with breast cancer chemoresistance. Gao et al.[459] detected 3093 circRNAs and identified 18 of them that are differentially expressed between MCF-7/ADM and MCF-7 cells. The circ_0006528-miR-7-5p-Raf1 axis plays a regulatory role in ADM-resistant breast cancer.

6.8.4 Bladder Cancer

Hypoxia contributes to chemoresistance through hypoxia-inducible factor 1α (HIF-1α)-mediated autophagy in several types of cancer. HIF-1α is overexpressed in bladder cancer. Gemcitabine-induced apoptosis during hypoxia is reduced. Hypoxia activates autophagy and enhances gemcitabine-induced autophagy. Combined treatment using gemcitabine and an autophagy inhibitor (3-methyladenine) under hypoxia increases gemcitabine cytotoxicity. Hypoxia-activated autophagy depends on the HIF-1α/BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (BNIP3)/Beclin-1 signaling pathway. Suppressing HIF-1α inhibits autophagy, BNIP3, and Beclin-1, and enhances gemcitabine-induced apoptosis in bladder cancer cells under hypoxic conditions.[460]

Xiao et al.[461] measured the chemosensitivity of five bladder cancer (BCa) cell lines to seven commonly used chemotherapeutic drugs, identified the most sensitive (5637) and most tolerant cell lines (H-bc) and conducted a multigroup test, selecting miR-22-3p as a target. *miR-22-3p* and neuroepithelial cell transforming 1 (NET1) are involved in BCa multichemoresistance.

6.8.5 Prostate Cancer

Castration-resistant prostate cancer (CRPC) occurs after the failure of androgen deprivation therapy and is the leading cause of deaths in prostate cancer patients. The lncRNA *HOXD-AS1* is highly expressed in CRPC cells and correlates with Gleason score, T stage, lymph node metastasis, and progression-free survival. Knockdown of *HOXD-AS1* inhibits the proliferation and chemoresistance of CRPC cells. Several cell cycle, chemoresistance, and castration resistance-related genes (*PLK1, AURKA, CDC25C, FOXM1,* and *UBE2C*) are activated transcriptionally by HOXD-AS1. HOXD-AS1 recruits WDR5 to directly regulate the expression of target genes by mediating histone H3 lysine 4 trimethylation (H3K4me3).[462]

BRD4 plays a major role in the transcription networks orchestrated by the androgen receptor (AR) in castration-resistant prostate cancer (CRPC). Several BET inhibitors (BETi), which displace BRD4 from chromatin, are being evaluated in clinical trials for CRPC. The mechanisms behind acquired resistance to BETi are amenable to targeted therapies in CRPC. BETi-resistant CRPC cells display cross-resistance to a variety of BETi in the absence of gatekeeper mutations, exhibit reduced chromatin-bound BRD4, and are less sensitive to BRD4 degraders/knockdown, suggesting a BRD4-independent transcription program. Reactivation of AR signaling due to CDK9-mediated phosphorylation of AR results in sensitivity to CDK9 inhibitors and enzalutamide. Increased DNA damage associated with PRC2-mediated transcriptional silencing of DDR genes leads to PARP inhibitor sensitivity. BETi resistance suggests the potential use of combination therapies in treating CRPC.[463]

TSPYL5 is a putative tumor suppressor gene that belongs to the nucleosome assembly protein family. The chromosomal location of the *TSPYL5* gene is 8Q22.1, and its role in prostate cancer etiology remains unclear. *TSPYL5* is differentially expressed in nontumorigenic prostate epithelial cells (RWPE-1), androgen-independent (DU145), and androgen-dependent (LNCaP) prostate carcinoma cells and tissues. There is an inverse relationship between DNA methylation and expression leading to silencing of the *TSPYL5* gene. In prostate carcinoma cells, in which TSPYL5 is absent or low (DU145 and LNCaP), treatment with the demethylating agent 5-aza-2′-deoxycytidine upregulates its expression in these cells. TSPYL5 protein levels are very low in tumors and *TSPYL5* overexpression in LNCaP cells increases cell sensitivity to chemotherapy drugs, such as docetaxel and paclitaxel.[464]

Prostate cancer relapse as a result of antiandrogen therapies can exhibit variant histology with altered lineage marker expression, suggesting that lineage plasticity facilitates therapeutic resistance. *Rb1* loss facilitates lineage plasticity and metastasis of prostate adenocarcinoma initiated by Pten mutation. Additional loss of *Trp53* causes resistance to antiandrogen therapy. Both mouse and human tumors exhibit increased expression of epigenetic reprogramming factors, such as *Ezh2* and *Sox2*. Clinically relevant Ezh2 inhibitors restore androgen receptor expression and sensitivity to antiandrogen therapy.[465]

6.8.6 Chronic Myeloid Leukemia

Tyrosine kinase inhibitors (imatinib) fail to induce a long-term response in some cases of chronic myeloid leukemia (CML). Nuclear-cytoplasmic trafficking of proteins plays a key role in the development of leukemia and drug resistance. KPT-330 (selinexor), an inhibitor of chromosome region maintenance 1 (CRM1, nuclear receptor exportin 1, XPO1), has been found to demonstrate activities against a few hematological malignancies. KPT-330 inhibits proliferation, cell cycle arrest, and apoptosis of IM-resistant CML K562G. KPT-330 inhibits CRM1 and increases the nuclear/cytoplasm ratio of BCR-ABL and P27. p-AKT is downregulated, while p-STAT1 and caspase-3 are upregulated. KPT-330 has shown an antileukemic effect in primary IM-resistant CML with *T315I* mutation in a CRM1-dependent manner. In a K562G xenograft mouse model, KPT-330 inhibits tumor growth and sensitizes K562G to IM in vivo.[466]

6.8.7 Acute Myeloid Leukemia

Drug resistance is daily encountered in acute myeloid leukemia (AML), leading to high mortality. Göllner et al.[467] identified loss of the histone methyltransferase EZH2 and subsequent reduction of histone H3K27 trimethylation as a novel pathway of acquired resistance to tyrosine kinase inhibitors (TKIs) and cytotoxic drugs in AML. Low EZH2

protein levels correlate with poor prognosis in AML patients. Suppression of EZH2 protein expression induces chemoresistance in AML cell lines. Low EZH2 levels result in derepression of *HOX* genes, and knockdown of *HOXB7* and *HOXA9* in resistant cells is sufficient to improve sensitivity to TKIs and cytotoxic drugs. The endogenous loss of *EZH2* expression in resistant cells and primary blasts from a subset of relapsed AML patients has been found to result from enhanced CDK1-dependent phosphorylation of EZH2 at Thr487. This interaction was stabilized by heat shock protein 90 (HSP90) and followed by proteasomal degradation of EZH2 in drug-resistant cells. Inhibitors of HSP90, CDK1, and the proteasome prevented EZH2 degradation, decreased *HOX* gene expression, and restored drug sensitivity. Patients with reduced EZH2 levels at progression to standard therapy tend to respond well to the combination of bortezomib and cytarabine, concomitant with the reestablishment of *EZH2* expression and blast clearance. Restoration of EZH2 protein is a viable approach to overcome treatment resistance in AML.

In acute myeloid leukemia (AML) Brown et al.[468] identified MEF2C S222 phosphorylation as a specific marker of primary chemoresistance. *Mef2c S222A/S222A* knockin-mutant mice engineered to block MEF2C phosphorylation exhibit normal hematopoiesis, but they are resistant to leukemogenesis induced by MLL-AF9. MEF2C phosphorylation is required for leukemia stem cell maintenance, which is induced by MARK kinases in cells. Treatment with the selective MARK/SIK inhibitor MRT199665 causes apoptosis and confers chemosensitivity in MEF2C-activated human AML cell lines and primary patient specimens, but not those lacking MEF2C phosphorylation. Kinase-dependent dysregulation of transcription factor control is a determinant of therapy response in AML.

Epigenetic regulators are recurrently mutated and aberrantly expressed in acute myeloid leukemia (AML). Targeted therapies designed to inhibit these chromatin-modifying enzymes, such as the histone demethylase lysine-specific demethylase 1 (*LSD1*) and the histone methyltransferase *DOT1L*, have been developed as novel treatment modalities for these refractory diseases. The LSD1 inhibitor GSK-LSD1 and the DOT1L inhibitor EPZ4777 show differential effects. GSK-LSD1 treatment causes global gains in chromatin accessibility, whereas treatment with EPZ4777 causes global losses in accessibility. Diminished expression of *PU.1* or genetic deletion of *C/EBPα* in MLL-AF9 cells generates resistance in these leukemias to LSD1 inhibition.[469]

Recurrent somatic mutations in DNA methyltransferase 3A (*DNMT3A*), most frequently at arginine 882 *(DNMT3AR882)*, have been observed in acute myeloid leukemia (AML) and in individuals with clonal hematopoiesis in the absence of leukemic transformation. Patients with *DNMT3AR882* AML have an inferior outcome when treated with standard dose daunorubicin-based induction chemotherapy, suggesting that *DNMT3AR882* cells persist and drive relapse. *Dnmt3a* mutations induce hematopoietic stem cell expansion, cooperate with mutations in the FMS-like tyrosine kinase 3 gene (*Flt3ITD*) and the nucleophosmin gene (*Npm1c*) to induce AML in vivo, and promote resistance to anthracycline chemotherapy. In patients with AML the presence of *DNMT3AR882* mutations predicts minimal residual disease, underscoring their role in AML chemoresistance. DNMT3AR882 cells show impaired nucleosome eviction and chromatin remodeling in response to anthracycline treatment, which results from attenuated recruitment of histone chaperone SPT-16 following anthracycline exposure. This defect leads to an inability to sense and repair DNA torsional stress, which results in increased mutagenesis.[470]

Homeobox (*HOX*) genes are frequently dysregulated in leukemia. Aberrant *HOX* gene expression accompanies leukemogenesis and affects disease progression and leukemia patient survival. Patients with acute myeloid leukemia (AML) bearing the *PML-RARα* fusion gene have a distinct *HOX* gene signature from other subtypes of AML patients. There is an association between the mRNA levels of *HOX* genes and those of the histone demethylases JMJD3 and UTX in PML-RARα-positive leukemia patients. Release of the PML-RARα-mediated block in PML-RARα-positive myeloid leukemia cells increases both *JMJD3* and *HOX* gene expression, while inhibition of JMJD3 using the specific inhibitor GSK-J4 reverses the effect. Gene expression levels are inversely correlated with alterations in H3K27me3 histone marks localized at *HOX* gene promoters. The combination of GSK-J4 and all-trans retinoic acid (ATRA) increases PML-RARα-positive cell apoptosis compared with ATRA treatment alone. This effect is also observed in ATRA-resistant NB4 clones, which may provide a new therapeutic opportunity for patients with acute promyelocytic leukemia (APL) resistant to current treatment.[471]

Growth factor independent 1 transcriptional repressor (GFI-1) is a zinc finger transcriptional repressor that binds histone deacetylases to allow transcriptional repression. AML patients who have a low level of GFI-1 show poor prognosis and panobinostat resistance. Heme oxygenase-1 (HO-1) is one of the main factors leading to chemotherapy sensitivity to AML. AML patients who have lower expression of GFI-1 have higher levels of HO-1, HDAC1, HDAC2, and HDAC3, which result in poor prognosis in AML. Knocking down *GFI-1* by siRNA can eliminate panobinostat-induced cell apoptosis. With *GFI-1* knockdown the phosphorylation of Akt and PI3K can be activated and resistance can be reverted by HO-1 inhibitors.[472]

6.8.8 Acute Lymphoblastic Leukemia

Mutations in *SETD2*, encoding histone 3 lysine 36 trimethyltransferase, are enriched in relapsed acute lymphoblastic leukemia and MLL-rearranged acute leukemia. *SETD2* mutations lead to resistance to DNA-damaging agents, cytarabine, 6-thioguanine, doxorubicin, and etoposide, but not to the non-DNA damaging agent, L-asparaginase. H3K36me3 localizes components of the DNA damage response (DDR) pathway, and *SETD2* mutation impairs DDR, blunting apoptosis induced by cytotoxic chemotherapy. Treatment with JIB-04, an inhibitor of the H3K9/36me3 demethylase KDM4A, restores H3K36me3 levels and sensitivity to cytarabine. SETD2 alterations represent a mechanism of resistance to DNA-damaging chemotherapy, consistent with a local loss of DDR.[473]

6.8.9 Lymphoma

TP53 mutations occur in over 50% of all human tumors. *p53* missense mutations are predictive of refractoriness to chemotherapy/radiotherapy in different types of cancer. Mutant p53-targeting agents for restoring p53 function are under development. Through gene expression profiling of *p53R172*-mutant lymphomas, Larsson et al.[474] identified retinoic acid receptor gamma (RARγ) as an actionable target and demonstrated that pharmacological activation of RARγ with a synthetic retinoid sensitizes resistant *p53*-mutant lymphomas to p53 restoration, while additively improving outcome and survival in inherently sensitive tumors.

Schlafen 11 (*SLFN11*) is a putative DNA/RNA helicase and a dominant genomic determinant of response to DNA-damaging agents. Histone deacetylase (HDAC) inhibitors can be used to release SLFN11 and sensitize SLFN11-inactivated cancers to DNA-targeted agents. *SLFN11* expression is suppressed in a broad fraction of common cancers and cancer cell lines. In cancer cells not expressing *SLFN11*, transfection of *SLFN11* sensitizes cells to camptothecin, topotecan, hydroxyurea, and cisplatin but not to paclitaxel. SLFN11 mRNA and protein levels are strongly induced by class I (romidepsin, entinostat) but not class II (roclinostat) HDAC inhibitors. *SLFN11* expression is also enhanced in peripheral blood mononuclear cells of patients with circulating cutaneous T cell lymphoma treated with romidepsin. Camptothecin and class I HDAC inhibitors are synergistic in many cell lines.[475]

6.8.10 Multiple Myeloma

Thalidomide and its derivatives, lenalidomide and pomalidomide (IMiDs), have changed the treatment landscape of multiple myeloma, and the discovery of cereblon (CRBN) as their direct biological target has led to a deeper understanding of their complex mechanism of action. To examine whether IMiD resistance is potentially reversible, Dimopoulos et al.[476] established lenalidomide-resistant (LR) and pomalidomide-resistant (PR) human myeloma cell lines from two IMiD-sensitive cell lines, OPM2 and NCI-H929. Acquired IMiD resistance is associated with an increase in genome-wide DNA methylation and an even greater reduction in chromatin accessibility. Transcriptome analysis confirmed that resistant cell lines are mainly characterized by a reduction in gene expression, identifying *SMAD3* as a commonly downregulated gene in IMiD-resistant cell lines. These changes are potentially reversible, as a combination of 5-azacytidine and EPZ-6438 restores accessibility changes and the expression of *SMAD3*, and resensitizes resistant cells to both lenalidomide and pomalidomide. The simultaneous inhibition of DNA methyl transferases and EZH2 can lead to extensive epigenetic reprogramming, which allows myeloma cells to regain sensitivity to IMiDs.

6.8.11 Pancreatic Carcinoma

The oncogene *KRAS* plays a crucial role in pancreatic ductal adenocarcinoma (PDAC) development and progression. RKIP is a tumor repressor that is reduced in PDAC. *KRAS* expression is inversely correlated with *RKIP* expression in PDAC fresh tissue regardless of KRAS-mutant status. *KRAS* overexpression and RKIP downregulation are associated with poor clinical outcomes. The MAPK-ERK pathway is involved in the regulation of RKIP. *KRAS* knockdown increases *RKIP* expression and inhibits metastasis and chemoresistance. KRAS inhibits the tumor suppressor RKIP. Targeting RKIP might be a strategy to overcome KRAS-induced tumor metastasis and chemoresistance in PDAC.[477]

6.8.12 Colorectal Cancer

Ultraviolent irradiation resistance-associated gene (*UVRAG*), a component of the Beclin-1/autophagy-related 6 complex, regulates autophagy initiation and plays a role in DNA damage response. *UVRAG* is frequently mutated

in various cancer types, and mutations of *UVRAG* increase sensitivity to chemotherapy by impairing DNA damage repair. *UVRAG* expression is increased in cells treated with histone deacetylase (HDAC) inhibitors, such as valproic acid and suberoylanilide hydroxamic acid. Downregulation of HDAC1 enhances *UVRAG* expression in colorectal cancer cells. The inhibition of HDAC1 reduces the activation of caspase-3 and cytotoxicity in 5-fluorouracil (5FU)-treated cancer cells. In contrast, *UVRAG* overexpression inhibits caspase activation and cell death in 5FU-treated cells. Upregulation of UVRAG by HDAC1 inhibition potentiates DNA damage-mediated cell death in colorectal cancer cells.[478]

6.8.13 Tongue Carcinoma

lncRNAs play pivotal roles in tumor metastasis and progression. The chemotherapy-induced lncRNA 1 (*CILA1*) has been found to regulate chemosensitivity in tongue squamous cell carcinoma (TSCC) cells. Upregulation of *CILA1* promotes EMT, invasiveness, and chemoresistance in TSCC cells, whereas the inhibition of *CILA1* expression induces mesenchymal-epithelial transition (MET) and chemosensitivity and inhibits the invasiveness of cisplatin-resistant cells. CILA1 functions by activating the Wnt/β-catenin signaling pathway. High *CILA1* expression levels and low levels of phosphorylated β-catenin are closely associated with cisplatin resistance and advanced disease stage and are predictors of poor prognosis in TSCC patients.[479]

SOX8 is overexpressed in chemoresistant patients with TSCC and is associated with higher lymph node metastasis, advanced tumor stage, and shorter overall survival. Stable knockdown of *SOX8* in cisplatin-resistant TSCC cells inhibits chemoresistance, tumorsphere formation, and EMT. The Wnt/β-catenin pathway has been found to mediate cancer stem-like properties in cisplatin-resistant TSCC cells. SOX8 binds to the promoter region of Frizzled-7 (*FZD7*) and induces FZD7-mediated activation of the Wnt/β-catenin pathway.[480]

Cancer-associated fibroblasts (CAFs) play important roles in the carcinogenesis and progression of tongue squamous cell carcinoma (TSCC), influencing chemotherapy resistance. During exposure to cisplatin, TSCC with CAFs exhibit increased cell viability and reduced apoptosis. Cisplatin increases the LC3-II and Beclin-1 levels of TSCC cocultured with CAFs. Beclin-1 siRNA decreases cisplatin-induced autophagy. CAFs contribute to cisplatin resistance in tongue cancer via autophagy activation.[481]

6.8.14 Hepatocellular Carcinoma

lncRNA *HOX* transcript antisense RNA (*HOTAIR*) exhibits oncogenic activity in several types of cancer, including hepatocellular carcinoma (HCC). Knockdown of *HOTAIR* expression in HCC Huh7 cells results in decreased cell proliferation and increased chemosensitivity to cisplatin. The expression levels of ATP-binding cassette subfamily B member 1 (*ABCB1*) mRNA and protein are decreased in Huh7 cells upon *HOTAIR* knockdown. *HOTAIR* knockdown reduces the levels of phosphorylated signal transducer and activator of transcription 3 (STAT3), and the inhibition of STAT3 phosphorylation reduces HOTAIR-mediated *ABCB1* expression. HOTAIR might serve as a novel potential therapeutic target to reverse multidrug resistance in HCC.[482]

6.8.15 Osteosarcoma

miRNAs act as key regulators of gene expression in diverse biological processes including the multichemoresistance of cancers such as osteosarcoma (OS). The miR-20a-5p level is higher in G-292 cells than in SJSA-1 cells. Forced expression of *miR-20a-5p* counteracts OS chemoresistance in both cell culture and tumor xenografts in nude mice. *SDC2* is a miR-20a-5p target mediating the miR-20a-5p-induced repression of OS chemoresistance.[483]

miR-34a-5p is implicated in the tumorigenesis and progression of several types of cancer. miR-34a-5p promotes osteosarcoma (OS) multichemoresistance via its repression of the Delta-like ligand 1 (*DLL1*) gene and a ligand of the Notch pathway. siRNA-mediated repression of the *DLL1* gene suppresses cell apoptosis and desensitizes G-292 and MG63.2 cells, while overexpression of *DLL1* sensitizes SJSA-1 and MNNG/HOS cells to drug-induced cell death. The activity of the ATF2/ATF3/ATF4 signaling pathway is altered by forced reversal of miR-34a-5p or DLL1 levels in OS cells. DLL1 is a target of miR-34a-5p and negatively regulates the multichemoresistance of OS. miR-34a-5p, DLL1, and ATF2/ATF3/ATF4 signaling pathway-associated genes are potential diagnostic and/or therapeutic targets for the effective chemotherapy of OS.[484]

6.8.16 Esophageal Carcinoma

Resistance to chemotherapy is a major obstacle in the treatment of esophageal squamous cell carcinoma (ESCC). Several miRNAs represent the miRNA signatures of resistant ESCC (hsa-miR-125a-5p, hsa-miR-130a-3p, hsa-miR-1226-3p, hsa-miR-148a-3p). miR-130a-3p sensitizes cells to cisplatin in 100% of cell lines, miR-148a-3p in 83%, miR-125a-5p in 67%, and miR-1226-3p in 50%. miR-130a-3p sensitizes 83% of cell lines to 5-FU, and miR-148a-3p/miR-125a-5p/miR-1226-3p only 33%. *Bcl-2* is a direct target of miR-130a-3p and miR-148a-3p, and *p53* is a target of miR-125a-5p. All miRNAs decrease migration and adhesion, except miR-130a-3p, and increase apoptosis. Simultaneous manipulation of two miRNAs exhibit additive sensitizing effects to cisplatin in 50% (miR-125a-5p/miR-148a-3p), and 75% (miR-148a-3p/miR-130a-3p) of cell lines.[485]

6.8.17 Renal Cancer

Patients with advanced renal cell cancer (RCC) have the potential to improve when treated with inhibitors of the PI3 kinase (PI3K)-AKT-mTOR axis, but treatment resistance is a major problem. Resistant cells are cross-resistant to mTOR inhibitor AZD2014 and PI3K-mTOR kinase inhibitor NVP-BEZ235 (BEZ235, dactolisib). Sensitivity can be regained after 4 months of drug withdrawal, and resistance can be partially suppressed by HDAC inhibition. BEZ235-resistant cells upregulate and/or activate numerous proteins including MET, ABL, Notch, IGF-1R, INSR, and MEK/ERK. However, resistance is not reversed by inhibiting or depleting these pathways, suggesting that many induced changes are passengers not drivers of resistance. BEZ235 blocks the phosphorylation of mTOR targets S6 and 4E-BP1 in parental cells and 4E-BP1 remains phosphorylated in resistant cells, suggesting BEZ235-refractory mTORC1 activity. Resistant cells overexpress the mTORC1 component *RAPTOR* at the mRNA and protein level. BEZ235 resistance is suppressed by RAPTOR depletion or by the allosteric mTORC1 inhibitor rapamycin. RAPTOR upregulation contributes to PI3K-mTOR inhibitor resistance.[486]

6.8.18 Melanoma

Induction of the histone methyltransferase Ezh2 controls several tumor cell-intrinsic and extrinsic resistance mechanisms. T cell infiltration selectively correlates with high EZH2-PRC2 complex activity in human skin cutaneous melanoma. During anti-CTLA-4 or IL-2 immunotherapy in mice, intratumoral tumor necrosis factor-α (TNF-α) production and T cell accumulation result in increased *Ezh2* expression in melanoma cells, which in turn silence their own immunogenicity and antigen presentation. Ezh2 inactivation reverses this resistance and synergizes with anti-CTLA-4 and IL-2 immunotherapy to suppress melanoma growth. These antitumor effects depend on intratumorally accumulating interferon-γ (IFN-γ)-producing PD-L1 $CD8^+$ T cells and PD-L1 downregulation in melanoma cells. Ezh2 serves as a molecular switch controlling melanoma escape during T cell-targeting immunotherapies.[487]

6.8.19 Coronary Artery Disease

Nitrates are widely used to treat coronary artery disease, but their therapeutic value is compromised by nitrate tolerance, owing to the dysfunction of prostaglandin I2 synthase (PTGIS). miRNAs repress target gene expression and are recognized as important epigenetic regulators of endothelial function. Nitrates induce nitrovasodilator resistance via miRNA-dependent repression of *PTGIS* gene expression. In cultured HUVECs, NO donors induce *miR-199a/b* endogenous expression of and downregulate *PTGIS* gene expression, both of which are reversed by carboxyl-PTIO or by silencing the serum response factor. The seed sequence of 976–982 in the 3′-UTR of *PTGIS* mRNA is a target of miR-199a/b. Gain-of-function mutations of *miR-199a/b* resulting from chemical mimics or adenovirus-mediated overexpression increase *PTGIS* mRNA degradation in HEK293 cells and HUVECs. GTN-decreased *PTGIS* gene expression is prevented by *miR-199a/b* antagomirs or is mirrored by the enforced expression of *miR-199a/b* in HUVECs. In *Apoe*−/− mice, GTN induces the ectopic expression of *miR-199a/b* in the carotid arterial endothelium, decreases *PTGIS* gene expression, and instigates nitrovasodilator resistance, all of which are abrogated by miR-199a/b antagomirs or LNA-anti-miR-199. The effects of miR-199a/b inhibition are abolished by adenovirus-mediated PTGIS deficiency. The enforced expression of miR-199a/b represses *PTGIS* gene expression and impairs the responses of aortic arteries to GTN/sodium nitroprusside/acetylcholine/cinaciguat/riociguat, whereas the exogenous expression of the *PTGIS* gene prevents nitrovasodilator resistance in Apoe−/− mice subjected to GTN infusion or miR-199a/b overexpression. Indomethacin, iloprost, and SQ29548 improve vasorelaxation in GTN-infused *Apoe*−/− mice, while U51605 induces nitrovasodilator resistance. In humans the increased expression of *miR-199a/b* is closely associated

with nitrate tolerance. Induced ectopic expression of *miR-199a/b* in endothelial cells is required for nitrovasodilator resistance via the repression of *PTGIS* gene expression. miR-199a/b is a novel target for the treatment of nitrate tolerance.[488]

6.9 PHARMACOEPIGENETIC PREDICTORS OF DRUG EFFICACY AND SAFETY

6.9.1 Bevacizumab

Some epigenetic biomarkers predicting response to bevacizumab in breast cancer have been proposed. Methylation of at least one cytosine in 26 gene regions was significantly associated with progression-free survival (PFS). Two methylation signatures of 3 and 9 genes can discriminate between responders and nonresponders to a bevacizumab-based therapy.[489]

6.9.2 Trastuzumab

Trastuzumab is a standard treatment for HER2-positive ($HER2^+$) breast cancer, but some patients are refractory to therapy. miRNAs have been used to predict the therapeutic effects for various cancers. Li et al.[490] identified 13 differentially expressed miRNAs in the serum of $HER2^+$ MBC patients who respond differently to trastuzumab, and four miRNAs were selected to construct a signature to predict survival using a LASSO model. miR-940 is mainly released from tumor cells and miR-451a, miR-16-5p, and miR-17-3p are mainly released from immune cells. These four miRNAs directly target signaling molecules that play crucial roles in regulating trastuzumab resistance.

Breast cancer resistance to the monoclonal erbB2/HER2 antibody trastuzumab (or herceptin) may be related to dysregulation of miRNA expression. Knockdown of the long noncoding RNA, urothelial cancer associated 1 (*UCA1*), can enhance the sensitivity of human breast cancer cells to trastuzumab. *UCA1* knockdown upregulates *miR-18a* and promotes miR-18a repression of Yes-associated protein 1 (*YAP1*). Reciprocal repression of *UCA1* and *miR-18a* are Argonaute 2 dependent. Knockdown of *YAP1* recapitulates the effect of *UCA1* silencing by reducing the viability of trastuzumab-treated breast cancer cells, whereas inhibition of miR-18a abrogates *UCA1* knockdown-induced improvement of trastuzumab sensitivity in breast cancer cells. The UCA1/miR-18a/YAP1 axis plays an important role in regulating the sensitivity of breast cancer cells to trastuzumab.[491]

Patients with breast cancer who achieved a pathological complete response (pCR) to therapy are associated with excellent disease-free survival. Genome-wide DNA methylation analysis of these patients identified eight genomic regions specifically methylated in patients with pCR. Of these, *HSD17B4* encoding type 4 17β-hydroxysteroid dehydrogenase is differentially methylated the most. *HSD17B4* methylation is an independent predictive factor. Combination with ER status and *HSD17B4* methylation improves specificity up to 91%.[492]

6.9.3 Platinum

A major limitation of treatment with platinum is the resistance that most oncotissues develop. Some epigenetically regulated miRNAs are biomarkers of platinum resistance in lung and ovarian cancers that have the highest ratios of chemoresistance. Vera et al.[493] identified four miRNAs (*miR-7, miR-132, miR-335,* and *miR-148a*) whose deregulation might be a common signature in the development of resistance to cisplatin in both tumor types. The basal methylation status of miR-7 before treatment may be a potential clinical epigenetic biomarker, a predictor of chemotherapy outcome to CDDP in ovarian cancer patients.

Glutathione peroxidase 3 (GPX3, plasma glutathione peroxidase) is a key component of cellular antioxidant regulation, and its gene has been reported to be methylated in specific tumor types. GPX3's role in oxidative damage has been associated with sensitivity to platinum. *GPX3* promoter region methylation is present in one-third of CRC samples, and *GPX3* methylation leads to reduced *GPX3* expression and increased oxaliplatin and cisplatin sensitivity. In contrast, in cell lines with high baseline levels of *GPX3* expression or with the ability to increase *GPX3* expression, platinum resistance is increased. The cisplatin IC50 in GPX3-methylated cell lines is approximately sixfold lower than that in GPX3-unmethylated lines. Knockdown cell lines with essentially no *GPX3* expression require *N*-acetylcysteine to survive in culture, underscoring the importance of GPX3 in redox biology. In vivo, *GPX3* methylation predicts tumor xenograft sensitivity to platinum by the regression of *GPX3* knockdown xenografts.[494]

6.9.4 Cisplatin

PAX5 is a novel gene methylation marker in esophageal squamous cell carcinoma (ESCC). *PAX5* methylation is a tumor-specific event in squamous cell carcinogenesis of the head and neck. Highly methylated cases are associated with downregulated *PAX5* expression and poor recurrence-free survival. *PAX5* knockdown cells exhibit increased cell proliferation and cisplatin resistance. *PAX5* gene methylation can predict poor survival outcomes and cisplatin sensitivity in ESCCs.[495]

Therapeutic options to treat advanced muscle-invasive bladder cancer (MIBC) include cystectomy and chemotherapy. Neoadjuvant cisplatin-based combination chemotherapy is effective in MIBC; however, it has not been widely adopted by the community because many patients do not respond to neoadjuvant chemotherapy, and no biomarker currently exists to identify these patients. Xylinas et al.[496] have studied potential cisplatin resistance patterns in preclinical models of bladder cancer and tested whether treatment with the epigenetic modifier decitabine is able to sensitize cisplatin-resistant bladder cancer cell lines. The methylation status of the *HOXA9* promoter is associated with response to cisplatin-based chemotherapy in bladder cancer cell lines and in metastatic bladder cancer. Bladder cancer cells resistant to cisplatin chemotherapy can be sensitized to cisplatin by the DNA methylation inhibitor decitabine.

6.10 TOXICOEPIGENETICS

There are over 100,000 synthetic chemicals readily available on the market. Recent large-scale human population studies have associated exposure to certain chemicals with increased health risk. Epidemiological studies in humans suggest that many different chemicals affect prenatal growth, thyroid function, glucose metabolism, obesity, puberty, fertility, and carcinogenesis mainly through epigenetic mechanisms.[497]

A total of 25 epigenetic toxicity pathway components (SET1, MLL1, KDM5, G9A, SUV39H1, SETDB1, EZH2, JMJD3, CBX7, CBX8, BMI, SUZ12, HP1, MPP8, DNMT1, DNMT3A, DNMT3B, TET1, MeCP2, SETDB2, BAZ2A, UHRF1, CTCF, HOTAIR, and ANRIL) have been identified as potentially involved in human cellular transformation.[498]

Crosstalk between the nuclear epigenome and mitochondria, both in normal physiological functions and in responses to environmental toxicant exposures, is a developing subfield of environmental and molecular toxicology. Most mitochondrial proteins are encoded in the nuclear genome, and programmed communication among nuclear, cytoplasmic, and mitochondrial compartments is essential for maintaining cellular health. The two main signaling systems are anterograde nuclear signaling to the mitochondria (for the regulation of oxidative phosphorylation (OXPHOS) and mitochondrial biogenesis in response to environmental signals received by the nucleus) and retrograde mitochondrial signaling to the nucleus.[499]

Genotoxicity and mutagenicity analyses have a significant role in the identification of hazards posed by therapeutic drugs, cosmetics, agrochemicals, industrial compounds, food additives, natural toxins, and nanomaterials for regulatory purposes.[500]

Several studies have documented the influence different environmental toxicants have on human health with the mediation of epigenetic mechanisms. Exposure to many different substances is under scrutiny: such substances as dioxins, polychlorinated biphenyls, organochlorine pesticides, perfluoroalkyl substances, phthalates, bisphenol A, and methylmercury.[501]

Altered expression profiles of lncRNAs have been explored after exposure to environmental chemicals (ECs). Various kinds of ECs have been reported to disturb the expression of lncRNAs. Dysregulated lncRNAs can affect the expression of target genes directly or indirectly via regulating the level of miRNAs. The network among lncRNAs, miRNAs, and mRNAs can initiate or impede specific signaling pathways and lead to adverse outcomes upon exposure to ECs. Recovery of the lncRNA level by overexpression or knockdown technology diminishes the effect induced by ECs.[502]

The serotonin transporter gene (*SLC6A4*) is a stress-related gene that has well-documented implications for behavioral and socioemotional development. It has been shown to be susceptible to transcriptional regulation via epigenetic mechanisms. Its association with adverse exposures, *SLC6A4* methylation, and developmental outcomes has been reported. *SLC6A4* methylation has been investigated in humans in association with a number of prenatal and postnatal adverse exposures, including maternal depression during pregnancy, perinatal stress exposure, childhood trauma and abuse, and environmental stress. SLC6A4 is a relevant biomarker of early adverse exposures.[503]

6.10.1 Drugs

6.10.1.1 Morphine

Morphine is one of the most effective analgesics in medicine. Its use is associated with the development of tolerance and dependence. There are epigenetic changes in the brain after exposure to opiates. Barrow et al.[504] studied epigenetic changes in 10 regions of the rat brain following acute and chronic morphine exposure. DNA methylation was assessed in six nuclear-encoded genes implicated in brain function (*Bdnf, Comt, Il1b, Il6, Nr3c1*, and *Tnf*) and three mitochondrially encoded genes (*Mtco1*, *Mtco2*, and *Mtco3*). Differential methylation of *Bdnf* and *Il6* was observed in the pons, *Nr3c1* in the cerebellum, and *Il1b* in the hippocampus in response to acute morphine exposure. Chronic exposure was associated with differential methylation of *Bdnf* and *Comt* in the pons, *Nr3c1* in the hippocampus, and *Il1b* in the medulla oblongata. Global 5mC levels decreased in the superior colliculus and increased in the hypothalamus following chronic exposure. Chronic exposure was also associated with increased global 5hmC levels in the cerebral cortex, hippocampus, and hypothalamus, but decreased in the midbrain.

6.10.1.2 Heroin

Studies investigating transcriptional and epigenetic profiling of postmortem human brain specimens from a homogeneous European Caucasian population of heroin users revealed marked impairments related to glutamatergic neurotransmission and chromatin remodeling in the human striatum. Hyperacetylation of lysine 27 of histone H3 shows dynamic correlations with heroin use history and acute opiate toxicology. Targeted investigation of *GRIA1*, a glutamatergic gene implicated in drug-seeking behavior, verified the increased enrichment of lysine 27-acetylated histone H3 at discrete loci, accompanied by enhanced chromatin accessibility at hyperacetylated regions in the gene body. Bromodomain inhibitor JQ1, which blocks the functional readout of acetylated lysines, reduces heroin self-administration and cue-induced drug-seeking behavior.[505]

6.10.1.3 Cocaine

Natural antisense transcripts (NATs) are an abundant class of long noncoding RNAs that have recently been shown to be key regulators of chromatin dynamics and gene expression in nervous system development and neurological disorders. Some drug-induced transcriptional responses are mediated by perturbations in NAT activity. About 22% of genes contain NATs, and the expression of *Homer1* natural antisense transcript (*Homer1-AS*) is altered in the nucleus accumbens (NAc) of mice following repeated cocaine administration. Homer1-AS depletion leads to an increase in expression of the corresponding sense gene, indicating a potential regulatory mechanism of *Homer1* expression by its corresponding antisense transcript.[506]

Paternal environmental perturbations including exposure to drugs of abuse can produce profound effects on the physiology and behavior of offspring via epigenetic modifications. Adult drug-naive male offspring of cocaine-exposed sires have memory formation deficits and associated reductions in NMDA receptor-mediated hippocampal synaptic plasticity. Reduced levels of the endogenous NMDA receptor coagonist D-serine are accompanied by increased expression of the D-serine-degrading enzyme D-amino acid oxidase (Dao1) in the hippocampus of cocaine-sired male progeny. Increased *Dao1* transcription is associated with enrichment of permissive epigenetic marks on histone proteins in the hippocampus of male cocaine-sired progeny, some of which are enhanced near the *Dao1* locus. Hippocampal administration of D-serine reversed both memory formation and synaptic plasticity deficits. Paternal cocaine exposure produces epigenetic remodeling in the hippocampus leading to NMDA receptor-dependent memory formation and synaptic plasticity impairments only in male progeny, which has significant implications for the male descendants of chronic cocaine users.[507] Cocaine strongly impairs the consolidation of extinction memory. Cocaine interferes with memory processing independently of incentive salience by directly altering DNA methylation dynamics.[508]

Chromatin regulation, in particular ATP-dependent chromatin remodelers, modulate reward-related behaviors in animal models of mental illnesses. BAZ1A is an accessory subunit of the ISWI family of chromatin-remodeling complexes that is downregulated in the nucleus accumbens (NAc) of mice exposed repeatedly to cocaine and in cocaine-addicted humans. Viral-mediated overexpression of *BAZ1A* in mouse NAc reduces cocaine reward and increases cocaine-induced locomotor activation. There are extensive nucleosome occupancy and shift changes across the genome in response to cocaine exposure.[509]

Neuroinflammation plays a critical role in the development of reward-related behavior in cocaine self-administration rodents. Cocaine activates microglia. miR-124, a microglia-enriched miR, functions as an antiinflammatory regulator that maintains microglia in a quiescent state. Cocaine exposure decreases miR-124 levels in both BV-2 cells and rat primary microglia. The molecular mechanisms underlying these effects involve cocaine-mediated

increased mRNA and protein expression of DNMTs in microglia. Cocaine increases the promoter DNA methylation levels of miR-124 precursors (pri-miR-124-1 and -2), but not that of pri-miR-124-3. Cocaine exposure increases the DNA methylation of *miR-124* promoter resulting in its downregulation, which leads to microglial activation.[510]

6.10.1.4 Amphetamines

Methamphetamine, one of the most frequently used illicit drugs worldwide, can induce psychosis in a large number of abusers. Methamphetamine induces DNA hypomethylation of the promoter regions of *DRD3, DRD4, MB-COMT,* and *AKT1* that are associated with increased expression of the corresponding genes in patients with methamphetamine psychosis. Methamphetamine dependency is associated with reduced DNA methylation and corresponding increase in expression of several key genes involved in the pathogenesis of psychotic disorders.[511]

Histone deacetylase (HDAC) activities modify the structure of chromatin and play a role in learning and memory during developmental processes. HDACs are involved in neural network remodeling in brain repair. Liu and Liu[512] studied *HDAC5* expression in a preclinical model of amphetamine-induced sensitization (AIS) of behavior. Naive C57black6 mice that experience acute exposure to amphetamine show expression of both total and phosphorylated (S259) HDAC5 antigens in GFAP+ and GFAP− cells. SPION-miD2861 enhances *HDAC5* expression in the lateral septum and the striatum after amphetamine, where neuroprogenitor cells coexpress *NeuN* and *GFAP*.

6.10.1.5 3,4-Methylenedioxymethamphetamine (MDMA)

The recreational drug of abuse 3,4-methylenedioxymethamphetamine (MDMA) produces neurotoxic damage and long-lasting changes in several brain areas. Serotoninergic and dopaminergic systems and nociceptin/orphaninFQ (N/OFQ)-NOP and dynorphin (DYN)-KOP systems are involved in neuronal adaptations evoked by MDMA. MDMA exposure affects body weight gain and induces hyperlocomotion. Gene expression analysis shows downregulation of the N/OFQ system and upregulation of the DYN system in the nucleus accumbens (NAc), highlighting opposite system regulation in response to MDMA exposure. Histone modifications have been strongly associated with addiction-related maladaptive changes. The study of two permissive (acH3K9 and me3H3K4) and two repressive transcription marks (me3H3K27 and me2H3K9) at opioid gene promoter regions has revealed that acute MDMA increases me3H3K4 at the *pN/OFQ*, *pDYN*, and *NOP* promoters. Acute MDMA administration causes an acH3K9 increase and a me2H3K9 decrease at the *pDYN* promoter. Activation of the DYNergic stress system together with inactivation of the N/OFQergic antistress system contribute to the neuroadaptive actions of MDMA.[513]

6.10.1.6 Phencyclidine

A deficit in parvalbumin neurons is found in schizophrenia and psychotic disorders. Phencyclidine (PCP) administration results in changes in DNA methylation in rat *Pvalb* promoter. PCP causes hypermethylation at one of two *Pvalb* CpG sites in both the prefrontal cortex and hippocampus, while no significant differences are found in long interspersed nucleotide element-1, a global measure of DNA methylation.[514]

6.10.1.7 Cannabis and Δ9-Tetrahydrocannabinol (THC)

The incidence of prenatal cannabis exposure (PCE) is increasing in developed countries. Unlike fetal alcohol spectrum disorder, there is no phenotypic syndrome associated with PCE. PCE causes lifelong cognitive, behavioral, or functional abnormalities, and/or susceptibility to subsequent addiction. The usage of marijuana during pregnancy perturbs the fetal endogenous cannabinoid signaling system (ECSS), which is present and active from the early embryonic stage, modulating neurodevelopment and continuing this role into adulthood. The ECSS is present in virtually every brain structure and organ system, and there is also evidence that this system is important in the regulation of cardiovascular processes. Endocannabinoids (eCBs) influence a broad spectrum of processes, including the early stages of fetal neurodevelopment and uterine implantation. Delta-9-tetrahydrocannabinol (THC), the psychoactive chemical in cannabis, enters the maternal circulation and readily crosses the placental membrane. THC binds to CB receptors of the fetal ECSS, altering neurodevelopment and possibly rewiring ECSS circuitry. Perturbations of the intrauterine milieu via the introduction of exogenous CBs alter the fetal ECSS, predisposing the offspring to abnormalities in cognition and altered emotionality.[515]

Adolescent THC exposure induces alterations in selective histone modifications (H3K9me3), impacting the expression of genes closely associated with synaptic plasticity. Changes in both histone modification and gene expression are more widespread and intense after adolescent treatment, suggesting specific adolescent susceptibility. Adolescent THC exposure increases Suv39H1 levels, which might account for enhanced H3K9me3. Pharmacological blockade of H3K9me3 during adolescent THC treatment prevents THC-induced cognitive deficits, suggesting a relevant role played by H3K9me3 in THC-induced effects.[516]

6.10.1.8 Glucocorticoids

Chronic exposure to glucocorticoids (GCs) can lead to psychiatric complications through epigenetic mechanisms. Approximately 70% of differentially methylated regions (DMRs) in both brain and blood lost methylation following GC treatment. Of the 3095 DMRs that mapped to the same genes in both tissues, 1853 underwent DNA methylation changes in the same direction. Only 209 DMRs (<7%) overlapped in genomic coordinates between brain and blood, suggesting tissue-specific differences in GC-targeted loci. DMR-associated genes are members of pathways involved in metabolism, immune function, and neurodevelopment. Separation of the cortex into neuronal and nonneuronal fractions and the leukocytes into T cells, B cells, and neutrophils showed that GC-induced methylation changes primarily occur in neurons and T cells, with blood tissue also undergoing a shift in the proportion of constituent cell types, while the proportion of neurons and glia in the brain remains stable.[517]

6.10.1.9 Oral Contraceptives

Li et al.[518] studied the association of telomerase activity (TA) and telomere length (TL) in granulosa cells (GCs) with IVF outcomes of polycystic ovary syndrome (PCOS) patients and the effects of oral contraceptive pill (OCP) pretreatment on these two parameters. Shorter TL was found in PCOS patients. TA levels did not change significantly in PCOS patients. PCOS patients with a lower TA level and shorter telomeres had an earlier onset of infertility symptoms. No predictive value was found for TA and TL in terms of embryo quality or IVF outcomes in PCOS patients, and no effect of OCP pretreatment was observed on either TA or TL.[518]

The developmental origin of health and disease is the name given to the concept that early exposure to toxicants or nutritional imbalances during perinatal life induces changes that enhance the risk for developing noncommunicable diseases in adulthood. An experimental model with an adult chronic germ cell death phenotype resulting from exposure to a xenoestrogen showed a reciprocal negative feedback loop that involved a decreased EZH2 protein level and increased *miR-101* expression. Knockdown of *EZH2* induced an apoptotic process in germ cells through increased levels of apoptotic factors (BIM and BAD) and DNA repair alteration via topoisomerase 2B deregulation. Increased miR-101 levels observed in animal blood might indicate that miR-101 may be a part of a circulating mark of germ cell death. According to Siddeek et al.[519] miR-101-EZH2 pathway deregulation might represent a novel pathophysiological epigenetic basis for adult germ cell disease which has environmental and developmental origins.

6.10.1.10 Estradiol-17β

Endocrine-disrupting chemicals (EDCs) interfere with the natural hormone balance and may induce epigenetic changes through exposure during sensitive periods of development. Van der Weijden et al.[520] studied the effects of short-term estradiol-17β (E2) exposure on various tissues of pregnant sows (F0) and on day 10 blastocysts (F1). In F0, perturbed tissue-specific mRNA expression of cell cycle regulation and tumor suppressor genes was found at both low- and high-dose exposure, being most pronounced in the endometrium and corpus luteum. The liver showed the most significant DNA hypomethylation in three target genes (*CDKN2D, PSAT1, RASSF1*). For *CDKN2D* and *PSAT1*, differential methylation in blastocysts was similar to that observed in F0 liver. While blastocysts showed hypomethylation the liver of 1-year old offspring showed subtle, but significant hypermethylation.

Estrogen signaling modulates the vasoactive and metabolic pathways in endothelial cells. Estrogen modulates the miRNA profile in human endothelial cells. The expression of 114 miRNAs is modified after E2 exposure, which affects the pathways involved in cell death and survival, lipid metabolism, and reproductive system function. miR-30b-5p, miR-487a-5p, miR-4710, and miR-501-3p increase while miR-378h and miR-1244 decrease in response to E2.[521]

Long-term estradiol (E2) replacement affects social behavior and gene expression in brain nuclei. Gene expression has revealed that the supraoptic nucleus had the greatest number of gene changes caused by E2: *Oxt*, *Oxtr*, and *Avp* were increased and *Drd2, Htr1a, Grin2b*, and *Gabbr1* were decreased. No genes were affected in the prefrontal cortex, but several genes were affected in the paraventricular nucleus (*Pgr*), bed nucleus of the stria terminalis (*Oxtr, Esr2, Dnmt3a*), and medial amygdala (*Oxtr, Ar, Foxp1, Tac3*).[522]

6.10.1.11 Experimental Hepatotoxicants: Clofibrate and Phenobarbital

The experimental hepatotoxicants clofibrate (CF) and phenobarbital (PB) cause dose-dependent increases in relative liver weights, hepatocellular hypertrophy and proliferation, and increases in *Cyp2b1* and *Cyp4a1* transcripts. These changes are associated with altered histone modifications within the regulatory units of cytochrome genes, *LINE-1* DNA hypomethylation, and altered miRNA profiles.[523]

6.10.1.12 Oxytetracycline

Oxytetracycline (OTC) is largely employed in zootechnical and veterinary practices to ensure the wellness of farm animals. It is partially absorbed within the gastrointestinal tract where it deposits in several tissues. Potential OTC toxicity is relevant when considering the putative risk derived by the entry and accumulation of such a drug in the food chains of humans and pets.

Human peripheral blood mononuclear cells (PBMCs) express DNA damage features (activation of ATM and p53, phosphorylation of H2AX, and modification of histone H3 methylation of lysine K4 in the chromatin) after in vitro exposure to OTC. These changes are associated with an inflammatory response reflected by increased expression of interferon (IFN)-γ and type 1 superoxide dismutase (SOD1).[524]

6.10.1.13 Permethrin

Permethrin (PERM) is a drug used for the treatment of scabies and lice. It is also used as an insecticide in agriculture, textile industry, aviation, and domestic insect control. PERM exposure during neonatal brain development leads to its accumulation long after exposure. In adolescent animals an increase in DNA methyltransferases (DNMTs) (DNMT1, DNMT3a), tyrosine hydroxylase, and monomeric and aggregated α-synuclein protein levels has been detected in the striatum. Adult animals show enhanced DNMT3b and α-synuclein aggregation, and aged animals exhibit a reduction in all biomarkers. There is a strong binding interaction between PERM and its metabolite 3-phenoxybenzoic acid with the nuclear orphan receptor Nurr1, and interference with the dopaminergic neuron pathway may occur during PERM accumulation in the brain.[525]

6.10.1.14 Antiepileptic Drugs

Prenatal exposure to antiepileptic drugs (AEDs) may cause severe complications in the fetus. Fujimura et al.[526] reported that (i) prenatal exposure to valproic acid (VPA), carbamazepine, and phenobarbital increases the risk of congenital malformations in a dose-dependent manner, and (ii) prenatal exposure to VPA increases the risk of brain function impairment including intellectual disabilities and autistic spectrum disorders in offspring. Prenatal exposure to specific AEDs causes microscopic structural abnormalities in the fetal brain. Prenatal exposure to VPA inhibits the differentiation of neural progenitor cells during the early to middle phases of neuronogenesis, leading to an increased number of projection neurons in the superficial layers of postnatal neocortices in mice.

6.10.1.15 Tetanus Vaccination

Vaccinations have been suggested to be associated with increased risk of allergic diseases. Tetanus vaccination is one of the most frequently administered vaccines in wound management and was also found to be associated with increased serum IgE levels. Tetanus vaccination is associated with decreased methylation of *cg14472551* and increased methylation of *cg01669161*. Both CpGs are associated with a decreased risk for asthma. *cg14472551* is located in an intron of *KIAA1549L*, the protein of which binds to a B cell commitment transcription factor; and *cg01669161* is located between an antisense regulator of the proteasome assembly chaperone (*PSMG3*) and a pseudogene (*TFAMP1*). Increased methylation of *cg01669161* is also associated with decreased serum IgE levels.[527]

6.10.2 Metals

Exposure to several metals (arsenic, lead, mercury, cadmium, antimony, tungsten, and others) has been shown to induce epigenetic changes associated with human disease.[528]

6.10.2.1 Arsenic and Related Compounds

Over 200 million people in 70 countries are exposed to arsenic through drinking water. Chronic exposure to this metalloid has been associated with the onset of many diseases, including cancer. Epidemiological evidence supports its carcinogenic potential. Susceptibility to the toxic effects of arsenic is influenced by alterations in genes involved in arsenic metabolism, as well as biological factors, such as age, gender, and nutrition. Chronic arsenic exposure results in several genotoxic and epigenetic alterations tightly associated with the arsenic biotransformation process, resulting in increased cancer risk.[529]

Exposure to inorganic arsenic (iAs) via drinking water represents a significant global public health threat, with chronic exposure associated with cancer, skin lesions, neurological impairment, and cardiovascular diseases. Prenatal and early-life iAs exposures are associated with long-term effects, many of which display sexually dimorphic

responses. Changes to the epigenome may play a key mechanistic role underlying many iAs-associated health outcomes.[530]

Arsenic exposure has been associated with male reproductive dysfunction by disrupting steroidogenesis. Histone H3 lysine 9 (H3K9) methylation is involved in steroidogenesis disturbance in mouse Leydig cells (MLTC-1) as a result of arsenic exposure. The mRNA and protein expression levels of 3β-hydroxysteroid dehydrogenase (*3β-HSD*) are upregulated, while other key genes involved in steroidogenesis are downregulated. Arsenic exposure decreases the histone H3K9 dimethylation and trimethylation (H3K9me2/3) levels in MLTC-1 cells. The H3K9me2/3 demethylase (JMJD2A) inhibitor quercetin attenuates the decrease of H3K9me2/3 and increase of *3β-HSD* expression induced by arsenic. Arsenic exposure induces 3β-HSD upregulation by suppressing the H3K9me2/3 status.[531]

Epigenetic modifications are associated with arsenical carcinogenicity. Trivalent inorganic arsenite (iAs(III)) induces histone H3K9 dimethylation (H3K9me2) and trimethylation (H3K9me3), histone H3S10 phosphorylation (H3S10p), histone H3T11 phosphorylation (H3T11p), and histone H3K9S10 trimethyl-phosphorylation (H3K9me3S10p). iAs(III) increases H3S10p and H3K9me3S10p in the *FOS* promoter around the SRE/ELK1-binding site (−400 to −200) and CRE-binding site (−50). In contrast, histone H3 around the *EGR1* promoter of the SRE/CRE-binding site (−200 to −50) is modified to H3S10p and H3K9me3S10p by iAs(III). The SRE/ELK1 site is important for iAs(III)-mediated FOS induction and the SRE/CRE site for EGR1 induction. iAs(III) induces histone H3 modifications around the transcription factor-binding sites of *FOS* and *EGR1* promoters, and these modifications seem to be important in transcriptional activation of these genes.[532]

The NRF2/KEAP1 pathway is inactivated in response to chronic arsenic exposure. Global DNA methylation is elevated in occupationally exposed workers, and As(III) levels are associated with the expression of *TXNRD1, GSTP1, HMOX1*, and *PRDX1*. The *NRF2* mRNA level is positively correlated with the expression of *NRF2*-target genes.[533]

Arsenic-exposed individuals show promoter hypermethylation of *MLH1* and *MSH2* and downregulation of H3K36me3, with no apparent effect on the expression of *SETD2* or the methyltransferase of an H3K36me3 moiety.[534]

Arsenic exposure impairs cognitive ability and alters the expression of neuronal activity-regulated genes. Total arsenic concentrations in the cortex and hippocampus are increased in a dose-dependent manner. The reduction in 5-methylcytosine (5 mC) and 5-hydroxymethylcytosine (5hmC) levels and downregulation of DNA methyltransferases (DNMTs) and ten-eleven translocation (TET) expression suggest that DNA methylation/demethylation processes are suppressed in brain tissues. The expression of indicators of oxidative/antioxidative balance and the tricarboxylic acid (TCA) cycle is also deregulated.[535]

Developmental exposure to a low level of arsenic (50 ppb) alters the epigenetic processes that underlie deficits in adult hippocampal neurogenesis leading to aberrant behavior. The master negative regulator of neural lineage REST/NRSF controls the precise timing of fate specification and differentiation of neural stem cells (NSCs). Early in development there is an increased expression of *Rest*, its corepressor *CoREST*, and the inhibitory RNA-binding/splicing protein Ptbp1, and altered expression of mRNA-spliced isoforms of *Pbx1* that are directly regulated by these factors in the male brain in response to prenatal 50-ppb arsenic exposure. These increases are concurrent with decreased expression of *miRNA-9 (miR-9), miR-9**, and *miR-124*, which are REST/NRSF targets. Exposure to arsenic decreases the formation of neuroblasts in vitro from NSCs derived from male pup brains. The female response to arsenic is limited to increased expression of *CoREST* and *Ptbp2*, an RNA-binding protein that allows for appropriate splicing of genes involved in the progression of neurogenesis. These changes are accompanied by increased neuroblast formation in vitro from NSCs derived from female pups. Unexposed male mice express transcriptomic factors to induce differentiation earlier in development than unexposed females. Arsenic exposure delays differentiation of NSCs in males, while potentially inducing precocious differentiation in females early in development. Arsenic-induced dysregulation of the regulatory loop formed by REST/NRSF, its target microRNAs *miR-9* and *miR-124*, and RNA-splicing proteins *PTBP1* and *PTBP2* leads to aberrant programming of NSC function that is perhaps perpetuated into adulthood.[536]

Arsenic-containing hydrocarbons (AsHCs) are a subgroup of arsenolipids found in fish and algae with toxic effects in various human cell lines. Müller et al.[537] studied the effects of two AsHCs (AsHC 332 and AsHC 360) on the expression of 44 genes covering DNA repair, stress response, cell death, autophagy, and epigenetics via RT-qPCR in human liver (HepG2) cells. Both AsHCs affect gene expression and, after treatment with AsHC 360, flap structure-specific endonuclease 1 *(FEN1)*, xeroderma pigmentosum group A complementing protein (*XPA*), and (cytosine-5)-methyltransferase 3A (*DNMT3A*) show time- and concentration-dependent alterations in gene expression, thereby indicating an impact on genomic stability. AsHC 360 increases global DNA hydroxymethylation levels. Both AsHCs are biotransformed and their metabolites include not only the respective thioxoanalogs of the two AsHCs, but also several arsenic-containing fatty acids and fatty alcohols.

Arsenic trioxide (As_2O_3) induces cell death in a variety of cancer cell types. The tyrosine kinase receptor (Trk) family comprises three members, namely TrkA, TrkB, and TrkC. *TrkA* and *TrkC* expression is associated with good prognosis,

while *TrkB* overexpression can lead to tumor cell growth and invasive metastasis. As_2O_3 can inhibit the growth and proliferation of a human neuroblastoma (NB) cell line and can also affect *N-Myc* mRNA expression.[538]

Studies of arsenic trioxide (As_2O_3), cisplatin (DDP), and etoposide (Vp16) on anticancer effects and P-glycoprotein (P-gp) expression in neuroblastoma (NB) cells have shown that As_2O_3, DDP, and Vp16 inhibit the growth and survival of SK-N-SH cells at different concentrations. As_2O_3 at low concentrations leads to enhanced accumulation of cell populations in the G2/M phase as a result of increased exposure time, as well as increased levels of apoptosis. Following pretreatment of SK-N-SH cells with As_2O_3 the expression of P-gp is not increased. Exposure to As_2O_3 reduces the expression of P-gp, whereas DDP and VP16 upregulate P-gp expression.[539]

6.10.2.2 Lead

Many adults are exposed to the neurotoxic effects of high levels of lead (Pb) during childhood. Childhood lead exposure may raise the risk for adult neurodegenerative disease, particularly dementia, through a variety of possible mechanisms including epigenetic modification, delayed cardiovascular and kidney disease, direct degenerative CNS injury from lead remobilized from bone, and lowered neural and cognitive reserve.[540]

Prenatal exposure to Pb decreases fetal growth and probably also affects postnatal growth. Imprinted genes are regulators of growth and energy utilization and may be vulnerable to environmental Pb exposure. Prenatal Pb exposure alters the DNA methylation of imprinted genes resulting in lower birth weight and rapid growth. Children born to women with Pb levels in the upper tertile show hypermethylation of the regulatory region of the *MEG3* DMR imprinted domain. Pb levels are also associated with lower birth weight and rapid gains in adiposity by children aged 2–3 years.[541] Early-life exposure to Pb results in epigenetic drift in H3K9Ac consistent with latent global gene repression.[542] Over a lifetime early developmental exposure to lead and prenatal stress (PS) are followed by multiple varied behavioral experiences. Posttranslational histone modification (PTHM) profiles differ by sex, brain region, and time point of measurement following developmental exposure to Pb ± PS.[543] Developmental exposure to Pb and PS impair cognition, which might derive from their joint targeting of the hypothalamic-pituitary-adrenal (HPA) axis and the brain mesocorticolimbic (MESO) system, including the frontal cortex (FC) and hippocampus (HIPP). Glucocorticoids modulate both FC and HIPP function. Developmental Pb + PS exposures alter glucocorticoid-related epigenetic profiles in brain MESO regions in the offspring of female mice exposed to 0- or 100-ppm Pb acetate drinking water. Both Pb and PS broadly impact brain DNA methyltransferases and binding proteins, particularly DNMT1, DNMT3a, and MECP2, with patterns that differ by sex and brain region.[544] Developmental Pb exposure results in persistent cognitive/behavioral impairment as well as an elevated risk for developing a variety of diseases in later life. Gender makes a significant contribution to the hippocampal methylome and effects of the Pb exposure level. The highest number of differentially methylated regions is found in females exposed to Pb at the lowest exposure level. Low-level Pb exposure alters gene-specific DNA methylation patterns in the brain in a sex-dependent manner.[545]

Oxidative stress and DNA damage are involved in lead toxicity in construction workers.[546]

SNPs located within the 3′-UTR of δ-aminolevulinic acid dehydratase (*ALAD*) can alter the risk for lead poisoning and *ALAD* gene expression. There is an association between *rs818708* and the risk for lead poisoning. *miR-545-5p* is influenced by the *rs818708* variant and might result in a significant change in *ALAD* expression.[547]

Novel intracisternal A particle (*IAP*) retrotransposons exhibiting regions of variable methylation have been proposed as candidate loci for environmental effects on the epigenome. Pb exposure induces global methylation changes in some of these retrotransposons in a tissue- and sex-dependent manner.[548]

6.10.2.3 Mercury

Occupational exposure to mercury (Hg°) affects the gene expression of antioxidant enzymes and the levels of selenoproteins.[549] Methylmercury (MeHg) is the causative substance of Minamata disease, which is associated with various neurological disorders such as sensory disturbance and ataxia. Low-level dietary intake of MeHg from MeHg-containing fish during gestation adversely affects the fetus. The levels of TH, the rate-limiting enzyme for dopamine synthesis, are decreased in neurons after MeHg exposure. Acetylated histone H3, acetylated histone H3 lysine 9, and trimethyl histone H3 lysine 9 levels at the *TH* gene promoter are not altered; however, trimethylation of histone H3 lysine 27 levels, related to transcriptional repression, are increased at the *TH* gene promotor after MeHg exposure. MeHg exposure during neuronal differentiation may lead to epigenetic changes that repress *TH* gene expression.[550]

Faroe Islanders consume marine foods contaminated with methylmercury (MeHg), polychlorinated biphenyls (PCBs), and other toxicants associated with chronic disease risks. Differential DNA methylation at specific CpG sites in cord blood was used as a surrogate biomarker of health impacts from chemical exposures to MeHg, major PCBs, other organochlorine compounds (hexachlorobenzene (HCB), *p*,*p*′-dichlorodiphenyldichloroethylene (*p*,*p*′-DDE), and *p*,*p*′-dichlorodiphenyltrichloroethane), and perfluoroalkyl substances. PCB congener 105 (CB-105) exposure was

associated with the majority of differentially methylated CpG sites (214 of a total of 250). In female-only analysis just 73 CB-105-associated CpG sites were detected, 44 of which were mapped to genes in the ELAV1-associated cancer network. In male-only analysis methylation changes were seen for perfluorooctane sulfonate, HCB, and *p,p′*-DDE at 10,598, 1238, and 1473 CpG sites, respectively, 15% of which were enriched in cytobands of the X chromosome associated with neurological disorders. The enrichment of specific X chromosome sites in males implies potential sex-specific epigenome responses to prenatal chemical exposure.[551]

Chronic exposure to MeHg causes epigenetic landscape modifications of histone H3K4 trimethylation (H3K4me3) marks in *Caenorhabditis elegans*. The modifications correspond to the locations of 1,467 genes with enhanced and 508 genes with reduced signals. Among the enhanced genes are those encoding glutathione-*S*-transferases, lipocalin-related protein, and a cuticular collagen. H3K4me3 marks are enhanced in these genes in animals exposed to MeHg in utero. In utero exposure enhances marks without altering mRNA expression except for the *lpr-5* gene. Knockdown of lipocalin-related protein gene *lpr-5*, which is involved in intercellular signaling, and cuticular collagen gene *dpy-7*, a structural component of the cuticle, by RNA interference (RNAi) results in increased lethality of animals after MeHg exposure.[552]

6.10.2.4 Chromium

Hexavalent chromium (Cr^{VI}) is a genotoxic environmental carcinogen with potential deleterious effects on the epigenetic machinery. Chronic Cr^{VI} exposure causes epigenetic dysregulation as evidenced by increased levels of histone H3-repressive methylation marks (H3K9me2 and H3K27me3) and related histone-lysine methyltransferases (HMTases). Pharmacological inhibition or knockdown of HMTases reduces H3-repressive methylation marks and malignant phenotypes of Cr^{VI}-transformed cells. Knockdown of HMTases in parental cells reduces chronic Cr^{VI} exposure-induced cell transformation. Knockdown of HMTases also decreases Cr^{VI} exposure-caused DNA damage.[553]

Hexavalent chromium compounds are well-established respiratory carcinogens used in industrial processes. While inhalation exposure constitutes an occupational risk affecting mostly chromium workers, environmental exposure from drinking water is a widespread gastrointestinal cancer risk, affecting millions of people throughout the world. Cr^{VI} is genotoxic, forming protein-Cr-DNA adducts and silencing tumor suppressor genes. Cr^{VI} disrupts the binding of transcription factors CTCF and AP-1 to their cognate chromatin sites. Chromium perturbs chromatin organization and dynamics. Cr^{VI} disrupts the accessibility of chromatin regions enriched for CTCF and AP-1-binding motifs, with a significant cooccurrence of binding sites for both factors in the same region. Over 30% of Cr^{VI}-enriched CTCF sites are located in promoters of genes differentially expressed from chromium treatment.[554]

6.10.2.5 Nickel

Nickel (Ni) is an environmental and occupational carcinogen, and exposure to Ni is associated with lung disease (chronic inflammatory airway diseases, asthma, fibrosis) and cancers. Ni induces epithelial-mesenchymal transition (EMT) and the mesenchymal phenotype remains irreversible even after termination of exposure. Ni-induced EMT is dependent on the irreversible upregulation of ZEB1, an EMT master regulator, via resolution of its promoter bivalency. *ZEB1* downregulates its repressors and the cell-cell adhesion molecule E-cadherin, resulting in cells undergoing EMT and switching to a persistent mesenchymal status. ZEB1 depletion in cells exposed to Ni attenuates Ni-induced EMT.[555]

Nickel displays weak genotoxicity and mutagenicity. Iron- and 2-oxoglutarate-dependent Tet dioxygenases are a class of epigenetic enzymes that catalyze the oxidation of DNA 5-methylcytosine (5mC). Nickel inhibits Tet protein-mediated oxidation of DNA 5mC in cells ranging from somatic cell lines to embryonic stem cells.[556]

6.10.2.6 Cadmium

Imprinted genes are defined by their preferential expression from one of the two parental alleles. This unique mode of gene expression is dependent on allele-specific DNA methylation profiles established at regulatory sequences called imprinting control regions (ICRs). Cadmium exposure alters the relative sensitivity of ICRs to methylation. In newborn cord blood and maternal blood, 641 and 1945 cadmium-associated DMRs have been identified, respectively. DMRs are more common at the 15 maternally methylated ICRs than at similar nonimprinted loci in newborn cord blood and maternal blood, suggesting a higher sensitivity of ICRs to cadmium. The top three functional categories for genes that overlapped DMRs in maternal blood are body mass index (BMI), blood pressure, and body weight. In newborn cord blood, the top three functional categories are BMI, atrial fibrillation, and hypertension.[557]

In vertebrates 5-methylcytosine (5mC) is sensitive to Cd exposure. Studies of 5mC content in DNA of the hepatopancreas of adult *Cantareus aspersus* confirmed its presence and provided evidence for Cd-induced changes in global 5mC levels in DNA of gastropods and mollusks. DNA methylation levels responded in a dose- and time-dependent

manner to dietary cadmium, with exposure dose having a much stronger effect than exposure duration. A strong association has been identified between Cd concentrations in the hepatopancreas and DNA hypermethylation levels in this organ. Total 5mC content in DNA of the hepatopancreas of land snails is responsive to sublethal Cd exposure.[558]

6.10.2.7 Titanium and Zirconium

miRNA expression is associated with personal levels of titanium (Ti) and zirconium (Zr) traced in hair samples. Ti and Zr materials are widely used for dental implants and other medical devices. Seven miRNAs (*miR-99b, miR-142-5p, miR-152, miR-193a-5p, miR-323-3p, miR-335, miR-494*) have been specifically associated with Zr levels. Some of these miRNAs are involved in inflammation, skeletal, and connective tissue disorders. Zr is more bioactive than Ti and activates miRNA-related molecular mechanisms sensitive to Zr exposure.[559]

6.10.3 Phthalates

Phthalates are endocrine disruptors to which the general population, including pregnant women, is ubiquitously exposed. In utero phthalate exposure alters the patterns of cord blood DNA methylation. Regional assessment identified 27 distinct DMRs, the majority of which were related to multiple phthalate metabolites. Most significant DMRs (67%) are observed in later pregnancy (26-week gestation). Over 50% of DMRs are associated with di-(2-ethylhexyl) phthalate metabolites. Hypermethylated genes are involved in inflammatory response (*IRAK4* and *ESM1*), cancer (*BRCA1* and *LASP1*), endocrine function (*CNPY1*), and male fertility (*IFT140, TESC*, and *PRDM8*).[560]

Phthalates cross the placenta and affect the fetal epigenome. lncRNAs are involved in the manifestation of EDC toxicity. Studies in patients with uncomplicated dichorionic diamniotic twin pregnancies at term show large variation in lncRNA levels, with no significant differences in lncRNA expression within twin pairs. Mono-(carboxynonyl) phthalate (MCNP) shows strong correlation with most lncRNAs. The strongest correlation is between MHiBP and LOC91450. Other strong correlations are between MiBP, DPP10, and HOTTIP. AIRN, DACT3.AS1, DLX6, DPP10, HOTTIP, LOC143666, and LOC91450 are strongly correlated with most phthalate metabolites.[561]

Phthalates are a chemical class of plasticizers that have a ubiquitous environmental distribution. They are contaminants associated with oxidative stress. Phthalic acid esters are antiandrogenic and may cause systemic effects in humans, particularly as a result of in utero exposure.

Huffman et al.[562] studied the effects of urinary phthalate metabolite and isoprostane concentrations on sperm mitochondria DNA copy number (mtDNAcn) and mitochondrial DNA deletions (mtDNAdel) in male partners undergoing assisted reproductive technologies (ART). Urinary monocarboxy-isononyl phthalate (MCNP) concentrations have been positively associated with mtDNAcn, whereas other urinary phthalate metabolite and isoprostane concentrations have not been associated with sperm mtDNAcn or mtDNAdel.

Epigenetic modification, such as DNA methylation, has been hypothesized to be an important mechanism that mediates certain biological processes and pathogenic effects of in utero phthalate exposure. DNA methylation levels at more than 450,000 CpG sites measured in cord blood samples identified 25 CpG sites where the methylation levels in cord blood correlated with prenatal di-(2-ethylhexyl) phthalate (DEHP) exposure of pregnant women during 28–36-week gestation. Genes involved in the androgen response, estrogen response, and spermatogenesis showed DNA methylation changes in response to exposure, especially the *PA2G4, HMGCR*, and *XRCC6* genes.[563]

Zebrafish embryos exposed to nonembryotoxic concentrations of the biologically active phthalate metabolite mono (2-ethylhexyl) phthalate (MEHP) and the DNA methyltransferase 1 inhibitor 5-azacytidine (5AC) have revealed a multitude of differentially methylated regions, strongly enriched in conserved nongenic elements for both compounds. The pathways involved in adipogenesis were enriched with the putative obesogenic compound MEHP. Exposure to 5AC resulted in enrichment of pathways involved in embryonic development and transgenerational effects on larval body length. Locus-specific methylation analysis of 10 differentially methylated sites revealed 6 to be differentially methylated in sperm sampled from adult zebrafish exposed during development to 5AC, and in first- and second-generation larvae. With MEHP, consistent changes were found at two specific loci in first- and second-generation larvae.[564]

6.10.4 Pesticides

Exposure to certain pesticides may increase the risk for particular cancers, mediated in part through global alterations in DNA methylation. Alexander et al.[565] evaluated alterations of *LINE-1* DNA methylation by pesticides in a

variety of classes. Increased exposure to five pesticides (imazethapyr, fenthion, EPTC, butylate, and heptachlor) were associated with increasing *LINE-1* DNA methylation, and increased exposure to three pesticides (carbaryl, chlordane, and paraquat) were associated with decreasing *LINE-1* DNA methylation.

Endosulfan is an organochlorine pesticide extensively used around the world that is known for its endocrine, neuroendocrine, and reproductive toxicity. α-Endosulfan promotes the viability of MCF-7 cells, upregulates the expression of DNA methyltransferases (DNMTs), and alters global DNA methylation. Total intracellular histone deacetylase (HDAC) activity is increased, correlating with upregulation of class I HDACs (HDAC 1 and 3). The expression and activity of the arginine and lysine methylation enzymes, protein arginine methyltransferase 5 (PRMT5) and enhancer of zeste homolog 2 (EZH2), respectively, are also modulated by α-endosulfan. Ghosh et al.[566] found increased expression of histones H3 and H4, trimethylated H3K27 (product of EZH2), symmetric dimethylation of H4R3 (product of PRMT5), and five different proteins with arginine residues that are symmetrically dimethylated (by increased level of PRMT5) in response to α-endosulfan. Overexpression of the basal level of estrogen receptor alpha (ERα) suggests the estrogenicity of α-endosulfan.

High pesticide exposure is genome-wide significantly associated with differential DNA methylation of 31 CpGs annotated to 29 genes. Of the 31 CpGs 20 were found in subjects with airway obstruction. Several of the identified genes (*RYR1, ALLC, PTPRN2, LRRC3B, PAX2*, and *VTRNA2-1*) are genes linked to either pesticide exposure or lung-related diseases. Of the 31 CpGs 7 were associated with gene expression levels.[567]

Intrauterine organochlorine pesticide (OCP)-dichlorodiphenyltrichloroethane (DDT) exposure can lead to epigenetic alterations by DNA methylation with possible important lifetime health consequences for offspring. Yu et al.[568] identified 1131 different CpG sites, which included 690 hypermethylation sites and 441 hypomethylation sites, in the DNA methylation level between cases and controls. The sites identified were located in 598 unique genes. The DNA methylation levels of the identified CpGs of *BRCA1* increased with increased exposure to dichlorodiphenyltrichloroethane (DDT), and the level of gene expression in the identified CpGs of *BRCA1* decreased with increased exposure to dichlorodiphenyltrichloroethane.

DDT and other environmental toxicants can induce the epigenetic transgenerational inheritance of disease through the germline. DNA methylation and ncRNA are altered in the sperm of each generation with the direct exposure of F1 and F2 generations being predominantly distinct from F3-generation epimutations.[569]

6.10.5 Herbicides

Glyphosate is a herbicide widely used in agriculture. Glyphosate induces DNA lesions, decreases global DNA methylation, and increases *p53* promoter methylation.[570]

Atrazine is a herbicide used on agricultural crops. It frequently contaminates potable water supplies and is a suspected endocrine-disrupting chemical causing morphological, hormonal, and molecular alterations as a result of developmental and adulthood atrazine exposure.

Atrazine decreases the activity of maintenance DNMT, and embryonic atrazine exposure decreases global methylation levels and the expression of *dnmt4* and *dnmt5*.[571]

6.10.6 Fungicides

Acylamino acid chiral fungicides (AACFs) are low-toxicity pesticides with potential toxicological effects on mammals by nongenotoxic mechanisms. AACFs affect methyltransferase activity resulting in modulating DNA methylation levels. *R*-Metalaxyl, *S*-metalaxyl, (*R*,*S*)-benalaxyl, and (*R*,*S*)-furalaxyl affect methylation levels. Global methylation is more susceptible to *S* enantiomers than to *R* enantiomers. The dependence of methylation inhibition on the chiral center of metalaxyl suggests considerable specificity of the AACF compound for DNA methyltransferases.[572]

Penconazole (PEN) and tebuconazole (TEB) are fungicides widely used in vineyards. Workers exposed to these fungicides have been found to accumulate both substances in their hair.[573]

Epigenetic transgenerational inheritance of disease and phenotypic variation can be induced by the antiandrogenic fungicide vinclozolin. This phenomenon can involve DNA methylation, ncRNA and histone retention, and/or modification of the germline. These epimutations can be transmitted to progeny. Vinclozolin alters DNA methylation and ncRNA in the sperm of each generation with different epimutations in the F1–F3 generations.[574,575,576]

6.10.7 Dioxin

Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)) is a ubiquitous by-product that is associated with a spectrum of diseases. TCDD activates cytochrome (CYP) p450 metabolic enzymes that are repressed by the transcription factor, Krüppel-like factor (KLF) 11, via epigenetic mechanisms. KLF11 antagonizes TCDD-mediated activation of *CYP3A4* gene expression and function in endometrial cells. When TCDD-exposed animals are treated with a HATi, *Cyp3* mRNA levels and protein expression decrease along with disease progression.[577]

6.10.8 *N,N*-Diethyl-*m*-Toluamide (DEET) and Fluocyanobenpyrazole (Fipronil)

Mitchell et al.[578] studied the effects of DEET and fipronil on lncRNAs. The insect repellent *N,N*-diethyl-*m*-toluamide (DEET) increased the transcript levels for 2 lncRNAs and lowered them for 18 lncRNAs, and the insecticide fluocyanobenpyrazole (fipronil) increased the transcript levels for 76 lncRNAs and decreased them for 193 lncRNAs. A mixture of DEET and fipronil increased the transcript levels for 75 lncRNAs and lowered them for 258 lncRNAs, indicating an additive effect on lncRNA transcript expression when the two chemicals were presented in combination. Dysregulated lncRNAs affect the innate and adaptive immune response and the p53 signaling pathway.[578]

Fipronil is a broad-spectrum insecticide with enantioselective toxicity in embryo development. *S*-(+)-Fipronil causes severe developmental toxicity in embryos. It dysregulates a higher level of genomic DNA methylation than *R*-(−)-fipronil. *S*-(+)-Fipronil affects in developmental processes. Compared with *R*-(−)-fipronil, *S*-(+)-fipronil disrupts seven signaling pathways (mitogen-activated protein kinases, tight junctions, focal adhesion, transforming growth factor-β, vascular smooth muscle contraction, and the hedgehog and Wnt signaling pathways) by hypermethylation of developmentally related genes, which further induce the downregulation of genes. Differences in DNA methylation may partly explain the enantioselectivity of fipronil to zebrafish embryos.[579]

6.10.9 Furan

Furan, a volatile heterocyclic organic chemical found in a wide spectrum of common human foods, is a liver toxicant and carcinogen in mice and rats. The carcinogenic effects of furan have been attributed to both genotoxic and nongenotoxic mechanisms. Of the nongenotoxic alterations induced by furan the most frequent are epigenetic aberrations.[580]

6.10.10 Sarin

In a mouse model of Gulf War illness (GWI), preexposure to the stress hormone corticosterone causes an increase in expression of specific chemokines and cytokines in response to diisopropyl fluorophosphate (DFP), a sarin surrogate and irreversible AChE inhibitor. The high physical and psychological stress of combat may have increased vulnerability to irreversible acetylcholinesterase (AChE) inhibitors leading to priming of the neuroimmune system. Transcriptional, histone modification (H3K27ac), and DNA methylation changes in genes related to the immune and neuronal system, potentially relevant to neuroinflammatory and cognitive symptoms, have been found to be present in GWI. Altered myelinating oligodendrocytes are also observed in the frontal cortex of GWI sufferers.[581]

6.10.11 Perfluorooctyl Sulfonate

Perfluoroalkyl substances (PFASs) are stable and persistent in the environment and can penetrate the placenta, affecting fetal growth. Prenatal exposure to perfluorooctanoic acid (PFOA), perfluorooctyl sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluoroundecanoic acid (PFUA) affects *Alu* methylation levels.[582]

6.10.12 1-Trichloromethyl-1,2,3,4-Tetrahydro-β-Carboline

1-Trichloromethyl-1,2,3,4-tetrahydro-β-carboline (TaClo) is a neurotoxic substance with carcinogenic properties. TaClo induces global DNA hypomethylation and transcriptional repression of critical tumor suppressor genes by increasing their promoter methylation. Enhanced cell proliferation, migration, and anchorage-independent growth are observed in cells exposed to TaClo.[583]

6.10.13 Benzene and Fuel-Related Pollutants

Benzene, a known human carcinogen, and methyl tert-butyl ether (MTBE) are fuel-related pollutants. Urinary benzene (BEN-U), *S*-phenylmercapturic acid, and MTBE levels are higher in petrol station workers than in controls, while trans,trans-muconic acid (tt-MA) is comparable in the two groups. Increased BEN-U is associated with increased *Alu-Y* and *Alu-J* expression, and increased tt-MA is associated with increased *Alu-Y*, *Alu-J*, and *LINE-1* (L1) 5′-UTR expression. Among repetitive element methylation, only L1-Pa5 is hypomethylated in petrol station workers compared with controls. While *L1-Ta* and *Alu-YD6* methylation is not associated with benzene exposure, a negative association with urinary MTBE has been observed. The methylation status of histone H3K9 was not associated with either benzene or MTBE exposure.[584]

Long-term treatment with low doses of hydroquinone (HQ), a benzene metabolite, may alter the epigenetic signature underlining *LINE-1* sequences. In the HL-60 cell line the transient instauration of the distinctive signature combining the repressive H3Lys27 trimethylation mark and the activating H3Lys4 trimethylation mark (H3K27me3/H3K4me3) indicates a tendency toward poised chromatin conformation. Minimum variations in DNA methylation and expression levels of *LINE-1* have been observed, despite a decrease in the protein levels of UHRF1, DNA methyltransferases, and histone methyltransferases.[585]

6.10.14 Sulfur Mustard

The chemical warfare agent sulfur mustard (SM) can cause long-term health effects that may exhibit years after a single exposure via epigenetic mechanisms.[586]

6.10.15 Endocrine-Disrupting Compounds (EDCs)

Endocrine-disrupting compounds (EDCs) are present in residential products and have the potential to disrupt hormone signaling and bring about epigenetic aberrations.[587] Some EDCs can cause aberrant lipid homeostasis and atherosclerosis in animals. EDCs can activate the nuclear receptor pregnane X receptor (PXR), which functions as a xenobiotic sensor to regulate host xenobiotic metabolism. Exposure to many EDCs can also induce epigenetic modifications.[588]

6.10.15.1 Bisphenol A (BPA)

Bisphenol A (BPA) is an endocrine-disrupting chemical widely used in the manufacture of polycarbonate plastic and epoxy resin to produce a multitude of consumer products, food and drink containers, and medical devices.[589] In utero exposure to BPA is associated with offspring obesity. As food intake/appetite is one of the critical elements contributing to obesity Desai et al.[590] studied the effects of in vivo maternal BPA and in vitro BPA exposure on newborn hypothalamic stem cells that form the arcuate nucleus appetite center. Maternal BPA increases hypothalamic neuroprogenitor (NPC) proliferation and differentiation in newborns, in conjunction with increased neuroproliferative (Hes1) and proneurogenic (Ngn3) protein expression. With NPC differentiation, BPA exposure increases appetite peptide and reduces satiety peptide expression. In vitro BPA-treated control NPCs show a shift toward neuronal vs glial fate as well as an increase in the epigenetic regulator lysine-specific histone demethylase 1 (LSD1). These results emphasize the vulnerability of stem cell populations that are involved in lifelong regulation of metabolic homeostasis to epigenetically mediated endocrine disruption by BPA during early life.[590]

Prenatal exposure to the endocrine disrupter BPA induces behavioral and neuronal disorders as a result of epigenetic changes in the brain. However, studies with low doses of BPA showed no methylation differences in 43,840 CpG sites in hippocampal DNA.[591]

Epigenetic alterations in liver tissue from adult mice offspring following perinatal BPA exposure at human physiologically relevant doses have been demonstrated. DNA methylation status is affected at Janus kinase-2 (*Jak-2*), retinoid X receptor (*Rxr*), regulatory factor x-associated protein (*Rfxap*), and transmembrane protein 238 (*Tmem238*).[592]

MEST mediates the impact of prenatal BPA exposure on long-term body weight development in offspring by triggering adipocyte differentiation.[593]

Perinatal BPA exposure is associated with higher body fat, impaired glucose tolerance, and reduced insulin secretion in first- (F1) and second-generation (F2) C57BL/6J male mouse offspring. BPA impairs insulin secretion in male but not female F1 and F2 offspring. Increased *Igf2* expression persists in the islets of male F1 and F2 offspring and is associated with altered DNA methylation. Maternal BPA exposure has dose- and sex-specific effects on pancreatic

islets of adult F1 and F2 mouse offspring. The transmission of these changes across multiple generations may involve either mitochondrial dysfunction and/or epigenetic modifications.[594]

BPA is similar to estradiol in structure and interferes in steroid signaling with different outcomes on reproductive health depending on doses, life stage, mode, and timing of exposure. BPA exerts epigenetic effects in both male and female reproduction. In males, BPA affects spermatogenesis and sperm quality and possible transgenerational effects on the reproductive ability of the offspring. In females, BPA affects ovary, embryo development, and gamete quality for successful in vivo and in vitro fertilization.[589]

6.10.16 Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are persistent organic environmental contaminants and known endocrine-disrupting chemicals (EDCs). Developmental exposure to the weakly estrogenic PCB mixture Aroclor 1221 (A1221) in Sprague-Dawley rats alters sexual development, adult reproductive physiology, and body weight. Prenatal A1221 exposure not only disrupts these endpoints within an exposed individual's (F1 generation) lifespan, but may also affect subsequent generations (F2–F3). A1221 descendants have higher body weight in the F2-maternal lineage throughout postnatal development, and in F3-maternal lineage animals after weaning. In females, generation- and lineage-specific effects of exposure are found for serum progesterone and estradiol. Reproductive and adrenal organ weights, birth outcomes, sex ratio, and estrous cycles are unaffected.[595]

6.10.17 Polybrominated Diphenyl Ethers

Polybrominated diphenyl ethers (PBDEs) are a group of ubiquitous reproductive toxins. PBDEs affect sperm DNA methylation. Perinatal exposure to 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) increases DNA methylation of epididymal sperm in genes, promoters, and intergenic regions for long periods of time.[596]

Polybrominated diphenyl ether-153 (BDE-153) induces neuronal apoptosis in rat cerebral cortex and primary neurons. Neurotrophins and cholinergic enzymes play critical roles in neuronal survival, maintenance, synaptic plasticity, and learning memory. Neuronal apoptosis induced by BDE-153 is dependent on p53 and on more calpain-2 than caspase-3 in the cerebral cortex of rats. Following BDE-153 treatment the protein contents and mRNA levels of *BDNF*, *GDNF*, *NGF*, *NT-3*, and *NT-4*, as well as AchE and ChaT activities are decreased in the cerebral cortex and primary neurons. When primary neurons are pretreated with the calpain inhibitor PD150606 or cyclin-dependent kinase (cdk5) inhibitor roscovitine, the neurotrophin contents and activities of ChaT and AchE are reversed, along with an improvement in neuron survival compared with BDE-153 treatment alone. Neurotrophins and cholinergic enzymes are regulated by calpain-2 activation and its downstream cdk5 pathway, which are affected by BDE-153 neurotoxicity.[597]

DE-71 is a commercial mixture of polybrominated diphenyl ethers widely used in flame retardants and has pervasive environmental contaminant effects. DE-71 exposure induces gene body-specific hypomethylation within the *Tbx3* locus, a transcription factor important in liver tumorigenesis and in embryonic and cancer stem cell proliferation. This nonpromoter hypomethylation is accompanied by upregulation of *Tbx3* mRNA and protein and by alterations in downstream cell cycle-associated marker expression. Exposure to DE-71 may facilitate tumor development by inducing epigenetic programs that favor the expansion of progenitor cell populations.[598]

6.10.18 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) comprise an important class of environmental pollutants that cause lung cancer in animals and are suspected lung carcinogens in humans. Fish et al.[599] assessed the patterns of genome-wide DNA methylation in lung tissues of adult offspring initiated in utero with the transplacental PAH carcinogens dibenzo[*def*,*p*]chrysene (DBC) or benzo[*a*]pyrene (BaP). Lung tumor incidence in 45-week-old mice initiated with BaP was 32%, much lower than that of DBC-exposed offspring at 96%. Male offspring is more susceptible to BaP than female. Distinct patterns of DNA methylation have been associated with exposure to PAHs.

Prenatal exposure to polycyclic aromatic hydrocarbons (PAHs) is a potential risk factor for adverse birth outcomes. Prenatal urinary 2-hydroxynaphthalene (2-OHNa) ($\sum$OHNa (1- and 2-OHNa)) and monohydroxy-PAH ($\sum$OH-PAHs) are associated with lower birth length, and prenatal urinary 2-OHNa and 1-hydroxyphenanthrene (1-OHPh) are associated with lower *Alu* and *LINE-1* methylation.[600]

6.10.19 Organophosphate (OP) Flame Retardants

Exposure to organophosphate (OP) flame retardants can alter DNA methylation in human sperm cells and thus affect offspring health. Soubry et al.[601] studied the sperm, urine, and urinary metabolites of a chlorinated OP (tris (1,3-dichloro-2-propyl) phosphate) and two nonchlorinated OPs (triphenyl phosphate and mono-isopropylphenyl diphenyl phosphate). They also studied sperm DNA methylation at multiple CpG sites of the regulatory differentially methylated regions (DMRs) of imprinted genes *GRB10, H19, IGF2, MEG3, NDN, NNAT, PEG1/MEST, PEG3, PLAGL1, SNRPN*, and *SGCE/PEG10*. Men with higher concentrations of urinary OP metabolites, known to originate from flame retardants, have a slightly higher fraction of sperm cells that are aberrantly methylated. Exposure to mono-isopropylphenyl diphenyl phosphate has been related to hypermethylation at *MEG3, NDN*, and *SNRPN* DMRs. Exposure to triphenyl phosphate was associated with hypermethylation at the *GRB10* DMR, and tris(1,3-dichloro-2-propyl) phosphate exposure was associated with altered methylation at *MEG3* and *H19* DMRs.

6.10.20 1,3-Butadiene

Baseline variability in chromatin organization and transcription profiles among various tissues and mouse strains influences the outcome of exposure to the DNA-damaging chemical 1,3-butadiene.[602]

6.10.21 Vinyl Chloride Monomer

Eight miRNAs are downregulated and seven miRNAs are upregulated in Chinese people highly exposed to vinyl chloride monomer (VCM). *miR-222-3p, miR-146a-5p*, and *miR-151a-5p* are downregulated, while *miR-22-3p* is upregulated in VCM-exposed subjects. The expression of miR-22-3p is upregulated in subjects with a high frequency of micronuclei.[603]

6.10.22 Crude Oil

Exposure to crude oil in *C. elegans* has been shown to demonstrate transgenerational toxicity and associated epigenetic changes. Reproductive function is reduced or inhibited, and defective reproduction is transgenerationally inherited. Decreased methylation of histone H3 (*H3K9*) is found in the parental generation exposed to crude oil of *C. elegans*. A heritable reduction in reproductive capacity occurs in wild-type N2 but not in a H3K9 histone methyltransferase (HMT) mutant [met-2(n4256)], suggesting a potential role for HMT in transgenerational toxicity.[604]

6.10.23 Asbestos

There is a robust association between exposure to asbestos and human lung cancer. *BEND4, ZSCAN31*, and *GPR135* are hypermethylated in lung cancer. DMRs in genes, such as *RARB, GPR135*, and *TPO*, and DVMCs in *NPTN, NRG2, GLT25D2*, and *TRPC3* are associated with asbestos exposure status in exposed vs nonexposed lung tumors. Hypomethylation is characteristic of DVMCs in lung cancer tissue from asbestos-exposed subjects.[605]

6.10.24 Tobacco and Cigarette Smoking

Tobacco smoke is a well-established lung cancer carcinogen. Five CpG sites are highly associated with pack-years of cigarette smoking. Smoking was negatively associated with methylation levels in *cg25771041 (WWTR1), cg16200496 (NFIX), cg22515201 (PLA2G6),* and *cg24823993 (NHP2L1)* and positively associated with the methylation level in *cg11875268 (SMUG1)*. The CpG-smoking association was stronger in lung squamous cell (LUSC) tissues than lung adenocarcinoma (LUAD). Of the five loci, smoking explained the greatest variation in methylation levels in *cg16200496*.[606]

Studies investigating the effects of cigarette smoking on sperm DNA methylation showed changes in 11 CpGs between cases and controls. Five of the eleven CpGs (*cg00648582*, *cg0932376*, *cg19169023*, *cg23841288*, and *cg27391564*) underwent deep bisulphite sequencing, in which *cg00648582* related to the *PGAM5* gene and *cg23841288* related to *PTPRN2* gene amplicons, and showed a significant increase in their DNA methylation level in more than one CpG in smokers. Hypomethylation was found at *cg19169023* and at CpGs in *TYRO3* gene-related amplicons.[607] Two CpGs (*cg07869343* and *cg19169023*) located in the *MAPK8IP3* and *TKR* genes are particularly

important. Significant differences in *MAPK8IP* (CpG3, CpG5, CpG6, CpG7, CpG8, and CpG21) and in *TKR* (CpG4) have been identified.[608]

Maternal smoking in pregnancy (MSP) has been associated with DNA methylation in specific CpG sites in infants and children. Over 70 CpGs are differentially methylated by MSP, with multiple CpGs mapping to *CYP1A1, MYO1G, AHRR,* and *GFI1*. MSP influences offspring DNA methylation in midlife and adulthood.[609] Intrauterine exposure to maternal smoking is linked to impaired executive function and behavioral problems in offspring. Maternal smoking is associated with reduced fetal brain growth and smaller volume of cortical gray matter in childhood, indicating that prenatal exposure to tobacco may impact cortical development and manifest as behavioral problems. Maternal smoking during pregnancy affects the global DNA methylation profiles of the developing dorsolateral prefrontal cortex (DLPFC) during the second trimester of gestation. Most differentially methylated regions (DMRs) are hypomethylated CpG Islands within the promoter regions of *GNA15* and *SDHAP3* of smoking-exposed fetuses. Developmental upregulation of *SDHAP3* mRNA is delayed in smoking-exposed fetuses. The DMRs identified affect *SYCE3*, *C21orf56/LSS*, *SPAG1*, and *RNU12/POLDIP3*.[610]

Huang et al.[611] conducted a joint metabolomic-epigenomic study to identify the patterns of epigenetic associations with smoking-related metabolites. It has been observed that in the 12 annotated smoking-related metabolites identified from a metabolome-wide association study, hypomethylation was associated with increased levels of *N*-acetylpyrrolidine, cotinine, 5-hydroxycotinine, and nicotine and hypermethylation was associated with an increased level of 8-oxoguanine.

Epigenetically regulated *NLRP10* affects Th17/IL-17 signaling during CS exposure. The *Nlrp10* promoter following cigarette smoke exposure suffers changes in active and repressive gene markers on histone 3 and histone 4. Alterations in the respective histone acetyltransferases and methyltransferases (PCAF, SET1, ESET, SUV20H1) correlate with histone modifications.[612]

Impaired placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) inactivates maternal glucocorticoids and is associated with poor fetal growth and a higher risk of chronic diseases in adulthood. Zhou et al.[613] studied the epigenetically regulatory mechanisms of nicotine on placental *11β-HSD2* expression. Prenatal nicotine exposure increases corticosterone levels in the placenta and fetal serum, disrupts placental morphology and endocrine function, reduces fetal body weight, and induces histone modification abnormalities (decreased acetylation and increased dimethylation of histone 3 Lysine 9) on the *HSD11B2* promoter, lowering the expression of 11β-HSD2. The expression of nicotinic acetylcholine receptor (*nAChR*) α4/β2, phosphorylation of extracellular regulated kinase 1/2 (*ERK1/2*) and Ets-like protein-1 (*Elk-1*), and expression of early growth response-1 (*Egr-1*) are increased by nicotine. In human BeWo cells, nicotine decreases *11β-HSD2* expression, increases *nAChRα9* expression, and activates ERK1/2/Elk-1/Egr-1 signaling. The antagonism of nAChRs, inhibition of ERK1/2, and *Egr-1* knockdown by siRNA are able to abrogate the effects of nicotine on histone modification and expression of 11β-HSD2.

Smoking is associated with peripheral blood DNA methylation. A total of 39 CpGs located at 27 loci, including *AHRR, F2RL3,* 2q37.1, and 6p21.33, are differentially methylated in smokers. Current smokers had the lowest methylation level. Two CpG sites, *cg06226150* (*SLC2A4RG*) and *cg21733098* (12q24.32), are particularly relevant.[614] Current smoking, cumulative smoking exposure (pack-years), and serum cotinine levels are strongly associated with 8-isoprostane (8-iso) levels. Of 151 smoking-related CpG sites, 71 loci are associated with 8-iso levels. Smoking-related epigenetic alterations are closely related to smoking-induced oxidative stress.[615] Alterations in DNA methylation and gene expression in blood leukocytes are potential biomarkers of harm and mediators of the deleterious effects of tobacco exposure. Urine cotinine levels are associated with methylation of 176 CpGs. Urine cotinine levels are also associated with the expression of 12 genes, including increased expression of *P2RY6*, a gene involved in the release of proinflammatory cytokines.[616]

Prenatal exposure to maternal cigarette smoking can result in postnatal global and gene-specific DNA methylation changes, with effects on the *CYP1A1* and *AHRR* genes involved in the detoxification of xenobiotic substances. For *AHRR*, maternal smoking has been associated with increased DNA methylation in the placentas of female fetuses in which mRNA expression was increased. For *CYP1A1*, maternal smoking was not associated with fetal DNA methylation changes, whereas mRNA expression increased in placentas and male fetal livers. First-trimester exposure to maternal smoking is associated with *CYP1A1* and *AHRR* DNA methylation and mRNA expression changes. Maternal smoking during pregnancy-mediated postnatal *CYP1A1* and *AHRR* DNA methylation changes are not imprinted during the first trimester.[617]

Prenatal smoke exposure (PSE) induces fetal programming of *Igf1r* and *Igf1*. PSE alters the promoter methylation of *Igf1r* and *Igf1* and deregulates their gene expression in lung and liver of fetal (E17.5) and neonatal (D3) mouse offspring. CpG site-specific aberrant methylation patterns are sex-, organ-, and time-dependent.[618]

Vaz et al.[619] defined how chronic cigarette smoke-induced time-dependent epigenetic alterations can sensitize human bronchial epithelial cells to transformation by a single oncogene. Smoke-induced chromatin changes include

initial repressive polycomb marking of genes, later manifesting abnormal DNA methylation by 10 months, when cells exhibit epithelial-mesenchymal changes, anchorage-independent growth, and upregulated RAS/MAPK signaling with silencing of hypermethylated genes, which normally inhibit these pathways and are associated with smoking-related nonsmall-cell lung cancer. These cells, in the absence of any driver gene mutations, now transform by introducing a single *KRAS* mutation and form adenosquamous lung carcinomas in mice.

Smoking tobacco is a known risk factor for the development of colorectal cancer and for mortality associated with the disease. Smoking is associated with changes in DNA methylation in blood and in lung tumor tissues. A total of 15 CpG sites within the *APC 1A* promoter are hypermethylated, and 14 CpG loci within the *NFATC1* gene body are hypomethylated in tumors of active smokers. The *APC 1A* promoter is hypermethylated in 7 of 36 tumors from never-smokers (19%), 12 of 47 tumors from former smokers (26%), and 8 of 13 tumors from active smokers (62%). Promoter hypermethylation is positively associated with duration of smoking and is confined to tumors, with hypermethylation never being observed in adjacent mucosa. The analysis of adjacent mucosa shows hypomethylation of four loci associated with the *TNXB* gene in tissue from active smokers. Hypermethylation of the key tumor suppressor gene *APC* is implicated in smoking-associated colorectal carcinogenesis.[620]

Studies investigating water pipe condensate-mediated and cigarette smoke condensate-mediated, dose-dependent growth-inhibitory effects measured in cultured respiratory epithelial cells have revealed that water pipe condensates and cigarette smoke condensates decrease histone H4 lysine 15 acetylation (H4K16ac) and histone H4 lysine 20 trimethylation (H4K20me3) levels in small-airway epithelial cells and human bronchial epithelial cells. A total of 873 genes are commonly affected by water pipe condensates and cigarette smoke condensates in small-airway epithelial cells, whereas a total of 1577 genes are commonly affected by water pipe condensates and cigarette smoke condensates in human bronchial epithelial cells. Of the 100 genes commonly affected by water/cigarette smoke condensates, the top three activated upstream regulators are cigarette smoke, benzo[*a*]pyrene, and nuclear factor (erythroid-derived 2)-like 2 (*NFE2L2, Nrf2*). The top three canonical pathways included xenobiotic metabolism signaling, aryl hydrocarbon receptor signaling, and nicotine degradation III.[621]

6.10.25 Alcohol

Parental chronic preconception alcohol consumption may have consequences for offspring health and development. The effects of paternal environmental exposure can be transmitted to the next generation via alterations to small ncRNAs in sperm. Chronic intermittent ethanol exposure alters several small ncRNAs from three of the major small RNA classes in sperm: tRNA-derived small RNA (tDR), mitochondrial small RNA, and miRNA. Chronic ethanol exposure affects posttranscriptional modifications to sperm small ncRNAs, increasing two nucleoside modifications in mitochondrial tRNA, and reduces epididymal expression of a tRNA methyltransferase (Nsun2) known to directly regulate tDR biogenesis.[622]

Alcohol consumption during pregnancy is associated with adverse outcomes in offspring, potentially mediated by epigenetic modifications. However, some studies have found no association between maternal alcohol consumption and offspring cord blood DNA methylation.[623]

There are strong associations between D2 receptor binding potential and neural responses to rewarding stimuli and substance use. Epigenetic alterations in the promoter region of the dopamine D2 receptor (*DRD2*) gene may be associated with cue-elicited activation of neural reward regions, as well as the severity of alcohol use behavior. *DRD2* promoter methylation is positively associated with responses to alcohol cues in the right accumbens, left putamen, right putamen, left caudate, and right caudate, suggesting that *DRD2* methylation is positively associated with robust activation in the striatum in response to reward cues.[624]

Hill et al.[625] hypothesized that the cross-generational effects of alcohol exposure could alter DNA methylation and expression of the *HRAS* oncogene and *TP53* tumor suppressor gene that drive cancer development. Controlling for both personal use and maternal use of substances during pregnancy, familial alcohol dependence has been associated with hypomethylation of CpG sites in the *HRAS* promoter region and hypermethylation of the *TP53* gene.

Alcohol-naive offspring of rats exposed to alcohol during adolescence exhibit altered gene expression profiles in the hypothalamus. Offspring of alcohol-exposed parents exhibit differential DNA methylation patterns in the hypothalamus. Differentially methylated cytosines (DMCs) in offspring are distinct depending on which parent was exposed to ethanol. Adolescent binge ethanol exposure causes altered genome-wide DNA methylation patterns in the hypothalamus of alcohol-naive offspring.[626]

Chronic alcohol exposure leads to behavioral changes and decreased expression of genes associated with synaptic plasticity. Chronic alcohol exposure impairs methionine synthase (Ms) activity leading to a decrease in the

S-adenosylmethionine/*S*-adenosylhomocysteine (SAM/SAH) ratio, which results in DNA hypomethylation. Chronic EtOH exposure decreases SAM levels, the SAM/SAH ratio, Ms, methylene tetrahydrofolate reductase, and betaine homocysteine methyltransferase (Bhmt) expression and increases methionine adenosyltransferase-2b (Mat2b) but not Mat2a expression in the liver. In contrast, chronic EtOH exposure decreases SAH levels, increases the SAM/SAH ratio and the expression of Mat2a and *S*-adenosylhomocysteine hydrolase, while the levels of SAM or Bhmt expression in cerebellum remain unaltered. In both liver and cerebellum, chronic EtOH exposure decreases the expression of Ms and increases Mat2b expression. All chronic EtOH-induced changes of 1-carbon metabolism in cerebellum, but not liver, return to near-normal levels during EtOH withdrawal.[627]

Alcohol affects two epigenetic phenomena, DNA methylation and DNA hydroxymethylation, and iron is a cofactor of ten-eleven translocation (TET) enzymes that catalyze the conversion from methylcytosine to hydroxymethylcytosine. Iron supplementation increases hepatic nonheme iron contents, and both alcohol and iron increase hepatic ferritin levels and decrease hepatic transferrin receptor levels. Alcohol reduces hepatic DNA hydroxymethylation, while iron supplementation does not change DNA hydroxymethylation. Unmodified cytosine levels are increased by alcohol, suggesting that alcohol increases the conversion from hydroxymethylcytosine to unmodified cytosine. Chronic alcohol consumption alters global DNA hydroxymethylation in the liver, but iron supplementation reverses the epigenetic effect of alcohol.[628]

6.10.26 Ochratoxin A

Ochratoxin A (OTA) is a fungal metabolite that induces cytotoxicity and apoptosis through the mechanism of oxidative stress. OTA-induced cytotoxicity and apoptosis is represented by decreased cell viability, increased LDH release, annexin V/PI staining, the *Bcl-2/Bax* mRNA ratio, and apoptotic nuclei in PK15 cells. OTA treatment upregulates ROS production, downregulates GSH levels, and activates DNA methyltransferase 1 (DNMT1) and histone deacetylase 1 (HDAC1). The DNMT1 inhibitor 5-Aza-2dc or the HDAC1 inhibitor LBH589 depress the upregulation of DNMT1 or HDAC1 expression, decrease GSH levels, and increase ROS production induced by OTA.[629]

6.10.27 Air Pollution and Particulate Matter

Long-term exposure to air pollution has been associated with several adverse health effects including cardiovascular diseases, respiratory diseases, and cancers. Long-term air pollution exposure levels, including NO_2, NOx, PM2.5, PMcoarse, PM10, and PM2.5 absorbance (soot), have been estimated. Exposure to NO_2 was associated with significant global somatic hypomethylation. Hypomethylation of CpG island shores and shelves and gene bodies has been found to be significantly associated with higher exposures to NO_2 and NOx.[630]

Traffic-related air pollution (TRAP) exposure is associated with changes in 5mC in *LINE-1*, *iNOS*, *p16CDKN2A*, and *APC*, together with H3K9ac. Season and/or sex may interact with air pollutants in affecting DNA methylation and H3K9ac.[631]

Particulate matter (PM < 2.5 μm) exposure during development is strongly associated with adverse cardiovascular outcomes in adulthood. In utero PM2.5 exposure can alter cardiac structure and function in adulthood.[632] Exposure to ambient particulate matter is associated with global DNA methylation and gene-specific methylation. Studies on PM2.5 species (Al, Ca, Cu, Fe, K, Na, Ni, S, Si, V, and Zn) and DNA methylation at 484,613 CpG probes in a longitudinal cohort of 646 subjects showed 20 CpGs for Fe, 8 for Ni, and 1 for V. Methylation at Schlafen Family Member 11 (*SLFN11) cg10911913* was positively associated with measured levels of all three species. The *SLFN11* gene codes for an interferon-induced protein that inhibits retroviruses and sensitizes cancer cells to DNA-damaging agents. Long-term exposure to specific components of ambient particle pollution, especially particles emitted during oil combustion, have been associated with methylation changes in genes relevant to immune responses.[633]

Genetic variation in miRNA-processing genes can modify the association of PM2.5 with DNA methylation age. Having at least one copy of the minor *rs4961280-AGO2* allele has been associated with a lower DNA methylation age. This association is weaker in homozygous carriers of the major *rs4961280-AGO2* allele. miRNA processing impacts DNA methylation-age relationships.[634]

Emissions from diesel vehicles and biomass burning are the principal sources of primary ultrafine particles (UFPs). Exposure to UFPs has been associated with cardiovascular and pulmonary diseases, including lung cancer. Diesel UFP exposure induces the secretion of biomarkers associated with inflammation (CCXL2, EPGN, GREM1, IL1A, IL1B, IL6, IL24, EREG, VEGF) and transcription factors (such as NFE2L2, MAFF, HES1, FOSL1, TGIF1) relevant for

cardiovascular and lung disease. Four genes (*STAT3, HIF1a, NFKB1, KRAS*) are major regulators of the transcriptional response of bronchial epithelial cells exposed to diesel exhaust.[635]

6.10.28 Ionizing Radiation

Studies of brain-fractionated exposure to low doses of ionizing radiation (FELDIR) have revealed increased levels of DNA damage, as reflected by increased occurrence of DNA strand breaks (SBs) and dysregulation of stress-response kinase p38. FELDIR also resulted in initial loss of global genomic methylation and altered expression of methyltransferases DNMT1 (downregulation) and DNMT3a (upregulation), as well as methyl-binding protein MeCP2 (upregulation).[636]

6.10.29 Laser Irradiation

Laser irradiation in plants induces heritable DNA methylation changes. Methylation changes and their heritability of the metastable epigenetic state can be verified by bisulfite sequencing of a portion of the retrotransposon *Tos17*, an established locus for assessing DNA methylation liability in rice. Various methylation-related chromatin genes are perturbed, especially two *AGOs* (*AGO4-1* and *AGO4-2*), and excisions of a *MITE* transposon (*mPing*) occur in laser-irradiated plants and their progenies. Studies have indicated that heritable DNA methylation changes can be induced by low-dose laser irradiation, accompanied by transpositional activation of transposable elements.[637]

6.10.30 Low-Frequency Magnetic Fields

Exposure to extremely low-frequency magnetic fields (ELF-MFs) has been associated with increased risk for neurodegenerative diseases. ELF-MFs induce an early reduction in the expression levels of *miR-34b* and *miR-34c* in SH-SY5Y human neuroblastoma cells and in mouse primary cortical neurons, by affecting transcription of the common *pri-miR-34*. This effect is attributed to hypermethylation of CpG island mapping within the *miR-34b/c* promoter. ELF-MFs alter the expression of α-synuclein, which is specifically stimulated upon ELF-MF exposure via both direct miR-34 targeting and oxidative stress.[638]

6.11 NUTRIEPIGENETICS

Nutrition plays a significant role in regulating the epigenome, and epigenetics may explain the transgenerational effects of nutrition.[639] Nutrients are crucial in the regulation of epigenetic modifiers. Many nutrients and their metabolites function as substrates or cofactors for epigenetic modifiers, and nutrition can modulate or reverse epigenetic marks in the genome as well as expression patterns.[640]

Dietary bioactive compounds from various sources, including green tea, soya, fruit and berries, cruciferous vegetables, whole grain foods, and fish, have been shown to target the enzymes involved in epigenetic gene regulation, including DNA methyltransferases, histone acetyltransferases, deacetylases, demethylases, and miRNAs. Gut microbiota and gut microbial metabolites might be important mediators of diet-epigenome interactions. Interindividual differences in the gut microbiome might affect the release, metabolism, and bioavailability of dietary agents and explain variability in response to dietary interventions.

Some microbial metabolites (folate, phenolic acids, *S*-(−)-equol, urolithins, isothiocyanates, short chain and long chain fatty acids) may affect diverse mechanisms in nutriepigenetics.[641]

The substrates used to modify nucleic acids and chromatin are affected by nutrient availability and the activity of metabolic pathways. Cellular metabolism constitutes a fundamental component of chromatin status and thereby genome regulation.[642] Metabolic states influence differentiation programs in different cell types. Metabolites, such as *S*-adenosylmethionine, acetyl-CoA, α-ketoglutarate, 2-hydroxyglutarate, and butyrate, have been shown to be donors, substrates, cofactors, and antagonists for the activities of epigenetic-modifying complexes and for epigenetic modifications. Nutrients processed through pathways, such as glycolysis, glutaminolysis, and 1-carbon metabolism, regulate metabolite levels to influence epigenetic events.[643] Transsulfuration is important in the conversion of methionine into cysteine using homocysteine (Hcy) as an intermediate. About 50% of the cysteine needed for hepatic glutathione synthesis is produced through this pathway, which produces *S*-adenosylmethionine (AdoMet).

Transsulfuration is interconnected with epigenetics, adenosine triphosphate (ATP), and glutathione synthesis, the polyol and pentose phosphate pathways, and detoxification.[644]

6.11.1 Maternal Diet

Many examples in recent years have illustrated the potential of maternal dietary supplementation as an epigenetic modifier protecting embryonic development. Maternal heat stress may induce epigenetic aberrations that result in the abnormal development of offspring embryos. Maternal environmental hyperthermia alters embryonic development via changes in the epigenetic status (global DNA hypomethylation and histone 3 lysine 9 hypoacetylation in the embryonic heart). Maternal dietary manganese supplementation increases the expression of heart antiapoptotic gene B cell CLL/lymphoma 2 under maternal hyperthermia and manganese SOD enzyme activity in the embryonic heart. Maternal dietary organic Mn supplementation can neutralize the consequences of maternal environmental hyperthermia on embryonic development, upregulating manganese *SOD* mRNA expression, reducing DNA methylation, and increasing histone 3 lysine 9 acetylation.[645]

Breastfeeding provides health benefits to infants and mothers. Epigenetic mechanisms are potential mediators of the effects of early-life exposures on later health outcomes. Breastfeeding might be negatively associated with promoter methylation of *LEP*, *CDKN2A*, and *SLC2A4* genes and positively with promoter methylation of the *NPY* gene.[646]

Maternal diets alter the transcriptome of fetal tissues. Maternal diet from mid to late gestation can shape the epigenome and transcriptome of fetal tissues.[647]

Fibroblast growth factor-21 gene (*FGF21*) undergoes peroxisome proliferator-activated receptor (PPAR) α-dependent DNA demethylation in the liver during the postnatal period. Reductions in *FGF21* methylation can be enhanced via pharmacologic activation of PPARα during the suckling period. The *FGF21* DNA methylation status once established in early life is relatively stable and persistent in adulthood. Reduced DNA methylation is associated with enhanced induction of hepatic *FGF21* expression after PPARα activation, which may contribute to the attenuation of diet-induced obesity in adulthood. *FGF21* methylation might be a form of epigenetic memory that influences the development of obesity programming.[648]

6.11.1.1 Body Weight and Lipid Metabolism

Nutrition affects the global DNA methylation status throughout the lifespan. Low-calorie diets appear to affect the epigenetic status of offspring more strongly if administered during the maternal pregestational period than the gestational and lactation period.[649]

Maternal and paternal periconceptional nutrition affects the likelihood of offspring developing chronic metabolic-related conditions as a result of epigenetic imprinting. Newborns from obese fathers have been shown to demonstrate altered methylation overall and hypomethylation at the *IGF2* gene. High maternal prepregnancy body mass index (BMI) is associated with altered DNA methylation levels of offspring, and gestational diabetes mellitus induces significantly increased methylation levels in offspring. Birth weight has been found to be higher in offspring exposed to famine in early gestation. Offspring born postmaternal bariatric surgery show a lower percentage of body fat and improved fasting insulin levels than siblings born prematernal bariatric surgery.[650] The available evidence suggests that poor maternal and paternal periconceptional nutrition can increase the risk of metabolic syndrome in offspring through epigenetic imprinting.

Subjects born with a low birth weight (LBW) display a more energy-conserving response to fasting than normal birth weight (NBW) subjects. Studies investigating the effects of short-term fasting on leptin (*LEP*) and adiponectin (*ADIPOQ*) DNA methylation and gene expression in subcutaneous adipose tissue from subjects with LBW and NBW have shown that fasting induces changes in DNA methylation in *LEP* and *ADIPOQ* promoters in NBW but not in LBW subjects.[651]

Overnutrition can alter gene expression patterns through epigenetic mechanisms that may persist through generations, and a high-fat diet alters the epigenetics and transcriptional activity of key hepatic genes controlling lipid homeostasis, contributing to the pathophysiology of obesity.[652] Postweaning diets may modify the epigenetic landscape to meet metabolic demands later in life. Compared with a lifelong high-fat diet, offspring exposed to a new postweaning control diet are able to remodel the hepatic epigenome. In this process 3,966 differentially methylated regions have been identified; 37% were mapped to gene bodies while 6% fell within promoter or downstream regions. Differentially methylated genes were clustered in the type II diabetes mellitus and the adipocytokine-signaling pathways.[653]

Dietary restriction (DR) increases most aspects of health during aging and extends lifespan in diverse species. Profiling genome-wide changes in DNA methylation, gene expression, and lipidomics in response to DR and aging in

female mouse liver shows that DR is strongly protective against age-related changes in DNA methylation. During aging with DR, DNA methylation becomes targeted to gene bodies and is associated with reduced gene expression, particularly of genes involved in lipid metabolism. The lipid profile of the livers of DR mice is correspondingly shifted toward lowered triglyceride content and shorter chain length of triglyceride-associated fatty acids, and these effects become more pronounced with age. DR remodels genome-wide patterns of DNA methylation so that age-related changes are profoundly delayed, while changes at loci involved in lipid metabolism affect gene expression and the resulting lipid profile.[654]

Keleher et al.[655] investigated maternal obesity in inbred SM/J mice by assigning females to a high-fat diet or a low-fat diet at weaning, mating them with low-fat-fed males, cross-fostering the offspring to low-fat-fed SM/J nurses at birth, and weaning the offspring onto a high-fat or low-fat diet. A maternal high-fat diet exacerbates obesity in high-fat-fed daughters, with higher serum levels of leptin as adults, accompanied by changes in gene expression and DNA methylation in their livers and hearts. Maternal diets affect genes involved in RNA processing, immune response, and mitochondria. Differentially expressed genes contain a differentially methylated region associated with maternal diets. Offspring high-fat diets reduce overall variation in DNA methylation, increase body weight and organ weight, increase long bone length and weight, decrease insulin sensitivity, and change the expression of 3908 genes in the liver. Maternal diets have epigenetic effects lasting through adulthood.[655]

The micronutrient status of parents can affect the long-term health of their progeny. The study of morphological, molecular, and epigenetic changes in mature offspring of parents that experienced a 1-carbon (1-C) micronutrient deficiency revealed that parental 1-C micronutrient deficiency results in increased lipid inclusion with 686 differentially expressed (downregulated) genes in offspring liver. Differential DNA methylation has been found at 2869 CpG sites, was enriched in promoter regions, and permutation analyses confirmed the association with parental feed.[656]

6.11.1.2 Brain Maturation and Function

The metabolic requirements of differentiated neurons differ from those of neuronal precursor and neural stem cells. Micronutrients (MNs) influence neonatal brain development, particularly neural migration and survival, neurite outgrowth, and process maturation. Metabolic reprogramming influences neuronal differentiation, and micronutrient signaling may be key to regulating these processes.[657]

There are concerns about the potential adverse intergenerational effects of excess fructose intake. Excess maternal fructose intake affects hippocampal function in offspring. Offspring from fructose-fed dams exhibit decreased brain-derived neurotrophic factor (*BDNF*) gene expression and DNA hypermethylation at the *BDNF* promoter. Increased methylation of the *BDNF* promoter region is maintained at least until rats reached maturity. Epigenetic changes associated with BDNF may underlie hippocampal dysfunction that is induced by early-life exposure to excess maternal fructose consumption.[658]

Nutrition during the prenatal and postnatal period of life (i.e., the first 1000 days of life, depending on the health lifestyle of mother and offspring) are relevant for the prevention of neurodegeneration later in life.[659]

Early exposure to nutrient and/or hormonal challenges can reprogram metabolism at adulthood. The hypothalamic arcuate nucleus (ARC) integrates peripheral and central signals to adequately regulate energy homeostasis. miRNAs participate in the control of gene expression of large regulatory networks including many signaling pathways involved in epigenetic regulations. Benoit et al.[660] identified over 400 miRNA species in the ARC of adult male rats, and 10 miRNAs specified by clusters *miR-96/182/183*, *miR-141/200c*, and *miR-200a/200b/429* as miRNAs of systematic and uncommonly high variation of expression. This uncommon variation of expression may underlie high individual differences in aging disease susceptibilities. The expression of 11 miRNAs was repeatedly impacted by a perinatal unbalanced environment.[660]

6.11.2 Enteral Feeding

Enteral feeding affects the intestinal epigenome and gene expression after preterm birth. Diet-dependent changes in DNA methylation and/or mRNA expression are related to innate immune response, hypoxia, angiogenesis, and the epithelial-mesenchymal transition pathways (TTC38, IL8, C3, HIF1A, VEGFR1).[661]

6.11.3 Vitamin B

Studies on the effects of vitamin B12 and/or folic acid supplementation on genome-wide DNA methylation revealed that vitamin B12 supplementation induces methylation changes (589 differentially methylated CpGs and

2892 regions) and B12 + folic acid supplementation also induce methylation changes (169 differentially methylated CpGs and 3241 regions). Methylation influences miR21 expression. *FTO, TCF7L2, CREBBP/CBP*, and *SIRT1* are targets of *miR21-3p*.[662]

6.11.4 Vitamin C

Vitamin C is essential to the functioning of epigenetic regulators that initiate the demethylation of DNA and histones. Vitamin C at physiological concentrations, combined with hypomethylating agents, may act synergistically to cause DNA demethylation through active and passive mechanisms, respectively.[663] Vitamin C is a dietary requirement for humans as an antioxidant and a cofactor for Fe^{2+}- and α-ketoglutarate-dependent dioxygenases (Fe^{2+}/α-KGDDs) which comprise a large number of diverse enzymes, including collagen prolyl hydroxylases and epigenetic regulators of histone and DNA methylation. Vitamin C can modulate embryonic stem cell (ESC) function, enhance the reprogramming of fibroblasts to induce pluripotent stem cells (iPSCs), and hinder the aberrant self-renewal of hematopoietic stem cells (HSCs) through its ability to enhance the activity of either Jumonji C (JmjC) domain-containing histone demethylases or ten-eleven translocation (TET) DNA hydroxylases.[664] Some vitamins intensify the erasure of epigenetic memory in naive embryonic stem cells. These effects are driven by complementary enhancement of TET demethylases. For instance, vitamin A stimulates *TET* expression, whereas vitamin C potentiates TET catalytic activity. Vitamin A and C cosupplementation synergistically enhances the reprogramming of differentiated cells to the naive state, but overuse may exaggerate the instability of imprinted genes.[665] Vitamin C enhances the activity of TET enzymes in embryonic stem (ES) cells, leading to DNA demethylation and activation of germline genes. Vitamin C induces a remarkably specific demethylation of histone H3 lysine 9 (H3K9me2) in naive ES cells. Vitamin C treatment reduces global levels of H3K9me2, but not other histone methylation marks. Vitamin C leads to widespread loss of H3K9me2 at large chromosomal domains as well as gene promoters and repeat elements. Vitamin C-induced loss of H3K9me2 occurs rapidly within 24 h and is reversible. Histone demethylases Kdm3a and Kdm3b are required for vitamin C-induced demethylation of H3K9me2. Vitamin C-induced Kdm3a/b-mediated H3K9me2 demethylation and TET-mediated DNA demethylation are independent processes at specific loci.[666]

It has been postulated that blueberries (*Vaccinium* spp.) would act via antioxidative and epigenetic modulation similarly to vitamin C. Urinary 8-OHdG levels are reduced by blueberry consumption. *MTHFR* methylation is decreased in blueberry consumers. There is a positive correlation between changes in urinary 8-OHdG and DNA methylation at *MTHFR* or *DNMT1*. According to these results reported by Kim et al.[667] blueberry juice shows similar antioxidative or antipremutagenic activity to vitamin C and has potential as a methylation inhibitor for *MTHFR* and *DNMT1* in humans.

Epigenetic memory (DNA methylation) is established during development in differentiating cells and must be erased to create naive pluripotent stem cells. TET enzymes can catalyze the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and further oxidize derivatives, thereby actively removing this memory. Retinoic acid (RA) or retinol (vitamin A) and ascorbate (vitamin C) act as modulators of TET levels and activity. RA or retinol enhances 5hmC production in naive embryonic stem cells by activating TET2 and TET3 transcription, whereas ascorbate potentiates TET activity and 5hmC production through enhanced Fe^{2+} recycling, and not as a cofactor. Both ascorbate and RA or retinol promote the derivation of induced pluripotent stem cells synergistically and enhance the erasure of epigenetic memory.[668]

6.11.5 Vitamin D

Vitamin D is an essential nutrient affecting brain, bone, heart, immune, and reproductive health. Maternal vitamin D deficiency leads to altered body weight and DNA methylation in two generations of offspring. Loci assayed in adult liver and sperm are mostly hypomethylated. There is no change in the total expression of genes adjacent to methylation changes in neonatal liver. Methylation changes are cell type-specific such that changes at the IG-DMR are present in sperm but not in liver. Some methylation changes are distinct between generations. Methylation changes at the H19ICR in second-generation liver are not present in first-generation sperm or liver. Some diet-dependent changes in body weight and methylation are seemingly influenced by parent of origin such that reciprocal crosses exhibit inverse effects.

The maternal vitamin D status plays a role in determining the DNA methylation state in the germline and soma. Detection of methylation changes in an unexposed second generation demonstrates that maternal vitamin D depletion can have long-term effects on the epigenome of subsequent generations. Differences in the vitamin D-dependent epigenetic state between cell types and generations indicate perturbation of the epigenetic landscape rather than a targeted, locus-specific effect.[669]

Vitamin D regulates mineral homeostasis through its activities in the intestine, kidney, and bone. Terminal activation of vitamin D3 to its hormonal form, 1α,25-dihydroxyvitamin D3 (1,25(OH)2D3), occurs in the kidney via the cytochrome P450 enzyme CYP27B1. Meyer et al.[670] identified a kidney-specific control module governed by a renal cell-specific chromatin structure located distal to *Cyp27b1* that mediates unique basal and parathyroid hormone (PTH)-, fibroblast growth factor 23 (FGF23)-, and 1,25(OH)2D3-mediated regulation of *Cyp27b1* expression. Selective genomic deletion of key components within this module in mice resulted in loss of either PTH induction or FGF23 and 1,25(OH)2D3 suppression of *Cyp27b1* gene expression. *Cyp27b1* is also expressed at low levels in nonrenal cells, in which transcription is modulated exclusively by inflammatory factors. Differential regulation of Cyp27b1 expression represents a mechanism whereby 1,25(OH)2D3 can fulfill separate functional roles, first in the kidney to control mineral homeostasis and second in extrarenal cells to regulate target genes linked to specific biological responses.[670]

High-serum 25-hydroxyvitamin D (25(OH)D) (>38.0 ng mL^{-1}) is inversely associated with breast cancer. SNPs in vitamin D-related genes (*CYP24A1*, *CYP27B1*, *CYP2R1*, *GC*, *DHCR7/NADSYN1*, *RXRA*, and *VDR*) may modify the association between serum 25(OH)D and breast cancer.[671]

Vitamin D may affect maternal and infant DNA methylation. Studies investigating the effects of vitamin D3 supplementation on DNA methylation in pregnant and lactating women and their breastfed infants have revealed that at birth intervention group mothers showed DNA methylation gain and loss at 76 and 89 cytosine-guanine (CpG) dinucleotides, respectively, compared with controls. Postpartum, methylation gain was noted at 200 CpGs and loss at 102 CpGs. Associated gene clusters showed the strongest biologic relevance for cell migration/motility and cellular membrane function at birth and cadherin signaling and immune function at postpartum. Breastfed 4- to 6-week-old infants of intervention mothers showed DNA methylation gain and loss in 217 and 213 CpGs, respectively, compared with controls. Genes showing differential methylation mapped most strongly to collagen metabolic processes and the regulation of apoptosis.[672]

6.11.6 Folic Acid

Folic acid is an essential component of 1-carbon metabolism, which generates methyl groups for DNA methylation. The disruption of genomic imprinting leads to biallelic expression which may affect disease susceptibility possibly reflected in high levels of *S*-adenosylhomocysteine (SAH) and low levels of *S*-adenosylmethionine (SAM). Tserga et al.[673] investigated the association between folic acid supplementation during pregnancy and loss of imprinting (LOI) of *IGF2* and *H19* genes in placentas and cord blood of 90 mother-child dyads in association with the methylenetetrahydrofolate reductase (*MTHFR*) genotype. The authors detected relaxation of imprinting (ROI) and LOI of *H19* in placentas not associated with differences in the methylation levels of the *H19ICR*. Placentas retained monoallelic allele-specific gene expression of IGF2, but 32.4% of cord blood samples displayed LOI of IGF2 and 10.8% showed ROI. High SAH levels were associated with low *H19* methylation. A positive association between the SAM/SAH ratio and high *H19* methylation levels has been detected among infants with low B12 levels.

Obesity and maternal folate deficiency are associated with increased risk for neural tube defects (NTDs). In women (obese vs normal) treated with folic acid (800 μg day^{-1}) for 8 weeks, Park et al.[674] studied serum folate concentration and changes in DNA methylation across 2098 CpG sites in 91 genes related to NTD risk and folate metabolism. The methylation of 56 and 99 CpG sites changed in response to supplementation, and most of these sites decreased in methylation. Gene ontology analysis revealed a response to supplementation in 61 biological processes from the selected genes. Changes in DNA methylation in genes related to NTD risk and folate metabolism in response to folic acid supplementation are different in women with normal weight vs obese women. Increased NTD risk and abnormal folate metabolism in obesity may be due to a distinctive epigenetic response to folate status in these genes.

Folate deficiency is implicated in the onset of insulin resistance by altering epigenetic processes on key regulatory genes. The calcium/calmodulin-dependent protein kinase kinase 2 (CAMKK2) is involved in the regulation of critical metabolic processes such as adiposity and glucose homeostasis. A total of 51 cytosine-phosphate-guanine sites have been associated with folate intake, including one located in the 5′-untranslated region of the *CAMKK2* gene. Subjects with total folate intake lower than 300 μg day^{-1} show more fat mass and higher levels of glucose, insulin, the homeostatic model assessment-insulin resistance (HOMA-IR) index, cortisol, and plasminogen activator inhibitor-1 than those consuming more than 300 μg day^{-1}. Folate deficiency is associated with lower *CAMKK2* methylation, and *CAMKK2* methylation negatively correlates with the HOMA-IR index.[675]

Male mice exposed throughout their lifetimes to both FD and FS diets have been shown to have decreased sperm counts and altered imprinted gene methylation with evidence of transmission of adverse effects to the offspring, including increased postnatal-preweaning mortality and variability in imprinted gene methylation.[676]

6.11.7 Methionine

Methionine restriction (MR) has been studied extensively for its role in altering metabolic hallmarks of disease. MR induces changes in metabolic flexibility with increases in energy expenditure, glucose tolerance, and lifespan. Hepatic fibroblast growth factor 21 links MR to several components of its metabolic phenotype. MR is associated with stress, metabolism, and lifespan extension with the involvement of novel epigenetic pathways and miRNA regulation.[677] Studies on genome-wide changes in H3K4me3 and gene expression in response to alterations in methionine availability in both normal mouse physiology and human cancer cells indicate that the location of H3K4me3 peaks is largely preserved under methionine restriction, while the response of H3K4me3 peak width encodes almost all aspects of H3K4me3 biology including changes in expression levels, and the presence of cell identity and cancer-associated genes.[678]

6.11.8 Niacin

Nicotinic acid and nicotinamide, collectively referred to as niacin, are nutritional precursors of the bioactive molecules nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). NAD and NADP are important cofactors for most cellular redox reactions and for maintaining cellular metabolism and respiration. NAD also serves as a cosubstrate for a large number of ADP-ribosylation enzymes with varied functions. Among the NAD-consuming enzymes identified to date are important genetic and epigenetic regulators such as poly(ADP-ribose)polymerases and sirtuins. There is a close connection between dietary niacin intake, NAD(P) availability, and the activity of NAD(P)-dependent epigenetic regulator enzymes. Dietary niacin intake is a central regulator of physiological processes, including maintenance of genetic stability, and of epigenetic control mechanisms modulating metabolism and aging.[679]

6.11.9 Antioxidants

Exposure to antioxidants and xenobiotics triggers the expression of a myriad of genes encoding antioxidant proteins, detoxifying enzymes, and xenobiotic transporters to offer protection against oxidative stress. This mechanism is regulated through cis-acting elements in an array of *Nrf2* target genes called antioxidant response elements (AREs), which play a critical role in redox homeostasis. Although the Keap1/Nrf2/ARE system involves many players, AREs hold the key to transcriptional regulation of cytoprotective genes.[680]

The epigenetic regulator SET domain-containing lysine methyltransferase 7 (Setd7) regulates the antioxidant Nrf2 pathway in prostate cancer (PCa) cells. Lentivirus-mediated shRNA knockdown of *Setd7* in LNCaP and PC-3 cells decreases the expression of downstream Nrf2 targets, such as NAD(P)H:quinone oxidoreductase 1 (Nqo1) and glutathione *S*-transferase theta 2 (Gstt2). Downregulation of Setd7 decreases the soft-agar colony formation ability of PCa cells. Knockdown of *Setd7* increases reactive oxygen species (ROS) generation. *Setd7* knockdown attenuates *Nqo1* and *Gstt2* expression in response to H_2O_2 challenge, whereas increased DNA damage is observed in *Setd7* knockdown cells. *Setd7* expression can be induced by the dietary phytochemicals phenethyl isothiocyanate (PEITC) and ursolic acid (UA). *Setd7* knockdown decreases H3K4me1 enrichment in the Nrf2 and Gstt2 promoter regions, while PEITC and UA treatments elevate the enrichment.[681]

6.11.10 Manganese

Manganese is an essential nutrient that may play a role in the production of inflammatory biomarkers. Kresovich et al.[682] studied the associations between estimated dietary manganese intake from food/beverages and supplements with circulating biomarkers of inflammation. Concentrations of IL-1β (46%), IL-6 (52%), and IL-8 (32%) were found to be increased by manganese. Estimated dietary manganese intake was additionally associated with changes in the DNA methylation of inflammatory biomarker-producing genes. Higher estimated intake was associated with higher methylation of NF-κβ member activator *NKAP* and *NKAPP1*. Estimated dietary intake of manganese at levels slightly above nutritional adequacy contributes to inflammatory biomarker production.

6.11.11 Zinc

The role of maternal dietary zinc supplementation in protecting the embryo from maternal hyperthermia-induced negative effects via epigenetic mechanisms was studied by Zhu et al.[645] using an avian model (*Gallus gallus*). Maternal

hyperthermia increases embryonic mortality and induces oxidative damage evidenced by the elevated mRNA expressions of heat shock protein genes. Maternal dietary zinc deficiency damages the embryonic development associated with global DNA hypomethylation and histone 3 lysine 9 hyperacetylation in the embryonic liver. Supplementation of zinc in maternal diets effectively eliminates embryonic mortality induced by maternal hyperthermia and enhances antioxidant ability with increased mRNA and protein expressions of metallothionein IV in the embryonic liver. Increased metallothionein IV mRNA expression is due to the reduced DNA methylation and increased histone 3 lysine 9 acetylation of the metallothionein IV promoter regardless of zinc source.

6.11.12 Omega-3 Polyunsaturated Fatty Acids

Omega-3 polyunsaturated fatty acids (n-3 FAs) have been found to have benefits for several health conditions. After supplementation with *n*-3 FAs obese subjects exhibit 308 CpG sites, assigned to 231 genes, that are differentially methylated. Of these pathways, 16 are related to inflammatory and immune response, lipid metabolism, type 2 diabetes, and cardiovascular signaling. Changes in the methylation levels of CpG sites within *AKT3*, *ATF1*, *HDAC4*, and *IGFBP5* correlate with changes in plasma triglyceride and glucose levels as well as with changes in the ratio of total cholesterol/HDL-cholesterol following supplementation.[683]

Diets that are high in saturated fatty acids (SFAs) or polyunsaturated fatty acids (PUFAs) have different metabolic responses. The epigenome of human adipose tissue is affected differently by dietary fat composition. SFA and PUFA diets increase the mean degree of DNA methylation in adipose tissue, particularly in promoter regions. Although the mean methylation is changed in 1797 genes (alpha-ketoglutarate-dependent dioxygenase (*FTO*), interleukin 6 (*IL-6*), insulin receptor (*INSR*), neuronal growth regulator 1 (*NEGR1*), and proopiomelanocortin (*POMC*)) by PUFAs, only 125 genes (adiponectin, *C1Q*, and collagen domain containing (*ADIPOQ*)) are changed by SFA overfeeding. SFA diets alter the expression of 28 transcripts (acyl-CoA oxidase 1 (*ACOX1*) and FAT atypical cadherin 1 (*FAT1*)), whereas PUFA diets do not affect gene expression. The mean methylation of 1444 genes, including fatty acid binding protein 1 (*FABP1*), fatty acid binding protein 2 (*FABP2*), melanocortin 2 receptor (*MC2R*), MC3R, PPARG coactivator 1α (*PPARGC1A*), and tumor necrosis factor (*TNF*), is changed in adipose tissue by overfeeding. The baseline DNA methylation of 12 CpG sites annotated to 9 genes (mitogen-activated protein kinase 7 (*MAPK7*), melanin concentrating hormone receptor 1 (*MCHR1*), and splicing factor SWAP homolog (*SFRS8*)) is associated with the degree of weight increase in response to extra energy intake.[684]

A large double-blind randomized placebo-controlled trial with docosahexaenoic acid (DHA) in pregnant mothers revealed minimum changes in global DNA methylation in their children at birth and at 5 years.[685] Prenatal and postnatal dietary omega-3 fatty acids alter white blood cell (leukocyte) DNA methylation of pig offspring. The methylation-enriched profile maps to 26% of the porcine genome. On chromosome 4 a 27.7-kb differentially methylated region downstream of *RUNX1T1* is hypomethylated, and hypermethylation is detected in intergenic regions of chromosomes 4 and 12. Some of these changes have been proposed to be regulated by methylation as a result of feeding eicosapentaenoic acid (EPA) and DHA during pregnancy.[686]

6.11.13 Hybrid Palm Oil

Hybrid palm oil contains higher levels of oleic acid and lower levels of saturated fatty acids than African palm oil and has been proposed to be equivalent to extra virgin olive oil. However, the biological effects of its consumption are poorly described. Dietary supplementation of marmosets with a hyperlipidic diet containing hybrid palm oil for 3 months does not modify plasma lipids levels, but does increase glucose levels, liver volume, liver fibrosis, hepatic total lipid content, and circulating transaminases. Liver miRNAs targeting genes involved in cell adhesion molecules and peroxisomal pathways are also affected by hybrid palm oil.[687]

6.11.14 Buttermilk and Krill Oil

The combination of two phospholipidic concentrates of krill oil (KOC) and buttermilk (BMFC) modulates the hippocampal expression of 119 miRNAs. *miR-191a-5p* and *miR-29a-3p* change in response to BMFC, whereas *miR-195-3p* and *miR-148a-5p* respond to a combination of both products. BMFC + KOC also induce a reduction in hippocampal ceramide levels.[688]

6.11.15 Olive Oil

Corominas-Faja et al.[689] studied the phenolic components of extra virgin olive oil (EVOO) that have been shown to be capable of suppressing the functional traits of cancer stem cells (CSCs) in breast cancer (BC). The secoiridoid decarboxymethyl oleuropein aglycone (DOA) can selectively target subpopulations of epithelial-like aldehyde dehydrogenase (ALDH)-positive and mesenchymal-like $CD44^+CD24^-$/low CSCs. DOA can potently block the formation of multicellular tumorspheres generated from single-founder stem-like cells. Pretreatment of BC populations with noncytotoxic doses of DOA dramatically reduces subsequent tumor-forming capacity in vivo. Mice orthotopically injected with CSC-enriched BC cell populations pretreated with DOA remain tumor free for several months. DOA binds and inhibits the ATP-binding kinase domain site of mTOR and the *S*-adenosyl-L-methionine (SAM) cofactor-binding pocket of DNMTs. DOA can act as an ATP-competitive mTOR inhibitor and as a blocker of SAM-dependent methylation activity of DNMTs.

6.11.16 Hydroxytyrosol

Hydroxytyrosol (HT) is the primary phenolic compound of olives, virgin olive oil, and their by-products. HT supplementation differentially affects the adipose and liver tissue proteome. Some oxidative stress-related proteins are modulated in both tissues, such as the multifunctional protein peroxiredoxin 1, which is consistently repressed by HT supplementation.[690]

6.11.17 Polyphenols

Diets rich in catechol-dominant polyphenols are reported to suppress enzyme activity and activate epigenetically silenced genes. Several dietary nutrients play a crucial role in 1-carbon metabolism including folate, cobalamin, riboflavin, pyridoxine, and methionine by directly affecting *S*-adenosyl-L-methionine. Soy polyphenols block DNA methyltransferases and histone deacetylases to reverse aberrant CpG island methylation. Organosulfur-rich compounds may help to normalize DNA methylation and activate *miR-140* expression, which represses *SOX9* and *ALDH1* and decreases tumor growth. Dietary components, such as nutritional polyphenols and flavonoids, are epigenetic mediators that influence epigenetic marks and control gene expression.[691]

A novel treatment with a select bioactive polyphenol preparation promotes resilience to stress-mediated depression/anxiety phenotypes in mice. Polyphenol treatment from both susceptible and naive donors alters global DNA methylation in the central nervous system and periphery and likewise has an effect on human blood cells after immune challenge. Select bioactive polyphenols may promote resilience to stress.[692]

Tea polyphenols are secondary metabolites of tea plants. Tea polyphenols can prevent cancer by modulating epigenetic aberrations taking place in DNA methylation, histone modifications, and miRNAs. By altering these epimutations they regulate chromatin dynamics and the expression of genes that induce or suppress cancer formation.[693]

Green tea extract (GTE) intake during lactation affects obesity-related fibrosis and inflammation in the kidney of high-fat-diet-fed adult offspring of protein-restricted-diet-fed dams during pregnancy and lactation. GTE intake during lactation attenuates tubulointerstitial fibrosis and macrophage infiltration by downregulating epigenetic modulators such as DNMT1, UHRF1, and G9a in the kidney of HF-diet-fed adult offspring programmed by maternal protein restriction.[694]

6.11.18 *Brassica* Derivatives

High consumption of vegetables belonging to the Brassicaceae family has been related to lower incidence of chronic diseases. These beneficial effects of broccoli, cabbage, or rocket (arugula) intake mainly have been attributed to sulfur-containing glucosinolates (GLSs), secondary plant compounds almost exclusively present in Brassicaceae, and in particular to their bioactive breakdown products including isothiocyanates (ITCs). Selected *Brassica*-derived ITCs exhibit health-promoting effects, including anticarcinogenic and antiinflammatory properties, by activating the redox-sensitive transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) that controls the expression of antioxidant and phase II enzymes.[695]

6.11.19 Coffee

Epigenome-wide DNA methylation levels are modified by coffee consumption. One CpG (*cg21566642* near *ALPPL2*) shows significant changes, and of the 10 additional CpGs affected 6 are located within 1500 bp of a transcriptional start site. The methylation levels of another 135 CpGs are also influenced by both coffee drinking and smoking. Coffee-associated CpGs are located near transcription factor-binding and protein kinase activity genes.[696]

6.11.20 Sucrose

The intake of high sucrose (HS) during pregnancy can change the vascular reactivity and dipsogenic behavior closely associated with an abnormal renin-angiotensin system (RAS) and thus increase the risk for hypertension in adult offspring. Maternal HS intake in pregnancy may further deteriorate Ang II-induced cardiovascular responses in aged offspring. Maternal HS intake during gestation increases basal blood pressure levels that respond to losartan treatment. Ang II levels are increased in blood vessels and there are enhanced AT1R-mediated vasoconstrictions in the aorta and mesenteric arteries of aged offspring. mRNA and protein expressions of the *AT1R* gene are also increased in both large and small blood vessels in association with potential histone modifications. Maternal HS intake during gestation may cause RAS-AT1R-related hypertension in aged offspring.[697]

6.11.21 Fruit vs Juice

Despite the general consensus that fruit and juice are nutritionally similar, epigenetic studies suggest that fruit- and juice-induced epigenetic changes are different. Fruit- and juice-specific epigenetic signatures are largely independent. Genes near the fruit-specific epigenetic signature are enriched among pathways associated with antigen presentation and chromosome or telomere maintenance, while the juice-specific epigenetic signature is enriched for proinflammatory pathways. Fruit- and juice-specific epigenetic signatures modulate macrophage (fruit) and B or T cell (juice) activities. These data suggest that these nutrients may not confer the same health benefits.[698]

6.11.22 Mediterranean Diet

The Mediterranean diet (MD) is a dietary pattern associated with various health benefits including protection against cardiovascular disease, diabetes, obesity, and various cancers.[699] Gonzalez-Nahm et al.[700] studied the association between adherence to a Mediterranean diet and infant methylation at the *MEG3*, *MEG3-IG*, pleiomorphic adenoma gene-like 1, insulin-like growth factor 2 gene, H19, mesoderm-specific transcript, neuronatin, paternally expressed gene 3, sarcoglycan, and paternally expressed gene 10 regions, and found that infants of mothers with low adherence to a Mediterranean diet had greater odds of hypomethylation at the *MEG3-IG* differentially methylated region.

6.11.23 Western-Style Dyslipidemic Diet

Monocytes and the recruitment of monocyte-derived macrophages to sites of inflammation play a key role in atherogenesis and other chronic inflammatory diseases linked to cardiometabolic syndrome and obesity. Metabolic stress promotes monocyte priming, such as enhanced adhesion and accelerated chemotaxis of monocytes in response to chemokines. Metabolic stress-induced monocyte dysfunction is in part caused by the *S*-glutathionylation, inactivation, and subsequent degradation of mitogen-activated protein kinase phosphatase 1. A Western-style dyslipidemic diet (DD), composed of high levels of saturated fat, cholesterol, and simple sugars, affects monocyte function in nonhuman primates (NHPs). Similar to the case with mice a DD enhances monocyte chemotaxis in NHPs parallel to the onset of hypercholesterolemia but prior to changes in triglycerides, blood glucose, monocytosis, or changes in monocyte subset composition. There is also a transitory decrease in the acetylation of histone H3 at the lysine residues 18 and 23 in metabolically primed monocytes, and monocyte priming correlates with the acetylation of histone H3 at lysine 27. According to Short et al.[701] the histone modifications accompanying monocyte priming in primates suggest a reprogramming of the epigenetic landscape, which may lead to dysregulated responses and functionalities in macrophages derived from primed monocytes that are recruited to sites of inflammation.[701]

6.11.24 Dietary Fiber

Higher intakes of dietary fiber (nondigestible carbohydrates (NDCs)) and the fermentation product butyrate are protective against colorectal cancer and may exert their prophylactic effects via modulation of the Wnt pathway. Dietary fermentable fiber generates short chain fatty acids (SCFAs) (butyrate) in the colonic lumen, which serves as a chemoprotective histone deacetylase inhibitor and/or as an acetylation substrate for histone acetylases. *n*-3 Polyunsaturated fatty acids (*n*-3 PUFA) in fish oil can affect the chromatin landscape by acting as ligands for tumor suppressor nuclear receptors. Combinatorial diets (fish oil + pectin) affect transcriptional profiles in the intestinal epithelium, upregulating lipid catabolism and beta-oxidation-associated genes. The chemoprotective fish oil + pectin combination diet uniquely induces global histone state modifications linked to the expression of chemoprotective genes.[702]

Dietary intake of fruit and vegetables is associated with lower incidence of hypertension, and a high-fiber diet and supplementation with the short chain fatty acid acetate may affect gut microbiota and cardiovascular function. High consumption of fiber modifies gut microbiota populations and increases the abundance of acetate-producing bacteria independently of mineralocorticoid excess. Both fiber and acetate decrease gut dysbiosis and increase the prevalence of *Bacteroides acidifaciens*. Both a high-fiber diet and acetate supplementation reduce systolic and diastolic blood pressures, cardiac fibrosis, and left ventricular hypertrophy. Transcriptome analyses show that the protective effects of high fiber and acetate are accompanied by downregulation of cardiac and renal Egr1, a master cardiovascular regulator involved in cardiac hypertrophy, cardiorenal fibrosis, and inflammation.[703]

6.11.25 Astragalus Polysaccharides

Paternal dietary astragalus polysaccharide (APS) supplementation can induce a transgenerational endotoxin tolerance-like effect in jejunum mucosa of broiler chickens. Nutriepigenetic modifications are crucial for this transgenerational effect. APS can induce a transgenerational endotoxin tolerance-like effect by activating the IFNα/SOCS1 pathway in chicks. In both jejunum and sperm the promoter methylation level of *SOCS1* is reduced, and paternal APS affects histone modification in the promoter region of *TRIF*.[704]

6.11.26 Epigenetic Nutraceuticals

Dietary compounds with properties that can alter epigenetic processes are gaining popularity as targets for cancer prevention studies. Soy-derived genistein (GEN) has been extensively studied for its role as an epigenetic modifier, especially as a DNA methyltransferase (DNMT) inhibitor; and the cruciferous-derived sulforaphane (SFN) is known to be a histone deacetylase (HDAC) inhibitor. Both compounds in conjunction have been tested in breast cancer prevention or therapy. These compounds display synergistic effects in decreasing cellular viability in breast cancer cell lines. Their combination is more effective than when given in single doses, acting as an HDAC and histone methyltransferase (HMT) inhibitor by increasing the rate of apoptosis and lowering the colony-forming potential of oncocells. This combination inhibits cell cycle progression to the G2 phase in MDA-MB-231 and the G1 phase in MCF-7 breast cancer cell lines, downregulates the levels of *HDAC2* and *HDAC3* both at the mRNA and protein level, and downregulates *KLF4* and *hTERT* levels.[705]

A series of novel epigenetic nutraceuticals have been developed by means of nondenaturing biotechnological processes from marine, vegetable, and animal sources.[706–711] These bioactive compounds target metabolic, immune, oncogenic, and neuronal effectors for the prevention and treatment of cardiovascular disorders and related risk factors (hypercholesterolemia, dyslipidemia), immune deficiency, cancer and neurodegenerative disorders.[706–711]

6.11.27 Age-Related Changes

Epigenetic biomarkers of age may predict all-cause mortality, chronic conditions, and age-related functional decline. Extrinsic epigenetic age acceleration (EEAA) exhibits associations with fish intake, moderate alcohol consumption, education, BMI, and blood carotenoid levels, whereas intrinsic epigenetic age acceleration (IEAA) is associated with poultry intake and BMI (see Chapter 5).[712]

6.11.28 Caloric Restriction

Caloric restriction (CR), defined as decreased nutrient intake without causing malnutrition, has been documented to increase both health and lifespan across numerous organisms, including humans. Many drugs and other compounds naturally occurring in our diet (nutraceuticals) have been postulated to act as mimetics of caloric restriction, leading to a wave of research investigating the efficacy of these compounds in preventing age-related diseases and promoting healthier, longer lifespans.[713]

Calorie restriction increases neurogenesis, improves memory function, and protects from age-associated neurological disorders. Epigenetic mechanisms, including DNA methylation, are vital to normal central nervous system cellular and memory functions and are dysregulated with aging. The beneficial effects of CR have been proposed to work through epigenetic processes. Lifelong CR prevents age-related hippocampal DNA methylation changes. Over 27 million CG and CH (non-CG) sites have been examined, and of the ~40,000 CG and ~80,000 CH sites differentially methylated with aging, over one-third were prevented by CR and were found across genomic regulatory regions and gene pathways. CR also caused alterations to CG and CH methylation at sites not differentially methylated with aging, and these CR-specific changes demonstrated a different pattern of regulatory element and gene pathway enrichment than those affected by aging. CR-specific DNA methyltransferase 1 and TET methylcytosine dioxygenase 3 promoter hypermethylation corresponded to reduced gene expression. CR attenuates age-related CG and CH hippocampal methylation changes, and in combination with CR-specific methylation may also contribute to the neuroprotective effects of CR.[714]

6.11.29 Chemopreventive Nutriepigenetics

Chemoprevention using dietary phytochemicals such as triterpenoids, isothiocyanates, and curcumin in the prevention of initiation and/or progression of cancer is gaining adepts. However, the extrapolation of preclinical data to humans is a risky venture.[715] The most suitable strategy for chemopreventive nutriepigenetics at the present time is the use of nutraceuticals specifically designed and clinically tested for defined pathologies.[706–711,716]

6.12 CONCLUSIONS

Many interesting epigenetic drugs have been developed during the past decade. The major targets for drug development are DNA methyltransferases, DNA demethylases, histone deacetylases, histone acetyltransferases, histone methyltransferases, histone demethylases, chromatin-associated proteins, bromodomains, chromodomains, and other components of the epigenetic machinery (Table 6.1). The most relevant epigenetic drugs developed so far belong to the categories of DNA methyltransferase inhibitors, histone deacetylase inhibitors, histone acetyltransferase inhibitors, histone methyltransferase inhibitors, histone demethylase inhibitors, ATP-dependent chromatin remodelers, polycomb repressive complex inhibitors, bromodomain inhibitors, and chromodomain inhibitors (Tables 6.4 and 6.10). The pharmacoepigenetic processors of epigenetic drugs are by-products of genes associated with the pathogenic, mechanistic, metabolic, transporter, and pleiotropic mechanisms responsible for drug efficacy and safety. All these genes encoding pharmacogenetic effectors are under the strict control of the epigenetic machinery for their efficient expression in an age-, sex-, and tissue-specific manner. Unfortunately, this is still a very primitive field, and fewer than 10% of epigenetic drugs have been sufficiently studied to understand their pharmacoepigenetic fate. In general, the pharmacoepigenetic machinery is promiscuous and redundant as a multilevel security system for drug biotransformation, metabolism, and elimination.[6,8,16,561] Most FDA-approved epigenetic drugs address neoplastic processes; they are not devoid of severe side effects, and drug-drug interactions in dual or multimodal therapeutic strategies may increase the risk for adverse drug events. Furthermore, the chemical structure of many epigenetic drugs does not allow satisfactory penetration in specific tissues, such as the central nervous system, as a result of difficulties in crossing the blood-brain barrier.[6,7]

Genomic defects in the pharmacoepigenetic apparatus, specific features of epigenetic drugs, and/or pathogenic complexities underpin the common phenomenon of drug resistance in which epigenetic aberrations are currently involved.

Since epigenetics is a universal language of communication between the environment and the genome, it is reasonable to assume that many environmental toxicants activate epigenetic pathways, which in part explains what the attractive field of toxicoepigenetics has revealed in recent studies. Likewise, nutriepigenetics is opening new avenues to understanding the positive or negative effects that nutritional factors may exert on health and disease, respectively.

Epigenetic drugs are opening up new possibilities for treatment of complex disorders and cases of drug resistance, despite their adverse drug reactions. Novel epigentics modifiers, with a wider therapeutic window and devoid of side effects are necessary in precision medicine. Finally, pharmacoepigenetics will help us to optimize therapeutic resources in terms of drug efficacy and safety.

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CHAPTER

7

Pharmacoepigenetics of Novel Nucleoside DNA Methyltransferase Inhibitors

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7.1 INTRODUCTION

DNA methylation induces heritable repression of gene expression and has a number of functions in the cell including gene regulation, control of cellular differentiation and development, preservation of chromosomal integrity, parental imprinting, and X-chromosome inactivation.[1] While DNA methylation dysregulation has been most thoroughly studied in cancer, it affects many other disease states.

Erroneous methylation of the promoters of tumor suppressor genes is one of three general mechanisms depleting gene products in cancer cells, the other two being mutational inactivation or loss of the gene by macrodeletions or microdeletions.[1] DNA methylation is highly dysregulated in cancer, with hypomethylation of distal regulatory regions and repetitive elements and hypermethylation of CpG (cytidine-phosphate-guanine dinucleotide) islands in promoter regions.[2] DNA methyltranferases (DNMTs) are a family of enzymes, the most prevalent of which is DNMT1, with DNMT3a and DNMT3b being barely detectable, while there are other minor forms, including DNMT2 and DNMT1b.[3] DNMT1 and DNMT3b overexpression can lead to inactivation of gene expression that suppresses tumorigenesis. The genes involved include[1] tumor suppressor genes,[1] genes that suppress apoptosis, metastasis, and angiogenesis,[2] genes that repair DNA, and[3] genes that express tumor-associated antigens. The molecular mechanism of gene expression silencing appears to be the result of the attachment of 5-methylcytosine-binding proteins to a methylated promoter, which blocks the action of transcription factors.[2, 4]

Since this epigenetic change is reversible it presents an interesting target for chemotherapeutic intervention. Both 5-azacytidine (5-azaC) and decitabine are approved in the United States for the treatment of myelodysplastic syndrome (MDS).[5, 6] More recently, decitabine has been approved in the European Union for the treatment of acute myeloid leukemia (AML).[7] These nucleosides were first synthesized in 1964.[8, 9] The lack of approved novel molecules in the interim attests to the difficulty in designing safe and effective molecules of this class. Both drugs produce remissions or clinical improvements in about half of patients treated for MDS. Responses include the requirement for multiple cycles of therapy and actual clonal elimination. Therapy optimization includes reducing the dose to favor hypomethylation over cytotoxicity and prolonging administration schedules. Molecularly, hypomethylation and gene reactivation have been shown and seem to be required for responses. Although the therapy is effective, with complete responses lasting months to years in some instances, for unknown reasons resistance seems to develop in the majority of patients.[10] Small retrospective series have looked at whether decitabine could provide salvage therapy after treatment failure of hematologic malignancies with 5-azaC and found little to no therapeutic benefit.[11, 12]

Data using currently approved drugs suggest that myeloid malignancies are the neoplasms most sensitive to DNMT inhibition; however, there is no known reason why solid tumors should not respond too.[13] A recent metaanalysis of decitabine and 5-azaC for the treatment of leukemia and MDS in elderly patients and in those who were not eligible for allogeneic stem cell transplantation demonstrated that DNMT inhibitors were superior to conventional care regimens. The overall survival rate was 33.2% vs 21.4% for DNMT inhibitors and conventional care, respectively (relative risk (RR) = 0.83, 95% confidence interval (CI): 0.71–0.98), and the overall response rate was 23.7% vs 13.4% (RR = 0.87, 95% CI: 0.81–0.93).[14] The benefit of DNMT inhibitors in solid tumors is not as well established, and in a

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recent metaanalysis of clinical trials in such patients DNMT inhibitors were shown to be able to improve clinical outcome, although overall response was limited.[15] One approach that has shown some promise is that of "episensitization," whereby patients with tumors that have become unresponsive to chemotherapy are treated with a DNMT inhibitor to effect acquired resistance.[16]

With the exception of some of the prodrugs discussed below, all therapeutic nucleosides need to penetrate cells through a transporter system, and subsequently be phosphorylated by kinases before incorporation into newly synthesized DNA and acting as a DNMT inhibitor. Fig. 7.1 provides the structures of the nucleosides discussed in this chapter, while Fig. 7.2 provides the structures of the prodrugs. Currently, the most effective DNMT inhibitors are those cytidines with a 5-azacytosine base. Decitabine has been found to be far more effective both in its DNA methylation inhibition and in its ability to reactivate methylation-silenced genes in cancer cells than nonnucleoside DNMT inhibitors.[17] Furthermore, when decitabine was compared with 5-fluoro-2′-deoxycytidine (FdCyd) in the human breast cancer cell line MDA-MB-231, it took up to 4 weeks of treatment with FdCyd for the expression of six of the eight originally silenced tumor suppressor genes, whereas these same six genes were expressed when the cells were treated with decitabine for 3 days.[18] When 5-aza-4′-thio-2′-deoxycytidine (aza-T-dCyd) and 4′-thio-2′-deoxycytidine (T-dCyd)

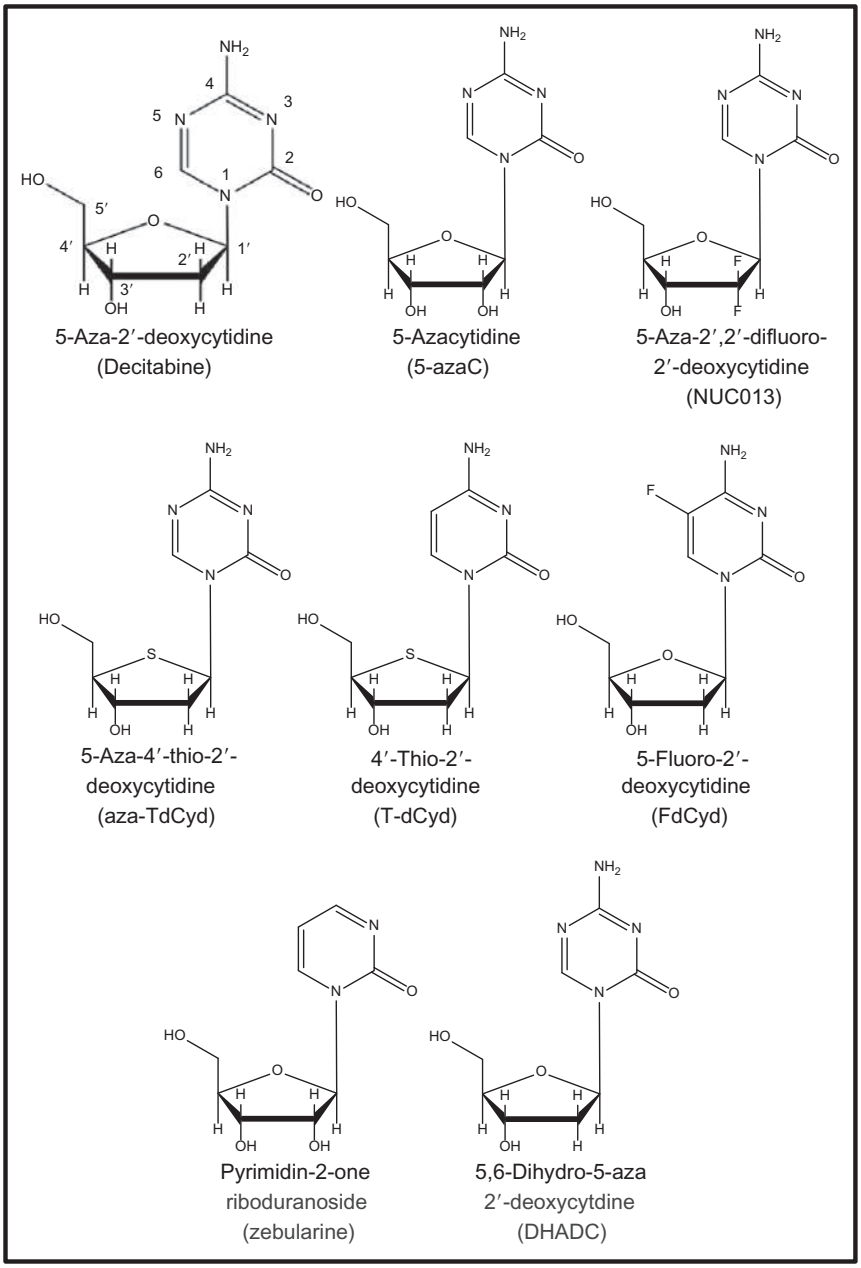

FIG. 7.1 Nucleoside DNMT inhibitors.

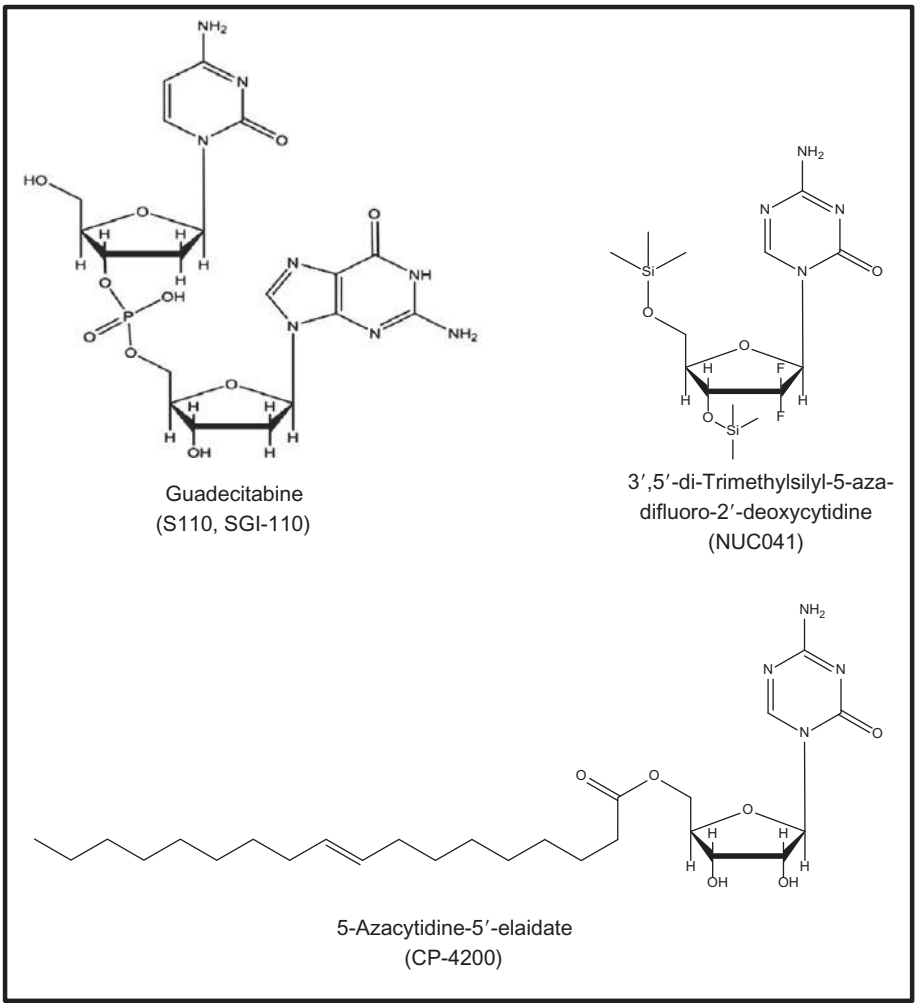

FIG. 7.2 Prodrugs of DNMT inhibitor cytidine analogs.

were compared for their capacity to inhibit DNMT1, aza-T-dCyd was found to be superior both in vitro and in vivo to T-dCyd.[19] Finally, decitabine has been found to be more effective as a DNMT inhibitor than low-dose zebularine.[20] The activity of 5-aza nucleosides was confirmed in a study comparing genome-wide demethylation, which found 5-azaC had a slightly greater demethylating effect than zebularine across the genome.[21]

Decitabine has a half-life lasting up to 35 minutes in humans, which is the result of deamination by cytidine deaminase (CDA).[22] Furthermore, the levels of CDA in patients in the clinic can affect the outcome of treatment using cytidines that are substrates for CDA.[23] Unfortunately, all the cytidine analogs under development are also substrates for CDA. Part of the rationale for developing nonaza cytidines as DNMT inhibitors relates to their stability in an aqueous environment. Nucleosides with a 5-azacytosine base are susceptible to hydrolysis between the C-5 and C-6 of the cytosine, complicating drug administration,[22] which is not the case for other cytidine analogs.

Because they are the two approved drugs most of the literature on DNMT inhibitors focuses on decitabine and to a lesser degree 5-azaC. It can be assumed that, within the constraints of their relative activities as DNMT inhibitors, what applies to one of these agents as an epigenetic drug applies to the others, although there are notable exceptions because they all have activities other than DNMT inhibition. For example, decitabine, FdCyd, and zebularine have been shown to be more effective against p53-mutant or null cells than wild-type (WT) cells,[24] while such is not the case for 5-aza-2′,2′-difluoro-2′-deoxycytidine, which in vitro is globally more active than decitabine and particularly so against p53 WT cell lines.[25] Not surprisingly though, decitabine and 5-azaC have demonstrated a substantial overlap of genes demethylated by both drugs, as 5-azaC needs to be reduced to decitabine before incorporation into DNA.[26]

The following sections provide a summary of some of the known activities of decitabine and how other nucleosides differ from decitabine in their mechanisms of action and activity.

7.2 APPROVED DNMT INHIBITORS

7.2.1 5-Aza-2′-Deoxycytidine (Decitabine)

Decitabine and 5-azaC have two main mechanisms of antitumor activity[1]: cytotoxicity as a result of incorporation into DNA (and RNA for 5-azaC) leading to induction of DNA damage response, and[1] DNA hypomethylation through the inhibition of DNA methyltransferase, enabling the restoration of normal growth and differentiation.[27] Decitabine acts by forming a covalent bond between a cysteine residue in the active site of the DNMT and the C-6 of cytosine in DNA to irreversibly inactivate the enzyme through formylation of a catalytic cysteine.[28] If cells are treated with decitabine, the formation of covalent methyltransferase-DNA adducts prevents DNA replication and transcription and induces DNA repair at a high level, including homologous recombination and inaccurate repair-like translesion synthesis. Depending on the amount of drug the cells may survive this phase or die, which explains the dose-dependent immediate toxicity of the drug. Eventually, the surviving cells are depleted of DNMT causing global hypomethylation of newly synthesized DNA.[29]

Two important outcomes of the treatment of cancerous cells by decitabine are derepression of tumor suppressor genes and tumor-associated antigens. Decitabine has been shown to increase the expression of p53 in TP53 WT cells.[30] TP53 is of vital importance in preventing human cancer development and progression, and is often referred to as the "guardian of the genome." Mutations of its gene are detected in approximately 50% of all types of human cancers, and the functions and stability of the p53 protein are often abrogated via posttranslational mechanisms, such as DNA methylation, in all other human cancers that harbor TP53 WT. p53 is often inactivated in cancer because it can trigger cell growth arrest, apoptosis, autophagy, or senescence, which are detrimental to cancer cells, and it impedes cell migration, metabolism, or angiogenesis, which are favorable to cancer cell progression and metastasis.[31] Despite having been shown to increase the expression of p53 in TP53 WT cells, Nieto et al. have reported that decitabine unexpectedly induced more apoptosis in TP53-mutant or null cells than in TP53 WT cells.[32–34] In a study of tumor samples involving predecitabine and postdecitabine administration to cancer patients, decitabine was also found to increase the expression of a number of other tumor suppressor genes, including FHIT, FUS1, WWOX, and PTEN.[35]

Selected cancer testis antigens (CTAs), human leukocyte antigens (HLAs), and accessory or costimulatory molecules required for efficient recognition of neoplastic cells by the immune system have been shown to be epigenetically silenced or downregulated in cancer. Decitabine induces the expression of CTA and HLA class I antigens, which lasts several weeks after treatment.[36] Decitabine has been shown to result in the expression of epitopes whose expression may be downregulated in cancer. This is particularly important in an era when immunotherapy is becoming an increasingly important part of the oncology armamentarium. For example, in patients with Epstein-Barr virus (EBV)(+) Hodgkin lymphoma, infused cytotoxic T cells (CTLs) targeting EBV-derived proteins can induce complete remissions. In relapsing patients up to 70% of tumors are EBV(−). For these patients an alternative is to target the CTA MAGE-A4 because a DNMT inhibitor can enhance MAGE-A4 expression in previously MAGE-negative tumors.[37] Decitabine was also found to upregulate MAGE-A in osteosarcoma.[38] In testicular cancer, SSX proteins comprise a set of CTAs whose expression has been found to be upregulated by decitabine.[39] Similarly, the homeobox protein, PEPP2, has been reported to be expressed in myeloid leukemic cells, and decitabine has been found to enhance PEPP2 expression in leukemic cells, but not in mononuclear cells from healthy donors.[40]

A possible therapeutic role for DNMT inhibitors extends beyond cancer. The most advanced of these other possible indications is for the treatment of sickle cell disease (SSD), but there are many others where epigenetics may play a role. Those that have been the subject of recent investigations will be mentioned below. Fetal hemoglobin (HbF, a2) decreases the polymerization of sickle hemoglobin and high levels correlate with decreased morbidity and mortality in SSD. Silencing the HbF gene is associated with DNA methylation. In clinical trials 5-azaC and decitabine have demonstrated the greatest efficacy of the various interventions used for HbF reactivation.[41] A recent phase I clinical trial of a regimen of an oral tetrahydrouridine (THU), an inhibitor of CDA, and decitabine in SSD demonstrated the feasibility of elevating HbF and total hemoglobin while maintaining an acceptable safety profile.[42]

Other possible applications for DNMT inhibitors include such diverse diseases as autoimmune diseases, alcoholism, and atherogenesis. Autoimmune-related epigenetic dysregulations, in particular DNA methylation, have been described in systemic lupus erythematosus (SLE), rheumatoid arthritis, and systemic sclerosis patients. Decitabine was tested in two murine models of experimental allergic encephalomyelitis (EAE). Decitabine treatment has been associated with a significant amelioration of clinical and histological hallmarks of EAE in both models, both in prophylactic and therapeutic regimens.[43] Mounting evidence points to a central role of epigenetic modifications in controlling gene expression and behavior in alcoholism. Indeed, a DNMT inhibitor has been found to reduce voluntary ethanol consumption in animal models. Decitabine-induced decreases in ethanol drinking were associated with global changes in gene expression, implicating roles in the regulation of cerebral blood flow, extracellular matrix

organization, and neuroimmune functions. In addition, in vivo administration of decitabine shortened ethanol-induced excitation of dopaminergic neurons in vitro, suggesting that decitabine reduced ethanol drinking via changes in the reward pathway.[44] Finally, DNMT inhibition could play a role in the prevention of atherogenesis. Disturbed blood flow is proatherogenic and induces the expression of DNMT1 both in vivo and in vitro. Decitabine has been found to reduce endothelial inflammation and inhibit the development of atherosclerosis in ApoE−/− mice.[45]

The most common side effects of treatment with decitabine all relate to bone marrow suppression and include myelosuppression, thrombocytopenia, febrile neutropenia, and anemia.[46]

7.2.2 5-Azacytidine

5-AzaC is the RNA analog of decitabine. Some 80%–90% of 5-azaC is incorporated into RNA as 5-azacytidine-triphosphate, while only 10%–20% is incorporated into DNA after conversion to 5-aza-2′-deoxycytidine-diphosphate by ribonucleotide reductase (RNR) and subsequent phosphorylation to 5-aza-2′-deoxycytidine-triphosphate.[47] As the major portion of 5-azaC is incorporated into RNA, it is not surprising that it has effects on RNA processing; in particular, it has been shown to result in the reduction of tRNA levels through the inactivation of tRNA methylase.[48] 5-AzaC has also been shown to be a potent inhibitor of RNR subunit RRM2. Hence the initial RNR-mediated 5-azaC conversion to decitabine is terminated through its own inhibition and persists over time.[47] This limits the utility of 5-azaC as a DNMT1 inhibitor. On the other hand, 5-azaC has been found to be an inhibitor of DNMT2. DNMT2 has been shown to have prominent tRNA methyltransferase activity, and 5-azaC can inhibit cytosine methylation in tRNA with considerable specificity, although the therapeutic implications of this finding are currently unclear.[49] That 5-azaC has limitations as a DNMT1 inhibitor was supported by a study investigating $CD34^+$ cells from MDS patients exposed to 5-azaC. This study found an increase in global transcription levels in all patients after 24 hours of 5-azaC exposure. DNA demethylation was also detected, although the degree of demethylation in each specific gene was very modest and thus of questionable biological relevance. DNA demethylation occurred in all genomic regions, but to a larger extent in nonpromoter regions, open-sea regions, and in regions annotated as heterochromatin, supporting the interpretation that this was not the direct cause of increased gene expression.[50] An oral version of 5-azaC is under clinical development.[51] The side effects of treatment with 5-azaC are similar to those observed with decitabine, with the most common being myelosuppression.[52]

7.3 DNMT INHIBITORS IN CLINICAL DEVELOPMENT

7.3.1 Guadecitabine

Guadecitabine (also known as S110 or SGI-110) is a dinucleotide that is a prodrug of decitabine. It consists of decitabine linked by a phosphodiester bond to deoxyguanosine. This linkage results in reduced susceptibility to immediate inactivation by CDA. However, following cleavage of the phosphodiester bond, free decitabine can undergo deamination.[53] Guadecitabine has similar stability in an aqueous solution to decitabine, and cytotoxicity is comparable.[54] In a phase I clinical trial the half-life of guadecitabine was found to be 0.59 to 1.44 hours following subcutaneous administration and 1.23 to 1.79 hours for decitabine derived from guadecitabine with an efficient release of decitabine.[55] This is longer than the half-life of decitabine following IV infusion.[6] In a phase II clinical trial of patients with AML and MDS, over half the patients in the subset with treatment-naive AML achieved a composite complete response with guadecitabine at all doses and schedules tested. The most common serious adverse events were febrile neutropenia, pneumonia, and sepsis.[56] In July 2018, Otsuka and Astex announced that the Phase 3 ASTRAL-1 study in treatment-naïve AML patients did not meet the co-primary endpoints of superiority of complete response rate or overall survival compared with the control arm of physician's choice of azacitidine, decitabine, or low-dose cytarabine. Clinical trials are ongoing in relapsed and refractory AML and MDS (https://www.otsuka.co.jp/en/company/newsreleases/2018/20180731_1.html). Other clinical trials have been completed or are ongoing for the treatment of germ cell tumors in combination with cisplatin, hepatocellular carcinoma, metastatic melanoma in combination with ipilimumab, ovarian cancer in combination with carboplatin, and small-cell lung cancer in combination with durvalumab or tremelimumab.[57]

7.3.2 5-Fluoro-2′-Deoxycytidine

5-Fluoro-2′-deoxycytidine (FdCyd) was first reported to have antineoplastic activity in the 1950s.[58] It was originally developed as a tumor-targeting CDA-activated prodrug for the thymidylate synthase inhibitors 5-fluorouracil (5-FU)

and 5-fluoro-2′-deoxyuridine (FdUrd).[59] Subsequently, it was shown that the cytotoxic events associated with FdCyd treatment might be related to any combination of the following: the incorporation of FdCyd into DNA, inhibition of DNA methylation, inhibition of thymidylate synthase, incorporation of FdUrd into DNA, or formation of 5-FU RNA.[60] In 1988 FdCyd was shown to covalently bind to prokaryotic DNMT[61] and subsequently to eukaryotic DNMT. In the latter experiments an oligodeoxynucleotide containing FdCyd as a suicide substrate captured the enzyme and the dihydrocytosine intermediate that leads to the production of 5-methyldeoxycytidine from deoxycytidine. Methylated pyrimidines were generated from dihydropyrimidine intermediates by elimination of the hydrogen atom at C-5 and the enzyme moiety at C-6 to regenerate the 5–6 double bond. This would not be possible when the pyrimidine being attacked is FdCyd, because 5-fluorine cannot be lost by elimination. For this reason nucleophilic attack and methyl group transfer of an FdCyd moiety will produce a stable complex comprising DNA and the enzyme.[62]

FdCyd appears to be a relatively weak inhibitor of DNMT.[18] In a study investigating the HCT-116 human colon cancer cell line, FdCyd induced cell cycle arrest at the G2-M phase and activated both the p53-signaling and DNA damage response pathways. FdCyd's capacity to induce G2-M arrest and suppress cancer cell proliferation appeared to be mediated by its role in activating the DNA repair pathway.[63] The half-life of FdCyd in humans administered IV is reported to be short, about 10 minutes, as a result of deamination by CDA. However, the administration of THU has been shown to improve the area under the curve (AUC) of the compound more than fourfold.[64] To increase the duration of exposure to the drug, FdCyd and THU are typically administered IV over 3 hours, and for patient convenience oral dosing is also being evaluated.[65] FdCyd and THU are currently in phase II clinical trials for the treatment of solid tumors.[66] The most important toxicities associated with treatment with FdCyd are reported to be anemia and lymphopenia.[67]

7.3.3 4′-Thio-2′-Deoxycytidine

4′-Thio analogs differ from natural nucleosides in that the 4′ oxygen atom in the deoxyribose ring is replaced by a sulfur atom. Both 4′-thio-2′-deoxycytidine (T-dCyd) and 5-aza-4′-thio-2′-deoxycytidine (aza-T-dCyd) were found to deplete DNMT1 in in vitro and in vivo models of cancer. Treatment with T-dCyd and aza-T-dCyd resulted in marked depletion of DNMT1 in two leukemia cell lines (CCRF-CEM and KG1a) at submicromolar concentrations. In solid tumor cell lines only aza-T-dCyd showed activity against two other cell lines, while both nucleosides also depleted DNMT1 in NCI-H23 cells. Aza-T-dCyd at concentrations of 1–10 μM depleted DNMT1 levels in HCT-116 and IGROV-1, whereas T-dCyd was not efficacious up to 100 μM. These results were confirmed in mice bearing CCRF-CEM tumors. On the day subsequent to 9 days of treatment, the tumors were removed and the level of DNMT1 protein was determined. DNMT1 levels were decreased in tumors of mice that were treated with both T-dCyd and aza-T-dCyd, although aza-T-dCyd was more effective than T-dCyd. T-dCyd resulted in the depletion of DNMT1 only near its MTD. These experiments demonstrated that aza-T-dCyd was a more potent DNMT1 inhibitor than T-dCyd. Studies in mouse xenografts with NCI-H23 lung cancer demonstrated the activity of T-dCyd and aza-T-Cyd under various doses and regimens. In some cases these regimens led to complete tumor regression, but some of the doses and regimens were also noted to be toxic. Efficacy was achieved despite a very short plasma half-life of T-dCyd in mice of 10–15 minutes. As the study authors pointed out, it remains unclear whether the activity seen in tumor growth inhibition is entirely the result of DNMT1 depletion.[19]

T-dCyd has been selected for clinical development and is currently in a phase I trial in patients with advanced solid tumors. Two patients on the trial have been reported as having developed *Pneumocystis jiroveci* pneumonia (PJP) resulting in all patients on the trial now being administered PJP prophylaxis.[68] This suggests that T-dCyd induces immunosuppression, and PJP in such patients tends to have a worse prognosis than in patients with HIV, although chemoprophylaxis is quite effective at preventing infection.[69, 70]

7.4 DNMT INHIBITORS IN PRECLINICAL DEVELOPMENT

7.4.1 5-Aza-2′,2′-Difluoro-2′-Deoxycytidine

5-Aza-2′,2′-difluoro-2′-deoxycytidine (NUC013) and its prodrug NUC041 are the most recent additions to the family of 5-aza nucleosides. NUC013 is comprised of the same base as 5-azaC or decitabine, hence ensuring good activity against DNMT when conjugated with a sugar of gemcitabine. It is interesting to note that among its various activities gemcitabine has been reported to induce or reactivate silenced genes in lung, mesothelioma, and prostate cancer cell

lines. While gemcitabine was found to directly inhibit DNMT, it did not affect global levels of DNA CpG methylation.[71]

NUC013 has been shown to inhibit DNMT1 in human leukemic cell lines and a human colon cancer cell line, HCT-116. In addition, like gemcitabine, it has been shown to inhibit RNR in HeLa cells. When its activity was compared with decitabine in the NCI 60 cell line panel, it was found to be active (defined as an $IC_{50} < 10$ μM) in significantly more cell lines than decitabine and to have such activity in at least one cell line from each tissue type tested. NUC013 has been found to be more active than decitabine in TP53-mutant or null cell lines and even more so against TP53 WT cell lines. The mechanism of action for such differences in activity may be tied to inhibition in the case of NUC013 or induction in the case of decitabine of p53R2, a p53-induced RNR. The inhibition of p53R2 would lead to decreased competition from endogenous nucleotides for NUC013 incorporation, while induction in the case of decitabine would lead to increased competition.[25]

The maximum tolerated dose (MTD) of decitabine administered IV for 3 consecutive days per week has been established as <5 mg kg^{-1} in mice, and that of NUC013 as >120 mg kg^{-1} with the same route and regimen. In mouse xenografts of human cancer NUC013 was shown to be significantly more effective and safer than decitabine in the treatment of human leukemia (HL60) and colon cancer (HCT-116).[25] Subsequently, NUC013 was shown to significantly inhibit tumor growth and improve survival in a mouse xenograft model of human nonsmall-cell lung cancer (NCI-H460).[72]

7.4.2 3′,5′-Di-Trimethylsilyl-2′,2′-Difluoro-5-Aza-2′-Deoxycytidine

Because decitabine is a cell cycle-specific agent, infusion of this agent for 1–4 hours only targets cancer cells in the S phase, whereas cells in the G1 and G2 phases escape the chemotherapeutic action of this analog during short-term treatment.[22] Attempts have been made to improve efficacy through continuous infusion, allowing greater incorporation into DNA, but these have been hampered by inconvenience and toxicity.[73, 74] NUC013 also suffers from a short half-life, as do the other nucleoside DNMT inhibitors, most likely as a result of deamination by CDA. To counter these undesirable pharmacokinetics a new prodrug approach has been taken, one that is formulation dependent. A hydrophobic prodrug (NUC041) has been developed for packaging in a hydrophobic vehicle to protect NUC013 from hydrolysis and deamination. In an aqueous environment hydrophobic moieties are readily hydrolyzed with the release of NUC013. This was achieved by conjugating NUC013 with trimethylsilanol (TMS) at the 3′ and 5′ position. The half-life of NUC013 administered IV in mice is 20.1 minutes, while that of NUC013 derived from NUC041 formulated in a PEG-phospholipid depot administered IM in a 50-μL volume was 3.4 hours.[72] As the half-life of a drug in a depot formulation is partially dependent on the volume that can be administered, it is anticipated that a substantially longer half-life could be achieved by IM or SQ administration of a depot formulation in humans, or perhaps with an IV formulation such as a PEG liposome.[75] Such a prolonged half-life will provide greater patient convenience and efficacy. Indeed, studies investigating a mouse xenograft of nonsmall-cell lung cancer (NCI-H460, p53 WT) have demonstrated that treatment with NUC041, leading to prolonged exposure to NUC013, results in tumor regression that is not observed with shorter exposure.[72] The balance between tumor cell proliferation, on the one hand, and cell apoptosis and senescence, on the other, is altered through such prolonged drug exposure. The mechanism of action is most likely mediated through derepression of p53 leading to the activation of natural killer cells and the death of senescent cancer cells.[76] Since nude mice lack an intact acquired immune system, it is anticipated that even greater activity would be observed in nonimmunocompromised hosts.

7.4.3 5,6-Dihydro-5-Aza-2′-Deoxycytidine

5,6-Dihydro-5-aza-2′-deoxycytidine (DHADC) was first described in 2005 as an antiretroviral for the treatment of HIV. DHADC was designed to reduce the fitness of HIV by hypermutating the viral genome.[77] In clinical trials the oral prodrug of DHADC (KP-1461) demonstrated safety and limited efficacy.[78] DHADC differs from decitabine in being stable to hydrolysis as a result of saturation of the C-5 to C-6 bond.

In leukemic cell lines DHADC was found to be a stronger inhibitor of the methylation level of THBS-1 and CDKN2B than zebularine at their respective IC_{50} values. Zebularine only reduced the methylation level by 40%, while decitabine or DHADC did so by up to 80%. These results were confirmed by analyzing hypomethylating activity on 24 different genes. Decitabine and DHADC were similarly effective in both the CCRF-CEM and HL-60 cell lines, resulting in a 50%–60% decrease in DNA methylation, while zebularine had only a 20%–25% hypomethylating effect. However, it should be noted that decitabine demethylated at 1 μM, while DHADC did so at 100 μM. Despite the 100-fold increased concentration of drug required to have the same effect, DHADC might have clinical applications as a DNMT

inhibitor because of its relative lack of toxicity. In vitro, while decitabine toxicity increases in a time-dependent manner, 100-fold higher concentrations of DHADC did not affect cell cycle progression. Perhaps more relevant, the toxicity of decitabine or 5-azaC when administered to patients appears to be greater than that of DHADC.[79]

7.4.4 Pyrimidin-2-One β-D-Ribofuranoside (Zebularine)

Zebularine was first mentioned in the medical literature in 1967 as a possible product of cytidine deamination.[80] Zebularine acts as an inhibitor of CDA[81] and forms covalent adducts with DNMT after nucleophilic attack of the cysteine residue at the C-6 of the aromatic ring, which is enhanced by the absence of a 4-amino functional group in the base.[82] Zebularine has shown activity in vitro and in vivo as a DNMT inhibitor in multiple tissues, including breast cancer,[83] cholangiocarcinoma,[84] colorectal cancer,[85] lung cancer,[86] and prostate cancer.[87] Zebularine has limited oral bioavailability when administered to monkeys (<1%) and a half-life in the range of 70–150 minutes after IV administration. Preclinical models have shown the reversibility of zebularine's DNMT inhibition and the need for tumors to have prolonged exposure to inhibit tumor growth. Based on the half-life of zebularine, it is likely that frequent dosing or continuous IV infusion would be necessary to maintain prolonged inhibition of DNMT.[88] Unfortunately, zebularine at doses compatible with plasma levels in the efficacious range induced mortality in monkeys, probably through renal or hepatic toxicity, and this has halted the possibility of its clinical development.[89]

7.4.5 5-Azacytidine-5′-Elaidate

CP-4200 is an elaidic acid prodrug of 5-azaC. CP-4200 has been shown to be less dependent on nucleoside transport than 5-azaC. Indeed, the cellular activity of CP-4200 persisted after hENT1 inhibition.[90] CP-4200 was also reported to likely be a poor substrate for CDA. It showed significantly higher antitumor activity, as measured by splenic weights, than 5-azaC in an orthotopic mouse model of acute lymphocytic leukemia.[91] This same technology was used to develop a prodrug of gemcitabine, CP-4126. CP-4126 was remarkable despite its very short half-life, reported to be between 0.05 and 0.07 hours, following administration in dogs.[92] While showing initial promise, it failed in a phase III clinical trial for the treatment of pancreatic cancer.[93]

7.5 CONCLUSION

Nucleosides with 5-azacytosine bases remain the most effective inhibitors of DNMT, although they are subject to hydrolysis, which complicates drug administration, and in some cases suffer from toxicity. All cytidine DNMT inhibitors have a short half-life because of deamination by CDA. This short half-life affects patient convenience, but more importantly impacts on efficacy; in some cases it has required combination treatment with the CDA inhibitor THU. A number of prodrug approaches are undergoing development to attempt to address these pharmacologic shortcomings, with 3′,5′-di-trimethylsilyl-2′,2′-difluoro-5-aza-2′-deoxycytidine having shown remarkable antitumor efficacy in vivo and offering the possibility of the drug remaining in circulation for days. There are nonaza cytidines under development, but their DNMT inhibition is relatively weak, and may only be a minor aspect of their repertoire of activities. In cancer there is a need for DNMT inhibitors that can act more robustly against solid tumors. The potential uses of DNMT inhibitors extend well beyond oncology. The major limitations to their use in other fields are concern about toxicity and to a lesser degree pharmacology. Hopefully, these can be addressed by newer DNMT inhibitors and their prodrugs that are under development.

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CHAPTER

8

Pharmacoepigenetics: Novel Mechanistic Insights in Drug Discovery and Development Targeting Chromatin-Modifying Enzymes

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8.1 INTRODUCTION

Epigenetics is a rapidly growing field describing genetic modifications in gene expression that do not involve DNA sequence variation. Pharmacoepigenetics research investigates how the epigenetic status affects drug response and how drugs affect the epigenetic status. Epigenetic gene regulation collaborates with genetic alterations in cancer development. These areas of study can provide valuable insights into histone deacetylase inhibitor (HDACi) responses and lead to new strategies for cancer management. Histone deacetylation is an important epigenetic event, implicated in the development and progression of cancer by regulating the accessibility of DNA for gene expression and transcription. The basic repeating unit of chromatin is the nucleosome, which is composed of DNA wrapped around a core of histone proteins.[1]

8.2 EPIGENETIC MECHANISMS

Epigenetic mechanisms are as important as genetic mechanisms for biological events and can also result in stable and heritable changes. However, the major difference between genetic and epigenetic regulation is that epigenetic mechanisms do not involve change in the DNA sequence, whereas genetic mechanisms involve the primary DNA sequence and changes or mutations to this sequence.[2] Chromatin is made of repeating units of nucleosomes, which consists of 146 base pairs of DNA wrapped around an octamer of four core histone proteins (H3, H4, H2A, and H2B). Epigenetic mechanisms that modify the chromatin structure can be divided into DNA methylation, covalent histone modifications, and noncovalent mechanisms, such as the incorporation of histone variants, nucleosome remodeling, and noncoding RNAs including microRNAs (miRNAs).

These modifications work together to regulate the way the genome functions by altering the local structural dynamics of chromatin, primarily regulating its accessibility and compactness. The interplay of these modifications creates an epigenetic landscape that regulates the way the mammalian genome manifests itself in different cell types, developmental stages, and disease states, including cancer. The distinct patterns of these modifications in different cellular states serve as guardians of cellular identity.[3]

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8.2.1 Epigenetics in Cancer

Epigenetic programming is crucial to mammalian development, and stable inheritance of epigenetic settings is essential for the maintenance of tissue- and cell type-specific functions.[4] Epigenetic mechanisms act to change the accessibility of chromatin to transcriptional regulation locally and globally via modifications in DNA and by modification or rearrangement of nucleosomes. Epigenetic gene regulation collaborates with genetic alterations in cancer development. The initiation and progression of cancer, traditionally seen as a genetic disease, is now realized to involve epigenetic abnormalities along with genetic alterations. Recent advances in the rapidly evolving field of cancer epigenetics have shown extensive reprogramming of every component of the epigenetic machinery in cancer.[5]

8.2.2 Histone Modification

Histone modification is covalent posttranslational modification (PTM) of histone proteins that includes methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation.[6] The PTMs of histones can impact gene expression by altering chromatin structure or recruiting histone modifiers. Histone proteins act to package DNA, which wraps around the eight histones, into chromosomes. Histone modification acts in diverse biological processes such as transcriptional activation/inactivation, chromosome packaging, and DNA damage/repair.

In most species histone H3 is primarily acetylated at lysines 9, 14, 18, 23, and 56, methylated at arginine 2 and lysines 4, 9, 27, 36, and 79, and phosphorylated at Ser10, Ser28, Thr3, and Thr11. Histone H4 is primarily acetylated at lysines 5, 8, 12, and 16, methylated at arginine 3 and lysine 20, and phosphorylated at serine 1. Thus quantitative detection of various histone modifications would provide useful information to get a better understanding of epigenetic regulation of cellular processes and the development of histone-modifying enzyme-targeted drugs. A complement of modifications is proposed to store the epigenetic memory inside a cell in the form of a "histone code" that determines the structure and activity of different chromatin regions.[7]

8.2.3 Histone Acetyltransferases

Histone acetyltransferases (HATs) are enzymes that acetylate conserved lysine residues on histone proteins by transferring an acetyl group from acetyl CoA to form ε-N-acetyl lysine. This modification neutralizes the positive charge of lysine and may thus disrupt the interaction between DNA and histone tails. HATs are traditionally divided into two different classes based on their subcellular localization.[8] Acetylated histones are generally associated with euchromatin and transcriptional activation. In contrast to histone acetylation, deacetylation restricts DNA accessibility by revealing the positive charge of lysine, permitting interaction between DNA and the histone tail and thus chromatin compaction.

8.2.4 Histone Deacetylases

The acetylation and deacetylation of histones provide a balance between an open conformation and a closed chromatin conformation for the regulation of transcription. This regulation is complex but is principally regulated by HATs. Histone deacetylases (HDACs) play a major role in the epigenetic regulation of gene expression through their effects on a compact chromatin structure.[9] Histone deacetylases are a class of enzymes involved in many biological pathways, and one of their best-known properties is their ability to remove acetyl groups from lysine residues on amino-terminal histone tails allowing the histones to wrap the DNA more tightly. HDACs are part of a vast family of enzymes that have crucial roles in numerous biological processes, largely through their repressive influence on transcription. In recent years HDACs have become promising therapeutic targets with the potential to reverse the aberrant epigenetic states associated with cancer. Alterations in acetylation levels and overexpression of various HDACs in many cancer cell lines and tumor tissues have been reported. The characterization of posttranslational modifications to histone H4 in a comprehensive panel of normal tissues, cancer cell lines, and primary tumors suggests that global loss of monoacetylation at Lys16 of histone H4 is a common hallmark of human cancer cells, implicating a critical role of HDAC activity in establishing tumor phenotypes. Thus far 18 HDAC enzymes have been identified, which are divided into zinc-dependent and NAD-dependent enzymes.[10] The aberrant activity of HDACs has been documented in several human cancers including lung cancer.[11] Histone modifications brought about by altered expression of HDAC enzymes have been implicated in carcinogenesis and cancer development in various types of solid cancer and hematologic malignancies.[12] It has very recently been reported that the contribution made by epigenetic changes to lung carcinogenesis is now well established in cancer development.[13]

8.2.5 Histone Modification Leads to Cancer

Histones are highly conserved alkaline proteins that can become posttranslationally modified in amino acid residues located on their N- and C-terminal tails. There are four core histones: histone 2A (H2A), histone 2B (H2B), histone 3 (H3), and histone 4 (H4), and one linker histone, histone 1 (H1). Approximately 146 base pairs of DNA are wrapped around each histone octamer, which consists of two copies of each of the core histones, in left-handed superhelical turns. H1, which is not included in the nucleosome "bead," serves as a linker and helps secure DNA that is wound around the nucleosome.[14] The addition or removal of posttranslational modifications from histone tails is fairly dynamic and is achieved by a number of different histone-modifying enzymes.[15]

8.2.6 Molecular-Targeted Therapies for Histone Deacetylase

Within the past decade our understanding of malignant cell growth and underlying molecular alterations has offered new opportunities for molecular-targeted cancer therapy. Studies investigating the molecular and cellular biology of lung cancer have gradually revealed the pathways and molecules driving cells to full-fledged lung cancer.[16] These studies include the identification of genetic and epigenetic changes of specific molecules resulting in the activation of signaling pathways important in carcinogenesis. Although remarkable progress has been achieved in the treatment of cancer, much remains to be learned about the delivery of therapeutic agents, particularly with regard to the optimal combination and timing of biologic agents with cytotoxic therapy.

The option of biologically targeted therapy has led to the development of novel agents that have driven innovation on the accurate measurement of clinical responses and relevant biologic parameters. The current use of agents targeting epigenetic changes exemplifies this trend in clinical research.[17] Encouraging response rates in patients receiving agents that target epigenetic marks drives continued efforts to identify key laboratory correlates that might help to deliver drugs in an optimal manner and to identify the key biologic features to elucidate a more exact mechanism of action. Much interest is now focused on the use of targeted therapies for the management of lung cancer.

8.2.7 Histone Deacetylase Enzymes as a New and Emerging Target for Cancer Treatment

Chromatin is composed of regular repeating units of nucleosomes in which deoxyribonucleic acid (DNA) has been conserved. The major components of chromatin are DNA and ribonucleic acid (RNA), which are negatively charged; associated proteins including histones, which are positively charged; and nonhistone chromosomal proteins, which are acidic at neutral pH.[18] Chromatin can be present in the nucleus as heterochromatin, which is highly compact and transcriptionally inactive, or as euchromatin, which is accessible to RNA polymerases for transcriptional processes and gene expression. A nucleosome comprises 146 nucleotide base pairs of DNA wrapped around the core histone octamer, which is composed of two copies each of H2A, H2B, H3, and H4 proteins. These proteins are basic as a result of having amino-terminal side chains rich in the amino acid lysine.[19] The balance between the acetylated/deacetylated status of the N-terminal tail of histones, crucial to modulating gene expression, is mediated by two different sets of enzymes: HATs and HDACs. The hypoacetylation of histones is associated with a condensed chromatin structure resulting in the repression of gene transcription, whereas acetylated histones are associated with a more open chromatin structure and the activation of transcription.[20] Both HATs and HDACs in turn regulate the transcription status of not just histones but also of other acetylated proteins such as p53, nuclear factor-YA (NF-YA), and globin transcription factor-1 (GATA-1).[21]

8.3 HISTONE DEACETYLASE INHIBITORS

HDACi are a class of compounds that interfere with the function of histone deacetylase. HDACi comprise structurally diverse compounds that can be grouped together as targeted anticancer agents. In general, these small-molecule inhibitors show higher sensitivity to transformed cells than to normal cells.[22] The overall number of genes regulated by HDAC is relatively small.[23] The genes induced by HDACi are mainly involved in cell growth, differentiation, and survival. Since HDAC enzymes are implicated in various diseases, there is significant interest in discovering whether HDACi could be used as potential therapeutic agents. In addition, the use of HDACi as part of combination therapy in the treatment of various cancer diseases has shown a lot of promise.[24] In cancer-pathological conditions where classical HDACs are overexpressed, HDACi have been found to be effective in reversing the malignant phenotype of transformed cells and have subsequently emerged as promising cancer-therapeutic agents. HDACi first came to the fore because of their ability to induce cellular differentiation.[25] This effect is associated with their

ability to cause cell cycle arrest in the G1 phase and/or the G2 phase, thus leading to the inhibition of cell growth. The concentrations necessary to cause growth inhibition correlate very well with those needed to induce the hyperacetylation of histones.[26] G1 cell cycle arrest in most cases is a result of the induction of the CDKN1A gene, which encodes the cyclin-dependent kinase inhibitor WAF1, p21.[27] The treatment of cells by HDACi can also bring about apoptosis. The inhibitors can initiate extrinsic (death receptor) and intrinsic (mitochondrial) pathways.[28] It has been shown that various members of the TNF receptor superfamily and ligands become transcriptionally activated upon HDACi treatment. One possibility is that HDACi cause global changes in gene expression that alter the balance of expression of proapoptotic and antiapoptotic proteins. It is also possible that HDACi can activate a defined protein or signaling pathway thus inducing the intrinsic apoptotic pathway. HDACi are able not only to target histones but have the ability to influence a variety of processes such as cell cycle arrest, angiogenesis, immune modulation, and apoptosis by targeting nonhistone proteins.

These effects also contribute to decreasing the nutrient supply of the metastasis, thus inhibiting metastatic spread of the tumor. Upregulation of the gene expression of metastatic suppressors and downregulation of genes that promote metastasis have also been described as being responsible for the antimetastatic effects of HDACi. One of the first identified HDACs was trichostatin A (TSA), an antifungal antibiotic that was isolated from *Streptomyces platensis* culture broth. TSA is a hydroxamic acid and is often used to investigate histone acetylation because its mechanism of inhibition is known to be due to chelating HDACi on the enzyme zinc ion.[29] As a result of this mechanism, TSA is a class I and II HDACi but not an inhibitor for class III HDACs since these enzymes do not contain a zinc ion.

8.3.1 Classification of Histone Deacetylase Inhibitors

HDACi are classified according to their chemical structures, hydroxamates, cyclic peptides, short chain fatty acids, and benzamides. TSA, which belongs to the hydroxamate group was the first natural product discovered to be an HDACi.[30] Another HDACi, depsipeptide (romidepsin), belonging to the cyclic peptide group, is a natural product extracted from *Chromobacterium violaceum*.[31] The aliphatic acid group contains HDACi, namely butyrate, phenylbutyrate, valproic acid, and their derivatives.[32] Benzamide HDACi, such as entinostat and tacedinaline, have also been established and are undergoing several phases of clinical trials for multiple cancers.[33] Up to now more than 20 HDACi have entered clinical studies, and to date four HDAC inhibitors, vorinostat, romidepsin, panobinostat, and belinostat, have successfully gained Food and Drug Administration (FDA) approval for treating distinct malignancies.[34] In addition to these four FDA-approved agents the butyrates, valproic acid, and compounds such as givinostat, mocetinostat, belinostat, and entinostat have been extensively studied in the clinic with varying results.

8.3.2 Histone Deacetylase Inhibitor Mechanisms of Action

HDACi can induce tumor cell apoptosis, growth arrest, senescence, differentiation, and immunogenicity as well as inhibit angiogenesis. Almost certainly, the biological effects and therapeutic outcomes depend on the genetic lesions driving the tumor of interest and the HDACi under investigation. While HDACi were historically identified on the basis of their ability to induce tumor cell differentiation,[35] the induction of tumor cell apoptosis is the biological outcome most often reported. However, divergent views exist regarding the importance of the intrinsic apoptotic pathway mediated by the interplay between proapoptotic and antiapoptotic Bcl-2 family proteins[36] vs the extrinsic pathway mediated by death receptors (e.g., TRAIL receptors, Fas) and their cognate ligands (e.g., TRAIL, FasL).[37] There is a link between altered gene expression and the induction of apoptosis with histone hyperacetylation observed at the promoters of apoptosis-inducing genes (such as TRAIL and proapoptotic Bcl-2 family member Bcl-2-modifying factor (BMF)) and changes in the activity of transcription factors as a result of acetylation (such as inhibition of SP1 and C/EBPα), leading to downregulation of the antiapoptotic protein Bcl-2[38] following HDACi treatment. It is therefore likely that both the threshold of apoptosis induction and the mechanism by which death can be triggered by HDACi are determined by the interaction between oncogenic lesions and the intrinsic apoptosis-signaling pathways active within each cell.

8.3.3 Histone Deacetylase Inhibitors in Clinical Trials

So far only four HDACi have been approved by the FDA for cancer treatment. Vorinostat ranks first among the approved inhibitors. It has been approved for cutaneous T cell lymphoma (CTCL). Romidepsin occupies the second rank and has been approved for CTCL and peripheral T cell lymphoma (PTCL). Belinostat occupies the third rank and

is approved for treating relapsed/refractory PTCL. Panobinostat, the last and fourth HDACi, gained approval for treating multiple myeloma (MM).[39] Although some of these agents often demonstrate more potent antitumor effects than vorinostat and romidepsin in preclinical testing, none has thus far demonstrated a novel mechanism of action, vastly superior clinical activity, or more favorable toxicity profiles and accordingly none has yet been registered for clinical use.[40]

8.4 DESIGNING PHARMACOPHORE-BASED HDAC INHIBITORS

However, the currently known HDACi exhibit limited isoform specificity, off-target activity, and undesirable pharmaceutical properties.[41] In the intensifying efforts to discover new, more therapeutically efficacious HDACi, molecular modeling-based rational drug design has played an important role in identifying potential inhibitors that vary in molecular structures and properties. Synthetic chemistry has also been used to design new compounds or to improve the efficacy or safety of drugs already in use for cancer therapy. So a second generation of HDACi need to be developed based on the common pharmacophore shared by all classes of HDACi, which can be broken down into four groups, namely: (a) a zinc-binding group (ZBG), which chelates zinc ion at the bottom of the pocket; (b) a linker (scaffold), usually hydrophobic, which occupies the narrow channel; (c) a connect unit (CU), which connects the SRM and linker; and (d) a surface recognition moiety (SRM), which interacts with residues on the rim of the active site.[42]

8.5 PEPTIDE-BASED MACROCYCLIC HISTONE DEACETYLASE INHIBITORS

Cyclic peptides represent the most structurally complicated and diverse class of HDACi.[43] Each subtype of the HDAC family performs a distinct role in gene expression, and cyclic peptides with their plentiful set of surface contacts, zinc-binding group, and macrocyclic cap, can target enzymes precisely by adequately modulating the amino acid configurational and structural assortment. This chapter summarizes the current status of different peptide-based macrocyclic compounds being developed as HDACi for the treatment of cancer. Cyclic peptide-based HDACi are among the most potent and most structurally complex class of HDACi. They generally fit the overall pharmacophore model of all HDACi and have been suggested to bind in a similar manner to long chain hydroxamate HDACi.[44] This realization inspired the identification of synthetic and semisynthetic analogs, in addition to other natural products. Variations within the cap group moiety of these HDACi modulate biological activity by enhancing isoform selectivity among different HDAC isoforms.[45] The synthesized structural based classification of novel HDACi under the classification of acids and peptides.

In recent decades much effort has been made to manage malignancies, and numerous anticancer agents have been developed from natural sources and from synthetic approaches. Unfortunately, the anticancer drugs presently available exert severe side effects. Hence the urgent need to discover potent anticancer agents capable of killing cancerous cells with minimum or no side effects. Chemotherapy and radiation therapy for cancer often have severe side effects that limit their efficacy. An efficient molecule to treat cancer is waiting to be discovered, and explorations to develop new entities are ongoing. Synthesizing drugs that have potential anticancer activity is a trend gaining popularity in many countries.[46] Anticancer drugs work by two mechanisms: they either kill cancer cells or modify their growth.

It is widely known that heterocyclic molecules play a critical role in health care and pharmaceutical drug design.[47] Currently a number of heterocyclic drugs have been approved by the FDA as anticancer drugs, such as Crizotinib, Ponatinib, Panobinostat, Lenvatinib, Palbociclib, Cabazitaxel, Afatinib,[48] and great efforts have been made to identify anticancer targets for novel drug discovery. Researchers are actively involved in the synthesis of various types of heterocyclic molecules and have evaluated their anticancer activity against various cell lines.[49] Evidence has been reported of the anticancer activity of heterocyclics, such as acridine,[50] benzimidazole,[51] indolylpyrimidine,[52] isatin,[53] isoquinoline,[54] pyrrole,[55] phenanthridine,[56] and camptothecine.[57]

Synthetic chemistry has also been used to design new compounds or to improve the efficacy or safety of drugs already in use for cancer therapy. Likewise, the urea functional group plays an important role in generating anticancer activity, and various urea-containing compounds exhibit admirable anticancer activity. Most urea derivatives include heterocyclic rings, such as oxadiazoles, thiadiazoles, triazoles, and pyrazoles, which possess valuable medicinal properties.[58] In addition, the nitrogen functionalities of urea and thiourea have been found to have a broad spectrum of biological effects, such as antidiabetic, antiinflammatory,[59] antiviral,[60] antibacterial, antifungal, and antidepressant,

on the central nervous system. Recently, some urea-based compounds have been shown to act as kinase inhibitors and as novel therapeutics in cancer treatment[61] as a result of their unique binding mode and kinase inhibition profile. Since urea-based derivatives have greater importance in pharmacological applications, this raises the possibility of designing more efficient urea-based derivatives as epigenetic modulators.

Thiourea derivatives are also of wide interest because of their anticancer activities against various leukemias and solid tumors.[62] Urea and thiourea derivatives show a broad spectrum of biological activities, such as antibacterial, antifungal, antiviral, anticancer, anticonvulsant, analgesic, and high-density lipoprotein (HDL) elevating.[63] Certain ureas have been described as having activity as serine-threonine kinase inhibitors and/or as tyrosine kinase inhibitors. In particular, the utility of certain ureas as active ingredients in pharmaceutical compositions for the treatment of cancer, angiogenesis disorders, and inflammatory disorders has been demonstrated.[64]

8.6 PHARMACOPHORE-BASED DESIGN AND DEVELOPMENT OF INHIBITORS TARGETING CHROMATIN-MODIFYING ENZYMES

Recently, some urea-based compounds have been shown to act as kinase inhibitors and as novel therapeutics in cancer treatment as a result of their unique binding mode and kinase inhibition profile.[61] As already mentioned, since urea-based derivatives have been shown to have great importance in pharmacological applications, this raises the possibility of designing more efficient urea-based derivatives as HDACi and antineoplastic properties. The compounds designed by the authors of this chapter share a common pharmacophore composed of four portions: (1) a zinc-binding group (ZBG), (2) a linker, (3) a connect unit (CU), and (4) a surface recognition moiety (SRM) toward class I, class II, and class IV HDAC isoforms (Fig. 8.1).

A novel class of urea-based derivatives are being used as HDACi[65] (Fig. 8.2). Epigenetic modifications reversibly alter gene expression and can contribute to the initiation and progression of cancer. The balance between histone acetylation and deacetylation is an epigenetic layer with a critical role in the regulation of gene expression. Dynamic regulation of acetylation is facilitated by two enzyme classes, namely HATs and HDACs. Reduced levels of histone acetylation as a result of aberrant HDAC activity have been detected in several human tumors and appear to repress tumor suppressor genes, thereby contributing to tumor onset and progression. HDACs are part of a vast family of enzymes that have crucial roles in numerous biological processes, largely through their repressive influence on transcription. Histone acetylation occurs in the ε-amino groups of evolutionarily conserved lysine residues located at the N-termini. Important positions for acetylation are Lys9 and Lys14 on histone H3 and Lys5, Lys8, Lys12, and Lys16 on histone H4. The mechanism of action of HDAC enzymes involves removing the acetyl group from histones comprising the nucleosome. Hypoacetylation results in a decrease in the space between the nucleosome and the DNA that is wrapped around it. HDACs regulate the activity of key cellular players involved in transcription, signal transduction, cell cycle, apoptosis, and other processes. This clearly indicates that HDACs regulate important cellular functions independent of their epigenetic role in controlling the chromatin structure. HDACi can reactivate gene expression and inhibit the growth and survival of cancer cells. HDACi are thought to possess anticancer activity because of their ability to halt the cell cycle and induce the expression of proapoptotic proteins that may correct the proliferative state of cancer cells. HDAC inhibition is a clinically validated therapeutic strategy for cancer treatment. Histone acetylation, particularly for the histones H3 and H4, is one of the most studied and best-understood covalent histone modifications. Acetyl groups are transferred to and from lysine residues on the N-terminal tail and on the surface of the nucleosome core.

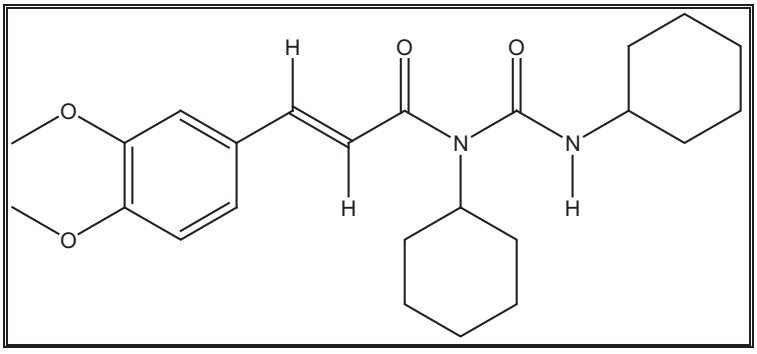

FIG. 8.1 Structure of *(E)-1, 3-dicyclohexyl-1-(3-(3,4-dimethoxyphenyl)acryloyl)urea.*

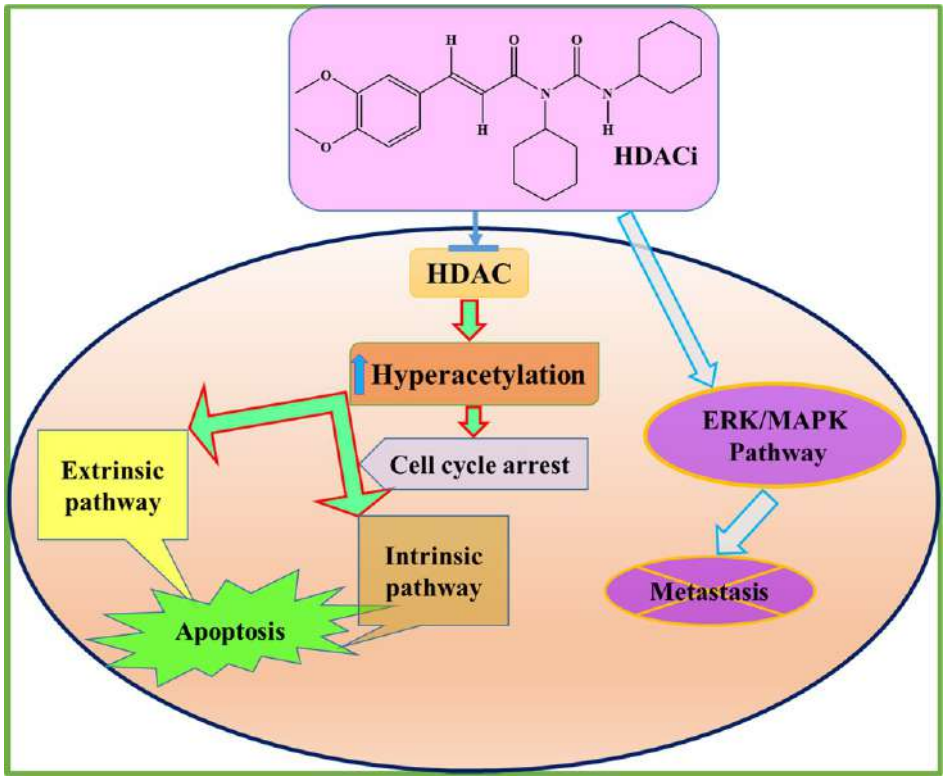

FIG. 8.2 Mechanism of *(E)-1,3-dicyclohexyl-1-(3-(3,4-dimethoxyphenyl)acryloyl)urea*.

8.7 CONCLUSION

(E)-1,3-dicyclohexyl-1-(3-(3,4-dimethoxyphenyl)acryloyl)urea is a potent HDACi that suppresses lung cancer cell growth. The mechanism of apoptosis has been shown to be carried by both extrinsic and intrinsic pathways. Antimetastatic activities have also been observed by inhibition of the MAPK pathway via suppression of ERK1/2, JNK, and p38 phosphorylation. Inhibition of this pathway resulted in decreased MMP-2 and MMP-9 expression which arrested metastasis. Our findings may validate the use of the novel compound, *(E)-1,3-dicyclohexyl-1-(3-(3,4-dimethoxyphenyl) acryloyl)urea*, as an effective epigenetic modulator for cancer treatment.

Abbreviations

CTCL	cutaneous T cell lymphoma
CU	connect unit
DNA	deoxyribonucleic acid
ERK/MAPK	extracellular signal-regulated kinase/mitogen-activated protein kinase
FDA	Food and Drug Administration
GATA-1	globin transcription factor-1
HAT	histone acetyltransferase
HDAC	histone deacetylase
HDACi	histone deacetylase inhibitor
HDL	high-density lipoprotein
MM	multiple myeloma
MMP	matrix metalloproteinase
NF-YA	nuclear factor-YA
PTCL	peripheral T cell lymphoma
PTM	posttranslational modification
RNA	ribonucleic acid
SRM	surface recognition moiety
TRAIL	tumor necrosis factor-related apoptosis inducing ligand
TSA	trichostatin A
ZBG	zinc-binding group

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CHAPTER

9

Pharmacoepigenetics of EZH2 Inhibitors

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9.1 POLYCOMB GROUP PROTEINS

First described in *Drosophila melanogaster*, polycomb group (PcG)[1] and later trithorax group (trxG)[2] genes were shown to serve in the preservation and maintenance of gene expression programs that modulate cellular differentiation, with roles as gene repressors and activators, respectively.[3] PcG proteins are most well known for their regulatory role of HOX gene expression, a group of genes involved in the differentiation process.[4] PcG gene mutations result in altered vertebrate body plan development, suggesting a role in homeotic gene regulation. PcG genes also have crucial roles in several cellular processes including imprinting, chromosome X inactivation, cell fate, and cell senescence.[5–9] PcG proteins are composed of two known chromatin-associating complexes: polycomb-repressive complex 1 (PRC1) and polycomb-repressive complex 2 (PRC2).[10]

PRC compositions vary between cell type and differentiation status of a particular cell or tissue.[11] The PRC1 is generally made up of the real interesting new gene 1 A/B (RING1A/B), B cell-specific Moloney murine leukemia virus integration site 1 (BMI1), and polyhomeotic 1 (PH1) and chromobox (CBX) subunits.[12] The PRC2 is primarily composed of enhancer of zeste homolog 1/2 (EZH1/2), suppressor of zeste 12 (SUZ12), embryonic ectoderm development (EED), and retinoblastoma (Rb)-binding protein 7 (RBBP7) subunits; again, these subunit compositions can vary.[13, 14] The composition of PRC and the levels of particular subunits are finely regulated during both development and cell differentiation.[11] Aberrations in components of either PcG or trxG chromatin modulators have been associated with disease including several cancer variants (discussed in further detail throughout this chapter) and implications in neurodevelopmental disorders like Weaver syndrome, ataxia-telangiectasia, autism spectrum disorders, Coffin-Siris syndrome, and Nicolaides-Baraitser syndrome.[15]

PRC1 and PRC2 have been shown to work cooperatively in a highly regulated fashion to modulate gene expression. Through a reaction catalyzed by the histone methyltransferase EZH2 (Fig. 9.1), PRC2 trimethylates lysine 27 of histone 3 (H3K27) and recruits PRC1 binding via the PHC subunit that recognizes the trimethylated H3K27 (H3K27me3) mark.[16–18] PRC1 monoubiquitylates lysine 119 of histone H2A (H2AK119ub1) via catalytic subunit BMI1, and in doing so prevents the initiation of transcription via prohibition of RNA polymerase II occupancy at the tagged site (Fig. 9.2).[19–21] There is evidence of further regulation by PRC2 via association with the DNA methyltransferases (DNMTs), DNMT1, DNMT3a, and DNMT3b (Fig. 9.3),[22] as well as by PRC1 through the recruitment and binding of DMNT1 and DNMT3b.[23] However, the recruitment of DNMT3a by EZH2 is not sufficient in activating the de novo functionality of DNMT3a.[24] Chromatin immunoprecipitation and bisulfite-sequencing analysis also suggest the binding activity of DNMTs to gene promoters repressed by EZH2 to be EZH2 dependent.[22]

9.2 EZH2

EZH2 is the catalytic subunit within PRC2 responsible for H3K27me3 and, with the aid of PRC1, EZH2 regulates transcriptional processes involved in cell proliferation, differentiation, and cell cycle progression.[25] EZH2 is 746 amino acids in length and composed of 2 primary domains: the CXC domain, composed of amino acid positions 503–604, and the catalytic SET domain, composed of amino acids 605–725.[26] The CXC domain is a highly conserved cysteine-rich

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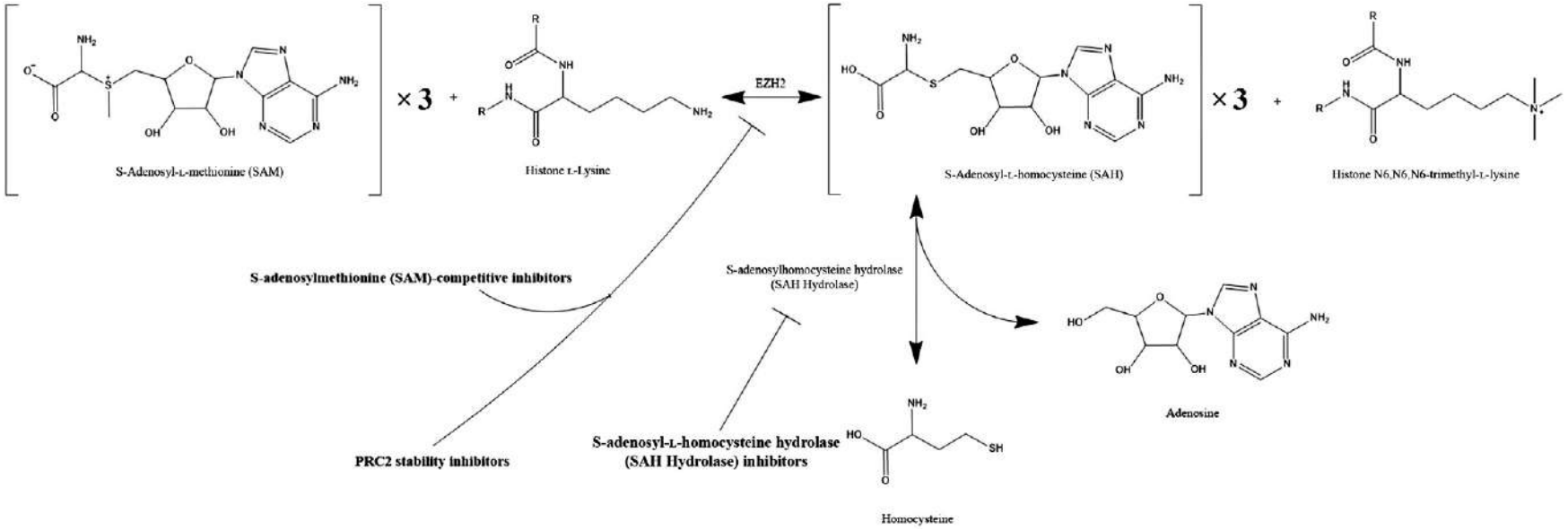

FIG. 9.1 Trimethylation reaction and mechanism of EZH2 inhibitors. *From top left*: A molecule of SAM serves as the substrate for the SET methyltransferase-binding pocket of EZH2 where a methyl group is transferred to the lysine residue of the histone protein. This process is repeated for the dimethylation and trimethylation of the final product of a trimethylated lysine residue. SAM-competitive inhibitors compete with SAM for the SET binding pocket of EZH2. PRC2 stability inhibitors prevent PRC2 formation and functionally inhibit methyltransferase function of PRC2. SAH hydrolase inhibitors target SAH hydrolase, resulting in an accumulation of SAH and negative feedback inhibition of SAM-dependent methyltransferases.

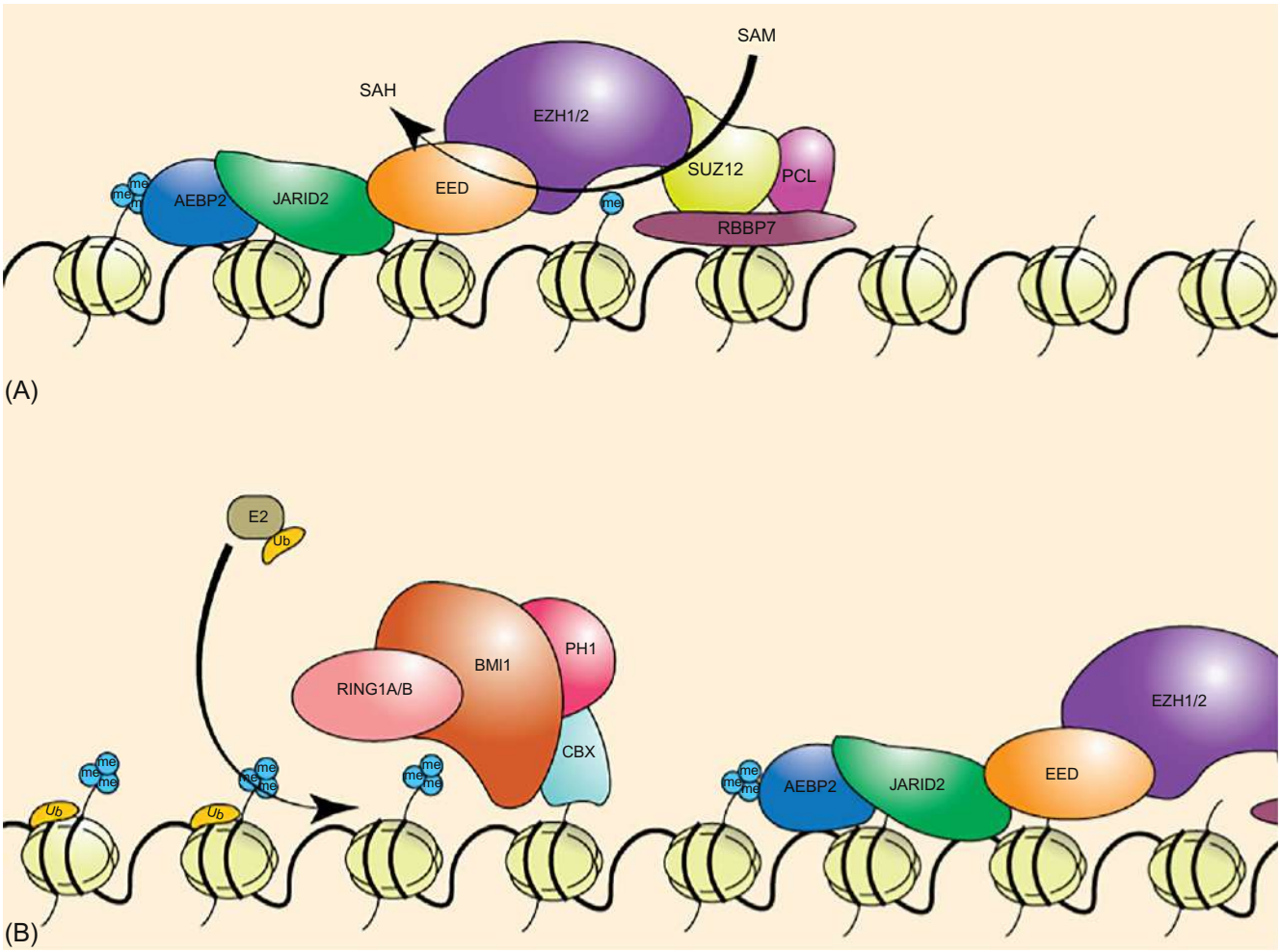

FIG. 9.2 PRC2 works in cooperation with PRC1 for gene silencing. (A) PRC2 binds the particular target of interest allowing EZH1/2 trimethylation of lysine 27 of histone 3. SAM serves as the substrate for the SET methyltransferase-binding pocket of EZH2 where methyl groups are transferred to the lysine residue of the histone protein. (B) PRC1 is recruited to the H3K27me3 mark and BMI1 monoubiquitinates lysine 119 of histone 2A, thereby stabilizing the repressive mark and preventing RNA polymerase II binding.

region containing three units of a C-X(6)-C-X(3)-C-X-C motif and is believed to be an integral domain to EZ-related proteins (Intropro IPR026489). The C-terminal SET domain contains the S-adenosyl-methionine (SAM)-binding pocket and region of methyltransferase activity.[26] There is strong evidence of conformational changes induced by EED and SUZ12 subunits being a requirement for adequate methylation of the H3K27 substrate.[26–30] The remaining PcG subunit RBBP7 acts as a histone-binding protein.[30] Often additional proteins, such as histone-binding protein retinoblastoma-binding protein 4 (RBBP4), DNA-binding protein JARID2, Zinc finger protein AE-binding protein 2 (AEBP2), and regulatory protein polycomb-like (PCL) can act as cofactors to PRC2 to further stabilize EZH2's catalytic activity (Fig. 9.2).[30–32]

EZH1, a paralog of EZH2, possesses similar catalytic ability to EZH2 as the enzymes share several overlapping target genes.[13] Unlike EZH2, EZH1 does not require the use of the cofactor S-adenosyl methionine (SAM); however, EZH1's catalytic ability is much weaker relative to EZH2.[13] The expressional patterns of EZH1 and EZH2 also differ in tissues. EZH1 can often be seen in abundance in differentiating cells, while EZH2 is primarily expressed in actively proliferating cells.[13] These expressional and efficacy differences between catalytic PRC2 subunits suggest variable roles within the cell during development. EZH1 may be responsible for the restoration of H3K27me3 profiles, while EZH2 is responsible for the establishment of H3K27me3-repressive marks.[33] The exact mechanism of gene targeting by EZH2 within the PRC2 is not fully understood; however, it is theorized that recruiting factors are a requirement directing PRC2 complexing to target genes.[34] A single transcription factor with target genes common to the PRC2 has not been identified. Instead, it is suggested that recruitment is cell specific and dependent on several transcription factors.[30, 35] One such transcription factor is YY1, which displays some overlap in PRC2 target genes in mouse stem cells.[36]

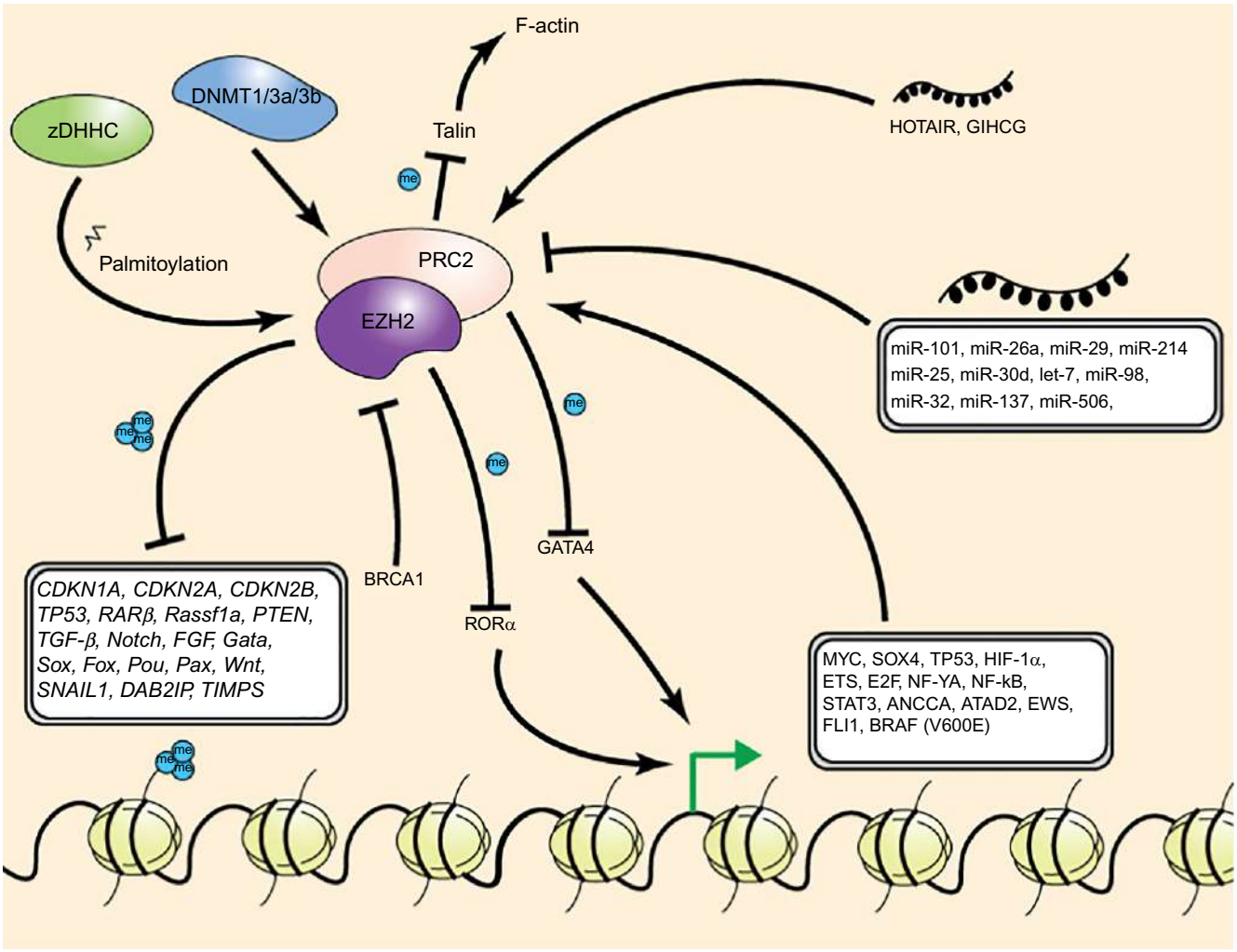

FIG. 9.3 Interactions of EZH2 within PRC2. *From top left*: Palmitoylation of EZH2 by zinc finger DHHC-type containing 5 (zDHHC) increases EZH2-mediated trimethylation of H3K27. PRC2 recruits and associates with DNA methyltransferases (DNMTs), DNMT1, DNMT3a, and DNMT3b. EZH2 directly methylates talin to prevent talin-F-actin binding. HOTAIR and GIHCG bind EZH2 and direct PRC2 to target genes. miR-101, miR-26a, miR-29, miR-214, miR-25, miR-30d, let-7, let-98, let-32, let-137, and let-506 target and downregulate the *EZH2* transcript. MYC, SOX4, TP53, HIF-1α, ETS, E2F, NF-YA, NFκB, STAT3, ANCCA/ATAD2, EWS/FLI1, and mutant BRAF (V600E) function as transcription factors of EZH2. EZH2 directly methylates cardiac transcription factor GATA4 at K299, silencing GATA4 gene targets. EZH2 directly methylates RAR-related orphan receptor alpha (RORα) to promote ubiquitination, resulting in RORα target gene silencing. Breast cancer susceptibility gene 1 (BRCA1) protein binds EZH2 and inhibits the binding of PRC2 to target genes. *CDKN1A*, *CDKN2A*, *CDKN2B*, *TP53*, *RARβ*, *RASSF1A*, *PTEN*, *TGFβ*, *NOTCH*, *FGF*, *NANOG*, *OCT4*, *Gata*, *SOX*, *FOX*, *Pou*, *PAX*, *WNT*, *SNAIL1*, *DAB2IP*, and *TIMP2* are known gene targets of PRC2.

TWIST-1 has shown the ability to encourage recruitment of EZH2 to the *ARK-INK4A* locus to silence the expression of both p14 and p16 proteins in mesenchymal stem cells[37]; additionally, MYCN is able to recruit EZH2 to the *CLU* promoter to promote tumorigenesis in neuroblastoma.[38]

It has been shown that histone protein H1 is necessary for the preferential binding of EZH2.[39] Chromodomain Y-like protein (CDYL) may play a role in the specific targeting of genes as well. In a genome-wide study of PRC2 target genes and CDYL target genes, significant overlap between the two was present.[40] Additionally, CDYL directly interacts with EZH2 and recognizes H3K27me2/3 modifications. When bound to the PRC2, CDYL enhances the enzymatic methyltransferase activity of EZH2 at their common target promoters.[40] A member of the Jumonji C family and DNA-binding subunit of the PRC2, JARID2, is suggested to play a role in PRC2 modulation.[41–44] The mechanism of modulation is not well understood, as knockdown studies have resulted in both increased and decreased H3K27me3, depending on observed genes and cell type.[41–44] PRC2 recruitment to target genes can also be mediated by long noncoding RNAs (lncRNAs) in a tissue-specific manner (Fig. 9.3).[45,46] HOTAIR is one such lncRNA that has direct evidence of interaction with PRC2, binding EZH2, and encouraging recruitment to the *HOXD* locus in human fibroblasts.[46] Breast cancer

susceptibility gene 1 (BRCA1) protein is also able to take advantage of the same binding domain as HOTAIR, instead having the opposite effect and inhibiting the binding of PRC2 to target genes (Fig. 9.3).[47]

The aberrant expression of EZH2 has been highly associated with carcinogenesis in several cancer types.[33, 48] However, the ways in which EZH2 functioning has been altered is variable in different cancers. Often EZH2 overexpression is described in solid tumors, activating or inactivating mutations are present in hematologic cancers, and a Lys27Met (K27M) in the gene encoding histone H3.3 (H3F3A) mutation is frequently present in pediatric gliomas (reviewed in Ref. 33).

9.2.1 Regulation of EZH2 From Transcription to Translation

Several transcription factors have been implicated in the regulation of EZH2 expression (Fig. 9.3). The most well-known transcription factors that have been shown to directly induce transcription of EZH2 include MYC, TP53, ETS, SOX4, HIF-1α, and E2F in various cancers including prostate,[49–52] bladder,[53] small-cell lung cancers,[54] breast,[55–57] melanoma,[58] and head and neck squamous cell carcinomas (HNSCC).[59] NF-YA, NFκB, STAT3, ANCCA/ATAD2, EWS/FLI1, and mutant BRAF (V600E) have also been shown to regulate EZH2 expression by a currently unknown mechanism in various other cancers including ovarian,[60] T cell leukemia,[61] colorectal,[62] breast,[51] and Ewing sarcoma.[63] The MEK-ERK1/2-ELK1 pathway has also been demonstrated to regulate EZH2 overexpression in pancreatic cancer,[64] triple-negative, and ERBB2-positive breast cancers,[65] as well as lung cancers.[66]

Once transcribed, EZH2 mRNA can be bound at the 3′ UTR by various microRNAs (miRs, miRNAs) that have the ability to modulate mRNA integrity and, ultimately, its translation into protein (Fig. 9.3).[67] In certain cancers the downregulation of specific miRs has led to the overexpression of EZH2 and associated tumor progression secondary to H3K27me3-mediated gene silencing. As reviewed by Benetatos et al.[68] EZH2 is negatively regulated by several miRs including miR-101, miR-26a, miR-29, and miR-214. As expected, the downregulation of these particular miRs is frequently implicated in the progression of disease. In nasopharyngeal carcinoma, miR-26a directly targets and suppresses EZH2 translation.[69] Similar results were shown in glioblastoma cells, where downregulation of miR-101 promoted EZH2-mediated tumor angiogenesis, cell proliferation, and invasion by overexpressed cytoplasmic polyadenylation element-binding protein 1 (CPEB1).[70] The downregulation of miR-25, miR-30d, let-7, miR-98, and miR-29 have also been implicated in EZH2-mediated carcinogenesis of several cancers including thyroid carcinoma, prostate cancer, esophageal squamous cell carcinoma, and rhabdomyosarcomas.[71–74] miRNA-32, miR-137, and miR-506 also directly target the EZH2 transcript, thereby suppressing tumor proliferation, angiogenesis, and metastasis in melanoma, colon cancer, and glioblastoma.[75–77] Sensitization to chemotherapy was also seen upon increasing miR-126 and miR-138 in osteosarcoma and gastric cancer cells.[78, 79]

Translated EZH2 is able to undergo further modification and regulation by several enzymes (Fig. 9.4). Phosphorylation at S21 by AKT1 (Fig. 9.4A) allows EZH2 to act as a transcriptional coactivator for both androgen receptor-associated complexes, as well as STAT3, whose activation has led to the development and progression of castration-resistant prostate cancer (CRPC)[80] and enhanced tumorigenicity of glioblastoma stem-like cells (GSCs),[81] respectively. Cyclin-dependent kinases 1, 2 (CDK1/2) and cyclin E have also been shown to regulate EZH2 via phosphorylation (Fig. 9.4C). The location of modification, however, greatly changes EZH2 activity. Phosphorylation by cyclin E and CDK2 at T416 enhances EZH2 activity in triple-negative breast cancer, promoting tumor formation, growth, and migration.[82] Janus kinase 3 (JAK3) has also been shown to phosphorylate EZH2 on Y244, thereby switching EZH2 activity into a transcriptional activator and promoting the survival and proliferation of NK/T cell lymphoma cells (Fig. 9.4A).[83] NIMA-related kinase 2 (NEK2) has been shown to form a complex with and phosphorylate EZH2 in gliomas, protecting EZH2 from ubiquitination-dependent protein degradation (Fig. 9.4D).[84] Palmitoylation of EZH2 by gene-encoding zinc finger DHHC-type containing 5 (zDHHC) has displayed EZH2-mediated trimethylation of H3K27, thereby increasing tumorigenicity in GSCs (Fig. 9.3).[85] Phosphorylation by CDK1 and CDK2 at T345 of EZH2 allows binding of regulatory lncRNA HOTAIR to EZH2 and has shown the capacity to recruit PRC2 binding to target genes.[86] In contrast, phosphorylation at T492 and T350 by CDK1 and CDK2 greatly decreases EZH2 methyltransferase activity through the disruption of PRC2 formation and promotes ubiquitinylation-mediated degradation.[87, 88] JAK2-mediated phosphorylation of Y641 also directs the ubiquitination and degradation of EZH2.[89] Following DNA damage, poly ADP-ribose polymerase 1 (PARP1)-mediated PARylation of EZH2 induces PRC2 complex dissociation and EZH2 downregulation.[90] Phosphorylation of EZH2 at T487 has also been shown; however, there is conflicting evidence as to its regulatory role on EZH2, as it has been implicated in both the activation and inhibition of EZH2.[86, 87]

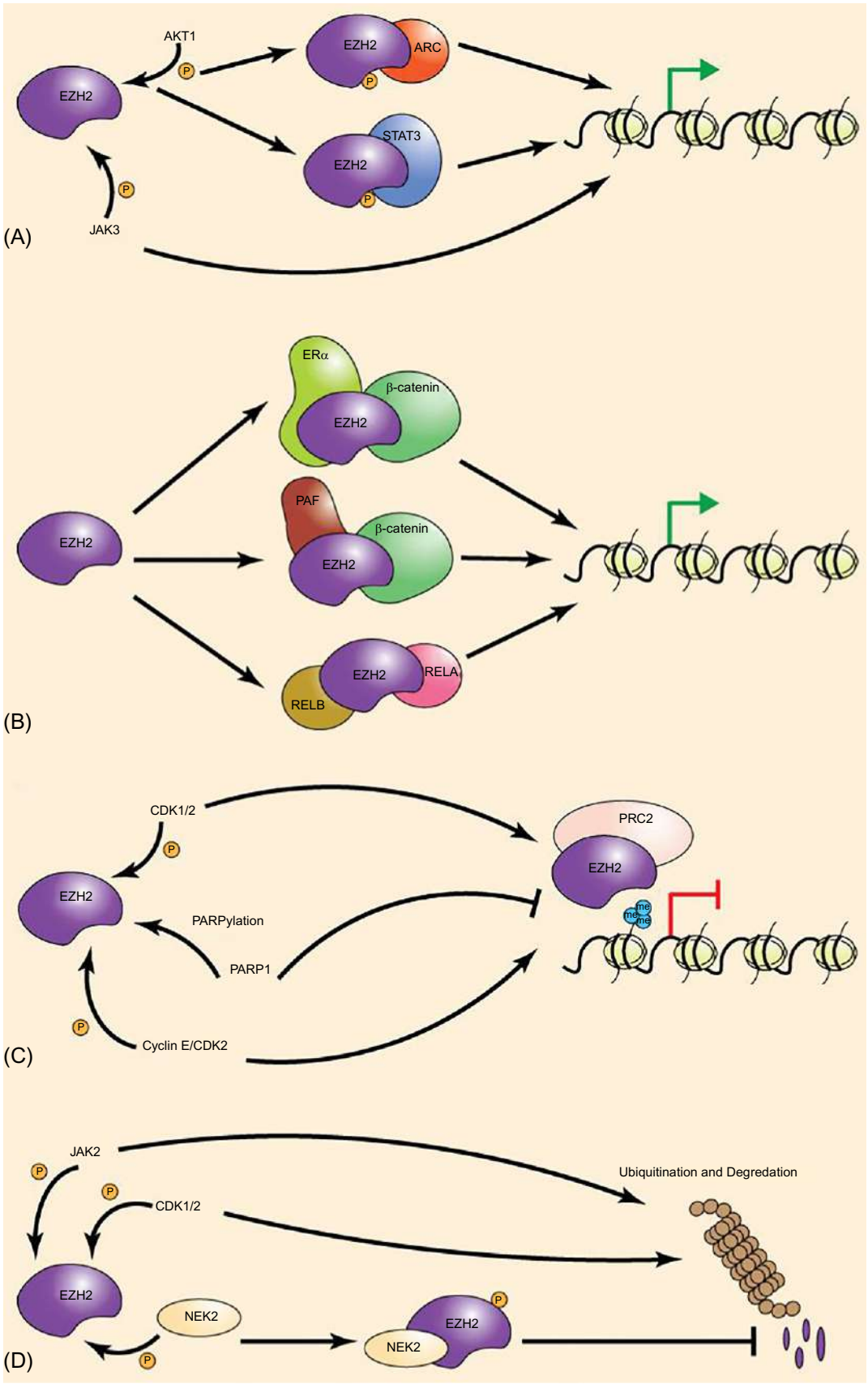

FIG. 9.4 EZH2 interactions outside PRC2. (A) AKT1 phosphorylates EZH2 at S21 which allows for the binding and transcriptional activation of androgen receptor-associated complexes (ARCs) or STAT3. Janus kinase 3 (JAK3) phosphorylates EZH2 on Y244, and EZH2 acts as a transcriptional activator. (B) EZH2 complexes with the estrogen receptor α and β-catenin or β-catenin and PCNA-associated factor (PAF) to enhance WNT pathway target genes. EZH2 can also interact with RELA and RELB to activate NFκB target genes. (C) Phosphorylation of T345 by CDK1 and CDK2 allows binding of regulatory lncRNA HOTAIR to EZH2, recruiting PRC2 binding to target genes. Poly ADP-ribose polymerase 1 (PARP1)-mediated PARylation of EZH2 induces PRC2 complex dissociation and EZH2 downregulation. Phosphorylation by cyclin E and CDK2 at T416 enhances EZH2 activity. (D) JAK2-mediated phosphorylation of Y641 promotes ubiquitination and degradation of EZH2. Phosphorylation of T492 and T350 by CDK1 and CDK2 greatly decreases EZH2 methyltransferase activity by disrupting PRC2 formation and promotes ubiquitinylation-mediated degradation of EZH2. NIMA-related kinase 2 (NEK2) complexes and phosphorylates EZH2, protecting EZH2 from ubiquitination-dependent degradation.

9.2.2 PRC2-Mediated EZH2 Targets

Mouse embryonic stem cell genome mapping of PcG target genes, denoted by increased H3K27me3-repressive marks, has revealed over 2000 different sites affecting numerous genes encoding key developmental regulators and signaling proteins.[91] Within normal cells EZH2 is partly responsible for the epigenetic regulation of transcriptional activity via silencing multiple genes, including those responsible for morphogenesis[92] and cell fate responses following DNA damage.[93] Solid tumors overexpressing EZH2 are frequently found to display an increased tumorigenicity, malignancy potential, and an overall poor clinical prognosis.[33] The primary mechanism behind EZH2-mediated oncogenesis is believed to be the result of the silencing of tumor suppressor genes, including *CDKN1A*, *CDKN2A*, *CDKN2B*, *TP53*, *RARβ*, *RASSF1A*, and *PTEN*.[94–102] However, given the widespread nature of PRC2 gene silencing,[91] evidence of PRC2 displaying a tumor suppressor role is also present.[103, 104] Its variable role is most likely to be dependent on which genes are being silenced.

PRC2 has also been shown to target, both directly and indirectly, numerous genes noted in regulating cell differentiation including *TGFβ*, *NOTCH*, *FGF*, *NANOG*, *OCT4*, *Gata*, *SOX*, *FOX*, *Pou*, *PAX*, and *WNT* (Fig. 9.3). The inhibition of these genes contributes to the maintenance of undifferentiated states and stem cell-like properties, in addition to the promotion of cell proliferation and tumorigenesis.[91, 105–108] There is also compelling evidence of EZH2 promoting epithelial-mesenchymal transition (EMT) in several cancer types, whereby cells develop a metastatic and invasive phenotype. Certain targets of EZH2 such as SNAIL1 in prostate and breast cancer cells,[109] DAB2IP silencing in colorectal cancer,[110] and TIMP2 in ovarian cancer cells[111] have been associated with EMT.

EZH2 may also cooperate with lncRNAs to silence target genes (Fig. 9.3).[46, 96, 112] As mentioned previously, phosphorylated T345 of EZH2 encourages the binding of HOTAIR, which encourages recruitment of PRC2 binding to target genes.[86] An additional lncRNA includes GIHCG, which is upregulated in hepatocellular carcinoma tissues.[113] GIHCG associates with both EZH2 and the promoter of miR200b/a/429, a miRNA involved in hepatocellular carcinoma tumor progression.[114] PRC2 methylation targets are not limited to histone proteins. EZH2 has been shown to directly methylate cardiac transcription factor GATA4 at K299, resulting in gene silencing of GATA4 gene targets,[92] RAR-related orphan receptor alpha (RORα) methylation at K38 to promote ubiquitination and lead to RORα target gene silencing,[115] as well as direct methylation of talin to prevent talin-F–actin binding (Fig. 9.3).[116]

9.2.3 EZH2 Targets Outside PRC2

EZH2 also has autonomous enzymatic activity independent of PRC2 (Fig. 9.4). When phosphorylated at S21, EZH2 can act as a coactivator for androgen receptor-associated complexes, as well as an activator of STAT3.[80, 81] In estrogen receptor-positive MCF-7 cells, EZH2 complexes with β-catenin and the estrogen receptor α to enhance WNT pathway signaling, including transcriptional activation of *CCND1* as well as *MYC*.[117] Similar coupling is present in colon cancers, where EZH2 complexes with β-catenin and PCNA-associated factor (PAF) to activate WNT target genes *MYC*, *CCND1*, and *AXIN2*.[118] EZH2 can also interact with RELA and RELB to activate NFκB target genes, including *TNF* and *IL6*, in estrogen receptor-negative MDA-MB-231 breast cancer cells.[119]

9.2.4 Common EZH2 Mutations

A frequently occurring EZH2 mutant in lymphomas is the Y641F mutant, located within the SET domain, which results in increased EZH2 activity.[120] Spleen cells containing Y641F mutants, in combination with Eμ-Myc expression, displayed the expected increase in H3K27me3 levels in vivo.[121] Additional mutants EZH2-A677 and EZH2-A687, also within the catalytic SET domain, display hypermethylated H3K27 representative of increased EZH2 activity in lymphoma models.[122–124] Additionally, two different single-nucleotide polymorphisms (SNPs) of *EZH2*, *rs6950683* and *rs3757441*, have been associated with a higher risk of lymph node metastasis in patients with hepatocellular carcinomas.[125]

9.2.5 Viral Interactions and EZH2

Viruses have been shown to display unique properties in various cell types, with viral proteins being the direct cause of disease including oncogenesis. Human papillomavirus (HPV) is one such virus and has been directly implicated in both cervical and HNSCC.[126] HPV-positive tumors are associated with increased EZH2 expression and genome-wide H3K27 hypermethylation.[127] This is the result of viral oncoprotein HPV-E7 interacting with the retinoblastoma (RB) protein, thereby releasing transcription factor E2F to transcribe downstream targets including EZH2.[59] The suppression of P53 by HPV-E6 may also provide a mechanism for EZH2 overexpression, as p53 activation leads to

repression of the EZH2 promoter.[59, 128] Hepatitis B virus oncoprotein HBx acts in a similar manner to HPV-E7 and releases E2F via binding to RB. This in turn activates EZH2 transcription.[129–131]

In vitro data suggest EZH2 may also have a regulatory role in the life cycle of human immunodeficiency virus-1 (HIV-1). In HIV-1-infected Jurkat cells, inhibition of EZH2 by GSK 343 and EPZ-6438 resulted in the reactivation of latent proviruses, suggesting their use as latency-reversing agents in the clinic.[132] Additionally, PRC2 subunit EED has been shown to directly interact with the HIV-1 proteins matrix (MA), integrase (IN), as well as regulatory protein NEF. The knockdown of EED resulted in a proviral reactivation of HIV-1 as well.[133–135]

The Epstein–Barr virus (EBV) utilizes components of PRC2 to repress numerous target genes affecting cell proliferation, differentiation, and apoptosis and has been implicated in nasopharyngeal carcinomas, gastric carcinomas, and a variety of B cell lymphomas.[136] Research on the Epstein-Barr nuclear antigen 3 proteins (EBNA3) reveals these proteins are capable of directly recruiting members of the PRC2 including EZH2 to target the tumor suppressor genes *CDKN2A*, *CDKN2B*, and *CDKN2C*, antiapoptotic *BCL2L11*, and the differentiation regulator gene *PRDM1*. Recently, EBV's EBNA3 proteins have been shown to repress *CXL9* and *CXL10* cytokine genes through a PRC2-mediated mechanism.[137]

9.3 CATEGORIES OF EZH2 INHIBITORS

Current strategies for disrupting the function and carcinogenic effects of EZH2 fall under three main categories: S-adenosyl-L-homocysteine hydrolase inhibitors, SAM-competitive inhibiting compounds, and inhibitors that disrupt PRC2 stability.

9.3.1 S-Adenosyl-L-Homocysteine Hydrolase Inhibitors

S-adenosyl-L-homocysteine (SAH) is a by-product of transmethylation reactions that utilize SAM as a methyl group donor. SAM is the most common donor for methyltransferases responsible for methylation of DNA, RNA, and numerous proteins including histones.[138] SAH is hydrolysed into adenosine and L-homocysteine through a reversible reaction catalyzed by S-adenosyl-L-homocysteine hydrolase (SAHH). Inhibition of SAHH results in increased cellular SAH levels, which repress the activity of SAM-dependent histone lysine methyltransferases through a negative feedback loop.[139] 3-Deazaneplanocin A (DZNeP) is a carbocyclic analog of adenosine and highly potent small-molecule inhibitor of SAH-hydrolase and one of the first discovered inhibitors of EZH2.[140] In vitro studies utilizing DZNeP have shown it to decrease c-Myc expression in GSC lines,[141] display apoptotic and antiproliferative activity in breast cancer cell lines,[142] impair growth and decrease the number of tumor-initiating epithelial cell adhesion molecule-positive cells in a hepatocellular carcinoma cell line,[143] inhibit proliferation, clonogenicity, tumorigenicity, and migration in malignant pleural mesothelioma cells,[144] induce apoptosis in acute myeloid leukemia cells,[145] and reduce cancer stem cell markers in prostate cancer cell lines.[146] Interestingly, HNSCC cell lines treated with DZNeP showed a reduction in H3K27me3 in HPV-negative cells, whereas HPV-positive cells were comparable with control groups.[147] In this same study DZNeP was able to demonstrate dramatic changes in gene expression regardless of HPV status. The study authors suggested the global effects on histone methyltranferases, rather than EZH2 alone, to be the reasoning behind their unique findings. In vivo, DZNeP has been shown to reduce tumor-mitigated angiogenesis without significant toxic effects in a glioblastoma xenograft model[148] and reduce tumor size and invasion in a prostate cancer model.[146] DZNeP was reported to result in toxicity at higher doses in animal models.[149]

9.3.2 S-Adenosylmethionine (SAM)-Competitive Inhibiting Compounds

SAM-competitive compounds are molecules that mimic the structure of SAM and competitively bind to EZH2 and other methyltransferases with various affinities and specificities, thereby interfering with their transmethylation efficiency. Current SAM-competitive inhibitors being researched include CPI-1205, EPZ005687, EPZ-6438, EPZ011989, GSK343, GSK126, GSK926, EI1, UNC1999, ZLD1039, and PF-06726304.

Treatment of a KARPAS-422 lymphoma xenograft model for 25 days with CPI-1205 achieved >97% tumor growth inhibition and was well tolerated in a 28-day toxicology study on Sprague-Dawley rats and beagle dogs.[150] CPI-1205 is one of two EZH2 inhibitors currently in the clinical trial stage of their implementation, with a phase I trial for its use for patients with progressive B cell lymphomas (NCT02395601) and phase I and II trials in combination with enzalutamide or abiraterone/prednisone in metastatic castration-resistant prostate cancer (NCT03480646).

Lymphoma cell lines harboring wild-type or mutant EZH2 showed a concentration-dependent reduction in H3K27 methylation when treated with EPZ005687. Furthermore, concentration-dependent apoptosis was also reported in mutant EZH2 cell lines.[151] However, EPZ005687 was unable to reduce H3K27me3 levels in HNSCC cell lines.[147]

GSK126 was able to inhibit growth and induce transcriptional activation of PRC2 target genes in a panel of mutant lymphoma cell lines as well as inhibit H3K27 methylation and tumor growth in a KARPAS-422 xenograft model.[152]

EPZ-6438 is an orally available SAM-competitive inhibitor of EZH2 which, like EPZ005687, showed reduction of H3K27 methylation in both EZH2-mutant and wild-type lymphoma cell lines, and apoptosis in EZH2-mutant cells.[153] The authors further report complete and sustained tumor regressions along with dose-dependent tumor growth inhibition in a non-Hodgkin lymphoma EZH2-mutant xenograft model. EPZ-6438 has been approved for FDA fast-track designation[154] and is currently undergoing several phase I and II clinical trials for lymphoma variants (NCT03010982, NCT03028103, NCT02889523, NCT01897571, NCT02875548, NCT03217253, NCT03213665, NCT03028103, NCT02889523, NCT02220842, NCT03456726, NCT03009344, NCT03155620), a phase II trial for grade I and II endometrial endometrioid adenocarcinomas, recurrent ovarian carcinomas, recurrent primary peritoneal carcinomas, and recurrent uterine corpus carcinomas (NCT03348631), a phase II trial for malignant mesotheliomas (NCT02860286), a phase I trial for atypical teratoid and malignant rhabdoid tumors, rhabdoid tumors of the kidney, and INI1-negative tumors (NCT02601937), and a phase II trial for malignant rhabdoid tumor of the ovary, relapsed/refractory synovial sarcomas, INI1-negative tumors or any solid tumor with a gain-of-function mutation for EZH2, renal medullary carcinomas, epithelioid sarcomas, and poorly differentiated chordomas (NCT02601950).

Similar to EPZ-6438, EPZ011989 is potent, orally available, and showed significant tumor growth inhibition in an EZH2-mutant lymphoma xenograft model.[155]

HNSCC cell lines treated with GSK-343 showed a dramatic reduction in H3K27me3 levels as well as gene expression changes.[147] Treatment of breast cancer, colon cancer, and leukemia cell lines with GSK343 resulted in significant selective gene expression changes.[156] Autophagy induction, enhanced drug sensitivity, and reduced viability have been reported in hepatocellular carcinoma cells treated with GSK343.[157] In 2015 Ding et al.[158] reported on GSK343's ability to reduce proliferation, invasion, and epithelial–mesenchymal transition of cervical cancer cells both in vitro and in vivo.

Little research has been carried out on GSK926, although early results suggest it shares GSK343's ability to lower H3K27 methylation in several breast and prostate cancer cell lines.[159]

EI1 was developed in 2012 and was reported to be specific in reducing H3K27 methylation selectively. In vitro assays showed EI1 to effectively reduce proliferation and induce cell cycle arrest and apoptosis in lymphoma cell lines either overexpressing wild-type EZH2 or harboring mutant EZH2.[95]

UNC1999 is an orally bioavailable SAM-specific inhibitor of both EZH2 and EZH1 that effectively reduced H3K27 methylation in cells and selectively killed heterozygous EZH2 Y641N-mutant lymphoma cells.[160]

Another orally available SAM-competitive inhibitor ZLD1039 is highly specific for EZH2 when tested across a panel of histone methyl transferases.[161] The authors (Song et al.) further report on ZLD1039's ability to reduce H3K27 methylation, upregulate tumor suppressor genes, reduce proliferation, and induce apoptosis in breast cancer cell lines. When mouse breast cancer xenograft models were treated with ZLD1039 they showed reduced tumor growth and metastasis.

PF 06726304, developed in 2016, has been shown to increase the expression of PRC2 target genes and inhibit tumor growth in a KARPAS-422 lymphoma xenograft model.[162, 163]

9.3.3 Inhibitors That Disrupt PRC2 Stability

Since its interaction with proteins of the PRC2 complex is a requirement for EZH2's ability to methylate H3K27, molecules that can disrupt these interactions offer an attractive option of EZH2 inhibition. Molecules of this class include astemizole, SAH-EZH2, and GNA022.

Astemizole is an FDA-approved drug that was reported in 2014 to have the ability to destabilize the PRC2 complex by binding to EED in a competitive manner with EZH2 and inhibit the proliferation of treated lymphoma cell lines.[164]

The EED-binding domain of EZH2 contains an alpha-helical structure that Woojin et al. exploited to develop a hydrocarbon-stapled peptide, SAH-EZH2, which successfully interrupts the EZH2-EED complex. Treatment with the peptide resulted in a reduction of EZH2 protein, while displaying growth arrest and differentiation of MLL-AF9 cells.[165]

Recently, Wang et al. reported on a molecule, GNA022, that has the ability to covalently bind the Cys668 residue within the SET domain of EZH2, thereby disrupting its ability to associate with PRC2.[166] Furthermore, the authors show that the covalent modification of EZH2 leads to its ubiquitin-mediated degradation (Table 9.1).

TABLE 9.1 Summary of Known EZH2 Inhibitors

Inhibitor	Mechanism	Findings	Clinical status	Clinical trials	References
3-Deazaneplanocin A (DZNeP)	SAHH inhibitor	Decreases c-Myc expression in GSC lines; induces apoptosis and antiproliferative activity in breast cancer cell lines; impairs growth; decreases the number of tumor-initiating epithelial cell adhesion molecule-positive cells in a hepatocellular carcinoma cell line; inhibits proliferation, clonogenicity, tumorigenicity, and migration in malignant pleural mesothelioma cells; induces apoptosis in acute myeloid leukemia cells; reduces cancer stem cell markers in prostate cancer cell lines; reduces H3K27me3 in HPV-negative cells, gene expression change in both HPV-positive and HPV-negative cells in HNSCC cell lines; reduces tumor-mitigated angiogenesis in glioblastoma in vivo; reduces tumor size and invasion in prostate cancer in vivo	Preclinical	N/A	140–149
CPI-1205	SAM-competitive inhibitor	Inhibits tumor growth in lymphoma xenograft models	Phase I/II	B cell lymphoma (NCT02395601); Metastatic castration-resistant prostate cancer (NCT03480646)	150
EPZ005687	SAM-competitive inhibitor	Reduces H3K27me3 in lymphoma cell lines harboring either wild-type or mutant EZH2; induces apoptosis in mutant EZH2-containing cell lines	Preclinical	N/A	151
EPZ-6438	SAM-competitive inhibitor	Reduces H3K27me3 in lymphoma cell lines harboring either wild-type or mutant EZH2; induces apoptosis in mutant EZH2-containing cell lines; induces sustained tumor regressions in non-Hodgkin lymphoma and EZH2-mutant xenograft models; reactivates latent proviruses in HIV-1-infected Jurkat cells	Phase I/II	Lymphomas (NCT03010982, NCT03028103, NCT02889523, NCT01897571, NCT02875548, NCT03217253, NCT03213665, NCT03028103, NCT02889523, NCT02220842, NCT03456726, NCT03009344, NCT03155620); Ovarian, endometrioid, and peritoneal cancers (NCT03348631); Mesotheliomas (NCT02860286); Tumors with rhabdoid features, synovial sarcomas, INI1-negative tumors, or any solid tumor with a gain-of-function mutation for EZH2, renal medullary carcinomas, epithelioid sarcomas, chordomas (NCT02601950, NCT02601937)	132, 153
EPZ011989	SAM-competitive inhibitor	Inhibits tumor growth in EZH2-mutant lymphoma xenograft models	Preclinical	N/A	155
GSK343	SAM-competitive inhibitor	Reduces H3K27me3 in HPV-positive and HPV-negative cells; induces gene expression changes in HNSCC cell lines; induces selective gene expression changes in breast cancer, colon cancer, and leukemia cell lines; induces autophagy, drug sensitivity; reduces viability in hepatocellular carcinoma cells; reduces proliferation, invasion, and epithelial–mesenchymal transition of cervical cancer cells both in vitro and in vivo; reactivates latent proviruses in HIV-1-infected Jurkat cells	Preclinical	N/A	132, 147, 156–158

GSK126	SAM-competitive inhibitor	Inhibits growth; induces transcriptional activation of PRC2 target genes in mutant lymphoma cell lines; inhibits H3K27 methylation and tumor growth in lymphoma xenograft models	Preclinical	N/A	152
GSK926	SAM-competitive inhibitor	Reduces H3K27me3 in breast and prostate cancer cell lines	Preclinical	N/A	159
EI1	SAM-competitive inhibitor	Reduces proliferation; induces cell cycle arrest, and apoptosis in lymphoma cell lines	Preclinical	N/A	95
UNC1999	SAM-competitive inhibitor	Reduces H3K27 methylation in cells; selectively kills heterozygous EZH2 Y641N-mutant lymphoma cells	Preclinical	N/A	160
ZLD1039	SAM-competitive inhibitor	Reduces H3K27 methylation; upregulates tumor suppressor genes; reduces proliferation; induces apoptosis in breast cancer cell lines; reduces tumor growth and metastasis in breast cancer xenograft models	Preclinical	N/A	161
PF-06726304	SAM-competitive inhibitor	Increases expression of PRC2 target genes; inhibits tumor growth in lymphoma xenograft models	Preclinical	N/A	162, 163
Astemizole	PRC2 disruption	Inhibits proliferation in lymphoma cell lines	Preclinical	N/A	164
SAH-EZH2	PRC2 disruption	Reduces EZH2 protein; inhibits growth arrest and differentiation in leukemia stem cell lines	Preclinical	N/A	165
GNA022	PRC2 disruption	Induces degradation of EZH2	Preclinical	N/A	166

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CHAPTER

10

Regulators of Histone Acetylation: Bromodomain Inhibitors

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10.1 INTRODUCTION

The acetylation of histones is an important posttranslational modification, together with methylation, phosphorylation, SUMOylation, and ubiquitination, and is regulated by so-called "writers" (such as histone acetyl transferases (HATs)), "readers" (such as bromodomains), and "erasers" [such as histone deacetylases (HDACs)), all of which also act on nonhistone proteins.[1] Acetylation has two primary functional roles—to neutralize positive charges and to act as an epigenetic marker—and thereby serves to modulate many DNA-dependent cellular processes, including transcription, chromatin remodeling, the cell cycle, cell differentiation, and DNA damage/repair. Dysfunction of these processes is involved in the pathogenesis of a diverse range of diseases, including cancers and inflammatory diseases. Therefore, small-molecule modulators of epigenetic regulators are expected to be candidates for epigenetic therapy.[2]

Neutralization of the positive charges on histone, which is controlled by writers and erasers, leads to nucleosome destabilization and the creation of an open chromatin architecture, and this facilitates the recruitment of transcription-associated factors, such as RNA polymerase, to nucleosomes. Notably, several inhibitors of epigenetic writers, including the HDAC inhibitors vorinostat (a.k.a. SAHA), romidepsin (a.k.a. FK228), belinostat (a.k.a. PXD101), and panobinostat (a.k.a. LBH-589), have already been approved for clinical use by regulatory authorities such as the US Food and Drug Administration (FDA) or are under clinical trial.

Acetylated lysine residue (Kac) is an epigenetic marker that is recognized by readers such as the bromodomain and YEATS domain. Although both the bromodomain and the YEATS domain bind to acetylated lysine residue (Kac), their structures are quite different.

The bromodomain was first identified as a result of studies on the Brahma gene in *Drosophila*,[3,4] and it is now known that 61 bromodomains are encoded in the human genome.[5] They are present in 46 diverse proteins, including histone acetyltransferases (HATs) (such as PCAF, CREBBP, and P300), ATP-dependent chromatin-remodeling complexes (such as BAZ1A), histone methyltransferases (such as ASH1L), and transcriptional regulators (such as the bromodomain and extraterminal domain (BET) family). The bromodomain is an ~110 amino acid module that forms an evolutionarily conserved left-handed up-and-down four-helix bundle (helices α_z, α_a, α_b, and α_c) with a pronounced cleft between two loops (termed the ZA loop and the BC loop) (Fig. 10.1).[6] The bromodomain preferentially recognizes Nε-Kac of histones (Fig. 10.2) and serves as a regulator of protein-protein interactions in numerous cellular processes, including transcription and chromatin remodeling.[7–9] There have been many synthetic studies on bromodomain inhibitors, although BET bromodomain inhibitors are currently the most extensively studied; indeed, some of them are already under evaluation in clinical trials (see below).

The YEATS domain (the name is an acronym based on proteins containing this domain: Yaf9, ENL, AF9, Taf14, and Sas5) was identified as another epigenetic reader only recently.[10] It recognizes not only Kac, but also crotonoylated lysine residues on histones. Human YEATS domain family members, such as AF9, are strongly linked to cancer, and thus development of YEATS domain inhibitors is considered a potential new avenue for cancer chemotherapy.

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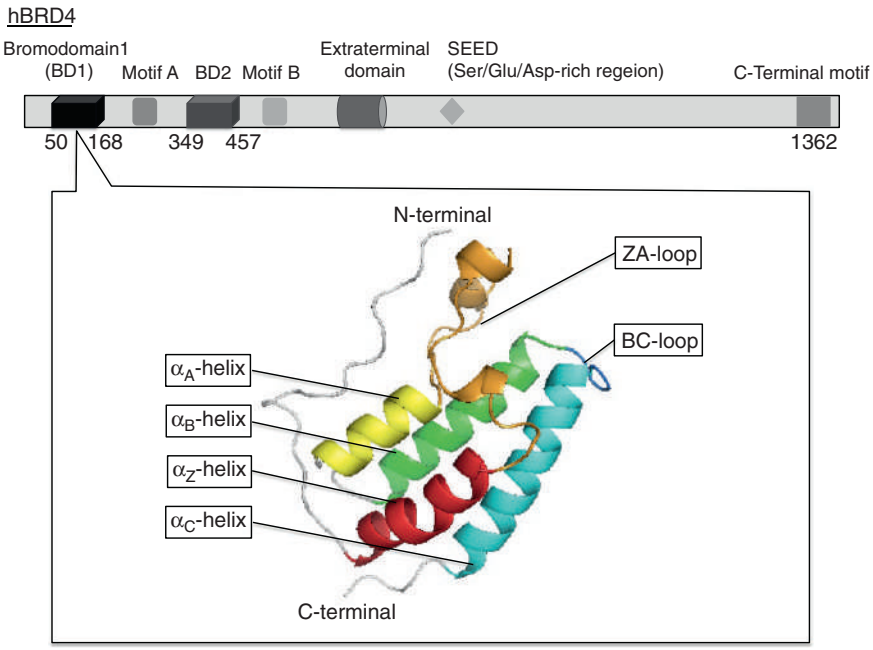


FIG. 10.1 Structure of bromodomain.

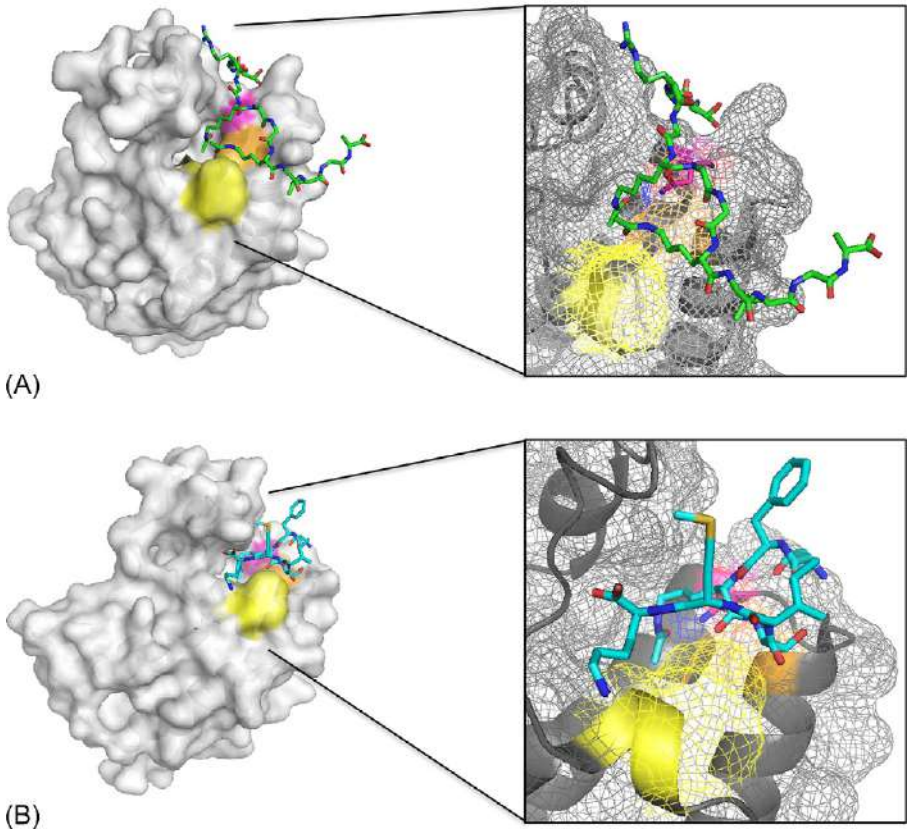


FIG. 10.2 Crystal structures of BRD4 BD1 complexes with (A) diacetylated histone 4 peptide (H4K5acK8ac) (PDB: 3UVW) and (B) acetylated RelA (PDB: 4KV1). *Yellow*, WPF shelf; *orange*, I146; *magenta*, N140.

10.2 BET FAMILY PROTEINS

10.2.1 Features of BET Family Proteins and Potential Value as Therapeutic Targets

The best-studied group of bromodomain-containing proteins is the BET family, which consists of four members, ubiquitously expressed BRD2, BRD3, and BRD4, and testis-specific BRDT. They each have amino-terminal tandem bromodomains (BD1 and BD2) and an extraterminal (ET) domain (Fig. 10.1), and are normally localized in the cell nucleus. There is a conserved amino acid sequence between the two bromodomains (i.e., BD1 and BD2) in each BET family member, and it acts as a nuclear localization signal.[11]

One of the BET proteins, BRD4, is found in diverse genomic regions, including enhancer, promoter, intergenic, and intragenic regions, and has multifaceted functions in transcription initiation and elongation of both protein-coding RNA and noncoding RNA called enhancer RNA. BRD4 therefore modifies nucleosomes and acts as a transcriptional cofactor of many cellular genes. Normally, BRD4 binds to Kac via its bromodomains (Fig. 10.2A) and recruits a range of chromatin-remodeling proteins, including nuclear receptor-binding SET domain protein 3 (NSD3), which belongs to a subfamily of H3K36 methyltransferases, via its ET domain.[12] Several mechanisms related to transcription coactivation of BRD4 have been reported; notably, they are not mutually exclusive but may cooperate closely. The most conventional model is the positive transcription elongation factor b (P-TEFb)-dependent mechanism: BRD4 interacts with Kac in histones such as H3 and H4 via its bromodomains, and then recruits P-TEFb, which is a heterodimer of cyclin-dependent kinase 9 (CDK9) and its regulator cyclin T, to a promoter, leading to transcriptional elongation through phosphorylation of serine 2 (Ser2) at the carboxyl-terminal domain of RNA polymerase II.[13–16] For example, BRD4 marks selected M/G_1 genes for transcriptional memory during mitosis, enabling prompt postmitotic transcription in daughter cells via direct interaction with P-TEFb.[17] Moreover, BRD4 is recruited to G_1 gene promoters during G_0–G_1 progression, increasing the binding of Cdk9, which is a component of P-TEFb, and RNA polymerase II to G_1 genes, and thereby stimulating G_1 gene transcription and promoting cell cycle progression to the S phase.[18,19] In addition, BRD4 binds *IgH* enhancers in MM cells, regulating *MYC* expression and transcriptional function, resulting in genome-wide regulation of Myc-dependent target genes.[20] The BRD4-P-TEFb interaction has been suggested to influence the growth and induction of differentiation of acute myeloid leukemia (AML) cells, osteoclasts, and osteoblasts. BRD4-P-TEFb also regulates expression of the differentiation-associated *SLC2A5* gene. SLC2A5 is the hexose transporter generally called glucose transporter 5 (GLUT5), and is linked to disorders such as type 2 diabetes mellitus and obesity.[21] Furthermore, BRD4 stimulates Tat-independent HIV-1 transcription in a P-TEFb-dependent manner.[22]

Recently, P-TEFb-independent mechanisms have also emerged: BRD4 functions as a histone chaperone at enhancers and on gene bodies, and facilitates the passage of RNA polymerase II elongation complexes through nucleosomes by interacting with acetylated histones via its bromodomains, leading to transcriptional elongation. BRD4 also accumulates at enhancer clusters (so-called superenhancers).[23] A superenhancer is a small set of large enhancer regions bound by master transcriptional factors at genes with prominent roles in cell identity, and contains an exceptionally high density of transcriptional apparatuses, including RNA polymerase and chromatin regulators. Superenhancers are acquired by tumor cells and drive the expression of key oncogenes. Therefore, BRD4 regulates the expression of genes such as MYC and other survival genes via its bromodomains in cancer cells, such as multiple myeloma (MM) cells.

BRD4 also binds to the Kac of nonhistone protein RelA (Fig. 10.2B), which is one of the subunits of the NFκB transcriptional complex, thereby activating transcription of NFκB and NFκB-dependent inflammatory genes, including *IL-6* and *IL-8*, through the P-TEFb-dependent mechanism described above.[24] BRD4 also regulates P-TEFb-independently the proliferation and tumorigenesis of NFκB-driven cancers by preventing the ubiquitination and degradation of RelA through binding to acetylated RelA via its BDs.

In addition, BET bromodomains coregulate various nuclear receptors. BRD2 normally corepresses peroxisome proliferator-activated receptor PPARγ in a ligand-independent fashion and reduces PPARγ-driven adipogenesis.[25] BRD2 might be a promising target for the treatment of diabetes and obesity, although indirect induction of insulin production by overactive BRD2 might worsen diabetes. On the other hand, BRD4 serves as a transcriptional coactivator of nuclear receptors such as estrogen receptor (ER), retinoic acid receptors (RARs), and androgen receptor (AR); it has been reported to regulate ER-dependent gene transcription, to function in retinoid-mediated differentiation of neuroblastoma, and to bind directly to the amino-terminal domain of AR.[26, 27]

Overall, BET family proteins have attracted interest as candidate therapeutic targets for the treatment of diverse disorders, including inflammatory disorders, diabetes mellitus, bone diseases, and cancers such as MM, AML, prostate cancer, breast cancer, and NFκB-driven cancers.[28, 29]

10.2.2 Discovery of BET Bromodomain Inhibitors

Academic and commercial interest in bromodomain inhibitors has increased dramatically since the first small-molecule BET bromodomain-selective inhibitors, I-BET762 (a.k.a. GSK525762) and (+)-JQ1, were reported in 2010.[30,31] These inhibitors both displace BET bromodomains from chromatin by competing with Kac in the Kac-binding pocket. They have anticancer and antiinflammatory activities, and I-BET762 is currently under clinical development. (+)-JQ1 itself is not currently considered a drug candidate as a result of its short half-life.

A GlaxoSmithKline (GSK) compound, the triazolodiazepine I-BET762 (Fig. 10.3A), was identified in an antiinflammatory phenotypic-screening search for small-molecule upregulators of apolipoprotein A-1 (ApoA1). I-BET762 modulates the expression of several cancer- and inflammatory-related proteins such as c-Myc, p-Erk1/2, IL-6, p-STAT3, and consequently induces G_1 cell cycle arrest and apoptosis in cancer cells, including prostate cancer cells, pancreatic cancer cells, and myeloma cells.[32,33] It also suppresses the production of inflammatory cytokines associated with tumor growth and inflammation. I-BET762 is under phase I clinical trial for treatment of NMC and other cancers. In addition, I-BET762 in combination with fulvestrant, an estrogen receptor (ER) antagonist and a selective ER degrader (SERD), is in phase II clinical trial for the treatment of breast cancer (Fig. 10.5).

The chemical structure of (+)-JQ1 (Fig. 10.3B), a triazolothienodiazepine, was inspired by and modified from those of similar BET inhibitors patented by Mitsubishi Tanabe Pharma, and (+)-JQ1 is widely used in laboratory applications. It has various biological activities, and potential applications of (+)-JQ1 in the treatment of cancers, cardiovascular diseases, HIV infection, and inflammatory diseases have been proposed. (+)-JQ1 directly targets the bromodomains in the oncoprotein BRD4-NUT (nuclear protein in testis), a driver of NUT midline carcinoma (NMC).[31] It also upregulates autophagy and lysosomal gene expression and induces differentiation, G_1 arrest, and apoptosis in NMC cells, thereby suppressing tumorigenesis and proliferation. On the other hand, it downregulates *MYC* oncogene expression in MM cells, and thus represses *MYC*-dependent target genes. In addition, (+)-JQ1 influences several nuclear receptors, including ER and AR. For example, (+)-JQ1 inhibits the interaction between BRD4 and the N-terminal domain of AR, functioning downstream of AR. In AR signaling-competent human castration-resistant prostate cancer (CRPC) cell lines, it suppresses BRD4 localization to AR target loci and decreases AR-mediated gene transcription.[34]

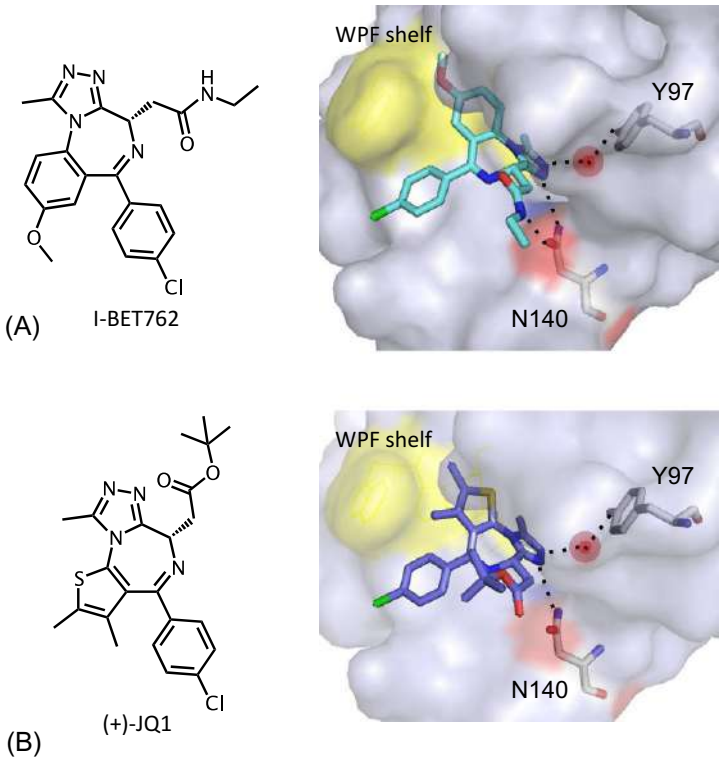


FIG. 10.3 Crystal structures of BRD4 BD1 complexes with (A) I-BET762 (PDB: 3P5O) and (B) (+)-JQ1 (PDB: 3MXF).

10.2.3 Kac-Binding Site Structure

There are well-conserved residues and features in the Kac-binding sites of diverse bromodomains that are important for the Kac binding and selectivity of inhibitors. The Kac-binding site is hydrophobic in nature, and contains residues of the BC loop and ZA loop. There is a hydrophobic shelf as well as a gatekeeper at the entrance of the Kac-binding site, and a large cavity filled with water molecules is located at the other end of the Kac-binding pocket. For example, in the Kac-binding site of BRD4 BD1 the hydrophobic WPF shelf (tryptophan (W81), proline (P82), and phenylalanine (F83)) in the ZA loop and the gatekeeper residue (isoleucine (I146)) are located at the entrance.

Kac recognition is mediated through direct formation of a hydrogen bond between the acetyl oxygen in Kac and the amino hydrogen of an evolutionarily conserved asparagine (Asn, N) residue in the BC loop (Fig. 10.2A). This Asn residue exists in 48 of the 61 known bromodomains.

10.2.4 Kac Recognition Motifs for BET Bromodomain Inhibitors

The BET bromodomain inhibitors developed so far bind to the Kac-binding site. The 3-methyl-1,2,4-triazole substituent of (+)-JQ1 acts as a Kac-mimetic structure (Fig. 10.3): one nitrogen of the 3-methyl-1,2,4-triazole binds directly to the amide hydrogen of the conserved Asn (N140 in BRD4 BD1) and the other nitrogen forms a water-mediated hydrogen bond with the hydroxyl hydrogen of the conserved Tyr (Y97 in BRD4 BD1). Introduction of the chlorophenyl substituent of (+)-JQ1 onto a shelf formed by W81, P82, and D145, and introduction of the dimethyl-substituted thieno moiety of (+)-JQ1 onto a shelf formed by W81, P82, and L92 stabilize the interaction between the 3-methyl-1,2,4-triazole substituent and N140. In addition, the methyl group occupies the hydrophobic pocket that recognizes the methyl group of Kac. (+)-JQ1 and I-BET762 form similar interactions with the BRD4 BD1, but I-BET762 forms an additional hydrogen bond between the carbonyl oxygen of N140 and its amide hydrogen in the side chain. These crystallographic findings have been utilized to develop other types of BET inhibitors with Kac-mimetic structures, including derivatives of isoxazole, pyridone, quinazoline, dimethylisoxazole, and purine (Fig. 10.4).[35–38] For example, purine derivatives can bind to BET bromodomains: an amino purine derivative has been reported to interact directly with the conserved N140 in BRD4 BD1 through a purine nitrogen. Structure–activity relationships have also been examined: N^6-(2,4,5-trimethoxybenzoyl)adenine was the most potent inhibitor of the N^6-benzoyladenine derivatives, being 50-fold more potent than N^6-benzoyladenine.

10.2.5 BET Bromodomain Inhibitors as Candidate Therapeutic Agents

Mutation, misexpression, and oncogenic fusion in some bromodomain-containing proteins have been reported to be associated with certain cancers, and therefore several BET inhibitors are currently under clinical development as

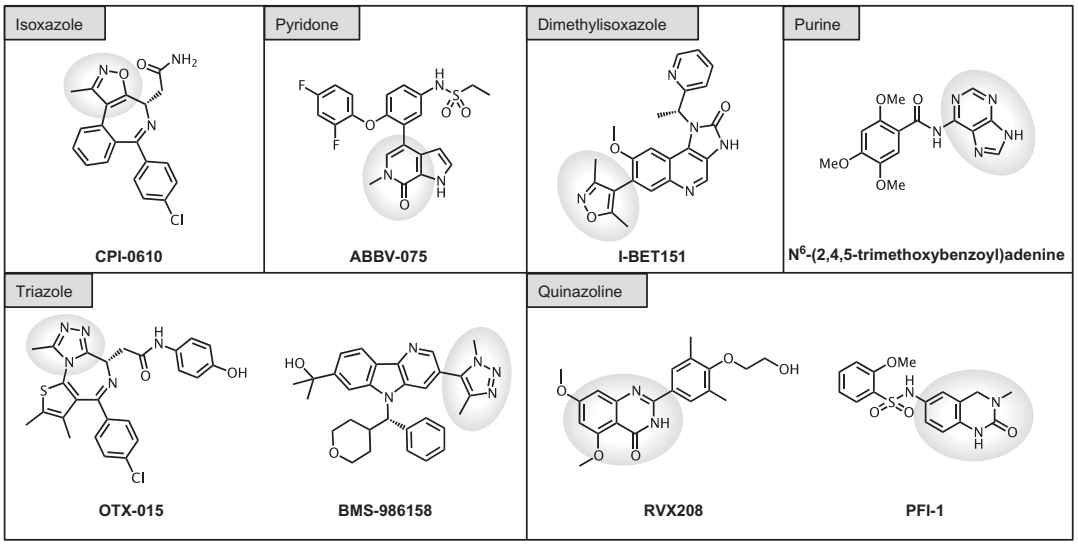


FIG. 10.4 BET inhibitors with Kac-binding motifs.

BET inhibitor	Condition (Phase and status)
I-BET762 (GSK525762)	ER positive breast cancer (combination with fluvestrant) (Phase 2, recruiting), Hematologic malignancies (Phase 1, recruiting), CRPC (combination with androgen deprivation therapy) (Phase 1, recruiting), NMC and other cancer (Phase 1, active but not recruiting)
OTX-015	AML, DLBCL (Phase1, active but not recruiting), Glioblastoma Multiforme (Phase 1 and 2, terminated), AML, diffuse Large B-cell Lymphoma (DLBCL), acute Lymphoblastic Leukemia, MM (Phase 1, completed), NMC, CRPC, TNBC, Non-small Cell Lung Cancer (NSCLC) (Phase1, terminated)
CPI-0610	Peripheral Nerve Tumors (phase 2, recruiting), Lymphoma (Phase 1, active not recruiting), Acute leukemia, myelodysplatic syndrome (MDS), myelodysplatic/myeloproliferative neoplasm, MM, myelofibrosis (phase 1, recruiting)
TEN-010 (RO6870810)	AML, MDS, solid tumors (Phase 1, completed), Ovarian cancer, MM, TNBC, DLBCL (phase 1, recruiting)
RVX208	Type 2 diabetes mellitus, coronary artey disease, cardiovascular diseases (Phase 3, recruiting), diabetes, atherosclerosis, coronary artery disease, dyslipdemia, acute coronary syndrome (Phase 2, completed), Fabry disease, kidney failure (Phase 1 and 2, not yet recuriting),
ZEN003694	CRPC (phase 1, recruiting)
GSK2820151	Soild tumors (Phase 1, recruiting)
ABBV-075	Advanced cancer, breast cancer, nonsmall cell lung cancer, AML, MM, prostate cancer, SCLC, nonhodgkins lymphoma (Phase 1, recruiting)
INCB057643	Soild Tumors and Hematologic Malignancy (Phase 1 and 2, recruiting)
BMS-986158	Multiple indications cancer (Phase 1 and 2, recruiting)
FT-1101	AML, MDS (Phase 1, recruiting)

FIG. 10.5 Clinical trials evaluating epigenetic BET bromodomain-targeted therapies.

therapeutic agents for cancers, as summarized in Fig. 10.5. For example, BRD3 and BRD4 have been reported to form fusions with the *NUT* gene, and I-BET762 is under evaluation in phase I clinical trials for the treatment of NMC. Furthermore, the Abbvie compound ABBV-075, a pyridone, is in phase 1 clinical trials for the treatment of AML, MM, breast cancer, and prostate cancer. The Bristol-Myers Squibb compound BMS-986158, a triazole, is in phase II clinical trials for the treatment of solid tumors. In addition, the OncoEthix compound OTX-015, a triazolothienodiazepine resembling (+)-JQ1, licenced from Mitsubishi Tanabe Pharma, is in phase II clinical trials for recurrent glioblastoma multiforme. Constellation Pharmaceuticals' compound CPI-0610, an isoxazole, is in phase II clinical trials for myelofibrosis, a myeloproliferative and fibrotic disorder. The ResVerlogix compound RVX-208 (a.k.a. RVX000222), a quinazolone, is under phase III clinical trials in high-risk type 2 diabetes mellitus patients with coronary artery and cardiovascular diseases. Interestingly, RVX-208 appears to bind selectively to the BD2 of BRD4.

10.3 POLYPHARMACOLOGY

Resistance to BET inhibitors has been reported in leukemia cell lines such as K562, ovarian carcinoma cell lines such as OVCAR-5, and breast cancer cells such as triple-negative breast cancer (TNBC) cells.[39,40] It is characterized by rapid restoration of MYC transcription caused by activated WNT signaling. To overcome this issue a polypharmacological approach, in which a single drug simultaneously modulates multiple disease-associated targets, has attracted interest as a therapeutic strategy. Advantages could include the relatively easy prediction of absorption, distribution, metabolism, and excretion (ADME) characteristics, as compared with classical combination therapy. Polypharmacological BET inhibitors targeting multiple proteins might therefore be potential therapeutic agents for overcoming resistance to BET inhibitors and/or treating diseases with complex pathogenic mechanisms, including cancers and inflammatory diseases.

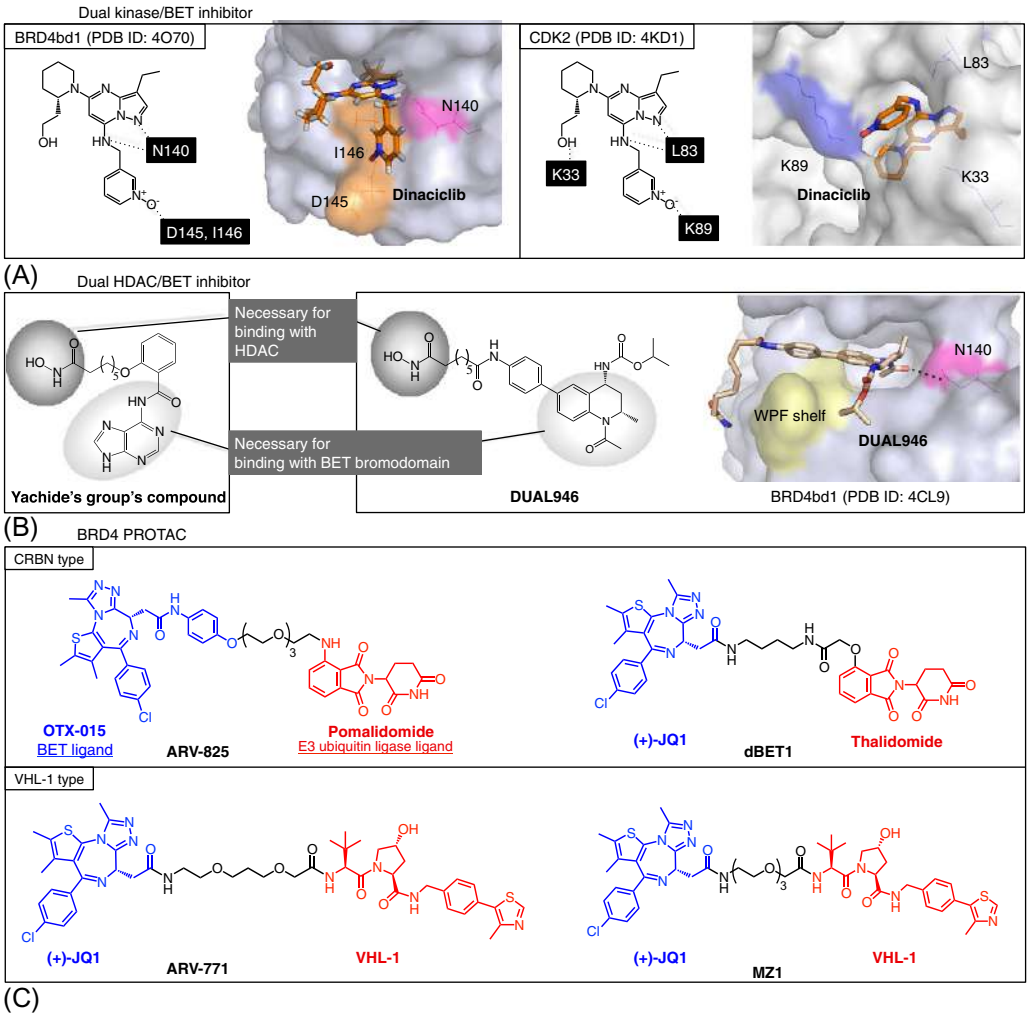


FIG. 10.6 Polypharmacological agents for which the BET bromodomain is a target: (A) dual kinase/BET inhibitors, (B) dual HDAC/BET inhibitors, and (C) BRD4 PROTACs.

10.3.1 Polypharmacological Agents Targeting Multiple Proteins

10.3.1.1 Dual Kinase/BET Inhibitors

Several clinically advanced kinase inhibitors, such as dinaciclib (a.k.a. SCH-727965), which is a cyclin-dependent kinase (CDK) inhibitor, volasertib (a.k.a. BI 6727), which is an ATP-competitive polo-like kinase (PLK) inhibitor, fedratinib (a.k.a. TG-101348), which is an ATP-competitive Janus kinase (JAK) inhibitor, and tideglusib, which is a non-ATP-competitive glycogen synthase kinase (GSK)-3 inhibitor, show BET bromodomain-inhibitory activity with therapeutically relevant potencies.[41] Dinaciclib binds to the Kac-binding site of BRD4 BD1.[42] As shown in cocrystal structures (Fig. 10.6A) the pyrazolopyrimidine moiety of dinaciclib forms two hydrogen bonds with N140, and the pyridine oxide interacts with the gatekeeper residue I146. The ethyl group lies deep inside the cavity, whereas the hydroxyl group is at the entrance of the pocket. Dinaciclib is under clinical trials for the treatment of various cancers, including leukemia, MM, breast cancer, pancreatic cancer, and solid tumors. The results of these studies will have important implications for the future of polypharmacological dual kinase/BET inhibitors.

10.3.1.2 Dual HDAC/BET Inhibitors

HDAC and BET inhibitors have been reported to induce similar genes and to have similar biological effects. For example, vorinostat and (+)-JQ1 both induce apoptosis-related genes, such as p53-regulated genes, including *Trp53inp1*, *Gadd45a*, and *Bbc3*. Also, they both show antitumor effects, including antiproliferative, caspase-dependent

apoptosis-inducing, cell cycle arrest-inducing, and differentiation-inducing activities. A synergistic antitumor effect between HDAC and BET inhibitors has been reported: the combination of panobinostat or vorinostat with (+)-JQ1 enhanced the apoptosis of AML and pancreatic ductal adenocarcinoma (PDAC) cells.

DUAL946 and N^6-[2-(7-hydroxyamino-7-oxoheptyloxy)benzoyl]adenine have been developed as dual HDAC/BET inhibitors.[43,44] They contain a hydroxamic acid group for binding with HDAC and a Kac-mimetic moiety for binding with BET (i.e., the 1-formyl-1,2,3,4-tetrahydroquinoline moiety or N^6-benzoyladenine moiety) (Fig. 10.6B). As shown in Fig. 10.6B the formyl oxygen of DUAL946 binds directly to the amide hydrogen of N140 in BRD4 BD1. DUAL946 is effective against immune and cancer cells. Interestingly, N^6-[2-(7-hydroxyamino-7-oxoheptyloxy)benzoyl]adenine shows growth-inhibitory activity toward BRD4 inhibitor-resistant cells, such as the K-562 and OVCAR-5 cell lines. Resistance to BET inhibitors may be alleviated or prevented by the use of dual HDAC/BET inhibitors in the future.

10.3.2 Polypharmacological Agents Targeting Multiple Functional Mechanisms

10.3.2.1 Inducers of BET Degradation

Proteolysis-targeting chimeras (PROTACs), which degrade specific target proteins via the ubiquitin proteasome system in contrast to conventional approaches that regulate functions elicited by the target protein, have attracted attention as a novel strategy.[45–47] Posttranslational degradation of a target protein can be achieved through the recruitment of E3 ubiquitin ligases with PROTACs, which bear an E3 ligase recognition structure. So far MZ1, dBET1, ARV-771, and ARV-825 have been developed for PROTAC-induced BRD4 degradation (Fig. 10.6C).[48–50] For example, ARV825 is a hybrid compound consisting of the BET inhibitor OTX-015 and pomalidomide, a ligand of E3 ligase cereblon (CRBN),[51,52] coupled via a polyethylene glycol (PEG) spacer moiety.[48] ARV825 induces rapid and extensive CRBN-mediated and proteasome-dependent BRD4 degradation, leading to robust and persistent downstream c-Myc suppression. It results in more effective inhibition of cell proliferation and induction of apoptosis than the corresponding BET inhibitor OTX-015 alone in Burkitt lymphoma (BL), which is an MYC-driven malignancy. Degradation of BET family proteins by MZ1, which utilizes a different E3 ligase system, has also been reported.[49] MZ1 consists of (+)-JQ1, a BET inhibitor, coupled via a polyethylene glycol (PEG) spacer moiety to VHL-1, a ligand of the E3 ligase von Hippel-Lindau protein (VHL). Interestingly, MZ1 rapidly induces long-lasting and selective degradation of BRD4 over BRD2 and BRD3, even though (+)-JQ1 is a pan-BET inhibitor. This preferential degradation of BRD4 results in differential gene modulation between MZ1 and (+)-JQ1: cancer-related genes responsive to MZ1 have been shown to coincide with BRD4-dependent genes such as *MYC*, *P21* and *AREG1*, but not BRD2-dependent genes such as *FAS*. Pharmaceutical studies of PROTACs are proceeding rapidly, and their future looks promising.

10.4 NON-BET BROMODOMAIN INHIBITORS

Non-BET bromodomain inhibitors have recently been reported and should be useful tools to understand the physiological functions of non-BET bromodomains, as well as being candidate therapeutic agents. The hydrophobic cavity in the ZA loop located at the entrance to the Kac-binding pocket, the region known as the WPF shelf in BET bromodomains, influences selectivity. The features of hydrophobic cavities targeted by typical inhibitors of non-BET bromodomains, including the ATAD2 bromodomain, BAZ2A/BAZ2B bromodomain, BRD7/BRD9, BRPF bromodomain, CBP/p300 bromodomain, CECR2 bromodomain, PCAF bromodomain, and SMARCA4 (BRG1) bromodomain, are shown in Fig. 10.7.[53]

10.5 CONCLUSIONS AND PROSPECTS

The bromodomain serves as a reader that recognizes acetylated lysine residues in both histone and nonhistone proteins. The human genome encodes 61 bromodomains in 46 diverse proteins, and epigenetic regulation of histone acetylation by bromodomains contributes to many DNA-dependent cellular processes. Several small-molecule BET inhibitors are already in trials for the treatment of various diseases, including cancer. Furthermore, polypharmacological agents such as dual kinase/BET inhibitors and dual histone deacetylase (HDAC)/BET inhibitors offer great promise, in addition to agents that degrade BET family proteins, such as proteolysis-targeting chimeras (PROTACs). Inhibitors of non-BET bromodomains, including PCAF, CBP/p300, and BRD7/9, have also recently been reported. Monospecific bromodomain inhibitors and multitarget BET inhibitors will likely be rapidly developed as candidate therapeutic agents for cancers, inflammatory diseases, immune deficiency diseases, diabetes, and cardiovascular diseases.

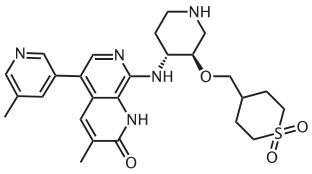
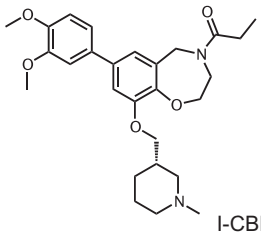
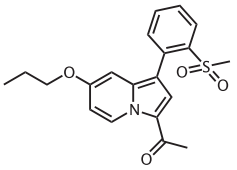
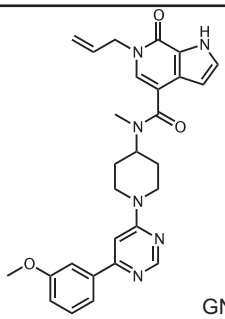
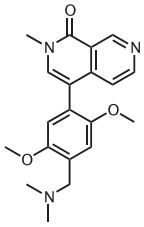
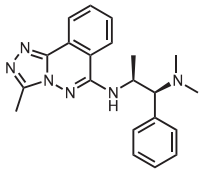
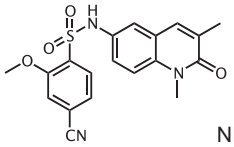
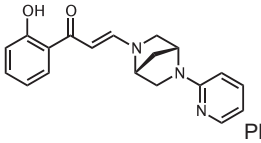
Non-BET protein (hydrophobic cavity)	Chemical structure of non-BET inhibitor	Non-BET protein (hydrophobic cavity)	Chemical structure of non-BET inhibitor
ATAD2 (Arg, Val, Phe)		CBP (Leu, Pro, Phe)	 I-CBP112
BAZ2A/B (Trp, Pro, Phe)	 GSK2801	CECR2 (Trp, Pro, Phe)	 GNE-886
BRD9 (Gly, Phe, Phe)	 BI-9564	PCAF (Trp, Pro, Phe)	 L-Moses
BRPF1 (Asn, Ile, Phe)	 NI-57	SMARCA4 (BRG1) (Glu, Val, Ile)	 PFI-3

FIG. 10.7 Structures of non-BET inhibitors and amino acid residues involved in the hydrophobic cavity of target proteins.

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CHAPTER

11

Bromodomain Inhibition and Its Application to Human Disease

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11.1 THE ROLE OF HISTONES IN CHROMATIN-SIGNALING PATHWAYS

11.1.1 Histones and Their Posttranslational Modifications

In each human cell 2 m of DNA are organized within a 10-μm-wide nucleus. Obviously, this DNA has to be carefully structured and organized to maintain its integrity and the correct expression of its genes, which define *in fine* the cellular identity and functions.[1] Numerous proteins interact with the genomic DNA to form a nucleoprotein structure named chromatin. Its fundamental unit is the nucleosome, formed by 147 base pairs of DNA wraped around a histone octamer, made of the proteins H2A, H2B, H3, and H4.[2] Chromatin organization conditions the functionality of the nucleus and is a key determinant of transcriptional regulation. It can adopt different states, leading to the activation or repression of gene expression. Heterochromatin has a compact conformation that silences gene expression, while euchromatin has a more open structure that promotes gene expression by making the DNA available to the transcription machinery.[3] Dynamic regulation of the transcription program involves complex signaling pathways, in which chromatin is a crucial interface. One of the most important determinants of chromatin structure is the deposition, removal, and interpretation of the post-translational modifications (PTMs) of histones.

The first histone PTMs, acetylation and methylation, were reported by Allfrey et al. in 1964.[4] Since this seminal publication a series of histone PTMs have been discovered including the ubiquitination, sumoylation, butyrylation, propionylation, and crotonylation of lysine residues, the methylation, ribosylation, and citrullination of arginine residues, and the phosphorylation/glycosylation of serine and threonine residues.[5] PTMs, which can occur within the central globular domain of histones (the histone fold) or the histone tails (*N*- or C-terminal) that extend out from the nucleosome, regulate the incorporation of histones into nucleosomes as well as the overall conformation of chromatin.[6, 7] PTMs found on the histone fold mainly affect the affinity of histones for DNA (exemplified by the acylation of H3K122) and modulate histone binding to molecular chaperones (exemplified by the acylation of H3K56).[8] PTMs also can act as docking sites that recruit a variety of proteins involved in nucleosome remodeling and gene transcription.[7]

11.1.2 Installation and Removal of Histone Modifications

PTMs are regulated and operated upon by three classes of enzymes or protein factors, which install, remove, or recognize these modifications and are commonly referred to as writers, erasers, and readers. As the names imply, writers are responsible for depositing the PTMs, erasers catalyze the removal of PTMs, and reader domains specifically and selectively recognize modified residues. Reader proteins are found in combination with writers, erasers, or other

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reader modules, forming large multiprotein complexes that make up key chromatin regulation machinery. The recognition of a PTM by its corresponding reader is achieved through a direct interaction between the modified histone residue and the ligand binding pocket of the reader protein, as well as by secondary contacts involving the flanking histone sequence.[9]

Typical writers include histone acetyltransferases (HATs) and histone methyltransferases (HMTs). HATs and HMTs have corresponding erasers, histone deacetylases (HDACs) and histone lysine/arginine demethylases (KDMs/RDMs), which are responsible for reversing the effects of the writer enzymes.

11.1.3 Drugging Epigenetic Writers and Erasers

Writers and erasers of acetylation and methylation, the most abundant histone PTMs, have been studied extensively in drug discovery programs due to their important roles in cancer and other diseases.[10]

Mutations in certain epigenetic enzymes can directly cause a disease state thus making them obvious targets for drug discovery campaigns.[11] However, perhaps the more interesting aspect of epigenetic drug discovery is the ability to indirectly target the main drivers of diseases that are "undruggable," such as altering the levels of oncogenic gene expression. For instance, if the epigenetic protein is involved in the overexpression of oncogenes, then inhibition of the epigenetic protein can reduce expression of the improperly regulated gene.[12] It should also be noted that a recent study revealed that approximately 1% of all FDA-approved drugs already display significant epigenetic activity, silencing promoters in colon cancer cells.[13]

Epigenetic proteins have increasingly been investigated as drug targets due to their roles in human disease. Two recent reviews by Jones et al. and Di et al. discuss the development and FDA approval of epigenetic cancer treatments, including the HDAC inhibitors vorinostat, belinostat, and romidepsin.[12, 14]

11.2 BROMODOMAINS AND THEIR INHIBITORS

11.2.1 Structure and Functions of Bromodomains

Histone acetylation is often associated with increased DNA accessibility and increased transcription.[15] Acetylation of the histone masks the positive charge on lysine residues and slightly changes the histone structure. Both phenomena weaken DNA-histone associations, thus making DNA more available for transcription.[16] In addition, histone acetylation marks recruit transcription/remodeling factors that lead to an increase in transcriptional activity. These factors are typically recruited by bromodomains (BRDs). BRDs are reader domains that recognize ε-N-acetylated lysine residues (Kac) on histones.[17–19] BRDs were first discovered in, and subsequently named after, the *Drosophila* protein Brahma.[20, 21] BRDs can be found in numerous chromatin-associated proteins, such as HATs (as in Gcn5 and p300/CBP), HMTs (as in Hrx/All-1), transcription initiation factors (as in Taf1), and ATP-dependent helicases (as in Snf2L2).[22, 23]

All human BRDs (hBRDs) share a similar structure, in which a left-handed bundle of 4 α-helices (Z, A, B, C) is linked together by 2 loops (the ZA loop and BC loop). The ZA loop is a large loop linking αZ and αA, while the BC loop is a relatively small loop that links αB and αC (Fig. 11.1). These two loops form a hydrophobic binding pocket located at one end of the bundle of α-helices that can recognize Kac. In most BRDs the Tyr residue on the ZA loop and the Asn residue on the BC loop are crucial for Kac recognition. The Asn residue on the BC loop binds directly to the Kac, or acetyl lysine mimetic, while the Tyr residue from the ZA loop binds to structural water located in the binding pocket (Fig. 11.2).

Five water molecules have been conserved in most of the BRD X-ray crystal structures reported to date. These water molecules can directly interact with BRD ligands, and therefore should be considered in BRD drug design.[3] Recent molecular dynamic simulations have revealed that the structurally conserved water in BRDs is vital for ligand recognition and selectivity.[24] However, a water molecule may be displaced by the incoming ligand, possibly resulting in increased BRD affinity.[25]

Alignment of the available BRD structures reveals that the ZA and BC loops vary considerably in both sequence and structure. This could explain why BRDs differ in ligand selectivity. Filippakopoulos et al.[23] comprehensively investigated BRD ligand selectivity.[23] A study of 33 hBRD binding affinities for known Kac sites of human histones using a SPOT peptide array found that in vitro BRD affinities for a range of acylated peptides were relatively weak (K_D values in the micromolar to millimolar range), suggesting that high affinity in vivo depends on additional binding interactions. In vitro affinity of BRDs for histone peptides bearing Kac marks was also sensitive to other modifications such as

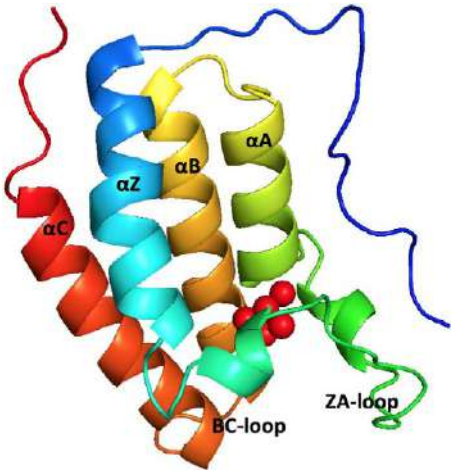


FIG. 11.1 Bromodomains (here exemplified by Brd4 BD1 with ligand removed) typically consist of a bundle of four conserved α-helices (Z, A, B, C) connected by the two loops (ZA and BC) which make up the Kac ligand binding pocket (PDB: 3mxf).

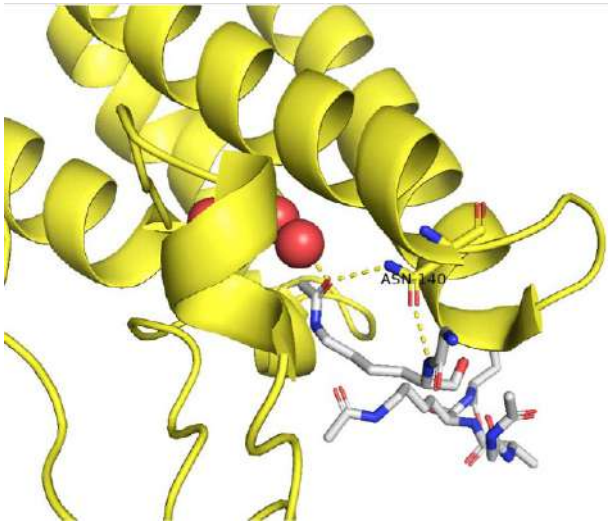


FIG. 11.2 Brd4-BD1 bound to diacetylated histone 4 peptide (H4K12acK16ac). Hydrogen bonds between ligand and key Asn residue are represented with dotted lines (PDB: 3uvx).

phosphorylation and trimethylation. The wide variety of PTMs that influence the affinity of BRDs with Kac, coupled with the observation that some BRDs display a cooperative binding mode with peptides having multiple Kac marks,[26] points to the complex nature of molecular recognition between BRDs and their native ligands.

The 61 distinct hBRDs are divided into eight major families according to sequence identity and structural homology (Fig. 11.3). Over half of hBRDs have reported atomic resolution structures, many of which have been deposited by the Structural Genomics Consortium (SGC).[22] To date, the most heavily investigated BRDs reside in a subclade of family II, named the bromodomain and extraterminal (BET) BRDs.

11.2.2 The Discovery of Bromodomain Inhibitors

While high-throughput screening and cellular phenotypic assays have been utilized in discovery programs aiming to develop BRD inhibitors, fragment-based drug discovery (FBDD) has also been a successful method for developing BRD inhibitors. Arguably, the first small-molecule inhibitors for BRDs were identified in a fragment-screening campaign targeting p300/Creb-binding protein (CBP) BRD. In this study a focused library composed of small Kac mimetics was explored to identify inhibitors via medium-throughput NMR-binding studies.[27]

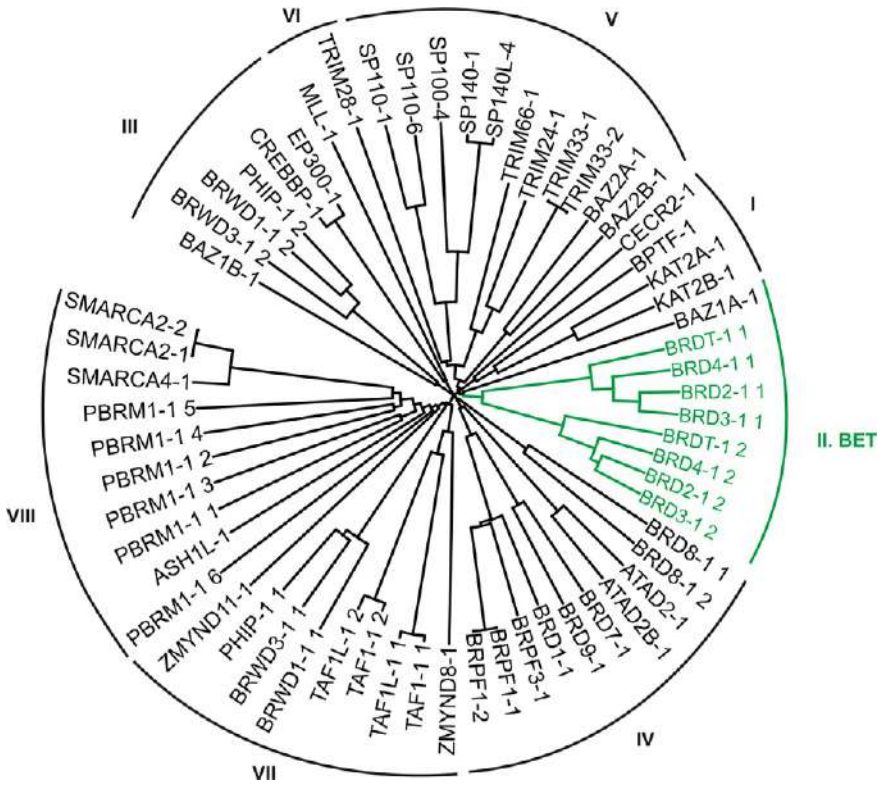


FIG. 11.3 BRD phylogenetic tree containing all 61 BRDs found in the human genome. BRD families are indicated as described by Filippakopoulos et al.[23]

Sensitive methods used to detect direct binding, like ^{15}N-NMR, are often utilized to detect the weak interactions typical of BRD binding to small ligand fragments. However, biochemical assays that measure the displacement of the acylated histone tails from the BRD binding pocket, such as AlphaScreen or FRET-based assays (HTRF), have also proven useful for this purpose.[28] Conserved Asn and Tyr in the Kac binding pocket can serve as anchor peptides capable of binding small ligand fragments, enabling successful development of several selective BRD inhibitors via FBDD endeavors.[29] FBDD has also recently been utilized to identify hit fragments for BRDs that are considered less druggable. For example, FBDD was used to identify inhibitors for the BRD adjacent to zinc finger domain protein 2B (Baz2b). In that study the hexahydro-1*H*-pyridoindole fragment initially bound the BRD with low potency (IC_{50} 37 μM) but was optimized through structure-activity relationship (SAR) studies and fragment merging to have an IC_{50} of 9 μM (Fig. 11.4).[30] Baz2b is considered to be one of the least "druggable" BRDs due to its small Kac binding pocket, making this activity an impressive achievement.[31]

Virtual screening has also been a common method for developing high-affinity ligands for BRDs,[3] aided by the large number of high-quality cocrystal structures of BRDs complexed with ligands that span a large region of chemical space. In 2018 Batiste et al. combined the use of FBDD and virtual screening while also considering synthetic feasibility. In this approach the authors utilized AutoCouple, a de novo computational ligand design protocol that "grows" an initial fragment with virtual chemical couplings.[32] Commercially available compounds suitable for robust cross-coupling reactions, such as Buchwald-Hartwig amination and Suzuki cross-coupling, were utilized as starting materials. An original in silico fragment hit was then virtually transformed into multiple derivatives and subsequently evaluated via a docking protocol. The top-scoring compounds were synthesized and tested. This approach culminated in a Kac mimetic with low nanomolar potency for Cbp (IC_{50} 19 nM), high selectivity against Brd4 BD1 (no activity at 200 μM), and cellular activity.

11.2.3 Inhibitors of BET Bromodomains

In 2010 two groups independently published the discovery of selective BET BRD inhibitors (BETi). The biological evaluation of (+)-JQ1 (referred to herein as JQ1), published by Filippakopoulos et al.,[33] and I-BET762,[34] first reported by Tarakhovsky et al., displayed the therapeutic potential of BETi, and stimulated renewed interest in epigenetic drug

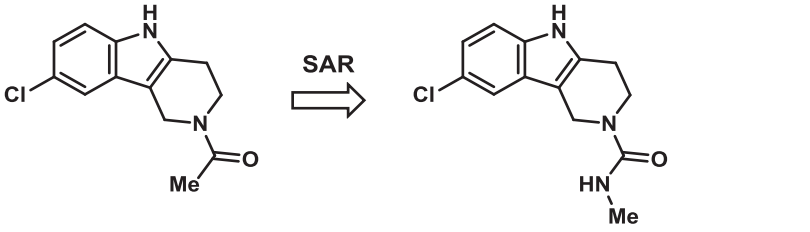


FIG. 11.4 Fragment-based drug discovery exemplified by Ciulli et al.[30]

development (Fig. 11.5). The BET family comprises Brd2, Brd3, Brd4, and the testis-specific Brdt. All four BET proteins contain two BRD modules, which are referred to as the first (BD1) and second (BD2) bromodomains. In Brd4, BD1 and BD2 are significantly less similar to each other (<45% sequence identity) than other BRD-containing proteins with multiple BRDs.

JQ1 and I-BET762 bind competitively with acetylated peptides and can displace Brd4 from chromatin in cellulo.[33] JQ1 was found to be selective for the BET family (K_D of 50 nM for Brd4 BD1 and 60–190 nM for the other members of the BET family). A cocrystal structure of Brd4 BD1 in complex with JQ1 reveals that the triazolothienodiazepine core forms hydrogen bonds with the anchor Asn residue as well as with the array of structural waters found in the binding pocket. This interaction mimics the Kac peptides that are the native ligands of BRDs. An illustration of the similarities between Kac binding and JQ1 is shown in Fig. 11.6.

JQ1 showed efficacy against NUT midline carcinoma (NMC), an aggressive form of squamous carcinoma, indicating the translational potential of BETi. I-BET762 (a.k.a. GSK525762) is another triazolodiazepine identified in a phenotypic assay monitoring apolipoprotein A-I (ApoA1) levels.[34] I-BET762 has a similar activity and selectivity profile to JQ1. I-BET762 downregulated inflammatory genes in cell studies and reduced inflammation in an in vivo mouse model.[34] JQ1 and I-BET762 continue to be used as chemical probes to investigate the biological function of BET BRDs, and the triazolodiazepine pharmacophore is frequently a basis for new approaches to BET inhibition (see Sections 11.2.4 and 11.2.5).

3,5-Dimethylisoxazole was discovered to be a novel Kac mimetic by the SGC.[35] This inhibitor motif was originally identified in a screen of methyl-bearing heterocycles after it was found that dimethylsulfoxide (DMSO) inhibits BRDs. The hit was optimized using structure-guided SAR analysis and follow-up SAR studies.[35–37] This resulted in several submicromolar inhibitors selective for BET BRDs.[35] Another well-established BETi, I-BET151, incorporates a 3,5-dimethylisoxazole pharmacophore (Fig. 11.5). It shows similar activity and selectivity to diazepine I-BET762, but has better pharmacokinetics and has an extended half-life in animal studies.[38]

While these BETi show selectivity for the BET family of BRDs, none is highly selective for one of the BET BRDs. The quinazolone compound RVX-208 (Fig. 11.5) was first spotted in a phenotypic screen designed to identify compounds that affect ApoA1 expression levels,[39] and subsequently shown to be a BETi.[40] RVX-208 shows remarkable selectivity for a single Brd4 BRD ($K_D = 140$ nM for Brd4 BD2, 1.1 μM for Brd4 BD1). This selectivity is believed to arise from a structural rearrangement of the BD2 binding pocket induced by RVX-208 binding in which His433 of Brd4 BD2 moves toward the front of the binding pocket and stabilizes the binding mode.[40]

A recent "bump and hole" approach, in which I-BET762 has a large substituent added (a "bump") and a Leu residue was replaced with a smaller Ala residue (a "hole"), was used to investigate the unique roles BD1 and BD2 play in Brd4.[41, 42] In that work it was shown that selectively inhibiting BD1 using this approach was able to displace Brd4 from chromatin.

11.2.4 Inducing Proteolysis With BET Inhibitors

Recently, several groups have investigated the use of proteolysis-targeting chimeras (PROTACs) as an approach to BET inhibition. That work has been recently reviewed.[43] Briefly, conjugation of a BETi to an E3 ubiquitin ligase inhibitor through a linker allows it to be tagged selectively with ubiquitin and subsequently degraded through a proteasome-mediated mechanism. Examples of these compounds are depicted in Fig. 11.7. PROTAC methods have been shown to be effective in murine models of leukemia,[44] in promotion of apoptosis in Burkitt lymphoma cells,[45] and in selective degradation of one specific BET protein.[46]

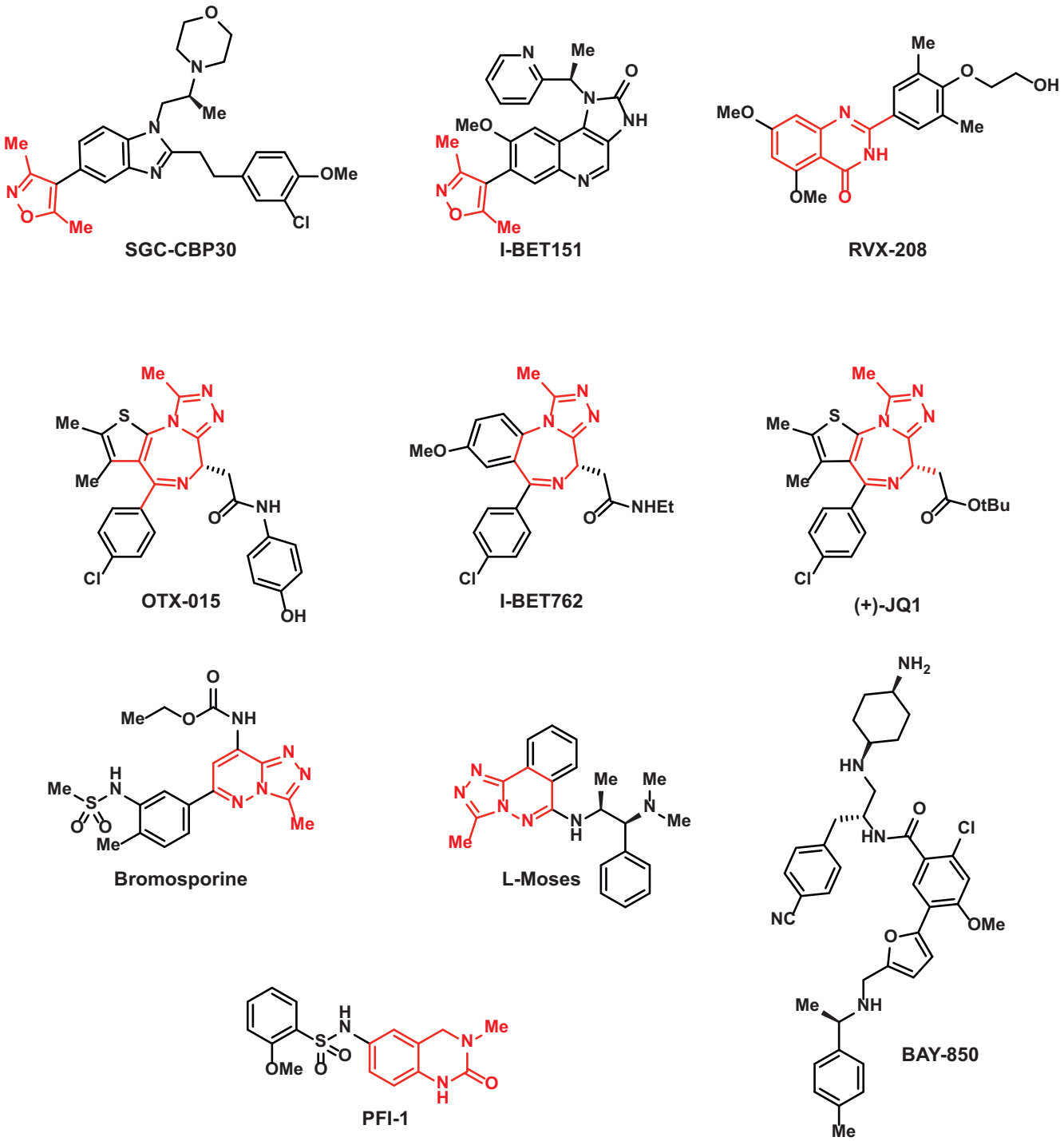


FIG. 11.5 A selection of bromodomain inhibitors. The Kac mimetic is highlighted in *red*.

11.2.5 Bivalent BET Inhibitors

Many BRD-containing proteins contain more than one BRD module, which often occur sequentially in tandem.[23] An inhibitor that simultaneously engages two BRDs is an attractive approach for the development of molecular probes or therapeutic agents with enhanced potency, which may benefit from the avidity effect (Fig. 11.8A).[47] Two main strategies have been realized (Fig. 11.8B). The first employs a single chemical entity that is capable of engaging both BRDs.[48–50] The second deploys a bivalent molecule that links two BETi.[51–53]

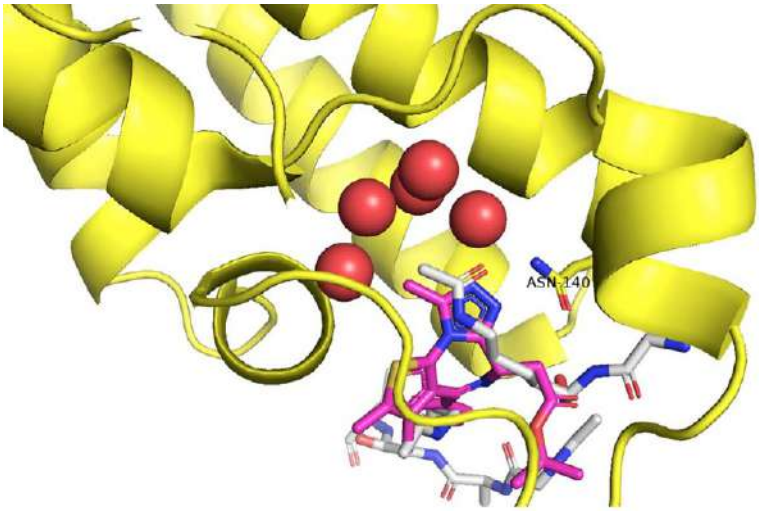


FIG. 11.6 JQ1 (*magenta*) mimics native Kac ligand (H4K12acK16ac, *gray*) bound to Brd4 BD1. The triazolothienodiazepine core forms hydrogen bonds with the anchor Asn residue. (PDB: 3mxf overlapped with PDB: 3uvx).

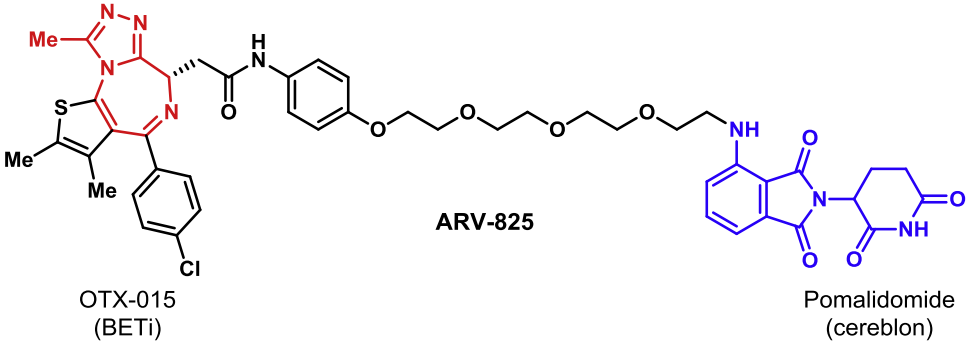
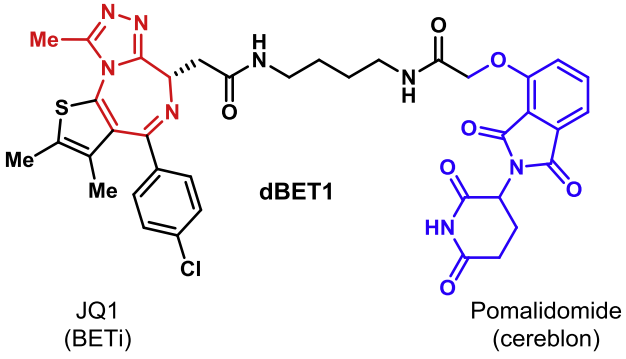


FIG. 11.7 PROTACs developed by Bradner et al. (dBET1) and by Crews et al. (ARV-825).



In 2016 researchers at AstraZeneca reported that AZD5153 (Fig. 11.8A), a small-molecule inhibitor incorporating two pharmacophores (triazolopyridazine and piperazinone), simultaneously binds to two BRDs.[48] AZD5153 was chosen for further development as a result of its pharmacokinetic profile and its high potency in vitro and in vivo effecting oncogene c-Myc downregulation.

During a campaign to identify compounds capable of downregulating the androgen receptor (AR), Waring et al.[49] discovered that the binding and cellular potency of compound **2.1** (Fig. 11.8A) had differed significantly. Compound **2.1** and related compounds produced downregulation of estrogen receptor-α (ERα) at concentrations similar to those seen with AR. The study authors speculated that BRD inhibition underlies both phenotypes. Further development of **2.1** led to the chemical probe biBET.[49] A battery of biophysical and cellular experiments, including elegant use of a

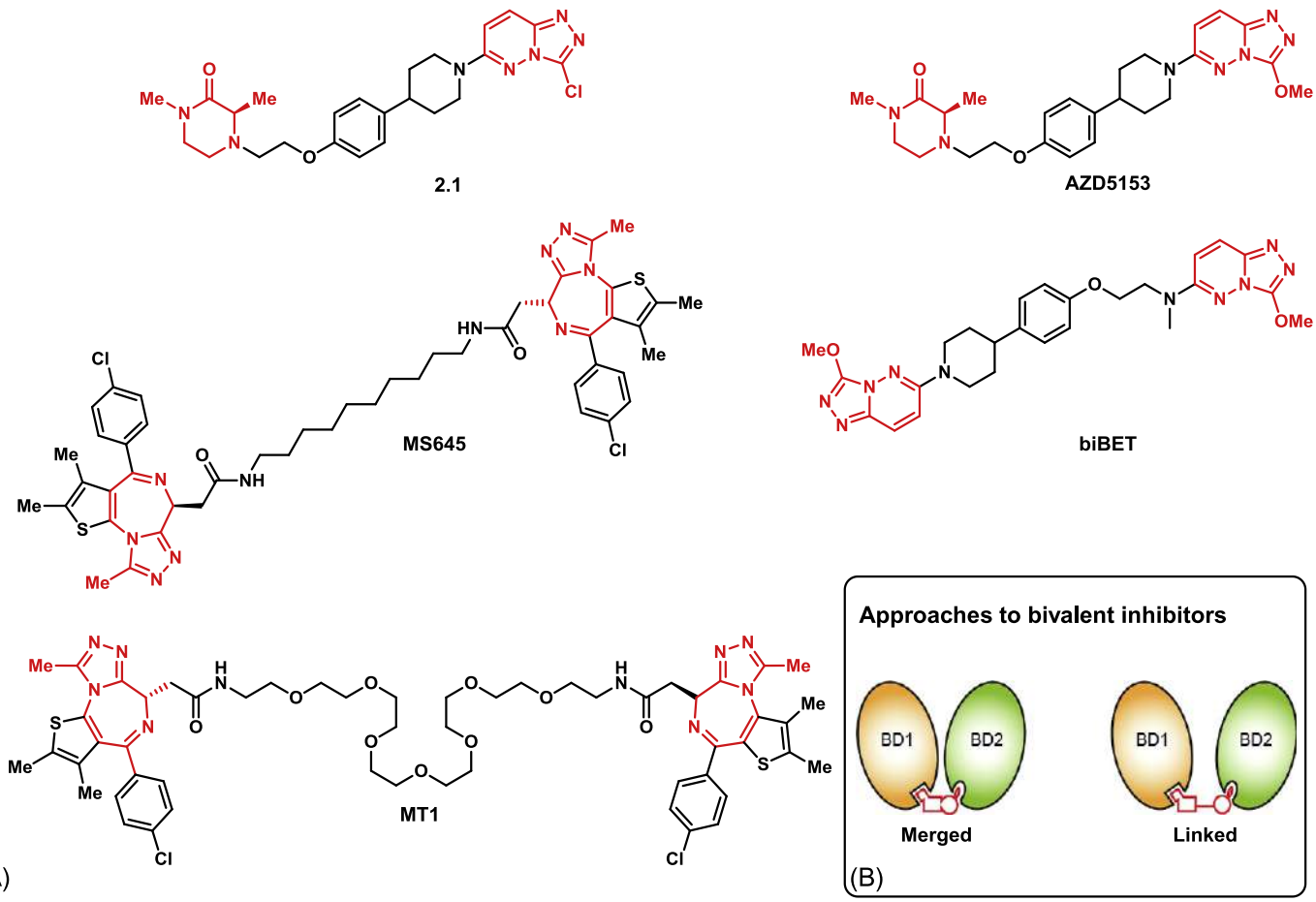


FIG. 11.8 (A) Depiction of bivalent BET inhibitors. (B) Merged and linked bivalent ligands as approaches to simultaneous BRD inhibition. The Kac mimetic is highlighted in *red*.

nanoBRET assay, revealed that biBET interacts with both BD1 and BD2 in Brd4. BiBET showed improved activity against both Brd4-sensitive and -insenstive cancer cell strains (MM.1S and RS4;11, respectively) relative to that of the monovalent BRD inhibitor I-BET762.

One of the most potent BETi to date, MT1 (Fig. 11.8A), is also a bivalent inhibitor, in which two molecules of JQ1 are connected by a PEG linker.[51] Biophysical and biochemical assays supported simultaneous binding of MT1 to both BRDs of Brd4. MT1 was over 100-fold more active in cellular assays than JQ1. A protein-ligand cocrystal structure confirmed that MT1 interacts with two units of Brd4 BD2, while conserving the JQ1-binding motif. Building on these results, Zhou et al. found that a thienodiazepine-based bivalent BETi, MS645 (Fig. 11.8a), blocked the proliferation of solid tumor cells.[52] RNA sequencing demonstrated that MS645 was markedly more effective than JQ1 in downregulating cell cycle control and DNA damage repair genes. Despite the large increases in potency provided by these bivalent compounds, they inevitably present challenges for translation to the clinic. Previously reported attempts to obtain cocrystallization data demonstrating simultaneous binding of these bivalent compounds to Brd4 BD1 and BD2 were not successful. These data may well be informative especially when it comes to the design of future inhibitors targeting both BRD binding sites.

11.2.6 Inhibitors Targeting Other BRD Families

The successful applications of JQ1 and I-BET762 (elaborated below) have also stimulated the search for potent and selective non-BET BRD inhibitors. This topic has been reviewed by several groups,[54–56] and the field continues to evolve rapidly. In 2016 alone more than 10 novel molecular probes/inhibitors targeting non-BET BRDs were identified.[57–67] The L-Moses[68] and BAY-850[69] small-molecule BRD inhibitors of p300/CBP-associated factor (PCAF) and of the ATPase family AAA domain-containing protein 2 (Atad2), respectively, were discovered the following year

(Fig. 11.5). These findings deserve specific mention because the identification of highly active (<100 nM) and selective (100 times greater than Brd4) BRD inhibitors for PCAF and Atad2 has been a long-standing challenge in BRD research. L-Moses and BAY-850 may be useful chemical probes to investigate the fundamental biological roles played by their respective BRD targets, and to validate PCAF and Atad2 as therapeutically relevant drug targets.

11.3 BET BROMODOMAIN INHIBITION IN PATHOLOGICAL CONTEXTS

11.3.1 BET Inhibitors in Cancer

As mentioned in Section 11.2.5, JQ1 has been investigated in an in vivo model of carcinoma and displayed in cellulo and in vivo inhibition of Brd4-NUT.[33] In this cancer type, named NMC, a genomic translocation generates a fusion between the proteins Brd4 and the nuclear protein in testis (NUT), which is the driving force behind one of the most aggressive solid malignant tumors known in humans.[70] Brd4-NUT has been shown to recruit factors that stimulate chromatin hyperacetylation, thereby recruiting more Brd4, which subsequently stimulates increased expression of prosurvival genes.[71, 72] Fluorescence recovery after photobleaching (FRAP) studies by Filippakopoulos et al.[33] revealed that JQ1 displaced Brd4-NUT from chromatin in cellulo.[33] In cellular assays JQ1 initiated differentiation and growth arrest in NMC cultures. In addition, JQ1 treatment led to tumor regression and improved survival rates in an in vivo mouse model of NMC.

After these initial reports, JQ1 and additional BETi were investigated in the context of other cancer types including leukemia, multiple myeloma (MM), and lymphoma. A majority of these approaches relied upon BETi that target Brd4. Brd4 recognizes and selectively binds to acetylated histones, where it recruits positive transcriptional elongation factor b (P-TEFb) to chromatin.[73, 74] P-TEFb, a hetereodimer of the cyclin-dependent kinase Cdk9 and a cyclin component, phosphorylates targets essential for transcriptional control, which results in the release of paused RNA polymerase II (Pol II) complexes and thus the elongation of transcription (Fig. 11.9).[75] When Brd4 associates with P-TEFb, it forces the kinase to remain in its active state by preventing its sequestration by the 7SK/HEXIM complex. This phenomenon allows Brd4 to directly regulate P-TEFb and consequently Pol II activity. BETi binding with Brd4 slows the transcription of genes associated with proliferation, which includes many protooncogenes.[71, 76] It has been pointed out that while Brd4 is a global transcription regulator, BETi activity on Brd4 can reduce gene expression selectively.[76, 77] Genes susceptible to BETi show significantly higher levels of Brd4 occupancy on some distal enhancers than on proximal gene promoters. These regions are referred to as superenhancers and are specific to cell types.

BETi have also been studied for treatment of several hematological malignancies. This topic has been extensively reviewed elsewhere.[78] I-BET151 was the first BETi to be investigated as a therapeutic approach in leukemia. I-BET151 induced early cell cycle arrest and apoptosis in both human and murine models of mixed lineage leukemia (MML-fusion).[38] I-BET151 administration downregulated oncogenic genes *MYC*, *CDK6*, *BCL2* via the displacement of BET BRDs and the disruption of transcription complexes SEC and PAFc. BETi, such as JQ1, I-BET151, and I-BET762, were

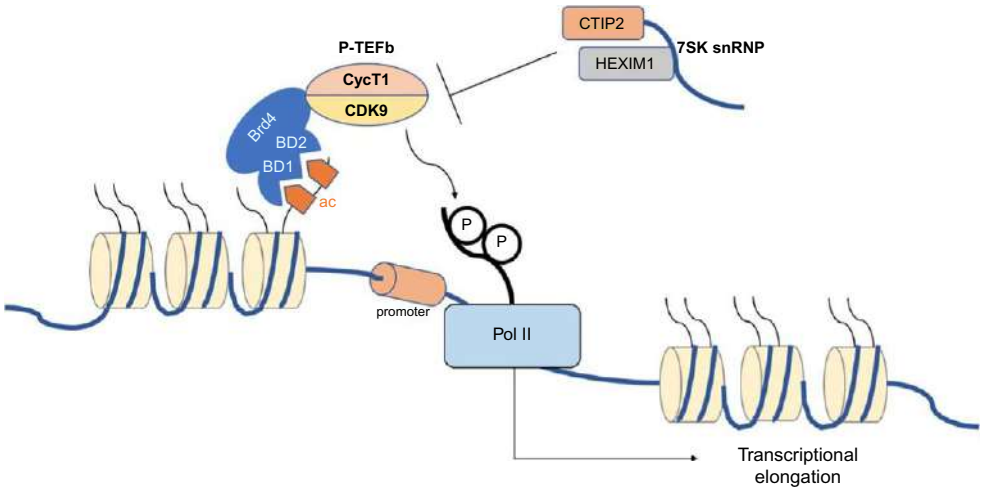


FIG. 11.9 Transcriptional function of Brd4. Brd4 binds acetylated histone tails through its BRDs and recruits P-TEFb to transcription start sites. Phosphorylation by P-TEFb of the Pol II and additional regulatory factors releases paused Pol II and prompts transcriptional elongation. The association between P-TEFb and Brd4 prevents P-TEFb from being inactivated by the 7SK/HEXIM1 complex.

also active in cellulo and in vivo models of multiple myeloma, where BETi downregulate the expression of *MYC* and *IRF4* while upregulating *HEXIM1*.[78]

11.3.2 BET Inhibitors in Inflammation

BETi have been explored in the context of inflammatory diseases. The initial publication describing the synthesis and activity of I-BET762 also reported that administration of this BETi suppressed proinflammatory gene expression of lipopolysaccharide (LPS)-stimulated bone marrow-derived macrophages.[34] I-BET762 suppressed the expression of many key proinflammatory cytokines and chemokines such as Il6, Ifnb1, Il1b, Il12a, Cxcl9, and Ccl12. I-BET762 also curtailed the expression of transcription factors Rel, Irf4, and Irf8, which the authors suggested could allow for I-BET762 to diminish the initial inflammatory gene expression. I-BET762 only had a small influence on general gene transcription in bone marrow-derived macrophages not treated with LPS and did not affect the transcription of housekeeping genes. I-BET762 selectively suppressed a specific subset of LPS-inducible genes, many of which were secondary-response genes. Promoters of LPS-inducible genes whose expression was not affected by the administration of I-BET762 exhibited higher basal levels of H3ac, H4ac, H3K4me3, and RNA Pol II. The study authors consequently hypothesized that the expression of these BETi-insensitive genes did not require BET BRD functionality. In support of this hypothesis, the administration of an HDAC inhibitor (which increases overall histone acetylation) converted BETi-sensitive into BETi-insensitive genes. The selective suppression of genes exhibited by I-BET762 raise interesting prospects for the use of BETi as an antiinflammatory therapeutic agent.

The translational potential of I-BET762 has been shown in a series of murine models. A single dose of I-BET762 (5 mg per kg i.p.) applied 1.5 h after LPS injection cured the mice. In addition, mice that suffered from polymicrobial peritonitis sepsis caused by caecal ligation and puncture were protected from death by injection of I-BER762 (30 mg per kg twice daily for 2 days).[34]

11.3.3 BET Inhibitors in Cardiovascular Diseases

BETi have also been studied as a strategy to treat cardiovascular disease. The research groups of Bradner and Haldar studied the use of BETi to treat heart failure (HF).[79, 80] Brd4 was found to coactivate stress-induced transcription pathways in HF. These pathways are induced during cardiac hypertrophy. Brd4 also coactivated NFκB and Gata4 (transcription factors known to be associated with HF progression). Gene expression profiling and genome-wide mapping by ChIP sequencing showed that Brd4 promotes transcriptional pause release and elongation in response to pathological stress.[79, 80] Common BETi, such as JQ1, I-BET151, I-BET762, and RVX-208, blocked cellular hypertrophy as well as lowered pathologic gene induction in a neonatal rat that had been treated with phenylephrine, an α1-adrenergic receptor agonist.[79, 80] In an in vivo mouse model JQ1 decreased the expression of hypertrophic marker genes. In this model JQ1 also helped prevent left ventricular hypertrophy, interstitial fibrosis, and systolic dysfunction.

In 2017 the same researchers jointly reported that BET inhibition could be beneficial in a more clinically relevant setting.[81] JQ1 had therapeutic effects during preestablished HF caused by prolonged pressure overload, as well as after anterior myocardial infarction in an in vivo mouse model. JQ1 blocked against hypertrophy in human-induced pluripotent stem cell-derived cardiomyocytes. JQ1 also did not interfere with physiological cardiac hypertrophy that occurs during stress in response to exercise. Cumulatively, these findings provide a strong foundation for further investigation of BETi as a therapeutic strategy for HF.

The BETi RVX-208 has been extensively evaluated as a treatment for atherosclerotic disease. Atherosclerosis progression can be inhibited by transporting cholesterol from artery walls to the liver for excretion. Key components of this pathway, known as reverse cholesterol transport (RCT), are ApoA1 and high-density lipoprotein (HDL) which are used by the body as acceptors and transporters for cholesterol. RVX-208, the first BETi to enter into clinical trials, was originally identified in a cellular assay employed to identify small molecules that would increase ApoA1 levels.[39]

In vivo studies revealed that oral administration of RVX-208 markedly increased the levels of ApoA1 and HDL cholesterol after a month of treatment. In addition, a series of clinical trials revealed that ApoA1, HDL cholesterol, HDL particle size, and total HDL particles were increased when RVX-208 was administered.[82] Two separate clinical trials also demonstrated that RVX-208 lowered major adverse cardiac events, particularly in patients with diabetes mellitus. A phase III clinical trial is currently under way to examine whether RVX-208 increases the time to major adverse cardiac events in high-risk type 2 diabetes mellitus patients with coronary artery disease (ClinicalTrials.gov identifier: NCT02586155).

Some researchers suggest that perhaps the reason for RVX-208 advancing into phase III clinical trials is its unique ability to selectively inhibit Brd4 BD2.[41] This example illustrates the need to develop small-molecule inhibitors that are highly selective for a single BRD to elucidate the biological roles of specific BRDs as well as to mitigate potential side effects that may be caused by pan-BETi (BETi that inhibit all BET BRDs) such as memory loss[83] and sterility.[84]

11.4 BROMODOMAIN INHIBITION AND PROTOZOAL INFECTIONS

Protozoal infections are believed to be responsible for over 1 million deaths a year.[85, 86] Malaria, caused primarily by two species of *Plasmodium*, accounts for most of these deaths. Other major diseases caused by invasive parasitic protozoa include African sleeping sickness, Chagas disease, and toxoplasmosis.[85, 86] The greater disease burden is localized in the subtropical regions of the world. No vaccines have been approved and available treatments are prone to decreased effectiveness resulting from drug resistance.[86] As a result some have argued for whole-cell screening of other approved drugs to identify new antiprotozoal agents.[86–88]

In a seminal 1996 publication Schmat et al. reported that the fungal metabolite apicidin exhibits broad-spectrum antiprotozoal activity on apicomplexan parasites and was orally and parenterally active in an in vivo malaria mouse model.[89] The observed antiprotozoal effect correlated with apicidin's activity toward the apicomplexan HDAC. This novel epigenetic approach to discovery of antiprotozoal agents was later validated with other HDAC[90–93] or KAT inhibitors.[94–97] Despite this early work, BRD inhibitors have only recently been investigated as antiprotozoal agents.

11.4.1 Bromodomains in Parasitic Protozoa

Recent genetic analysis of the common pathogenic protozoal species (*Toxoplasma gondii*, *Plasmodium falciparum*, *Trypanosoma brucei*, *Trypanosoma cruzi*) predicted the existence of 29 BRDs distributed into 6 clades, with 3 clades containing proteins from all 4 species.[98] While BRDs have been shown to play a key role in gene regulation in *Toxoplasma*[99] and *Trypanosoma*[100–103] species, perhaps most relevant to current epigenetic drug discovery efforts is the function of *P. falciparum* BRDs in malaria.[104] Duffy et al. discovered that *P. falciparum* expresses PfBdp1, a BRD-containing protein that plays a key role in parasite biology (Fig. 11.10). PfBdp1 regulates *P. falciparum*'s invasion of host erythrocytes by binding to the transcriptional start sites of invasion-related genes and activating their expression. PfBdp1 binds acetylated histone H3 and associates with a second BRD-containing protein, PfBdp2, signifying a potential mechanism by which PfBdp1 is recruited to acetylated nucleosomes at regulatory sites. Knockdown of PfBdp1 dramatically impaired erythrocyte invasion of red blood cells and parasite proliferation. This observation provides the biological basis for BRD inhibition as a potential strategy for antimalarial drug development.

P. falciparum contains a single Gcn5 (PfGcn5), a BRD-containing KAT protein, which preferentially associates with acylated H3 and is believed to be necessary for blood-stage replication.[96] PfGcn5 was investigated as a drug target in 2007 by Cui et al.[96] In that study curcumin inhibited the growth of several parasite strains with IC_{50} values in the 20–30-μM range. This activity was posited by the study authors to be caused by an increase in reactive oxygen species in the parasitic cell, as well as inhibition of the HAT activity of PfGcn5. Since that paper was published it has become widely acknowledged that curcumin and its derivatives are pan-assay interference compounds that can often produce misleading assay results, so future work should attempt to clarify the actual role of curcumin.[105] Notwithstanding this

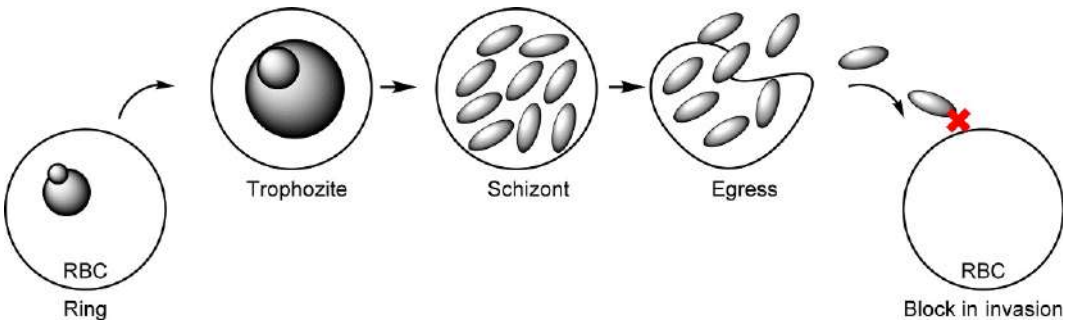


FIG. 11.10 Depiction of *Plasmodium falciparum* life cycle during Pfdbp1 knockdown experiments. The erythrocyte's ability to invade red blood cells (RBCs) is impaired, thus halting parasitic proliferation.

concern, the potential of various established KAT inhibitors as antiprotozoal agents appears to be well supported by other work.[94, 95, 97]

11.4.2 Inhibiting Bromodomains of Parasitic Protozoa

Andrews et al. tested BRD inhibitors (BRDi) in vitro against *P. falciparum* multidrug-resistant Dd2 parasites.[106] Compound selection was aided by the pharmacophore identification software LigandScout. In total, 38 candidates were identified, as well as 4 well-established BRD inhibitors. They were further evaluated in docking studies using the apo BRDs of both PfGcn5 and PfBdp1. While interesting interactions with the conserved Asn1436 of the PfGcn5 BRD were revealed, at the time of publication the only crystal structure of PfBdp1 available lacked the ZA loop, which is known to be critical for ligand recognition.[24, 33, 107] This point is underlined by the fact that, when these compounds were tested on isolates of *P. falciparum*, the known inhibitor SGC-CBP30 (Fig. 11.5) was more active than any of the designed candidates, albeit its activity (IC_{50} 3.2 μM) and selectivity (mammalian cell IC_{50}/PfDd2 IC_{50} 7) were modest. It is apparent that there is an opportunity for future work in this area. There is now a high-quality cocrystal structure of PfGcn5 in complex with L-Moses (PDB ID 5TPX), which could provide better predictive power in in silico drug discovery programs to develop protozoal-specific PfGcn5 inhibitors.[68]

BETi have also been tested on *T. brucei*, the cause of the parasitic disease African sleeping sickness. In 2015 Papavasiliou et al. reported that treatment of *T. brucei* cultures with I-BET151 downregulated genes required for bloodstream survival. The study authors attributed this activity to the inhibition of BRD-containing proteins TbBdf2 and TbBdf3.[102] Parasites treated with I-BET151 displayed slower growth, cell cycle defects and disrupted bloodstream form immune evasion mechanisms used to elude host antibody responses. Mice infected with *T. brucei* cultures that were pretreated in vitro with I-BET151 survived significantly longer than control mice. When the pretreatment of *T. brucei* was extended for 3 days, 80% of the mice did not develop detectable parasitemia. The omission of a concurrent treatment study was justified on the basis of the low affinity of I-BET151 for parasitic BRDs.

Strikingly, the crystal structure of the Bdf2 BRD complexing I-BET151 revealed a novel mode of binding in which the 3,5-dimethylisoxazole pharmacophore (Section 11.2.3) is positioned away from the Kac binding pocket (Fig. 11.11A).[102] Thus recognition of the conserved Asn is mediated by hydrogen bonds with the imidazoline motif. The study authors suggested that this unexpected binding mode is due to a steric clash between the pyridine of I-BET151 and the large gatekeeper residue (Trp92), which is not present in any of the 61 human BDs known (Fig. 11.11B).[102] The result gives credibility to the proposition of developing parasite-specific BRD inhibitors that could leave hBRD function relatively unaffected.

In 2016 it was reported that the hBRD inhibitors JQ1, I-BET151, and SGC-CBP30 inhibit *T. cruzi* replication.[103] The study authors posited that the inhibitors interact with the BRD of TcBdf3 on the basis that overexpression of this protein decreased parasite sensitivity to the compounds.

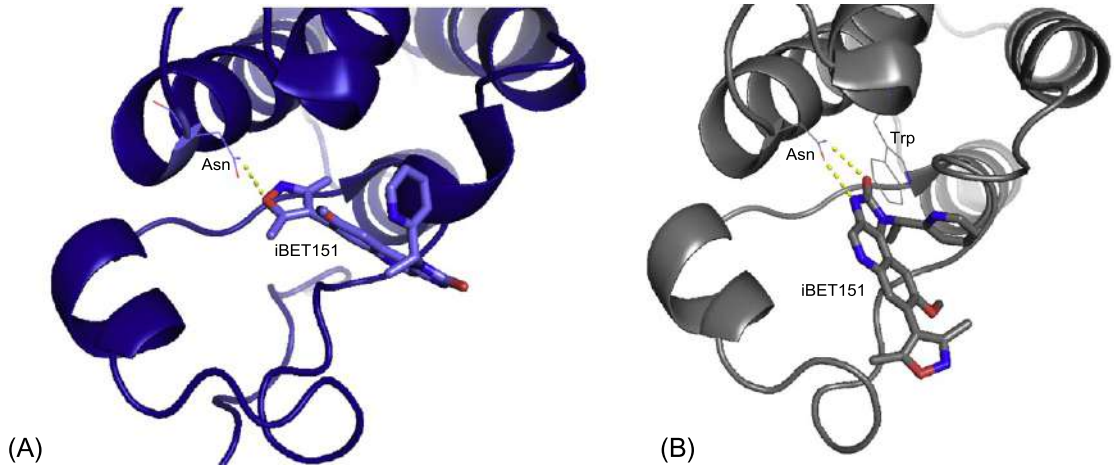


FIG. 11.11 Comparison between the cocrystal structures of Brd4 and TBdf2 in complex with I-BET151: (A) Brd4 BD1 in complex with I-BET151 (PDB 3ZYU); (B) *Trypanosoma brucei* Bdf2 in complex with I-BET151 (PDB: 4PKL). Hydrogen bonds are depicted using *dashes*.

The above findings suggest that BRD inhibition could be used to treat parasitic infections of a broad range of protozoa. The prospect is encouraging; however, future studies should focus on the development of potent (low nM) protozoal-specific BRD inhibitors that could be transitioned into in vivo models of infection.

11.5 BROMODOMAIN INHIBITION AND FUNGAL INFECTIONS

Opportunistic fungal infections are a substantial global health concern. There are roughly 2 million cases reported and over 800,000 deaths that are attributed to invasive fungal infections.[108, 109] While *Candida albicans* is the most common pathogenic fungi observed in the clinic, there has been a stark increase of *Candida glabrata* infections over the past 20 years.[110] There is a marked growth in the isolation of drug-resistant fungal strains in the clinic, and yet there is only a limited number of safe antimycotics available to treat severe fungal infections. These points illustrate the urgent need for novel antifungal agents.[108, 111–113]

11.5.1 Fungal BET Bromodomains

Bromodomain factor 1 (Bdf1) is a fungal BET protein that regulates the transcription of numerous genes. In *Saccharomyces cerevisiae*, Bdf1 binds acetylated histones H3 and H4[114, 115] (H3ac and H4ac) as well as the transcription factor TFIID.[116] Similar to Brd4 (the homologous human BET BRD), Bdf1 recognizes acetylated chromatin with its two BRDs (BD1 and BD2). Bdf1 plays an important role in euchromatin maintenance and antisilencing,[117] and is involved in chromatin compaction during sporulation.[118] In addition to Bdf1, *S. cerevisiae* also possesses Bdf2 (a second BET family gene that is functionally redundant with Bdf1).[119, 120] Disruption of *BDF1* causes morphological and growth defects, while simultaneous deletion of *BDF1* and *BDF2* is lethal to *S. cerevisiae*.[114–116] Point mutations that weaken chromatin recognition by ScBdf1 BD1 and BD2 cause growth and sporulation defects.[121] In contrast to *S. cerevisiae*, most pathogenic fungi (including *Candida* species) lack Bdf2 and have only one BET family gene.

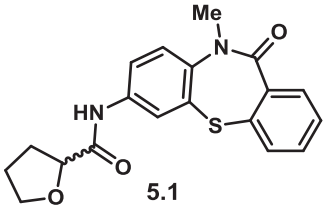
11.5.2 Inhibition of Fungal BET Bromodomains

A recent publication by Mietton et al. has explored the potential of BET BRD inhibition as an antifungal strategy.[122] The study authors showed that mutations of both BD1 and BD2 in *BDF1* that inactivate *C. albicans* Bdf1 (CaBdf1) functionality resulted in a loss of viability in vitro and decreased virulence in an in vivo model of disseminated candidiasis. Protein crystallographic studies demonstrated that the atomic structures of CaBdf1 BDs diverge from those of human BET proteins, highlighting that selective species-specific compounds could be developed. A high-throughput screen utilizing a miniaturized Homogeneous Time-Resolved Fluorescence (HTRF) assay was employed to identify BD1 and BD2 inhibitors (compounds **5.1**–**5.3** in Fig. 11.12) that can displace a polyacylated histone H4 tail from Bdf1 BRDs. Inhibitors **5.1**–**5.3** phenocopied the effect of BRD-inactivating mutations on the viability of *C. albicans*. Interestingly, none of the well-established hBRD inhibitors (JQ1, I-BET151, PFI-1, and bromosporine; Fig. 11.5) tested displayed cytotoxic effect in cultures of *C. albicans* despite showing small activity in the HTRF assay used to determine H4ac4 displacement from the BRDs (I-BET151 showed IC_{50} values of 303 and 1470 nM for CaBdf1 BD1 and CaBdf1 BD2, respectively).

Cocrystal structures of **5.1** and **5.3** complexed with CaBdf1 BD1 and BD2, respectively, revealed that the BRD binding pocket recognized the ligands through the conserved Asn residue. Inhibitor **5.3** showed a water-mediated hydrogen bond with the conserved Tyr of CaBdf1 BD2, presumably because the phenol of **5.3** displaced a conserved water molecule in the Kac binding pocket. The selectivity of molecules **5.1**–**5.3** toward the fungal BRDs was demonstrated to arise from steric clashes with key residues in the hBRD orthologs (Trp8 and Leu94 for Brd4-BD1 and Trp374, Leu387, and His437 for Brd4-BD2).

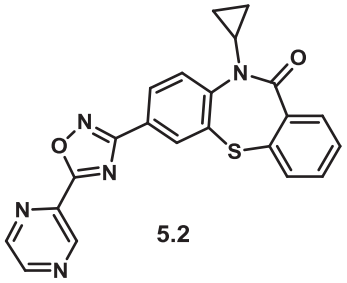
Several hurdles still need to be overcome for BET inhibition to become a viable treatment for systemic candidiasis. First, a compound that can inhibit both BRDs of *C. albicans* may be necessary to obtain a useful therapeutic effect. This could be realized by the use of "dual-warhead" or bivalent BRD inhibitors, as has been investigated for the treatment of cancer (discussed in Section 11.2.5). Second, the low cellular activity of these compounds relative to their in vitro potency must be addressed.

FIG. 11.12 Fungal-specific BRD inhibitors.



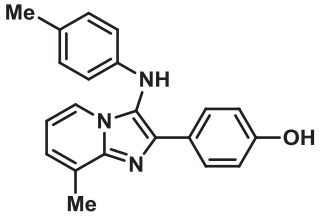
5.1

	IC_{50} (μM)
*Ca*Bdf1 BD1	1.7 ± 0.6
*Ca*Bdf1 BD2	>20
Brd4 BD1	12.5 ± 0.8



5.2

	IC_{50} (μM)
*Ca*Bdf1 BD1	4.3 ± 1.5
*Ca*Bdf1 BD2	>20
Brd4 BD1	>20



5.3

	IC_{50} (μM)
*Ca*Bdf1 BD1	>20
*Ca*Bdf1 BD2	1.1 ± 0.4
Brd4 BD2	>20

11.6 BROMODOMAIN INHIBITION AND VIRAL INFECTIONS

The role of BETi in HIV infection and persistence is an emerging area of research. While there are now highly effective means of controlling the viral infection, a cure remains to be discovered. The virus persists in certain cell types and reemerges if treatment ceases. Reactivation of the latent virus via inhibition of Brd4 has been studied. The HIV transactivator protein Tat requires Brd4 to recruit p-TEFb to the HIV promoter. Once Tat has been produced, both Tat and Brd4 compete to bind with p-TEFb. The negative regulator status of Brd4 means that HIV reactivation can be induced by BETi. Several researchers have investigated the use of JQ1 and I-BET151 to bring the HIV virus out of latency.[123–126] However, there has been varied success, with some patient cells not displaying any signs of HIV reactivation. The most effective strategy appears to be combining BETi with a second reactivating agent, like ingenol B, a protein kinase C agonist.[127] Despite some of the contradictory results, further investigation is warranted with the goal of adding BET inhibition to the anti-HIV arsenal.

BETi have been evaluated on human polyomavirus JC (JCV), which replicates in glial cells and causes the fatal disease progressive multifocal leukoencephalopathy (PML). Administration of JQ1 decreased late protein expression and virion production in a cellular assay of JCV infection of SVGA cells.[128] This activity was suggested to arise from disrupting the interaction of Brd4 and p65, halting p65 binding with chromatin and viral transcription. You et al. investigated the affect BETi have on the human papillomavirus (HPV) life cycle.[129] The study authors demonstrated that recruitment of p-TEFb by Brd4 was important for viral protein E2 transcription activation. E2 is the master regulator of HPV early gene transcription. The study authors also reported that JQ1 dissociated E2-Brd4 complexes from chromatin and potently reduced HPV transcription.[129, 130]

Park et al. recently reported a novel use of JQ1 to disrupt the replication of hepatitis C virus (HCV).[131] In these studies the authors noted that c-Myc, HIF-1a, and glycolytic enzymes were upregulated in HCV-infected hepatocytes. They also demonstrated that metabolic adaption of the host cell, which resembles that of cancer cells, is necessary for viral replication. The overexpression of c-Myc, HIF-1a, and glycolytic enzymes suggest joint regulation of these proteins. Interestingly, treatment of HCV-infected cells with JQ1 downregulated the expression of both Myc and glycolytic enzymes, resulting in inhibition of viral replication.

11.7 FUTURE DIRECTIONS

Since the discovery of the first selective BET BRD inhibitors in 2010, research to explore the therapeutic potential of BRD inhibitors has expanded dramatically. In a 2017 review Esteller et al. listed over 20 clinical trials in which BRD inhibitors are being evaluated for cancer therapy alone.[132] While the rapid translational progress of BRD inhibition is evident in some disease areas, in others further work is needed to potentiate a translational outcome. In particular, the discovery and development of a highly potent, species-specific, bioavailable, and safe BRDi approved for treatment of an invasive fungal or parasitic infection remains a challenge for the future, notwithstanding the intriguing proof-of-principle results reviewed in this chapter. Meanwhile, BET BRDi are proving to be useful as probes to elucidate epigenetic processes at the molecular level. In this regard inhibitors of non-BET BRDs and previously unidentified non-human BET BRDs may hold similar promise as well as affording new targets for therapeutic agents.

Acknowledgments

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CHAPTER

12

Epigenetic Drug Discovery for Cancer

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12.1 INTRODUCTION

Posttranslational modification (PTM), a major mechanism used to increase the diversity of protein species and their corresponding functions in cells, is widely distributed in cellular processes.[1] Apart from well-known PTMs, such as phosphorylation, glycosylation, and ubiquitination, another PTM called acetylation is crucial to regulating the biological activity of proteins in cells. The chief protein components of chromatin are histones. They are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes to play a role in gene regulation. Acetylation at the epsilon amino group of lysine residues was first discovered to be a PTM of histones more than 50 years ago.[2]

Lysine acetylation of histone proteins is a fundamental PTM that regulates chromatin structure and plays an important role in gene transcription, the aberrant levels of which are associated with the development of several diseases.[3] It is typically controlled by two groups of enzymes: lysine acetyltransferases (KATs) and lysine deacetylases (KDACs), a.k.a. histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. Lysine acetylation functions by catalyzing the addition of acetyl groups to lysine residues. In contrast, lysine deacetylation is catalyzed by a specific group of NAD^+-dependent KDACs, sirtuins (SIRTs), the function of which is to remove acetyl groups from lysine residues. In addition, lysine acetylation can alter chromatin structure and function by providing binding signals for reader proteins containing acetyl-lysine recognition domains, including the bromodomain (BRD).[4] Among the bromodomain-containing proteins, several chromatin-remodeling proteins, transcription factors, and KATs can be observed (Fig. 12.1).

Lysine acetylation is involved in maintaining the proper functioning of cells and has recently emerged as a significant PTM for cells, which may be relevant to the development of several diseases, including cancer.[5] Furthermore, BRDs, HATs, HDACs, SIRTs and so on appear to be potential drug-epigenetic targets, something that has encouraged the discovery and development of several small-molecule inhibitors in recent years.[6] In this chapter we focus on such small-molecule inhibitors of some targets associated with lysine acetylation to shed new light on the discovery of epigenetic cancer drugs.

12.2 BROMODOMAIN (BRD) PROTEINS AND THEIR INTERACTION WITH SMALL-MOLECULE INHIBITORS IN CANCER

BRD proteins, consisting of BRD2, BRD3, BRD4, and testis-specific BRDT members, are epigenetic readers and play a key role in the regulation of gene transcription, which has been identified to regulate lysine acetylation by inhibitors.[7, 8] A small-molecule inhibitor of BRD4, 9f, has been found to induce autophagy-associated cell death by blocking the BRD4-AMPK interaction and thus activating the AMPK/mTOR-ULK1-modulated autophagic pathway in breast cancer cells, which has displayed therapeutic potential in both breast cancer xenograft mouse and zebrafish models.[9] A compound called 13-d, a biased, potent inhibitor of the BRD of BRPFs that has excellent selectivity over nonclass IV

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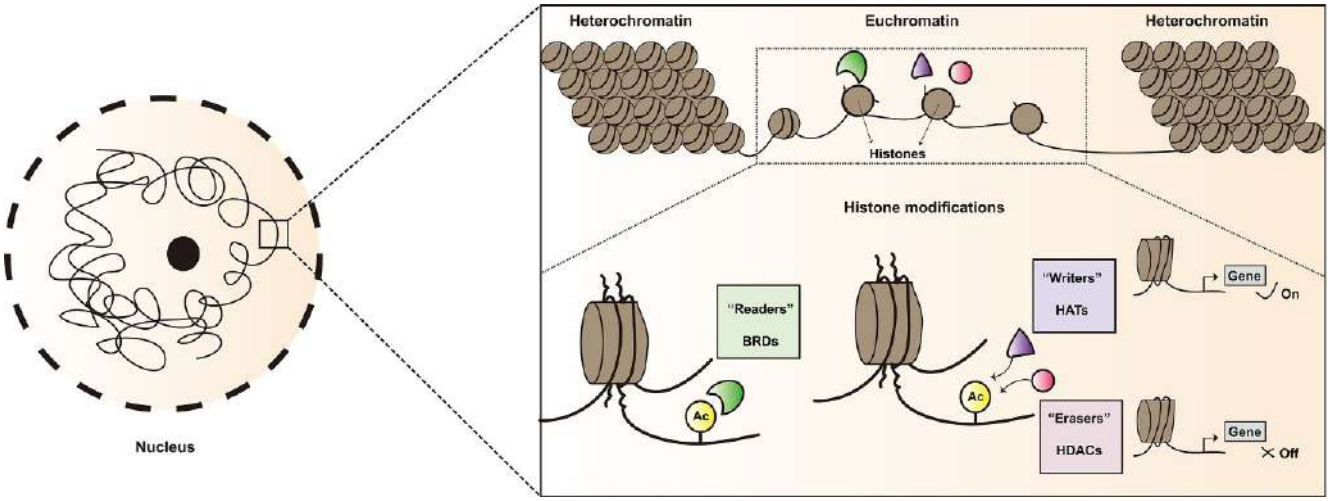

FIG. 12.1 Posttranslational modification of lysine acetylation of histones. Chromatin exists in two main states: an open relaxed conformation called euchromatin and a more compact dense state called heterochromatin. Chromatin modifications such as acetylation, which is laid down by epigenetic "writers," bound by epigenetic "readers," and removed by epigenetic "erasers," can switch from one state to the other. The main readers are proteins containing bromodomains (BRDs), writers are histone acetyltransferases (HATs), and erasers are histone deacetylases (HDACs).

BRD proteins, showed selective inhibition of proliferation in a subset of acute myelocytic leukemia (AML) lines. Pharmacokinetic studies have established that 13-d had properties compatible with oral dosing in mouse models of disease (Fpo 49%).[10] CPI-0610, a potent BET inhibitor currently undergoing phase I clinical testing, displayed potent cytotoxicity against multiple myeloma (MM) cell lines and patient-derived MM cells through G1 cell cycle arrest and caspase-dependent apoptosis. CPI-0610-mediated BET inhibition overcame the protective effects conferred by cytokines and bone marrow stromal cells. Moreover, the efficacy of CPI-0610 in vivo was confirmed in an MM xenograft mouse model.[11] I-BET151 has been shown to have preclinical activity against acute leukemia, including mixed lineage leukemia-related acute myeloid leukemia.[12] I-BET762 (GSK-525762A) had a favorable pharmacologic profile as an oral agent and was found to inhibit myeloma cell proliferation, resulting in increased survival advantage in a systemic myeloma xenograft model.[13] The first-in-class small-molecule inhibitor OTX015 (MK-8628) was found to specifically bind to bromodomain motifs BRD2, BRD3, and BRD4 of bromodomain and extraterminal (BET) proteins, inhibiting them from binding to acetylated histones, which were active in hematological preclinical entities including leukemia, lymphoma, and myeloma.[14] Moreover, based on OTX015, a proteolytic-targeting/BET inhibitor chimera, both ARV-771 and ARV-825 demonstrated rapid and prolonged BRD4 degradation in several Burkitt lymphoma cell lines, which had a superior durable effect on cell proliferation and induced apoptosis than the small-molecule reversible BET inhibitors JQ-1 and OTX-015.[15, 16] dBET1 has demonstrated a similar biochemical potency and selectivity toward the BET family of bromodomains as the parental small-molecule BET inhibitor JQ-1.[17] The efficacy of the BET protein degrader was further tested in an acute myeloid leukemia cell line, and it was demonstrated that complete BRD4 degradation was achieved within 2 h of cell treatment at concentrations as low as 100 nM.[18] The degrader, MZ1, was designed by linking JQ-1 to the recognition motif of E3 ubiquitin ligase VHL. By varying the polyethylene glycol linkers attached to the solvent-exposed group of JQ-1, it was possible to optimize the linker length with no significant penalties in binding affinities to BRD4.[1] The leading PROTAC candidate, MZ1, was able to degrade BRD4 effectively at the protein level in HeLa and U2OS osteosarcoma cancer cell lines.[19] ABBV-075 efficiently triggered apoptosis in acute myeloid leukemia (AML), non-Hodgkin lymphoma, and multiple myeloma cells.[20] CPI-203 has been found to synergistically induce cell death when combined with lenalidomide. Moreover, the addition of CPI-203 to lenalidomide therapy in mice further decreased the tumor burden (Fig. 12.2).[21]

12.3 HISTONE ACETYLTRANSFERASES AND THEIR INTERACTION WITH SMALL-MOLECULE INHIBITORS IN CANCER

Histone acetyltransferases (HATs) can function as writers to regulate lysine acetylation and as modulators of some small-molecule compounds, like inhibitors. The coenzyme A-containing compound, Spd(N1)-CoA, has been reported

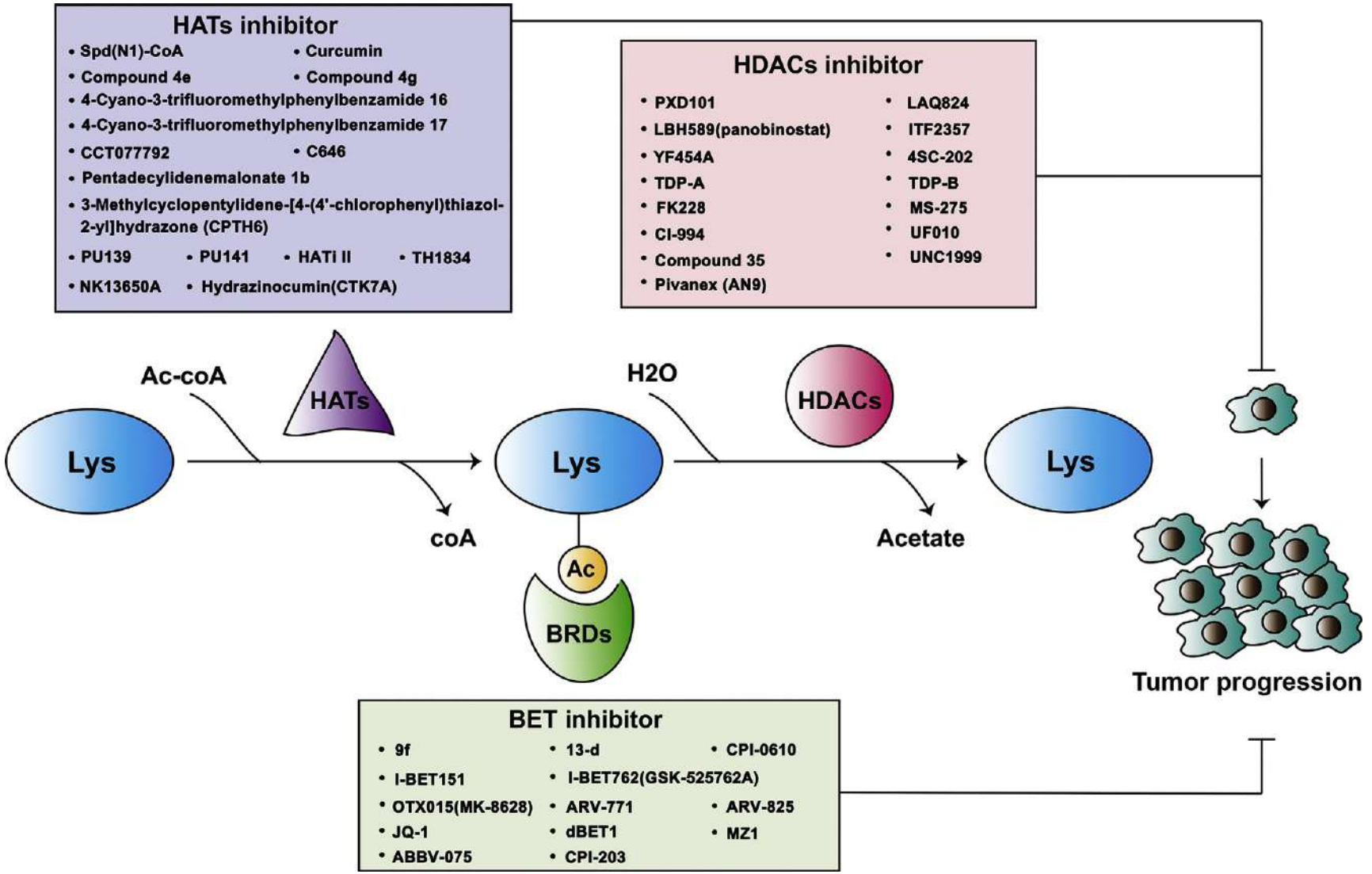

FIG. 12.2 The mechanism behind histone acetylation and corresponding targeted therapies. Lysine side chains are acetylated via acetyl-CoA (Ac-CoA)-dependent mechanisms by histone acetyltransferases (HATs), histone deacetylases (HDACs), and bromodomains (BRDs). Corresponding targeted therapies, such as HAT inhibitors, HDACi, and BET inhibitors are applied in cancer therapy.

to inhibit p300/CBP-mediated histone acetylation. Treatment of cancer cells with it results in increased sensitivity to cisplatin, 5-fluorouracil, and camptothecin, while healthy cells remained unaffected. Moreover, removal of much of coenzyme A led to a substructure that had enhanced chemosensitization effects. Owing to their partial polyamine structure, Spd(N1)-CoA and the corresponding substructure are internalized into mammalian cells via polyamine transporter uptake. This approach is a novel strategy for the treatment of cancer based on chemosensitization by selective small molecules.[22] Curcumin is the major curcuminoid component in the roots of spice turmeric *Curcuma longa* L. and was published as being a HAT inhibitor in 2004, after evaluation in several clinical trials for the therapy of cancer, Alzheimer disease, rheumatoid arthritis, cystic fibrosis, and psoriasis at the moment. However, it is unclear which of the many activities is really decisive for curcumin in vivo.[23, 24] HAT inhibition in vitro has been shown to correlate with their antiproliferative effects in a set of cancer cell lines including breast and cervical carcinoma, T cell lymphoma, and prostate adenocarcinoma. Moreover, compounds 4e and 4g have been reported as HAT inhibitors, along with a number of related structures. Interestingly, these compounds were nontoxic against a nonmalignant human fibroblast cell line.[25] 4-Cyano-3-trifluoromethylphenylbenzamides 16 and 17 were published as p300 inhibitors in vitro, the activities of which were found to be comparable with that of anacardic acid. Moreover, the short chain derivative 16 induces apoptosis of leukemia cells at 50 mM.[26] High-throughput screening of a large compound library has led to the discovery of 35 isothiazolones that are potent p300 and PCAF inhibitors. One of them, CCT077792, has been reported to significantly reduce the growth inhibition of human colon carcinoma cells in a time- and concentration-dependent manner.[27] Pentadecylidenemalonate 1b, a simplified analog of anacardic acid, was identified as the first mixed activator/inhibitor of histone acetyltransferases, the remarkable apoptotic effect of which together with the ability to selectively acetylate histone vs nonhistone substrates appointed it as a lead for the development of anticancer drugs.[28] C646, a selective inhibitor of p300 and CBP, inhibited cell viability and the cell cycle and promoted cell apoptosis in five gastric cancer (GC) cell lines (SGC-7901, MKN45, MGC-803, BGC-823, and KATO III).[29] 3-Methylcyclopentylidene-[4-(4′-chlorophenyl)thiazol-2-yl]hydrazone (CPTH6), a novel pCAF and Gcn5 histone acetyltransferase inhibitor, is a small molecule that preferentially targets lung cancer stem-like cells (LCSCs) derived from nonsmall-cell lung cancer (NSCLC) patients.[30] Two selected pyridoisothiazolone HAT inhibitors, PU139 and PU141, induced cellular histone hypoacetylation and inhibited the growth of several neoplastic cell lines originating from different tissues.[31] HATi II inhibited proliferation and induced apoptosis via the caspase-dependent pathway in human glioma cell lines, possibly by activating the p53-signaling pathway.[32] TH1834 has been found to specifically induce apoptosis and increase unrepaired DNA damage in breast cancer but not in control cell lines.[33] NK13650A, isolated from a production strain belonging to *Penicillium*, selectively inhibited the activity of p300 HAT but not that of Tip60 HAT. Moreover, NK13650A treatment inhibited the hormone-dependent and hormone-independent growth of prostate cancer cells.[34] A water-soluble small-molecule inhibitor, hydrazinocurcumin (CTK7A), has been found to inhibit the HAT activity of p300 and substantially reduce xenografted oral tumor growth in mice (Fig. 12.2).[35]

12.4 HISTONE DEACETYLASES AND THEIR INTERACTION WITH SMALL-MOLECULE INHIBITORS IN CANCER

In contrast to HATs, histone deacetylases (HDACs) can function as erasers. Histone proteins are responsible for assembling vast amounts of genomic DNA into a size and structure that can be easily accommodated in the eukaryotic nucleus. Histones receive a rich repertoire of posttranslational modifications, frequently referred to as the "histone code," which impacts both their intrinsic properties and interactions with chromatin-associated proteins and has significant consequences on the level of gene activity. According to the chemical structure, HDAC inhibitors from nature and synthetic derivatives can be divided into several types typically targeting the HDAC family, which comprises 11 members in humans.

Hydroxamic acid agents were among the first compounds to be identified as HDAC inhibitors (HDACi). TSA and SAHA are hydroxamic acid-based compounds, as are PXD101, LAQ824, LBH589 (panobinostat), and ITF2357.[36] YF454A, a highly potent candidate of a novel synthesized class of hydroxamate-based HDACi that has potent activities against breast cancer by the upregulation of acetyl-histone H3 and H4, downregulation of paxillin expression, and inhibition of tumor epithelial-mesenchymal transition, can overcome EGFR-TKI resistance in NSCLC in combined therapy with erlotinib through interfering with the multiple "bypass" receptor tyrosine kinase pathways.[37] Recently 4SC-202, a novel clinically validated inhibitor of class I histone deacetylases (HDACs), was found to efficiently block HH/GLI signaling. Remarkably, 4SC-202 treatment abrogates GLI activation and HH target gene expression in both SMOi-sensitive and SMOi-resistant cells.[38] The cyclic peptide class is a structurally complex group of HDACi, which

includes the natural product depsipeptide (FK-228) and the CHAPs group of molecules. Two new small-molecule HDACi TDP-A and TDP-B, analogs of the class I-biased HDAC inhibitor FK228, have been found to suppress cell viability and induce apoptosis at nanomolar drug concentrations. TDP-B showed the greatest similarity to the biological activity of FK228 with greater cytotoxic effects than TDP-A in vitro.[39]

The benzamide class of HDACi is exemplified mainly by MS-275 and CI-994. MS-275, a synthetic benzamide derivative, has presented antiproliferative and cytotoxic activity against a wide range of tumor cell lines in vitro. MS-275 is an orally active inhibitor currently in clinical trials. High-throughput screening (HTS) led to the discovery of a lead compound, UF010, that selectively inhibits HDAC1, HDAC2, and HDAC3 (class I HDACs) and features a previously unknown benzoylhydrazide scaffold as the pharmacophore for HDACi. UF010 and analogs have been found to inhibit cancer cell proliferation and thus bring about global changes in protein acetylation and gene expression, leading to the activation of critical tumor suppressor pathways (e.g., p53 and Rb), while inhibiting several dominant oncogenic mechanisms (e.g., MYC, MYCN, and KRAS), which are novel mechanisms of action for a potential anticancer agent.[40] Synthetic compound 35 has been found to effectively induce the apoptosis and autophagy of HCT116 cells, which may relate to enhanced antitumor activity and ultimately lead to cell death. Moreover, compound 35 showed excellent in vivo antitumor efficacy in HCT116 xenograft models.[41] Glioblastoma (GBM) is the most lethal and aggressive adult brain tumor, urgently requiring the development of efficacious therapeutics. The novel SAM-competitive EZH2 inhibitor UNC1999 exhibited low-micromolar cytotoxicity in vitro on a diverse collection of brain tumor-initiating cell (BTIC) lines, synergized with dexamethasone, and suppressed tumor growth in vivo in combination with dexamethasone. In addition, a unique brain-penetrant class I HDAC inhibitor, compound MS-275, exhibited cytotoxicity in vitro on a panel of BTIC lines and extended survival in combination with temozolomide (TMZ) in an orthotopic BTIC model in vivo.[42]

Members of the group of aliphatic acids, such as phenylbutyrates and its derivatives and valproic acid, tend to be relatively weaker HDACi than hydroxamic acids or cyclic peptide agents. Both valproic acid and phenylbutyrate are relatively old drugs but have recently been found to have inhibitory activity on HDAC. To obtain higher plasma levels, several prodrugs of butyric acid have been developed, like Pivanex (AN9) (Fig. 12.2).

12.5 SIRTUINS AND THEIR INTERACTION WITH SMALL-MOLECULE ACTIVATORS/INHIBITORS IN CANCER

Sirtuins are members of the class III histone deacetylase family of enzymes that play complex and important roles in cancer.[43] There are seven mammalian sirtuins (SIRT1-7), which are divided into three classes. SIRT1, 6, and 7 are in class I and predominantly localized in the nucleus; SIRT3-5 are in class II and reside within the mitochondria; and SIRT2 is limited to the cytoplasm.[44] As a result of the diversity of sirtuins in subcellular locations, when combined with different substrates individual family members possess diverse biological functions.

SIRT1 activators have been shown to have potential therapeutic applications in the treatment of age-related diseases. Resveratrol, a polyphenol in red wine, increases NAD^+ and the activity of SIRT1. The metabolic effects of resveratrol result from the inhibition of cAMP and the activation of Epac1, thus increasing intracellular Ca^{2+} levels and activating the CamKK/β-AMPK pathway.[45] A novel series of imidazo[1,2-b]thiazole derivatives has been reported as being effective SIRT1 activators, namely SRT1720, SRT2104, and SRT2379. A representative analog within this series is SRT1720. It enhanced SIRT1 activity by 750% at 10 μmol/L. SRT1720 highly effectively decreased the viability of basal-type MDA-MB-231 and BT20 cells and induced lysosomal membrane permeabilization and necrosis. SRT1720 also inhibited the growth of allograft tumors of SIRT1-proficient cells.[46] Moreover, resveratrol has been found to induce mitochondrial biogenesis and treatment in type 2 diabetic mice. Using high-throughput screening researchers have identified compounds with SIRT1-activating properties and confirmed the biological activity of these compounds, namely compound 1c and compound 1.[47, 48] Research has reported on pyrrolo[1,2-a]quinoxaline derivatives, the first synthetic SIRT6 activators. Biochemical assays have shown that UBCS038 and UBCS039 bind to the SIRT6 catalytic core and have demonstrated the potent activation of SIRT6-dependent deacetylation of peptide substrates and physiological protein substrates.[49]

Sirtinol has been shown to inhibit both human SIRT1 and SIRT2 activity in vitro with IC_{50} values of 131 and 38 μM, respectively. m-Sirtinol and p-sirtinol, two analogs of sirtinol, were two- to tenfold more potent than sirtinol against SIRT1 and SIRT2. Senescence-like growth arrest was induced by sirtinol in breast cancer MCF-7 and lung cancer H1299 cells.[50] Splitomicin showed rather weak inhibition on human SIRT1.[51] AGK2, a potent and selective SIRT2 inhibitor, binds to SIRT2 with an IC_{50} value of 3.5 μM. Treatment with AGK2 exacerbated H_2O_2-induced decreases in intracellular ATP levels, and increased the necrosis of PC12 cells without autophagy. Moreover, AGK2 induced caspase-3-dependent apoptosis in glioma cells.[52, 53] Cambinol inhibits human SIRT1 and SIRT2 deacetylase activity in vitro.

It has a calculated IC_{50} for SIRT2 of 56 and 59 μM, respectively. It inhibits SIRT5 to a lesser extent (42% at 300 μM). Cambinol is characterized as a chemically stable compound that shares a β-naphtol pharmacophore with sirtinol and splitomicin. Cambinol has been shown to reduce tumor growth in Burkitt lymphoma xenografts and induce apoptosis via hyperacetylation of BCL6, without inducing obvious toxicity in animals.[54] While screening for sirtuin activators, suramin was found to be a potent inhibitor of SIRT1 with an IC_{50} value of 0.297 μM. It was also a potent inhibitor of SIRT2 ($IC_{50} = 1.15$ μM) and SIRT5 ($IC_{50} = 22$ μM) to activate p53 and decrease tumor growth. However, there are many serious disadvantages of suramin, principal among which are severe neurotoxicity, systemic side effects in therapeutic treatment, and limited cellular uptake of these highly polar sulfonic acids.[55, 56] During screening to ascertain the ability of small molecules to activate p53, tenovin-1 and tenovin-6 were found to decrease tumor growth in vitro. Both compounds decreased tumor growth in vitro and delayed tumor growth in vivo. Tenovin-1 inhibits several sirtuins and has an IC_{50} value of 21 mM for SIRT1, 10 mM for SIRT2, and 67 mM for SIRT3.[57] Tenovin-1 elevated the levels of p53 and p53 downstream targets, but p53 was not essential for its long-term effect. Tenovin-6 is another small-molecule inhibitor of SIRT1 and SIRT2.[58] The effectiveness of tenovin-6 was slightly higher than tenovin-1.[57] MC2141 has been shown to be endowed with SIRT1-inhibitory properties that are both robust and selective and has demonstrated promising antiproliferative activity in human cancer cell lines.[59] Salermide has a strong in vitro inhibitory effect on SIRT1 and SIRT2. Salermide induces apoptosis in cancer but not in normal cells. It does so by reactivating proapoptotic genes by means of SIRT1-mediated deacetylation of K16H4. Another study using breast carcinoma cell lines and p53-deficient mouse fibroblasts reported that p53 is essential for salermide-induced apoptosis. In human nonsmall-cell lung cancer cells, research has found that the salermide effect can be mediated by upregulation of death receptor 5 expression (Fig. 12.3).[60] OSS-128167, a.k.a. SIRT6-IN-1, is a potent and selective SIRT 6 inhibitor with an IC_{50} value of 89 μM.[61]

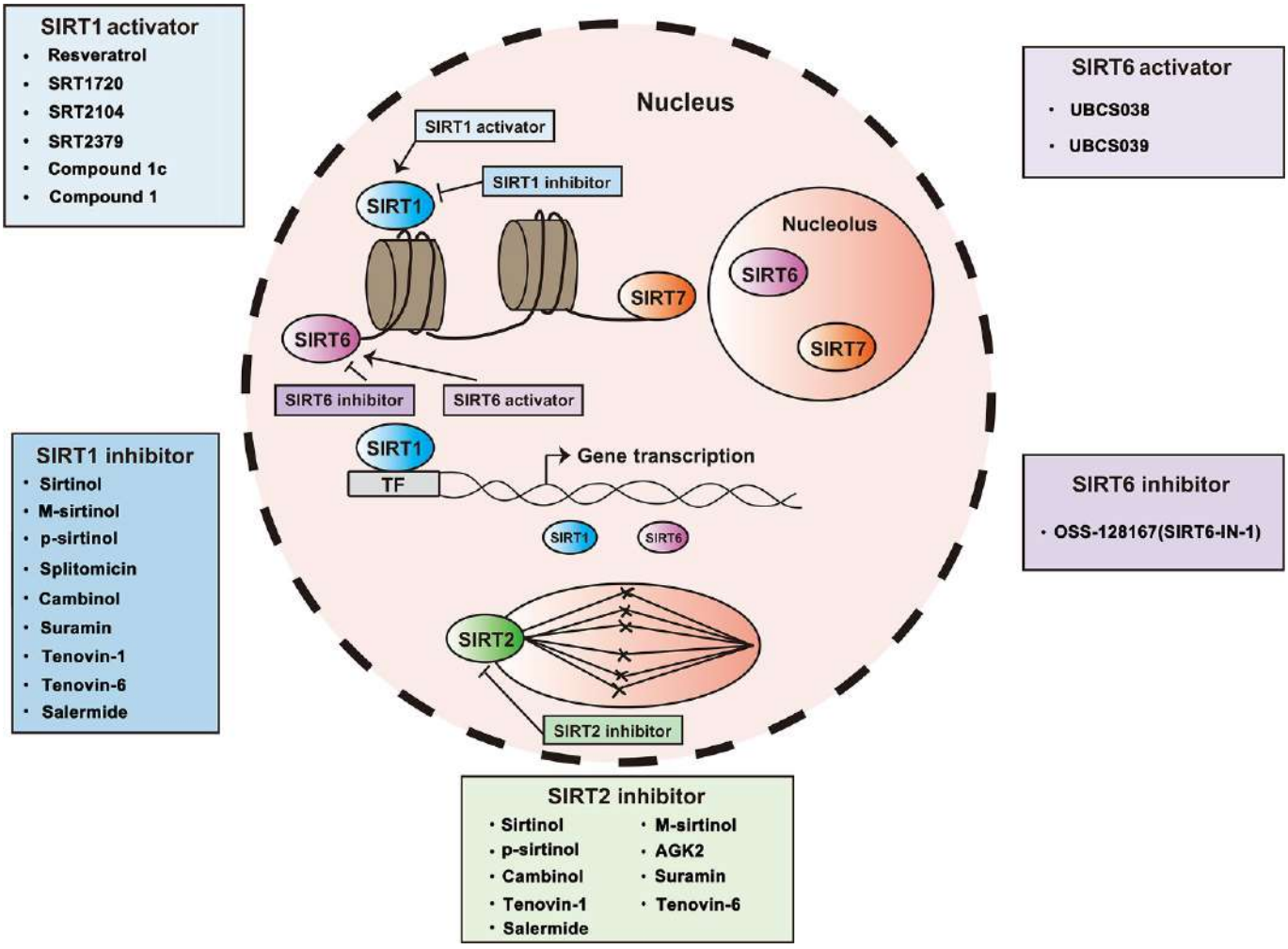

FIG. 12.3 NAD^+-dependent sirtuins (SIRTs) and targeted therapies. Lysine deacetylation can be catalyzed by one of a specific group of NAD^+-dependent sirtuins (SIRTs), the function of which is to remove acetyl groups from lysine residues. Targeted therapies, such as SIRT1 activator, SIRT1 inhibitor, SIRT6 activator, SIRT6 inhibitor, and SIRT2 inhibitor, are applied in cancer therapy.

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CHAPTER

13

Pharmacoepigenetics of Histone Deacetylase Inhibitors in Cancer

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13.1 INTRODUCTION

The process of histone acetylation plays a key role in gene expression. Transcriptional levels are usually elevated during acetylation, while deacetylated histones are often linked with gene downregulation. Histone deacetylases (HDACs) have enzymic action and regulate gene expression by removing the acetyl group from histones. There are also known additional acyl-lysine modifications, such as crotonylation, succinylation, and malonylation, which show the role of HDAC as acyl erasers. Mutation or inappropriate expression of various HDACs has often been observed in human diseases, particularly in cancer, making HDACs important therapeutic targets for anticancer therapies. The whole pattern of histone acetylation is deregulated in cancer. One research group reported that cancer cells undergo a loss of acetylation of histone H4 in place of lysine 16, suggesting that HDAC activity is crucial to formation of the cancer phenotype.[1, 2] In pathological conditions where classical HDAC are overexpressed, HDAC inhibitors (HDACi) could be best described as anticancer agents and their mechanism of action and clinical efficacy have shown very positive results in the last decade. Therefore, HDACi have emerged as promising anticancer therapeutic agents and continue to be at the center of clinical research for further pharmaceutical applications. To date, four HDACi have been approved by the US Food and Drug Administration (FDA) for anticancer therapy; vorinostat (SAHA, Zolinza) and romidepsin (FK228, Istodax) are used against T cell lymphoma; belinostat (Beleodaq) against peripheral T cell lymphoma; panobinostat (Farydak) against multiple melanoma (Table 13.1). Several HDACi are still at the clinical stage.[3] However, most HDACi have the disadvantage of lacking enzyme specificity and can cause a wide range of unwanted effects. In addition, it is worth mentioning that the contribution of HDAC to cancer may be through mechanisms other than overexpression, which may be related to truncating mutations and inactivating mutations. Also, HDAC may be inappropriately mobilized to target genes by interacting with fusion proteins, as is the case in some cases of leukemia. In this case it will be necessary to investigate the use of alternative therapeutic agents. The previously known role of HDAC in neoplastic diseases is presented in two respects: one concerning their expression in neoplastic patients and the other concerning their mechanism of action in cancer cell lines.

13.2 HISTONE ACETYLATION AND DEACETYLATION

Histone acetylation is catalyzed by histone acetyltransferases (HATs). Several families of HATs exist. The first family includes the general control nonderepressible 5 (Gcn5)-related *N*-acetyltransferases, such as GCN5, histone

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TABLE 13.1 US Food and Drug Administration (FDA)-Approved Histone Deacetylase Inhibitors (HDACi)

Drug name	Zolinza	Istodax	Beleodaq	Farydak
Year of approval	2006	2009	2014	2015
Chemical name	Vorinostat (SAHA)	Romidepsin (FK228)	Belinostat	Panobinostat
Classification	Hydroxamic acid	Cyclic peptide	Hydroxamic acid	Hydroxamic acid
Therapeutic target	Cutaneous T cell lymphoma	Cutaneous T cell lymphoma	Peripheral T cell lymphoma	Multiple melanoma

acetyltransferase 1 (HAT1), and p300/CBP-associated factor (PCAF). The second family includes the p300/CREB-binding protein (p300/CBP). The third family includes MYST protein founder members (MOZ, Ybf2/Sas3, Sas2, Tip60). The fourth family includes ATF2, TAF1, TFIIIC90, and Tip60, but a number of proteins with HAT activity still remain unclassified. Other proteins have also been reported to demonstrate HAT activity, and it should be expected that more similar proteins with HAT activity still remain to be identified and studied.[4–6]

Histone acetylation occurs by transfer of an acetyl moiety from acetyl coenzyme A onto the ε-amino group of lysine residues of protein tails and promotes transcriptional activation by neutralizing the positive lysine load, which results in reducing the degree of interaction with negatively charged DNA molecules.[4, 7, 8] As a result of this reduced interaction, chromatin formation is less dense and coiled, facilitating the access of transcription factors to DNA molecules making transcription possible. The mobilization of ATP-dependent chromatin rearrangement complexes, such as the SWI/SNF complex, further promotes this process and facilitates the binding of transcription factors by DNA descintillation.[9] The acetylation process deposits histones onto DNA segments, resulting in site-specific acetylation-related functions.[6] Histone acetylation is a reversible process and deacetylated histones are subsequently reacetylated with new patterns to enable a wide variety of functions.[10–12]

HDACs rapidly remove acetyl groups from protein tails. Deacetylation results in the restoration of the positive charge to lysine residues, alters the electrostatic interaction with the DNA molecule, and causes the transition of chromatin to a more dense conformation (Fig. 13.1). Deacetylation also suppresses DNA transcription.[13]

Of the above described effects of acetylation and deacetylation by HDAC the latter influence the epigenetic status of cells and consequently alter gene expression patterns in the absence of mutations to the genome itself.[14]

13.3 HISTONE DEACETYLASES

The 18 human HDACs (or more correctly lysine deacetylases) were first identified in the 1990s, and they are grouped in four classes within two families. Since HDACs were identified as the first substrates of these proteins that catalyse the removal of an acetyl segment, they were all named HDACs despite the fact that some of them do not actually target histones.

Classification based on class depends on cofactor dependency and sequence similarity to the yeast enzymes Rpd3 (reduced potassium dependency 3), Hdal and Sir2 (silent information regulator 2).[13, 15] Classes I, II, and IV belong to the family of "classic" HDACs, whereas class III enzymes are known as sirtuins and consist of a second and distinct family. Classical HDACs and sirtuins differ in both their biologic activity and structure. Classical HDACs carry a Zn^{2+} catalytic pocket on their base and can be inhibited by zinc-binding chelating agents. In contrast, sirtuins have a different catalytic mechanism, which requires the presence of nicotinamide-adenine dinucleotide (NAD) as a cofactor. Typically, the term HDACi refers mainly to substances that act against classical HDACs.

It is apparent from their name that the enzymatic activity of HDACs is the deacetylation of histone proteins. Through this activity, HDACs control the interaction of positively charged histones with negatively charged DNA, thereby altering chromatin modulation, access to transcription enzymes therein, and consequently transcriptional activity. The high activity of HDACs is associated with concentrated, inactive chromatin. Apart from this epigenetic function of HDACs, it is now recognized that certain HDACs also exhibit significant cytoplasmic function by controlling the acetylation state and functionality of various cytoplasmic proteins and transcription factors. As a result the term "lysine deacetylases" would have probably been more precise, as aforementioned.[16]

In general, more and more substrates for HDACs have been identified, such as p53, E2F, GATA1, Bcl-6, Stat3, HMG, HSP90, NFκB, tubulin, ibortine, nuclear hormone receptors, and β-vacuine.[17] HDACs regulate the activity of various

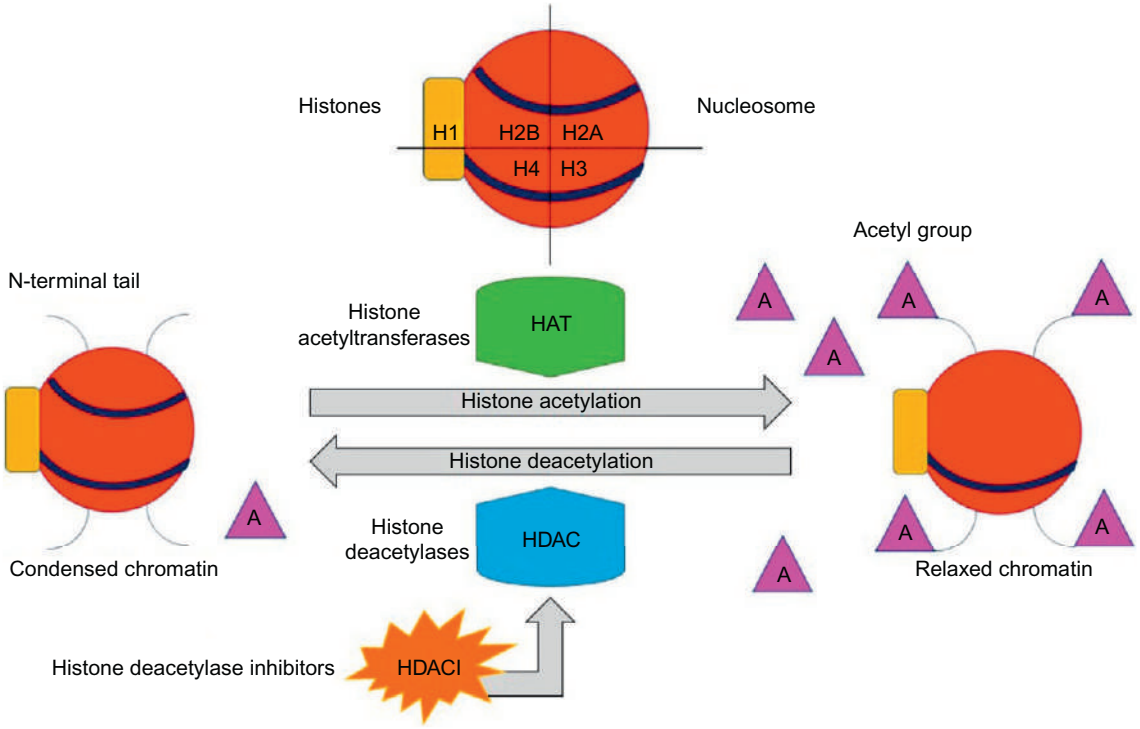

FIG. 13.1 Histone acetyltransferases (HAT) and histone deacetylases (HDAC) action.

agents of central importance for the cell that are involved in the regulation of transcription, intracellular signaling, cell cycle and apoptosis, among others. This clearly shows that HDACs regulate important cellular functions regardless of their epigenetic role in controlling the chromatin structure in the cell nucleus.[18, 19]

In summary, HDACs have emerged as crucial corepressors of transcription in a variety of physiological and pathophysiological systems. To date, 18 human HDACs have been identified and categorized (as described in Table 13.2).

13.3.1 Class I

Class I HDACs are expressed in all tissues and consist of subunits of polyprotein nuclear complexes that play a key role in transcriptional repression and epigenetic landscaping.[20] All class I HDACs are yeast Rpd3 homologs, and are composed primarily of a catalytic domain.[21–23] Their expression is considered to be ubiquitous and primarily nuclear

TABLE 13.2 Classification of Histone Deacetylases (HDAC)

Class I	Class II		Class III	Class IV
Classical			Sirtuins	Classical
HDAC1	IIa	HDAC4	SIRT1	HDAC11
HDAC2		HDAC5	SIRT2	
HDAC3		HDAC7	SIRT3	
HDAC8		HDAC9	SIRT4	
	IIb	HDAC6	SIRT5	
		HDAC10	SIRT6	
			SIRT7	
Zn^{2+} dependent			NAD dependent	Zn^{2+} dependent

in localization, and they commonly act in multiprotein complexes.[6] Aberrant expression of several class I HDACs in tumor samples has been reported,[24, 25] and class I HDACs are involved in a number of developmental processes.

HDAC1 and HDAC2 only exist in the nucleus and appear to have originated from independent gene duplication events in different lineages, also occurring in a common ancestor of all vertebrates.[15] Human HDAC1 and HDAC2 exhibit 82% structural similarity suggesting there should be a functional overlap, but studies in animal models show some distinct and nonduplicated functions.[26] The HDAC1 catalytic domain consists of two amino acid segments essential for interaction with the ubiquitin ligase, which regulates protein degradation.[27] HDAC1 also contains a specific C-terminal nuclear localization signal that is not found in HDAC2.[28] A C-terminal coiled coil domain specific to HDAC2 is thought to enable additional protein-protein associations.[15] HDAC1 can be acetylated and subsequently inactivated by p300.[29] Acetylation of HDAC1 also inactivates HDAC2.[30] Therefore, it is safe to consider that one deacetylase can coregulate the activity of another.[31] HDAC2 is regulated posttranslationally by tyrosine nitration and cysteine S-nitrosylation by nitric oxide/reactive nitrogen species, or reactive oxygen species (ROS)/aldehydes, serine phosphorylation, the heat shock protein HSP70, and a specific polyubiquitinylation and proteasomal degradation pathway limited to this HDAC.[32–35] HDAC1 and HDAC2 are components of the corest complex that inactivates the expression of neuronal genes in nonneuronal tissues,[36] while other complexes containing HDAC1 and HDAC2 are NURD- and SIN3-suppressive complexes.[37]

HDAC3 is found in the N-COR and SMRT complexes.[38] HDAC8 has so far not been found to be part of any repressive complexity, but that attaches particular importance to it. They are located in the cell nucleus and exert a strong catalytic effect on histone lysine residues. HDAC1 and HDAC2 show great similarity and they are involved in various cellular processes, such as proliferation, differentiation, cellular aging, apoptosis, and neoplasia.[31, 39–42] HDAC1 in particular forms a complex with RET finger protein and nuclear transcription factor Y (NF-Y), regulating the sensitivity of cancer cells to oxidative stress through the repression of thioredoxin-binding protein 2 (TBP-2) expression.[43] In murine in vitro models conditional HDAC1 or HDAC2 deletions showed murine embryonic stem cell differentiation is affected by loss of either HDAC1 or HDAC2, although proliferation remains intact, suggesting it is specific processes that are affected by loss of either homolog.[44] In contrast, deletion of both HDAC1 and HDAC2 is required in a number of cell types to generate a phenotype suggesting functional redundancy.[40, 45]

HDAC3 exists as a single protein in a large number of organisms from humans to *Drosophila*.[15] It is regarded as distinct from HDAC1 and HDAC2, as it is not present in either NuRD or Sin3 corepressor complexes.[46] Two reported isoforms of HDAC3 exist, HDAC3A and HDAC3C, differing at the N-terminus. Both isoforms are present in the nucleus and the cytoplasm. HDAC3A could be generated by posttranscriptional modifications, although it could also be an artifact formed during library construction. HDAC3C is either an alternatively spliced isoform of HDAC3 or it is transcribed from a different transcriptional start site.[47] HDAC3 is part of a multiprotein complex consisting of SMRT (silencing mediator for retinoid and thyroid receptor), and therefore it is essential to the function of class IIa HDACs.[48] HDAC3 binds to and regulates the human GCMa transcription factor. The transcriptional coactivator CBP enhances GCMa-mediated transcriptional activation and as a HAT acetylates GCMa to support its protein stability. HDAC3 plays a significant role in reversing GCMa acetylation.[49] HDAC3 also plays a role in response to DNA damage.[50]

HDAC8 has a similar structure to HDAC3.[21] It has undergone significant functional specialization compared with other class I HDACs and appears to be restricted to vertebrates.[15] It may have developmental or tissue-specific functions consistent with its apparently tissue-specific expression.[13] HDAC8 is highly expressed in the liver, but is present in almost all tissues. Northern blot analysis demonstrated two different transcripts, a common 1.7-kb form and a rarer 2.4-kb form; the latter being particularly prominent in neoplasmatic cell lines.[21] In normal human tissues HDAC8 is exclusively expressed by cells showing smooth muscle differentiation and is mainly detected in their cytosols.[51] HDAC8 has been identified in visceral and vascular smooth muscle cells, myoepithelial cells, and myofibroblasts with immunocytochemistry, strongly suggesting a cytoskeleton-like distribution of the enzyme.[51, 52] HDAC8 has not been described so far as part of any protein complex.[16]

13.3.2 Class II

Class II HDACs comprise the human analog of yeast HDA1 and HDA3 and are further divided into class IIA and IIB.[53, 54] They are present both in the nucleus and the cytoplasm,[55, 56] but class IIa members have distinct subcellular localizations. HDAC4 is mainly cytoplasmic in undifferentiated myoblasts, with accumulation in the nucleus upon differentiation into myotubes.[57] HDAC5 and HDAC7, which were located in the nucleus, relocate to the cytoplasm during differentiation into myotubes.[58, 59] Class IIa HDACs contain a long noncatalytic N-terminus and a C-terminal catalytic domain. Extended long N-terminal domains are characteristic of this subfamily, possessing conserved

amino acid motifs specialized for binding an array of proteins, and therefore important for class IIa function and regulation.[53, 54, 60, 61] This N-terminal adaptor region contains intrinsic nuclear import and export signals for nuclear-cytoplasmic trafficking.[58, 62, 63] The intracellular traffic of these HDACs is regulated by endogenous nuclear insertion and extraction signals, as well as by binding sites for 14-3-3 proteins. HDAC4, HDAC5, HDAC7, and HDAC9 contain three such positions. Binding of 14-3-3 proteins results in maintenance in the cytoplasm or extraction from the core of HDAC class IIA, in a phosphorylation-dependent manner, which regulates the activity of transcription factors such as MEF2.[16, 58, 64] Various signaling pathways regulate the phosphorylation of these 14-3-3 binding sites. These pathways include Ca^{2+}/calmodulin-dependent kinases (CaMKs),[16] protein kinase D,[65] kinases that regulate the affinity for microtubules,[66] salt-inducible kinases,[67] and kinase 1 of cell cycle checkpoints (CHK1).[68] It is likely that other distinct kinases may differentially regulate the subcellular localization of class IIa members. A recently identified protein was kinase D (PKD), which was shown to phosphorylate the 14-3-3 binding sites of class IIa HDACs and neutralize their repressive activity.[69, 70] PKD activation is also necessary for inactivation of class IIa HDACs during T cell apoptosis, cardiac hypertrophy, B cell receptor signaling, skeletal and cardiac muscle remodeling, and angiogenesis.[65, 69–75]

Class IIa HDACs are expressed in specific tissues and are involved in cell differentiation and growth. They exert their suppressive effect on transcription in striated and smooth muscles, myocardium, vasculature, bone, immune and central nervous system, among others. They also have a long regulatory N-terminal segment, through which their interactions are mediated by tissue-specific transcription factors and cotranscriptors.[16, 19] The catalytic activity of class IIa HDACs remains unclear, but it has been found to be part of the SMRT/N-CoR suppressor complex.[48, 76]

Class IIb HDACs—HDAC6 and HDAC10—are distinct from those of class IIa in several ways. They possess a second catalytic domain and are also resistant to the inhibitory effects of trapoxin B and butyrate.[77, 78] Both of these proteins interact with major cellular phosphatase protein phosphatase 1 (PP1) and could therefore be involved in the same regulatory networks,[79] but there is little functional overlap between the two class IIb HDACs.[77, 80] HDAC6 is predominantly found in the cytoplasm, where its main molecular target is a-tubulin.[16, 81] It contains two active deacetylase catalytic centers in series and a C-terminal zinc finger. HDAC6 has now been shown to regulate cell mobility, adhesion, and has chaperone activity. Its functions are not affected by its deacetylation activity. By ubiquitin binding via the zinc finger of its catalytic center, it regulates intracellular aggregates, autophagy, and function of heat shock factor 1 and platelet-derived growth factor (platelet-derived growth factor, PDGF).[82, 83] HDAC10 is structurally related to HDAC6 apart from a catalytically inactive protein domain. It is found in both the nucleus and the cytoplasm.[16, 81] The function of HDAC10 is largely unknown.

13.3.3 Class III

Sirtuins are widely expressed and have a very wide range of biological functions, such as regulating oxidative stress, DNA repair, metabolic regulation, and cell aging.[84, 85] Class III HDACs are members of the Sir2 family and their deacetylation activity depends on the presence of NAD.[86, 87] Sirtuins are located in different cell compartments: SIRT1 and SIRT2 can be found both in the nucleus and the cytoplasm; SIRT3, SIRT4, and SIRT5 are located in mitochondria; SIRT6 and SIRT7 are nuclear proteins.[88]

13.3.4 Class IV

HDAC11 is currently the only HDAC class IV member. It is structurally related to both class I and class II HDACs, and it was considered a class I HDAC until recently.[15] HDAC11 contains amino acid residues in the regions of catalytic active sites, which share both class I and class II HDACs.[89] The last of the zinc-dependent HDACs to be discovered, it resides in the nucleus and is considered an immunoregulator as a result of its role in the regulation of interleukin 10 (IL-10) expression.[90, 91] HDAC11 directly interacts at the chromatin level with a distal region of the IL-10 promoter in activated macrophages, and kinetics suggests there may be a secondary means of regulation by HDAC11.[91] Alteration of HDAC11 activity and consequently the abundance of IL-10 has been shown to modify the response of an organism from high immune function to anergy or tolerance.[90] Its expression is higher in the liver, brain, testicles, heart, and skeletal muscles. It has been associated with the development of oligodendrocytes and the immune response.[92, 93]

13.4 HISTONE DEACETYLASE INHIBITORS

As aforementioned, four classes of HDACs have been distinguished. Class I, II, and IV HDACs rely for their effect on zinc-dependent catalysis. Under these enzymatic conditions a hydrophobic pocket leads to the active catalytic

central position of zinc and most inhibitors come into contact with this center as a result of their ability to enter the hydrophobic pocket and thus block the access of enzyme substrates. Class III HDACs comprise sirtuins—SIRT1 to SIRT7—and use NAD^+ as a cofactor for their action.[94–96]

A number of compounds inhibiting the function of HDACs have been identified, leading to the hyperacetylation of histones and other nonspecific effects.[97] Several studies have demonstrated that HDACi are selective in their effects on gene expression, altering the expression of 2%–10% of the genes analyzed.[98] Many HDACi reversibly or irreversibly block access to the active site.[13] Based on their chemical structure, HDACi that act against zinc-dependent HDACs can be classified into several main groups.[99] They all share a common structural pattern consisting of a zinc-binding domain, a linker domain of appropriate length that mimics the substrate and occupies the active site channel, and a cap substructure that interacts with amino acids at the active site.[100–105]

In vitro studies have demonstrated that class I and II HDACi induce antiproliferative, prodifferentiative, or proapoptotic genes, resulting in cellular growth arrest, terminal differentiation, and cell death in transformed cells.[106–112] DNA microarrays using malignant cell lines cultured in the presence of an HDACi have shown that only a small number of genes had altered expression, as already mentioned.[108, 113–116] Approximately equal numbers of genes are activated or repressed following pharmacological inhibition of HDAC activity, providing evidence for a dual role of HDACs.[117] A number of HDACi are currently in preclinical and phase II/III trials. To date, it has been demonstrated that selecting different isoforms may contribute to the responsiveness of different cancer cell lines to the respective treatment.[118] Originally, it was thought that HDACi act in much the same way against HDACs, but it is now accepted that many HDACi show significant differences in activity against specific HDACs, and this could be utilized in the development of isoform-specific HDACi. Zinc-dependent HDACi are simple aliphatic carboxylic acids, hydroxamic acids, benzamides, cyclic peptides, and depsipeptides.[99] Hydroxamic acid-derived HDACi are universal inhibitors and target class I/II HDACs. At the same time, aliphatic acids, benzamides, and cyclic peptides have shown inhibitory activity against class I HDACs, but do not act against class II HDACs.[78, 103, 119, 120] Class II HDACs have been found to be fivefold less susceptible to inhibition by valproic acid than class I HDACs. HDAC4 is also comparatively less sensitive to inhibition by butyrate.[13] HDAC8 is partially resistant to Trichostatin A (TSA), SAHA, and MS-275.[102] HDAC6 is not sensitive to short chain fatty acids and cyclic tetrapeptide inhibitors with large cap groups, but it is inhibited by tubacin.[81, 121] HDAC6 was identified early on as an exception when it came to HDACi sensitivity, and it is resistant to inhibition by trapoxin.[103] FK228 strongly inhibits HDAC1 and HDAC2, but is weak in inhibiting HDAC4 and HDAC6.[122] Specific inhibitors have now been developed for some HDACs, employing the structural properties of HDACi.[100, 123–125] Table 13.3 summarizes the most important HDACi with anticancer effects.

13.4.1 Hydroxamic Acids

Hydroxamic acids form chelates with metallic atoms and can bind to the zinc ions necessary for the catalytic activity of HDACs. It has been shown that, with the exception of a few of them, they are generally well tolerated by the body. They can bind to the active center of deacetylases directly inhibiting catalytic action. The HDACi that fall into the hydroxamic acid class and have been more studied in various malignancies include TSA, vorinostat, panobinostat, and belinostat[126, 127] (Table 13.4). TSA is a natural HDACi.[128] The action of trichostatin has been studied in vitro in

TABLE 13.3 Most Important Histone Deacetylase Inhibitors (HDACi) With Anticancer Effects

HDACi	Class	Target HDAC
Trichostatin A (TSA)	Hydroxamic acids	Classes I, II
Panobinostat		Classes I, II
Vorinostat		Pan-inhibitor
Belinostat		Pan-inhibitor
Valproic acid	Short chain fatty acids (SCFAs)	Class I/IIa
Butyrate		Class I/IIa
Romidepsin	Cyclic peptides	Class I
Entinostat	Benzamides	Class I
Mocetinostat		Class I

TABLE 13.4 Hydroxamic Acids as Histone Deacetylase Inhibitors (HDACi)

Inhibitor	Target HDAC	Phase
Trichostatin A	All	Preclinical
Suberoylanilide hydroxamic acid (SAHA)	All	Approved for cutaneous T cell lymphoma
Belinostat	All	Approved for peripheral T cell lymphoma
Panobinostat	All	Approved for multiple myeloma
Givinostat	All	Phase II clinical trial
Resminostat	All	Phase I and II clinical trials
Abexinostat	All	Phase II clinical trial
Quisinostat	All	Phase I clinical trials
Rocilinostat	Class II	Phase I clinical trials
Practinostat	Classes I, II, and IV	Phase II clinical trial
CHR-3996	Class I	Phase I clinical trials

many cancer cell lines and in vivo using allografts in nude mice. However, as a result of its toxicity its clinical use has never been favored, and instead synthetic analogs such as SAHA are preferred.[129–132] Vorinostat is a synthetic substance that belongs like TSA to class I/II HDACI,[133] and has been approved by the FDA for the treatment of recurrent or reversible cutaneous T cell lymphoma.[134] TSA inhibits tumor cell growth, promotes apoptosis in cancer cell lines by activating the intrinsic mitochondrial and extrinsic/Fas/FasL system death pathways, increases histone H4 acetylation and the expression of p21, sensitizes cancer cells to chemotherapy in vitro, and exhibits antiproliferative activity.[135] In 2007 Arnold et al.[136] reported the effect of vorinostat on pancreatic cancer and described how vorinostat induces cell cycle arrest in the G1 phase by increasing p21 in BxPC-3 and COLO-357 cells, but not in gemcitabine-resistant PANC-1 cells. This inhibitor exhibited a synergistic effect with gemcitabine on BxPC-3 and COLO-357 cells and sensitized PANC-1 cells to gemcitabine.[136] The same year, a contradictory study by Kumagai et al.[137] showed that vorinostat therapy leads to inhibition of PANC-1 cell growth, induces p21 in these cells, and causes arrest in the G2/M transition instead of the G1 phase of the cell cycle.[137] Other recent studies have investigated the effect of vorinostat in combined therapies in gastric cancer, specific types of lymphoma, and nonsmall-cell lung cancer.[138–140] In vitro studies showed that the combination of gemcitabine, the proteasome inhibitor bortezomib, and vorinostat exhibits the greatest inhibitory effect on cell growth. This finding has not been confirmed in vivo on murine models, as no significant benefit of the triple combination versus gemcitabine with bortezomib was confirmed.[141]

In 2012 a phase I clinical study by Millward et al.[142] demonstrated a significant synergism of combining proteasome inhibitor marizomib and vorinostat in cancer cell lines in vitro with cells derived from nonsmall-cell lung cancer, melanoma, and pancreatic cancer. The initial results did not detect any tumor response to this treatment.[142] Limited encouraging results in preclinical and clinical studies exist supporting vorinostat's anticancer activity.[143] In an ongoing phase I/II clinical study the combination of vorinostat with radiotherapy and 5-FU has been considered in patients with locally advanced disease.[144] Another ongoing study has attempted to evaluate the efficacy of a vorinostat, capecitabine (a 5-FU prodrug), and radiotherapy combination in patients with nonmetastatic pancreatic carcinoma.[145]

Panobinostat inhibits all classes of deacetylases of zinc-dependent histones and is therefore called a universal inhibitor.[146] It was first studied against pancreatic cancer in 2008 by Haefner et al.[147] The results of this study showed that panobinostat interrupted cell cycle G2/M transition, upregulated p21, and induced in vitro apoptosis. Under in vivo conditions the substance significantly reduced tumor mass in nude murine models and regulated the efficacy of gemcitabine, but apoptosis was insignificantly increased and cell proliferation was insignificantly reduced.[147] Panobinostat has been tested in multiple phase II clinical studies in combination with bortezomib against different malignancies.[148–150] However, a recent study of panobinostat in combination with the inhibitors PI3K and mTOR BEZ235 demonstrated growth inhibition both in vitro and in vivo using allografts in nude murine models.[151]

Belinostat is a relatively new pan-inhibitor of HDACs that is already approved for peripheral T cell lymphoma.[146, 152] In 2010 it was studied in a phase I clinical study in combination with carboplatin and/or paclitaxel in patients with solid tumors.[153] Study results showed a partial response to belinostat combined with carboplatin. Similar to panobinostat, belinostat in combination with bortezomib have also been studied in a preclinical study, showing synergistic

activity against cell proliferation and in favor of apoptosis in pancreatic cancer and multiple myeloma.[154, 155] Two other studies have shown that belinostat induces cell growth inhibition both in vitro and in vivo in immunocompromised mice, either alone or in synergy with gemcitabine.[156, 157]

13.4.2 Short Chain Fatty (Aliphatic) Acids

Short chain fatty (aliphatic) acids (SCFAs) are weaker HDACi than hydroxamic acids, as they have no access to the Zn^{2+} ion found in the active center of HDACs.[158] SCFAs are considered to be a bacterial fermentation by-product of fiber foods as a result of the action of intestinal flora, and they could potentially protect the intestine from developing tumors. This hypothesis makes SCFAs extremely important in anticancer research. As far as pancreatic cancer is concerned the best studied and most promising SCFAs are valproic acid and butyrate. Valproic acid is a class I/IIa HDACi, used mainly as a medication against epilepsy, bipolar disorder, and migraines.[152] Its inhibitory effects on HDACs have since become evident.[159] In a phase I clinical trial of the effect of valproic acid and epirubicin on solid tumors, one patient presented with a partial response to this drug combination.[160] In vitro, valproic acid has also demonstrated significant downregulation of cell proliferation and the adhesion of cancer cells.[161]

Two distinct studies conducted in the same year by Iwahashi et al.[162, 163] on pancreatic cancer cell lines reported that valproic acid was incapable of inhibiting growth to any significant degree on its own. More recently a phase II study was conducted to evaluate the toxicity and efficacy of valproic acid in combination with gemcitabine and radiotherapy.[164] The long-term use of valproic acid as a drug for central nervous system disorders offers the advantage of valproic acid's well-known pharmacological profile and its effect on certain solid malignancies, mainly driven by elements of the nervous system.

Valproic acid seems to have a potential role in the treatment of medullary thyroid cancer, as it induces metabolic stress, activates AMP-activated protein kinase, and increases autophagic flux in thyroid cell lines.[165]

Butyric acid is a class I/II histone HDACi that induces apoptosis and prevents penetrance and infiltration in cancer cell lines.[166] It is believed to exert a significant influence on chemotherapy activity.[167, 168] Butyric acid is currently undergoing phase II clinical study, but its biotoxicity, half-life, and clearance in its first hepatic passage are problematic.[169] Butyrate prodrugs that pose better pharmacological features could offer an alternative therapeutic option to butyrate; for example, the prodrug tributyrin has been shown to inhibit cellular growth in pancreatic cancer cells.[170] Phenylbutyric acid is another promising SCFA and is currently in phase I clinical studies.[152] Further studies are necessary to evaluate the therapeutic value and pharmacological properties of butyrate-related substances.

13.4.3 Cyclic Peptides

Romidepsin is a pentapeptide that interacts with the Zn^{2+} ion of the active site of HDACs. It is a class I/II HDACi and has been approved by the FDA for cutaneous T cell lymphoma since 2009.[152, 171] Romidepsin disrupts the G1 or G2/M phase of the cell cycle and induces cell apoptosis in treatment-resistant pancreatic carcinoma.[172] In addition, romidepsin is reported to inhibit in vivo growth in allografts of pancreatic cancer.[173] In 2012 a phase I study by Jones et al.[174] investigated the effect of romidepsin combined with gemcitabine in solid tumors. In this study the disease was stabilized in 14 patients and there was a partial response in 2 patients, but the cumulative blood biotoxicity of this drug combination was also reported.[174] These results require further investigation through further focused studies.[174, 175]

13.4.4 Benzamides

Entinostat (MS-275-SNDX-275) is a characteristic synthetic benzamide derivative and a class I HDACi.[146] Saito et al.[176] first reported the potential anticancer activity of entinostat in 1999 and found that it exhibited significant antineoplastic activity against human cancers in nude mice.[176] Previous phase I studies on the effect of entinostat in patients with metastatic pancreatic cancer reported contradictory results.[177, 178] Entinostat is currently in different phases of clinical studies for a number of different cancer cell lines: hormone receptor-positive breast cancer, Hodgkin lymphoma, and nonsmall-cell lung cancer. Entinostat has shown promising results especially against advanced breast cancer and colon cancer.[179, 180] Mocetinostat (MGCD0103) is a class I/IV HDACi and is currently undergoing phase II clinical study of Hodgkin lymphoma.[152] It has also been reported to inhibit colon cancer cell proliferation by upregulating WNT ligand DKK-1 expression, a mechanism distinct from other benzamides.[180] Adverse effects have been recorded during its use, though. Tacedinaline is a class I HDACi currently in phase III clinical studies on nonsmall-cell lung cancer and pancreatic cancer.[152] A novel class I HDACi called 4SC-202 inhibits the survival and

proliferation of primary human colon cancer cells and established colorectal cancer lines.[181] However, 4SC-202 was not cytotoxic to colon epithelial cells and provoked apoptosis activation in colorectal cancer cells. 4SC-202 is also undergoing phase I clinical study of advanced hematological malignancies.[152]

13.4.5 Sirtuin Inhibitors

Sirtuin inhibitors include nicotinamide, a pan-inhibitor, and specific SIRT1 and SIRT2 inhibitors: sirtinol, cambinol, and EX-527. They have demonstrated potential for use against different types of neurodegenerations and cancer cell lines. Despite the fact that SIRT1 are potentially useful targets in battling cancer, chemical inhibition of SIRT1 with selective and potent inhibitors has not been shown to prevent the proliferation of multiple cancer cell lines.[182] Nicotinamide is an inhibitor of all class III HDACs and is currently undergoing phase III clinical trial for laryngeal cancer.[152]

Sirtinol and cambinol are SIRT1/2 HDACi and are still in preclinical phases of research.[152] Selistitat (EX-527) is a SIRT1 HDACi that functions in a concentration-specific manner.[183] Although it is still in preclinical phases of research on cancer cell lines, it is in clinical phases of trials on other nonneoplastic diseases.[152] Tenovins are weak micromolar inhibitors in biochemical deacetylase assays, showing slightly more selectivity to SIRT2 than SIRT1.[182]

13.5 HISTONE DEACETYLASE INHIBITORS: MECHANISM OF ACTION

HDACi can induce cell cycle arrest, differentiation, and cell death via different molecular pathways, depending partially on the degree cells are exposed to them and on the specific molecular features of each cell. They additionally reduce angiogenesis and alter immune response.

Normal cells exhibit relative resistance to HDACi-induced cell death, as proposed by the "epigenetic vulnerability of cancer cells" hypothesis of Dawson and Kouzarides.[152, 184–186] This hypothesis claims that normal cells multiply under epigenetic regulatory mechanisms while cancer cells do not. Therefore, HDACi may be essential for the survival and growth of cancer cells, but not of normal ones.[184]

The anticancer effects of HDACi are brought about by a number of different mechanisms depending on the specific type of cancer cell line, the characteristics of a given HDACi, its dose, and a number of other factors[187] (Fig. 13.2).

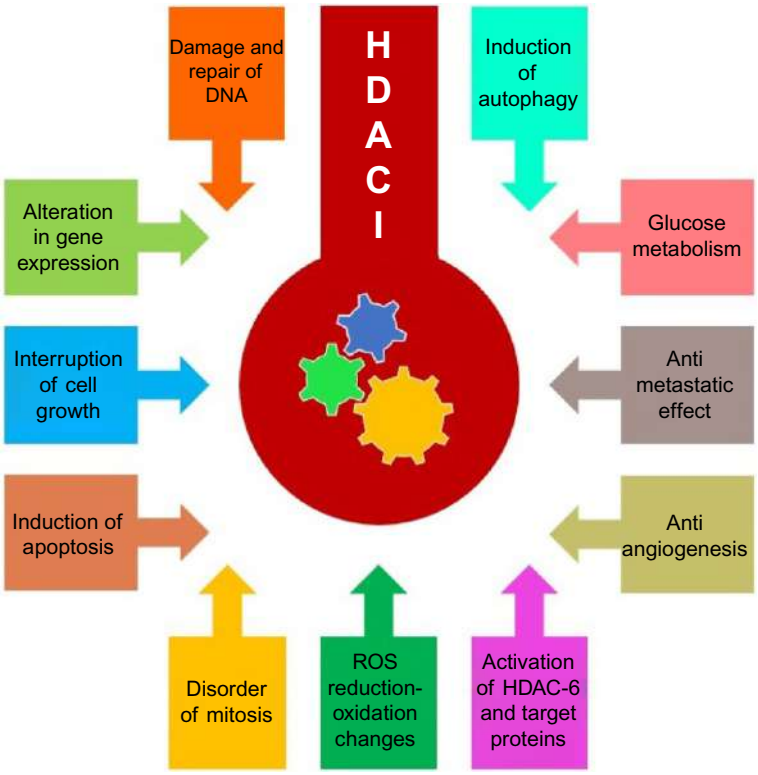

FIG. 13.2 Mecanisms of anticancer action of histone deacetylase inhibitors (HDACi).

13.5.1 Damage and Repair of DNA

To date, no published data exist to support the belief that HDACi directly cause mutations. HDACi-induced histone acetylation causes structural changes in chromatin that could potentially expose DNA to harmful mutagenic agents, including ultraviolet radiation, cytotoxic drugs, and oxygen radicals. The effects of mutagenic agents have been shown to lead to discontinuations of the double-helix structure.[18, 96, 188] HDACi can induce the buildup of reactive oxygen species and a phosphorylated form of H2AX, a double-helix disintegration marker.[185, 186, 189, 190] HDACi may also lead to downregulation of proteins related to DNA repair in homologous recombination (RAD51, BRACA1, and BRAC2) and to nonhomologous repair of double-helix disruption (Ku70, Ku86, and DNA-PKcs).[191–196] As DNA damage builds up, so do alterations in gene expression and apoptotic cell death. Affected cells have various defects in double-helix repair pathways, and they lack the ability to repair their own DNA structure. The synergy of HDACi and other DNA-damaging therapeutic modalities, such as cytotoxic drugs and radiotherapy, arises not only from the effect of HDACi on inhibiting DNA repair procedures, but from activating the endogenous and exogenous pathways of apoptotic cell death. According to some authors, cancer treatment with HDACi not only triggers widespread histone acetylation in cancer cells, but also increases ROS and DNA damage that are further increased following treatment with DNA-damaging chemotherapies. Despite the fact that the origins of ROS production are not well studied, some mechanisms have been reported, including inflammation and altered antioxidant signaling. While the mechanism by which ROS are generated may be an explanation, at least in part, for the source of DNA damage observed with HDACi treatment, DNA damage can also be independently induced by changes in DNA repair activity and chromatin-remodeling factors, as described earlier in this text. While DNA damage and stress responses could be of interest as markers for future clinical uses, they still have to be validated as markers for responses to HDACi treatment. Although the selective cytotoxic activity of HDACi just on cancer cells is promising, the mechanism remains unclear.[197]

13.5.2 Alteration in Gene Expression

HDACi alter gene transcription by promoting histone acetylation, in much the same way as transcription factors and other proteins regulate gene expression.[96, 198] Early studies of TSA-treated lymphatic cell cultures showed that only 2% of genes change their expression compared with untreated cells. This change has been defined either as an increase or a decrease in gene expression.[18, 199] More recent studies using cDNA sequences have demonstrated that 10%–20% of genes exhibit an altered expression in leukemia, multiple myeloma cell lines, and colon, kidney, prostate, and breast cancer cells, when treated with HDACi.[200–203] The duration of culture and concentration of HDACi affect the total number of genes that change their expression in an analogous manner. Some changes in gene expression are believed to be a direct result of HDACi, while others may be manifestations of subsequent points of the biochemical circuits that are affected by HDACi. The pattern of HDACI-induced changes in gene expression is similar among different inhibitors, although differences exist in induced changes in relation to the molecular status of cell lines.[200–202]

p21WAF1/Cip1, a cyclin-dependent kinase inhibitor (CDKi), is one of the most frequently HDACi-induced genes.[202] HDACi-induced expression of p21 is independent of p53. In ARP-1 cells vorinostat has been reported to change the acetylation and methylation patterns of lysines on H3 and H4 histones, which are associated with the proximal promoter region of the p21 gene.[204] The acetylation or methylation of histones in the promoter region of the expressed p27 (KIP1) or silent globin E in HDACi-treated ARP-1 cells were not altered. The expression of these genes was not reported. Vorinostat caused a significant decrease in HDAC1 and Myc as well as recruitment of RNA polymerase II to the protein complex, which is associated with the proximal promoter region of the p21 gene. There was only a small number of detectable changes in HDAC2, Brg1, GCN5, P300, and Sp1 proteins in the complex. These findings suggest that selective alteration of the transcription of a gene by HDACi may be determined by the composition of proteins involved in the transcriptional complex, including HDAC. HDACi can inhibit STAT5-mediated gene expression.[205] HDACi also suppress transcription of the androgen receptor (AR) gene and inhibit transcriptional activation of other genes mediated by AR.[206] HDACi and SAHA can also alter the expression of miRNA in cancer cells.[207] These miRNAs have gene targets related to angiogenesis, apoptosis, chromatin modifications, cell proliferation, and cell differentiation. HDACi can activate Sp1/Sp3-mediated induction of multiple response genes to cellular stress (such as *fos*, *Juh*, *egr1*, *egr3*, *a3*, *arc*, *mr4a1*, *mdrg4*, *Mt1B*, *MtiE*, *Mt1f*, and *ME1H*) that are associated with cellular apoptosis.[208]

13.5.3 Interruption of Cell Growth

HDACi can induce cell growth disruption in both normal and malignant cells according to cell culture results. Vorinostat primarily causes cell cycle disruption in the G1 phase at low concentrations and in the G1 and G2/M phases

at higher concentrations.[209] In culture cells treated with HDACi, elevated levels of CDKIs and decreased levels of cyclins may be a cause of decreased activity of CDKs, causing dephosphorylation of Rb and inhibiting E2F activity in gene transcription for G1 phase progression and transition from the G1 to S phase of the cell cycle.[210, 211] HDACi can affect both cell growth and nonproliferating transformed cells.[186, 212] This is in contrast to the action of many other chemotherapeutic drugs, which are active only against the transformed cells being divided.

13.5.4 Induction of Apoptosis

HDACi can induce the apoptosis of transformed cells by activating the exogenous and/or endogenous apoptotic pathways and by regulating proapoptotic and antiapoptotic genes.[17, 111, 213–220] Some mechanisms, such as caspase 3 activation, are common steps between the exogenous and endogenous pathways.[214]

The exogenous apoptotic pathway is activated by binding cell death receptors, such as Fas, TNF-1, LIGHT, TLA1, TRAIL (DR4 and DR5), DR3 (Apo3), and DR6, with their ligands, which results in activating caspase 8 and caspase 10.[218] Inhibition of these death receptors and their ligands stops HDACi-induced apoptosis.[111, 220–222] HDACi can upregulate not only cell death receptors but also their ligands both in vitro and in vivo in transformed cells but not in normal cells. In vivo xenograft studies using tumor cells with TRAIL and Fas suppressed by siRNA showed a significant decrease in apoptosis after treatment with VPA.[214] Moreover, treatment using vorinostat followed by TRAIL has been proven to target the multiple pathways of neoplastic, angiogenetic, and metastatic progression. HDACi can also cause TNF-dependent apoptosis by inhibiting the ubiquitin-dependent pathway, which may be the basis for the effectiveness of combining HDACi with the proteasome inhibitor in promoting tumor cell apoptosis.[218] Combined treatment of HDACI with agents that promote the exogenous apoptotic pathway is likely to be critical in the development of effective therapeutic strategies.

The endogenous apoptotic pathway is mediated by disorders of mitochondrial function and the release of mitochondrial transmembrane proteins, including AIF, Smac, and cytochrome *c*, which results in the activation of caspases.[17,18,96,223,224] HDACi promote the endogenous apoptotic pathway by deactivating or suppressing antiapoptotic proteins and activating proapoptotic proteins such as Bid (which inititates the endogenous pathway and affects the mitochondria of cancer cells), Bad, and Bim (which activates the intrinsic apoptotic pathway).[220, 225] High levels of Bcl-2 or Bcl-XL, which protect mitochondria, have been found in some malignant cells that are resistant to HDACi-mediated cell death.[215] Bcl-2 inhibition induced by a chemical agent may increase sensitivity to HDACi. HDACi reduce the antiapoptotic proteins of the same family as Bcl-2 (through activation of ERK), Bcl-XL, Bcl-w, and Mcl-1, as well as XIAP, the genetic inhibitor of apoptosis.[226] SAHA and entinostat increase the expression of TBP-2, which results in the inhibition of thioredoxin in LNCaP prostate cancer, T24 bladder cancer, and MCF7 breast cancer cell lines.[189] Thioredoxin is an intracellular antioxidant, hence treatment of tumor cells with this HDACi promotes ROS-dependent apoptosis.[227, 228] Valproic acid induces apoptosis more effectively under hypoxic conditions and overcomes hypoxia-induced resistance to cisplatin in high-risk neuroblastoma-derived cells, probably by bringing about HIF-1α degradation.[152]

13.5.5 Disorder of Mitosis

HDACi can induce inappropriate accumulation of acetylated histones in heterochromatin and centromeric areas, resulting in the death of neoplastic cells.[229–232] In TSA-transformed cell cultures, recently synthesized histones found in chromatin remain acetylated and disrupt the structure and the function of the centromere and pericentric chromatin by loss of attachment to heterochromatin-binding proteins. Histone acetylation also inhibits histone phosphorylation by disrupting the function of mitotic spindle and cell cycle checkpoint proteins, such as BubR1, hBUB1, CENP-F, and CENP-E. Cell lines that are sensitive to the activity of HDACi undergo early mitotic arrest prior to apoptosis, while HDACi-resistant cell lines complete mitosis after a short delay and arrest in G1.[233] Hence the accumulation of chromosomal disorders in the process of mitosis leads to cell death.

13.5.6 Reactive Oxygen Species (ROS) Reduction-Oxidation Changes

HDACi are responsible for the accumulation of ROS in transformed cells but not in normal cells.[234–236] This increase in intracellular ROS concentration occurs within 2 h of HDACi culture prior to mitochondrial disorder. *N*-Acetylcysteine and other free radical scavengers reduce HDACi-mediated apoptosis, suggesting that ROS production is an important factor in the death of cancer cells. Thioredoxin is a hydrogen donor required for the activation of

transcriptional factors, such as NFκB, and various proteins that are necessary for DNA synthesis. Reduced thioredoxin is a scavenger of ROS.[237] Vorinostat increases the expression of TBP-2 that binds and inhibits the activity of reduced thioredoxin, causing the downregulation of thioredoxin in malignant but not in normal cells.[186, 189] Thioredoxin is a kinase 1 inhibitor that regulates apoptosis through ASK1. The inhibition of thioredoxin by binding to TBP2 activates ASK1. This induces the SET1-JNK and MKK3/MKK6/p38 cataract-signaling pathways and enhances the expression of Bim apoptotic protein, resulting in cancer cell apoptosis.[238] SAHA and MS-275 have been found to arrest the growth of both normal human cells and transformed cells, and induce the rapid cell death of transformed but not normal cells. This is achieved by increases in their ROS levels and activation of the caspase pathway in transformed cells. The inhibition of caspase activation by pan-caspase inhibitors does not block the SAHA- or MS-275-induced death of transformed cells.[186]

13.5.7 Activation of HDAC6 and Target Proteins

HDAC6 is the only HDAC to have two catalytic sites and a ubiquitin-binding site.[82, 121, 239–244] It can bind directly to ubiquitinated proteins through a ubiquitin-binding domain (BUZ). HDAC6 has been shown to have a key role in aggresome formation and probably participates in regulating cell viability in response to misfolded proteins. HDAC6 has several specific nonhistone substrates, including a-tubulin, cortactin, heat shock protein 90 (HSP90), and other chaperone proteins, peroxiredoxins, and transmembrane proteins.

Overexpression of HDAC6 leads to the deacetylation of a-tubulin and to an increase in cellular mobility. HDAC6 can bind both monoubiquitinated and polyubiquitinated proteins, while promoting ubiquitination of itself. Specific inhibition of HDAC6 activity with tubacain or downregulation via siRNA causes the accumulation of acetylated a-tubulin, HSP90, peroxiredoxin, and other proteins that are related to its activity. Acetylation of HSP90 causes loss of its chaperone function and exposes the proteins that it affects—such as the survival and proliferation-related proteins Akt, Bcr-Abl, c-Raf, and Erb-2-involved in multiubiquitination and degradation via the proteasome pathway.[82, 245, 246] HSP90 is essential for the stability and function of various proteins that are involved in cell signaling pathways and cell homeostasis. Recent studies have demonstrated a direct interaction between HDAC6 and HSP90. They further showed that HDAC6 is a regulator of HSP90 activity through its deacetylation.[246, 247] HDAC6 can bind directly to PP1 and cause simultaneous changes in the phosphorylation and acetylation of cell proteins. As HSP90 affects a large number of proteins, numerous molecular changes may occur as a result of the inactivation of HSP90 via inhibition of HDAC6 by HDACi. As already mentioned, HDAC6 is a component of the aggresome, a cellular structure that is the major break point of defective protein aggregates that have a defective tertiary structure with respect to both ubiquitinated and nonubiquitinated proteins characterized by having a defective tertiary structure.[82, 245] These proteins are susceptible to the formation of cytotoxic aggregates that may adversely affect normal cellular function. HDAC6 acts as a bridge between the microtubule motors of dynein and the process of ubiquitination, leading polyubiquitinated proteins to the aggresome. The BUZ region of HDAC6 exhibits high affinity for the ubiquitin molecule and is involved in the transport of multiple labeled proteins. Loss of function of HDAC6 increases the sensitivity of transformed cells to stress associated with defective-forming proteins caused by proteasome inhibition. Overall, these findings are important for the development of therapeutic strategies that combine the use of HDACi and proteasome inhibitors, as well as HSP90 inhibitors, possibly in the treatment of certain types of cancer.[248]

13.5.8 Antiangiogenesis

HDACi exert their antitumor activity by inhibiting angiogenesis via the downregulation of angiogenic factors, leading to reduced blood supply to tumor cells.[249] Solid tumors are often highly dependent on angiogenesis, and angiogenesis is critical for all tumor growth and metastasis. Tumor angiogenesis is mediated either by secondary hypoxia of cell growth or by increased tumorigenicity. HIF-1A hypoxia factor, vascular endothelial growth factor (VEGF), its receptors (VEGFRs), and basic fibroblast growth factors are the most potent proangiogenic factors and are critical in tumor angiogenesis.[250] HDACi have been reported to inhibit angiogenesis by suppressing exactly these important factors in animal model experiments. Under normal conditions, HIF-1A binds to von Hippel-Lindau protein and is inactivated by ubiquitination and then deconstruction to the proteasome. Hypoxia conditions can enhance the transcription of HDAC1-3 in cancer cell lines, resulting in decreased expression of von Hippel-Lindau protein and increased expression of HIF-1A. This sequence can be controlled by HDACi, although HDACI can induce the degradation of HIF-1A through a mechanism independent of von Hippel-Lindau protein. Class II HDACs are in direct

association with HIF-1A and their selective inhibition by siRNA induces HIF-1A degradation. The disturbance of HSP90 synergistic function via acetylation exposes HIF-1A to signaling and degradation to the proteasome.[251–253] These observations support the development of combined HDACi therapies and drugs that restrict neovascularization.

13.5.9 Antimetastatic Effect

HDACi cause increased regulatory expression in genes that suppress metastasis, such as KAII, RECK protein, Ras homologs, RhoB, and TIMP-1. In contrast, the expression of metastasis-promoting genes may be regulatorily restricted by HDACi. Genes related to metalloproteinases (MMPs), integrin-α5, and forms of collagen are included in this group.[254] These findings suggest that HDACi may be effective in reducing the metastatic potential of some primary tumors, which is an area to be explored by future studies.

13.5.10 Glucose Metabolism

The therapeutic results of HDACi are mainly attributed to their ability to modify gene expression as a result of their acetylation of transcription factors and histones. HDACi have been reported to quantitatively inhibit glucose transporter 1 (GLUT1)-mediated glucose transport into cancer cells by both downregulation of GLUT1 and inhibition of hexokinase 1 (HXK1). This effect of HDACI may be important in the selective removal of nutrients from cancer cells, which contributes to the inhibition of cell growth and subsequently to the death of these cells. Inhibiting glucose utilization in this way is accompanied by an increase in amino acid catabolism with no increase in fatty acid oxidation. Based on published data, HDACi have multiple targets and they are involved in almost every cellular biochemical pathway that affects cell survival as well as differentiation, proliferation, migration, and death. Wardell et al.[255] have suggested that HDACi-induced change in carbon source preference could play a large role in the therapeutic efficacy of HDACI by creating a pattern of fuel utilization that is incompatible with rapid tumor cell growth and survival.[255] This pattern might also be the result of altered gene expression triggered by HDACi. In cell culture studies HDACi induce the death of transformed but not normal cells, which probably reflects the ability of normal cells to recover after exposure to HDACi reversible inhibition.

13.5.11 Induction of Autophagy

The existing literature supports the belief that HDACi induce autophagy in cancer cells.[256] Autophagy is a complex recycling mechanism through which a cell's proteins and organelles are sequestered in autophagosomes and degraded after fusing with lysosomes to recycle useful ingredients and to destroy potentially hazardous (for the cell) agents. Autophagy could also function as a tumor suppressor pathway by degrading cellular components that are damaged or no longer required.[3] Mice that are haploinsufficient for beclin 1 (an essential autophagy gene) present with a greater incidence of tumor and loss of at least one beclin 1 allele have been reported in some primary tumors.[257] Autophagy is also a mechanism of promoting cell survival despite the metabolic/hypoxic stress of an already existing cancer.[258] Thus, autophagy mediates cell death, but it is also a key mechanism of tumor resistance to HDACi.

13.6 CONCLUSION

HDACi comprise a group of anticancer agents that have great potential for application in the treatment of both blood and solid tumors. There is great interest among researchers in this drug category, which has led to informal competition between biomedical and pharmaceutical researchers as to the most effective composition of HDACi, the malignancies targeted, and additional uses in nonneoplastic diseases. Studies on newer and more effective HDACi and preclinical or clinical trials of already known agents are continuously being published. HDACi not only affect histones but also a broader category of proteins referred to as lysine deacetylases. Therefore, it is important to mention that the spectrum of cellular functions directly or indirectly affected by HDACi is very wide and includes the regulation of gene expression, cell proliferation, cell migration, and apoptosis. The regulation of gene expression modified by HDACi makes it evident that HDACi could potentially alter body mechanisms and processes that are outside the scope of this chapter. Body processes such as angiogenesis and immune response are also affected by the action of HDACi. When cell death is induced in modified cancer cells, HDACi are believed to simultaneously trigger multiple

molecular pathways. All the observed and published results on HDACi are based on the aforementioned molecular mechanisms.

Normal cells are more resistant to cell death induction by HDACi than cancer cells, something that could be explained by the accumulation of genetic lesions in neoplastic cells. Normal cells are much better at overcoming the hazardous effects that HDACi introduce to their biochemical processes. As far as clinical therapeutics is concerned, this means that the exposure of cells to these substances can be adjusted to take advantage of the therapeutic window resulting from this difference between normal and malignant cells without reaching the toxic threshold for normal cells. In other words, the transient and intermittent mode of administration can ensure minimal biotoxicity on non-cancerous cells.

Attempts to clarify the biochemical actions of HDACi are ongoing, since a number of their mechanisms of action have yet to be discovered. A major question in need of clarification is whether the use of universal inhibitors of deacetylase has a comparative clinical advantage over the administration of selective inhibitors of individual enzymes. Developing specific inhibitors that act not only on given proteins but also on their close isoforms will probably assist in clarifying this question. The HDACi used in studies were not selective for a given enzyme. Another point of interest in clinical research would be the discovery of biomarkers that could predict the potential response of a patient to HDACi administration, as well as the outcomes of HDACi treatment. This is of great importance as HDACi do not appear to be universally effective in all patients for any given diagnosis but only in a percentage of them. Furthermore, clinical pharmacology should optimize the pharmacokinetic properties of HDACi, especially in terms of their water solubility and choice of oral administration. These points once resolved would boost further development of HDACi as their clinical value would be greatly enhanced. The effect of HDACi on protein interactions coupled with catalytic enzyme inhibition should be studied and clarified further. Clarifying these interactions is of great importance to understanding what lies behind HDACi toxicity on normal cells and to better understanding their pharmacodynamics on cancer cells that have been noted to have multiple molecular lesions and complicated pathophysiology.

Clinical and preclinical studies have shown that HDACi are effective when administered in combination with other anticancer agents, other than those used in surgical treatment (i.e., cytotoxic chemotherapeutic agents, factors targeted, and radiotherapy).[259] As mentioned above, coadministration of HDACi with alternative antineoplastic treatment modalities will entail cooperation as well as a program in which these therapies can be implemented to achieve the best results possible. Combined therapies are expected to be more effective against cancer cells as a result of the various types of lesions that cancer cells carry. There is a need for new clinical studies investigating the coadministration of HDACI with other anticancer agents and thereby upgrading anticancer weaponry.

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CHAPTER

14

Pharmacoepigenetics of LSD1 Inhibitors in Cancer

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14.1 INTRODUCTION

Lysine methyltransferases and demethylases have been reported to be able to catalyze the *N*-methylation and *N*-demethylation of histone-specific lysine residues, respectively.[1, 2] Demethylases can be divided into two subgroups based on their catalytic mechanisms: the flavin adenine dinucleotide (FAD)-dependent LSD1/LSD2 family and the JmjC domain-containing JMJD family.[3] Prior to the discovery of the first demethylase LSD1 (e.g., KIAA0601, KDM1A, AOF2, and BHC110) in 2004,[4] histone methylation had long been recognized as an irreversible process. LSD1 specifically demethylates histone lysine residues H3K4me1/2 and H3K9me1/2 under diverse biological settings and targets other nonhistone substrates such as p53, E2F1, DNMT1, and STAT3,[5] while JmjC-type histone demethylases utilize Fe^{2+} and 2-oxoglutarate (2-OG) to oxidatively remove the methyl group of histone lysine residues.

Increasing evidence has demonstrated that LSD1 is implicated in many cellular signaling pathways and plays critical roles in regulating fundamental cellular processes.[6] Aberrant overexpression of LSD1 has been observed in various human cancers and is closely associated with cell proliferation, epithelial-mesenchymal transition (EMT), stem cell biology, malignant transformation of cells, and cell differentiation.[7] Dysfunction of LSD1 is believed to be responsible for the development of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL).[8–10] Inactivation by small molecules or downregulation of LSD1 inhibits cancer cell differentiation, proliferation, invasion and migration, and tumor growth in different types of animal models.[11] These findings underscore the biological importance of LSD1 as a promising therapeutic target for cancer therapy. To date, a large number of LSD1 inhibitors have been identified,[12–20] some of which are currently being assessed in clinical trials for the treatment of AML, SCLC, etc.

14.2 LSD1-MEDIATED DEMETHYLATION OF HISTONE LYSINES

FAD-dependent monoamine oxidase LSD1 has been reported to be able to specifically catalyze demethylation of histone lysine substrates H3K4me1/2 and H3K9me1/2.[21] As illustrated in Fig. 14.1, the dimethylated lysine of H3K4me2 is converted to an iminium cation through FAD-dependent single-electron oxidation in the presence of molecular oxygen, generating FADH2 and H_2O_2. After the addition of H_2O to the iminium cation the hydroxylated product generated is then subjected to oxidation and hydroxylation, forming formaldehyde and monomethylated H3K4 (H3K4me1). The H3K4me1 is then transformed into nonmethylated histone lysine (H3K4me0) following the same catalytic route. Unmodified histone lysines (H3K4/9me0) have proven to possess diverse biological functions. For example, the interactions between AIRE (autoimmune regulator) and H3K4me0 are important for targeting AIRE, which directs the expression of TRAs (tissue-restricted antigens) in medullary thymic epithelial cells.[22] Cao et al. reported that the PHD1 finger of KDM5B could recognize the H3K4me0 substrate during the demethylation of histone H3K4me2/3 by KDM5B, and that disruption of the KDM5B PHD1-H3K4me0 interaction decreases the cellular H3K4me2/3 demethylation activity of KDM5B and represses the transcription of tumor suppressor genes.[23]

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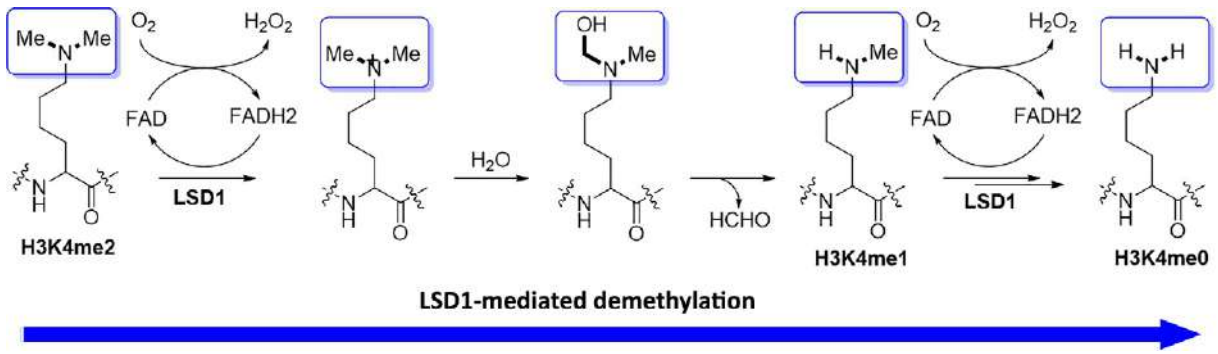


FIG. 14.1 LSD1-mediated demethylation process.

14.3 THE DEVELOPMENT OF LSD1 INHIBITORS FOR CANCER THERAPY

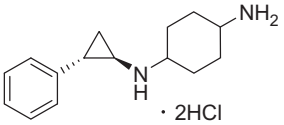
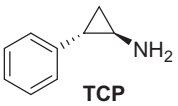
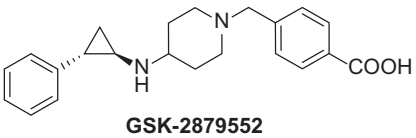
14.3.1 Irreversible Inhibitors

The MAO inhibitor tranylcypromine (TCP) was initially approved by the US Food and Drug Administration (FDA) for the treatment of mood and anxiety disorders in 1961[24] and subsequently was found to be able to moderately inhibit its homolog LSD1 by forming a covalent adduct with the flavin ring.[25, 26] The identification of TCP as an LSD1 inhibitor has inspired further extensive medicinal chemistry efforts to identify new TCP-based irreversible LSD1 inhibitors. To date, a large number of irreversible TCP-based LSD1 inhibitors have been discovered,[11, 18] of which TCP, RG6016 (e.g., ORY-1001 and RO7051790),[27] GSK-2879552,[28, 29] IMG-7289, CC-90011, INCB059872,[30, 31] and ORY-2001 alone or in combination with other therapeutic agents such as ATRA and azacitidine are currently undergoing clinical assessment at different phases for cancer therapy, such as acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), small-cell lung cancer (SCLC), etc. (Table 14.1). Note that clinical trials of GSK-2879552 for AML and relapsed/refractory SCLC, respectively, have been terminated because of the risk benefit in relapsed refractory AML and SCLC. Apart from applications in the field of oncology, LSD1 inhibitors ORY-1001, GSK-2879552, IMG-7289, and ORY-2001 (dual LSD1/MAO-B inhibitor) alone or in combination with other therapeutic agents such as ATRA and azacitidine are also being evaluated in clinical trials for the treatment of MDS, myelofibrosis, multiple sclerosis, and Alzheimer disease (Table 14.1).

ORY-1001 (e.g., RG6016 and RO7051790), developed by Oryzon Genomics, is presently being evaluated in clinical trials for the treatment of AML and solid tumors. Phase I clinical trials for relapsed, extensive stage disease SCLC treatment have been done (ClinicalTrials.gov identifier: NCT02913443). ORY-1001 potently inactivates LSD1 ($IC_{50} < 20$ nM) with high selectivity over other FAD-dependent aminoxidases (MAO-A/B, IL4I1, KDM1B (>100 μM), SMOX (7 μM)).[32] ORY-1001 time-dependently and dose-dependently induces H3K4me2 accumulation at LSD1 target genes and causes concomitant induction of differentiation markers (H3K4me2 and FACS CD11b at EC_{50} values < 1 nM) in THP-1 (MLL-AF9) cells. ORY-1001 induces apoptosis of THP-1 cells, inhibits proliferation and colony formation of MV(4;11) (MLL-AF4) cells ($EC_{50} < 1$ nM), and significantly reduces tumor growth in rodent MV(4;11) xenografts after daily oral administration of doses <0.020 mg/kg. ORY-1001 shows excellent oral bioavailability, target exposure, and activity in vivo and is stable in hepatocytes (CL_{int} <0.6 mL/min/g liver at 1 μM) without inhibition against CYP ($IC_{50} > 100$ μM) and hERG (<2% inhibitory rate at 10 μM) inhibition. Safety, pharmacokinetics (PK), and pharmacodynamics (PD) studies of ORY-1001 in acute leukemia show that ORY-1001 at the recommended dose is well tolerated and promotes differentiation of blast cells in 64% of patients.[33] ORY-1001 exhibits potent synergy with standard-of-care drugs (e.g., ATRA, cytosine arabinoside, and quizartinib), selective epigenetic and targeted inhibitors (e.g., EPZ5676, SGC-0946, decitabine, azacitidine, SAHA, and ABT-737) in both AML (MV(4;11) and MOLM13) and ALL (MOLT4) cell lines, reduces growth of an AML xenograft model, and prolongs survival in a mouse PDX (patient-derived xenograft) model of T cell acute leukemia.[34, 35] Additionally, ORY-1001 shows better growth inhibition against a panel of classic SCLC cell lines than variant ones with IC_{50} values ranging from subnanomolar to nanomolar.[36] ORY-1001 treatment inhibits xenograft growth of the response signature-positive cell line NCI-H510A, but is less sensitive to NCI-H526 xenografts.

GSK2879552 treatment causes local changes near the transcriptional start sites of genes whose expression increases with LSD1 inhibition without affecting the global levels of H3K4me1/2 and increased cell surface expression of CD11b and CD86 in AML cell lines. GSK2879552 treatment shows potent antiproliferative effects in some AML cell lines and inhibits the formation of AML blast colonies in marrow samples derived from primary AML patient samples.[28, 37, 38]

TABLE 14.1 LSD1/KDM1A Inhibitors Being Evaluated in Clinical Trials

Drugs	Structure	Sponsor	Phase	Trial number	Diseases	Status
ORY-1001/ RG6016	 ORY-1001/RG6016	Oryzon/Roche	Phase I/II	NA[a]	AML	
			Phase I	NCT02913443	SCLC	Completed
			Preclinical	NA[a]	AML; solid tumors	
TCP/ATRA	 TCP	University of Miami	Phase I	NCT02273102	AML; MDS	Recruiting
		Martin-Luther-Universität Halle-Wittenberg	Phase I/II	NCT02261779	Relapsed/ refractory AML	Unknown
TCP/ATRA/ cytarabine		Ulrike Kohlweyer	Phase I/II	NCT02717884	Non-M3 AML	Recruiting
GSK-2879552	 GSK-2879552	GlaxoSmithKline	Phase I	NCT02034123	Relapsed/ refractory SCLC	Terminated
				NCT02177812	AML	Terminated
GSK-2879552/ azacitidine			Phase II	NCT02929498	High-risk MDS	Recruiting
INCB059872	Undisclosed	Incyte Corporation	Phase I/II	NCT02712905	Solid tumors; hematologic malignancy	Recruiting
IMG-7289	Undisclosed	Imago BioSciences	Phase I	NCT03136185	Myelofibrosis	Recruiting
IMG-7289/ ATRA		Imago BioSciences	Phase I	NCT02842827	AML; MDS	Recruiting
CC-90011	Undisclosed	Celgene Corporation	Phase I	NCT02875223	Relapsed/ refractory solid tumors; non-Hodgkin lymphomas	Recruiting
ORY-2001	Undisclosed	Oryzon Genomics	Phase IIa	NA[a]	Multiple sclerosis	Recruiting
					Alzheimer disease	Recruiting

[a] *NA means the related data are not available at the https://clinicaltrials.gov website and hence are excerpted from the Oryzon Genomics website. Accessed 4 April 2018.*

Additionally, SCLC cell lines and primary samples with DNA hypomethylation are sensitive to GSK2879552 treatment. Over 80% of tumor growth inhibition (TGI) is observed in mice engrafted with SCLC lines after GSK2879552 treatment.[39] INCB059872 inactivates LSD1 by forming covalent FAD adducts. It potently and selectively inhibits proliferation of a panel of SCLC cell lines with EC_{50} values ranging from 47 to 377 nM. Oral administration of INCB059872 inhibits tumor growth in NCI-H526 and NCI-H1417 human SCLC xenograft models, induces FEZ1 and UMODL1 genes in these models, and significantly reduces serum levels of the neuroendocrine marker pro-GRP in NCI-H1417 human SCLC xenograft models.[31] INCB059872 significantly inhibits tumor growth in human AML xenograft models and prolongs the median survival of MLL-AF9-expressing leukemic mice. Mechanistic studies demonstrate that INCB059872 induces cell differentiation of murine blast cells, reduces blast colonies, and normalizes clinical hematological parameters to those of nonleukemic mice.[30] IMG-7289, developed by Imago BioSciences, or in combination with ATRA has advanced into phase II clinical trials for AML in Australia (data excerpted from Adis Insight). The orally available LSD1 inhibitor CC-90011 is currently being evaluated in phase 1 clinical trials for the treatment of relapsed/refractory solid tumors and non-Hodgkin lymphoma (data excerpted from the NCI website).

The discovery of these irreversible LSD1 inhibitors in clinical trials and their clear modes of action reveal that LSD1 is a promising epigenetic target for cancer therapy, particularly for AML and SCLC. TCP has been recognized as a privileged scaffold for designing potent LSD1 inhibitors. For detailed advances in TCP-based irreversible inhibitors, see our recent reviews.[11, 18]

14.3.2 Reversible Inhibitors

Apart from the above mentioned TCP-based irreversible LSD1 inhibitors, numerous reversible LSD1 inhibitors have been reported to date.[12, 13] These inhibitors can be categorized into natural products and nonnatural ones. Only part of the excellent work done in this area is discussed here as a result of space constraints.

14.3.2.1 Natural Products

Natural products have been recognized as rich sources for identifying new anticancer agents. Over 100 drugs used in the clinic are natural products or natural product-derived compounds.[40, 41] Several natural epigenetic modulators are currently being assessed in clinical trials. The cytidine analog azacitidine (5-AC), an irreversible DNMT inhibitor, has been approved by the FDA for the treatment of myelodysplastic syndrome (MDS).[42, 43] The class I HDAC inhibitor romidepsin isolated from *Chromobacterium violaceum* has been approved for the treatment of cutaneous T cell lymphoma (CTCL).[44] To date, some natural products, such as resveratrol, curcumin, quercetin, α-mangostin, and polymyxins (Fig. 14.2), have proven to inactivate LSD1.[15, 45–49] However, most of these natural products inhibit LSD1 at the micromolar level, and only the natural cyclic peptides polymyxin B and E (Fig. 14.2) exert promising anti-LSD1 activity with K_i values of 157 and 193 nM, respectively.[47] Possibly as a result of the poor permeability of polymyxin E across plasma membrane, no remarkable effects on either the cell growth of MV4-11cells or H3K4/H3K9 methylation (either globally or at a specific LSD1 target gene) are observed after polymyxin E treatment, thereby limiting cellular efficacy. The cocrystal structures of polymyxins E and B/LSD1-CoREST complexes reveal that polymyxins E and B bind to their circular peptide moieties at the entrance of the H3 tail-binding cleft through electrostatic interactions. Polymyxins E and B are relatively distant from flavin (>5 Å). Baicalin reversibly and moderately inactivates LSD1 with an IC_{50} value of 3.01 μM and inhibits the growth of MGC-803 cells (IC_{50} = 8.78 μM). The sugar moiety

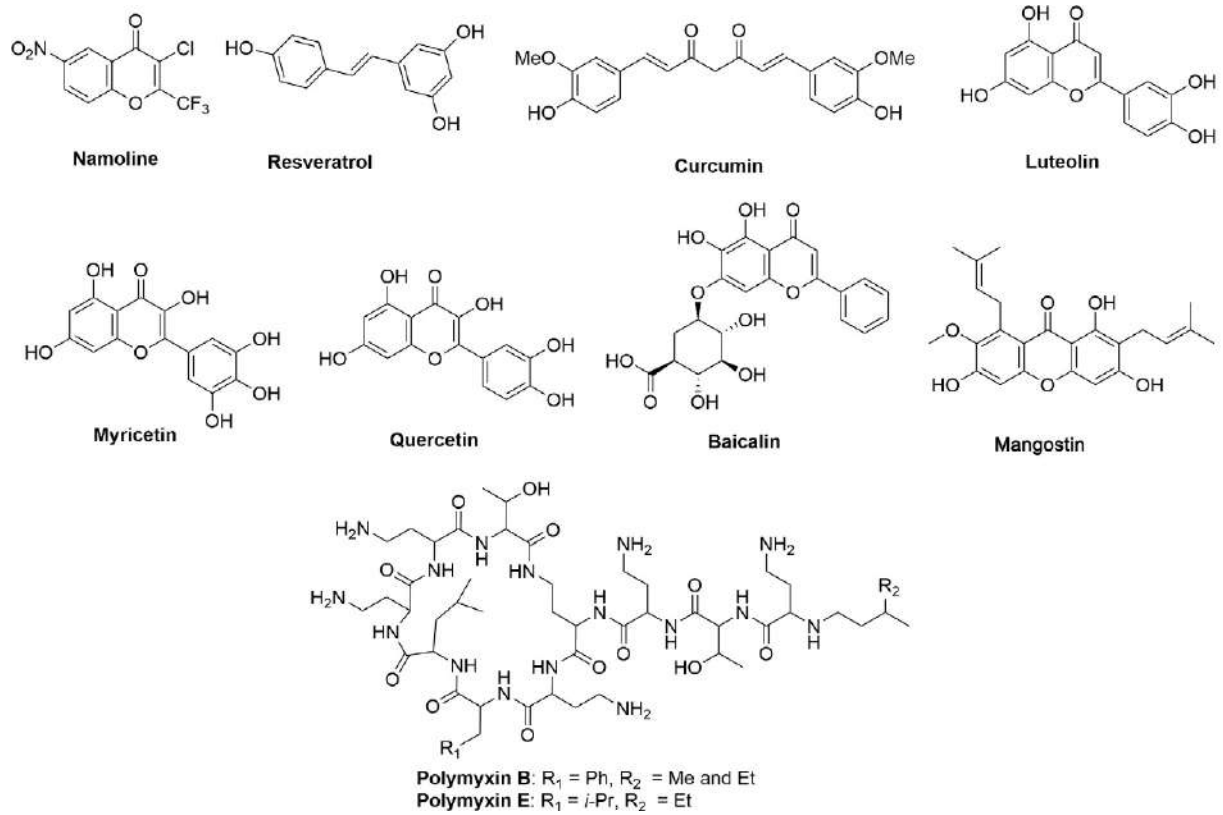


FIG. 14.2 Representative natural LSD1 inhibitors.

attached is crucial for anti-LSD1 activity.[15] Baicalin induces the accumulation of H3K4me2, significantly increases the expression of cellular biomarker CD86 mRNA, and inhibits the migration of MGC-803 cells.

14.3.2.2 *Nonnatural Inhibitors*

SP-2509 (e.g., HCI2509 and LSD1-C12), identified from the compound library (ca. 13 million compounds) based on the high-throughput virtual screening, has been found to potently inactivate LSD1 ($IC_{50} = 13$ nM) in a reversible and noncompetitive manner[50] and is highly selective to LSD1 over MAOs ($IC_{50} > 300$ μM). Inspired by the high potency and selectivity of SP-2509 (Fig. 14.3), SP-2509 analogs have also been designed and synthesized by different research groups.[51–54] SP-2509 inhibits the survival of a panel of cancer cells at low micromolar levels with minimal inhibition against CYPs and hERG. Combining SP-2509 with chloroquine increases the protein levels of LC3-II in uterine serous carcinoma ARK2 cells and synergistically inhibits proliferation of ARK2 cells via caspase-dependent apoptosis. The synergistic effect of SP2509 and chloroquine in cancer cells is also observed in animal models with xenografted tumors.[55] SP-2509 increases the levels of H3K4me2/3 in a concentration-dependent manner and shows an increase in the H3K4me3 mark on the gene promoters of KLF4, HMOX1, p57, and p21 in AML blast progenitor cells (BPCs). SP-2509 attenuates the binding of LSD1 with CoREST, which is accompanied by increased levels of p16, p21, and p27 in AML BPCs, and inhibits the suspension and colony growth of AML BPCs. SP-2509 also induces C/EBPα expression and features of morphologic differentiation in cultured and primary AML BPCs. Additionally, the pan-HDAC inhibitor panobinostat (PS) enhances SP-2509-induced chromatin effects and differentiation of AML cells. Cotreatment of PS with SP-2509 synergistically induces apoptosis of cultured AML cells, sensitizes AML cells to ATRA-induced differentiation, and significantly induces loss of viability in primary AML BPCs but not in normal $CD34^+$ cells. Treatment with SP-2509 demonstrates improved survival of NOD/SCID mice and NSG mice with established human AML, without exhibiting any toxicity.[56, 57] SP-2509 has been reported to possess potent antitumor activity in Ewing sarcoma.[58] Its clinical formulation SP-2577 entered a phase I trial in patients with Ewing sarcoma in 2017.[59] SP2509 is effective in inducing cell death, Δψm dissipation, and caspase 3/7 activity in MOLM-13 cells at low micromolar concentrations, while GSK2879552, tranylcypromine, and ORY-1001 have no effect at all even at much higher concentrations. However, the cytotoxic activity of SP-2509 observed is found to be independent of LSD1 inhibition. The off-target effects of SP-2509 may dominate cellular response to the agent because of the potential promiscuity of SP-2509 as a pan-assay interference compound (PAINS).[8, 60] Undoubtedly, SP-2509 potently inhibits LSD1 in vitro and shows effectiveness against tumor cells, but the potential target responsible for the interesting anticancer effects of SP-2509 needs to be identified.[60] Very recently, Sehrawat et al.[61] reported that SP-2509, an allosteric inhibitor of LSD1, blocked important demethylase-independent functions and suppressed

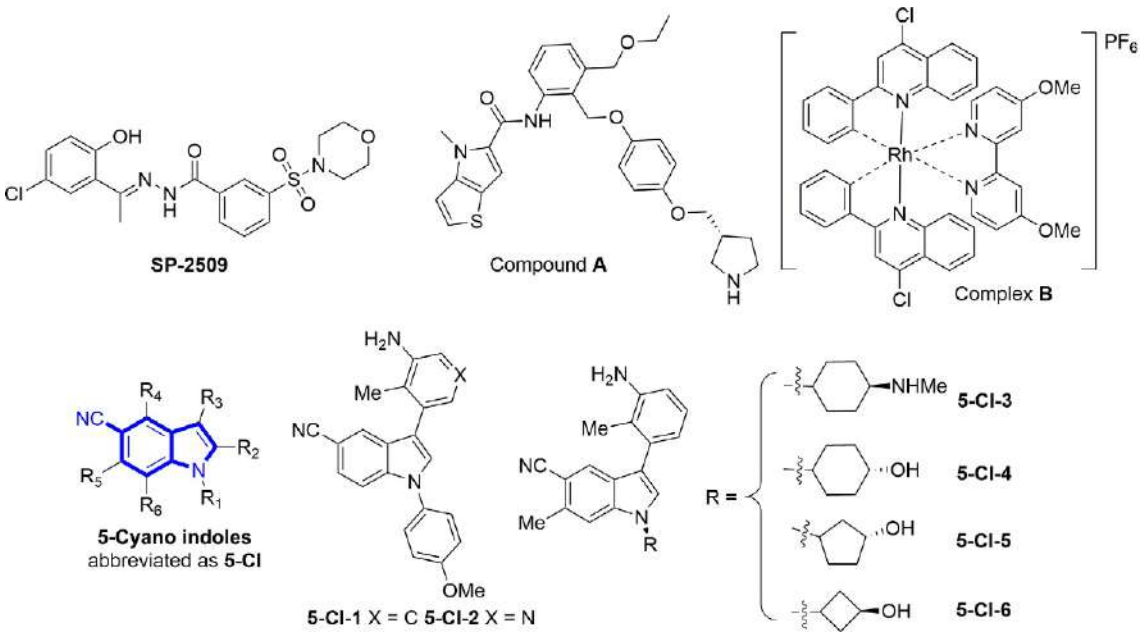


FIG. 14.3 Selective reversible LSD1 inhibitors.

the viability of castration-resistant prostate cancer (CRPC) cells.[61] The data suggest that strategies targeting LSD1 protein stability or important coactivators like ZNF217, rather than LSD1's demethylase function, may be feasible to control prostate cancer.

Very recently, a time-resolved fluorescence resonance energy transfer (TR-FRET)-based high-throughput screening (HTS) campaign was initiated by Vianello et al.[62] to identify new reversible LSD1 inhibitors from a compound collection containing 34,000 small molecules.[62, 63] Of the 115 hit compounds identified, the thieno[3,2-b]pyrrole scaffold was chosen as a starting point to develop new LSD1 inhibitors, followed by extensive medicinal chemistry efforts. Compound A (Fig. 14.3) was identified to possess extremely high potency against LSD1 ($IC_{50} = 7.8$ nM) and high selectivity to LSD1 over LSD2 and MAO-A/B ($IC_{50} = 12.9$, 41.3, and >100 μM, respectively). Compound A inhibits 70% of colony formation at 1 μM in THP-1 cells, transcriptionally affects the expression of CD14, CD11b, and CD86 genes, and shows significant anticlonogenic effects on MLL-AF9 cells. The ethyloxymethyl chain interacts with Gln358, which is responsible for conformational restraints on the ortho longer chain and further induces a U-shaped conformation. The cocrystal structures of small-molecule ligand-LSD1/CoREST complexes reported reveal underexploited binding regions that are suitable for designing new LSD1 inhibitors.

The rhodium (III) complex B (Fig. 14.3), the first metal-based LSD1 inhibitor reported to date, potently inhibits LSD1 ($IC_{50} = 40$ nM, $K_i = 0.57$ μM) and is highly selective over other histone demethylases such as LSD2, KDM7, and MAOs.[64] Complex B downregulates GLUT1 expression, suppresses H3K4me2 demethylation, which is accompanied by increased amplification of p21, FOXA2, and BMP2, and inhibits the growth of PC-3 cells at low micromolar levels. Additionally, complex B enhances the amplification of LSD1-regulated promoters, interrupts the interaction between LSD1 and H3K4me2 in PC3 cells, and induces G0/G1 arrest but not apoptosis in PC3 cancer cell lines. Collectively, complex B could be considered as a potential scaffold for designing potent and selective metal-based LSD1 inhibitors for the treatment of prostate cancers.

Novartis AG recently filed a patent relating to the use of 5-cyano indole derivatives (5-CI series) as a new class of reversible LSD1 inhibitors (Fig. 14.3).[65] This work is highlighted by Ahmed F. Abdel-Magid in a paper in *ACS Medicinal Chemistry Letters*.[7] This series of compounds potently inhibit LSD1 ($IC_{50} < 20$ nM) and also inhibit the proliferation and colony formation of Molm13 cells at low nanomolar levels; hence they could be used for the treatment of LSD1-mediated diseases and disorders, such as different types of cancers.

14.4 CONCLUSIONS

Since the discovery of LSD1 by Yang Shi in 2004, the biological roles of LSD1 have been extensively investigated. The findings indicate that LSD1 is implicated in diverse biological processes and its dysfunction is closely related to the development of cancers, particularly AML and SCLC, in which aberrant overexpression of LSD1 is always observed. Pharmacological inhibition or RNAi-mediated downregulation suppresses cancer cell differentiation, proliferation, invasion, migration, etc. Therefore, LSD1 holds great promise as an therapeutical target for cancer therapy. To date, numerous LSD1 inhibitors with different chemotypes have been reported. Presently, just TCP-based irreversible LSD1 inhibitors (alone or in combination with other therapeutic agents) are being evaluated in clinical trials for cancer treatment (Table 14.1). These trials indicate that TCP scaffolds hold great promise in the design of new potent and selective LSD1 inhibitors. In addition, a large number of reversible LSD1 inhibitors have also been reported. However, none of them has advanced into clinical trials despite their high potency and selectivity. Interestingly, some natural products (Fig. 14.2) have shown potential for LSD1 inhibition. Polymyxin B and E, which inhibit LSD1 at nanomolar levels, are the most potent natural LSD1 inhibitors identified to date. The identification of rhodium (III)-based LSD1 inhibitor B may suggest that metal-based complexes could be used in the design of new LSD1 inhibitors. SP-2509 has proven to be effective against tumor cells. However, the cellular response of SP-2509 may be due to its potential promiscuity as a pan-assay interference compound (PAINS) independent of its LSD1 inhibition. The potential target responsible for the interesting anticancer effects of SP-2509 needs to be identified. The development of dual inhibitors and strategies in which LSD1 inhibitors can be combined with other therapeutical agents may have potential for cancer therapy.

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Pharmacoepigenetics

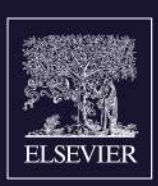
Edited by
Ramón Cacabelos

The last decade has seen a revolution in human genomics, both in technological innovation and the discovery of genetic biomarkers associated with disease. In parallel, steady progress in epigenetic research has demonstrated how epigenetic change and misregulation can have damaging effects resulting in chronic disease, cancer, age-associated illness, heart disease, neuropsychiatric disorders, and many other disease types. In response, interest from the pharmaceutical industry to leverage pharmacoepigenetics for more effective and efficient clinical drug development is now stronger than ever before.

With *Pharmacoepigenetics*, Ramón Cacabelos has compiled a comprehensive volume on the role of epigenetics and epigenomics in drug discovery and development, providing a detailed but accessible view of the field from basic principles to application in disease therapeutics. Here, leading international researchers from across academia, clinical settings, and the pharmaceutical industry offer guidelines and approaches for applying pharmacoepigenetics in drug development, drug efficiency, and safety and learn to adopt the latest epigenetic advances to realize new precision therapies. Throughout the book, chapter authors offer a balanced and objective discussion of the future of pharmacoepigenetics and its crucial contribution to the growth of precision and personalized medicine.

Key Features

- Fully examines the influence of epigenetics and epigenomics in human pathology; epigenetic biomarkers for disease prediction, diagnosis, and treatment; current epigenetic drugs; and the application of epigenetic procedures in drug development.
- Discusses the pharmacoepigenetics of cardiovascular disease, neuropsychiatric disorders, rheumatic disease, age-related disease, and numerous cancers.
- Features chapter contributions from leading international experts.
- Instructs researchers, students, and clinicians in how to better interpret and employ pharmacoepigenetics in drug development, drug efficiency, and safety.



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