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■ ■ETHICAL CONSIDERATIONS In all of these applications, ethical considerations are of utmost impor tance; it is never acceptable to deceive or provide false information to the patient. Within these bounds, however, there is much we can do to improve patients' mindsets and expectations. Consider the nocebo effects resulting from informing patients about side effects. While it is not ethical to withhold this information from patients, providers could either provide more realistic expectations about the likelihood of side effects and set more adaptive mindsets about their meaning. In one study of children undergoing oral immunotherapy treatment (OIT) for peanut allergies, half were randomized to receive a typical warning message: side effects are negative outcomes, unrelated to treatment efficacy, that need to be managed and endured. The other half were given messages aimed to instill the mindset that some mild symptoms are often a sign that the treatment is working and signal desensitization. Compared with families informed that symptoms are negative side effects, families informed that "symptoms are positive signs of treatment efficacy" experienced significantly less anxiety, fewer symptoms during the highest doses, and improved levels of IgG4, an immune marker of allergic tolerance. Similar effects of this messaging have proven to reduce anxiety and side effects for those receiving the COVID-19 vaccine. THE FUTURE OF PLACEBO EFFECTS We are entering a new era of understanding about placebo effects, one in which they are not viewed as treatment alternatives or as something to subtract, but as psychological, social, and biological mechanisms that can be considered an integral component of the overall treatment effect in medicine. Work in this field is proliferating, and translation of the findings to clinical trials and clinical care is important for optimiz ing placebo effects to improve existing treatments while minimizing nocebo effects to reduce harm. ■ ■FURTHER READING Colloca L et al: Placebo Effects through the Lens of Translational Research. New York, NY: Oxford University Press, 2023. Evers AW et al: Implications of placebo and nocebo effects for clinical practice: Expert consensus. Psychother Psychosom 87:204,

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Brian C. Capell, Shelley L. Berger

The Role of Epigenetics

in Disease and Treatment The term epigenetics was coined by Conrad Waddington in 1942, as he sought to explain how changes in phenotype could occur throughout development independent of any changes to genotype. Appending the prefix epi- (Greek, meaning “over, outside of, around”) to genet ics aptly describes the numerous mechanisms by which gene expres sion and phenotypes are influenced—and sometimes even inherited through cell division—independent of any changes to the underlying DNA sequence. Today, epigenetics occupies one of the most exciting topics in biology and medicine, offering profound opportunities for discovery, as well as promise for the development of new therapies for disease. Interdisciplinary by nature, the field crosses virtually all areas of science and medicine: chemistry and genetics, development and dif ferentiation, immunology, cancer, aging, and neuroscience. The continuous introduction of ever more powerful technologies for interrogating the epigenome has led epigenetics to become one of the most innovative fields within the biomedical sciences. Given the vast expanse of the topic and limitations of space, in this chapter, we provide a broad but brief overview of the field and then highlight key areas across the landscape of biomedicine where epigenetics has been revealed to play critical roles in physiology and disease, and impor tantly, where epigenetics-based therapies have demonstrated success in clinical medicine. CHAPTER 497 ■ ■THE BIOCHEMICAL BASES OF EPIGENETICS Fundamental to epigenetic regulation is the intricate organization into chromatin of each cell’s genome (Chap. 479). The fundamental unit of the packaging into chromatin is the nucleosome, consisting of 147 base pairs of DNA wrapped around an octamer of 8 histone proteins (two copies of each of the four core histone proteins: H2A, H2B, H3, and H4), and nucleosome assembly into a regular repeating spaced array along the DNA polymer. The presence of nucleosomes and level of compaction of this basic chromatin array determine the accessibility of the DNA strand to transcription factors, to DNA repair machinery, and to other DNA-binding

entities. Thus, compaction has a profound influence on gene expression levels and on local DNA mutation rates. Open regions of chromatin (euchromatin) tend to be transcriptionally active, whereas compacted chromatin (heterochromatin) tends to be transcriptionally repressed. Higher order three-dimensional chromatin architecture such as folding and looping further contribute to epigen etic gene regulation and cellular phenotypes. The Role of Epigenetics in Disease and Treatment Histones include the four core histones, which are the most abun dant and most frequently found throughout the genome, and the vari ant histones of H2A, H2B, and H3. The individual protein structures of both core and variant histones include amino- and carboxyl-terminal "tails," which are extended and unstructured, and highly conserved globular domains. The x-ray crystal structure of the nucleosome par ticle has illuminated the dynamic alterations of chromatin by an aston ishing range of regulatory mechanisms, summarized below. The three main processes that regulate chromatin compaction, and thus access to the DNA template, include direct methylation modifi cations (and oxidized derivatives of methylation) of the DNA strand itself, posttranslational modifications of histones, and remodeling of nucleosomes to alter their location and composition with variant histones (Fig. 497-1). The major modification of DNA is cytosine methylation of CpG dinucleotides (5-mC), associated with gene repres sion and catalyzed by the DNMT1, DNMT3A, and DNMT3B enzymes. DNMT3A and 3B catalyze the addition of methyl groups on unmeth ylated DNA de novo at CpG dinucleotides that are typically located throughout transcribed genes and in intergenic regions, but lacking at promoters, while DNMT1 is critical for the maintenance of the meth ylation state after DNA replication and after transcription during the

Tonsils Thymus Bone marrow Lymph nodes Spleen Appendix IMMUNE SYSTEM Chromosome PART 20 Emerging Topics in Clinical Medicine DEVELOPMENT AGING METABOLISM CANCER FIGURE 497-1 Epigenetic pathways influence multiple physiologic and disease pathways. As depicted in the center of the illustration, epigenetics refers to the chemical modifications of DNA and histones, which influence chromatin structure, gene expression, and susceptibility to mutations. These molecular pathways, in turn, play important roles in development, cancer, metabolism, aging, neural function, and behavior, and in the immune system. ETC, electron transport chain; TCA, tricarboxylic acid. S phase of the cell cycle. To further alter and to remove methylation, the TET enzymes (TET1–3) progressively oxidize 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC), to 5-formylcytosine (5-fC), and to 5-carboxylcytosine (5-caC), which are unable to be recognized by DNMT1 but can be removed by additional enzymes. Hence, these are mechanisms to passively lose 5-mC following DNA replication or to actively remove 5-mC, both potentially returning to unmethylated cytosine. Histone posttranslational modifications (hPTMs) are rich sources of diverse signaling to, and marking of, the chromatin template, includ ing at least 60 different covalent chemical modifications on the histone N- and C-terminal tails and within the globular domains. The hPTMs are added (written) and removed (erased) by enzymes and also serve as sequence- and PTM-specific binding surfaces for effector proteins and complexes (readers) to carry out a wide range of downstream actions including transcription, replication, DNA repair, and recombination.

BRAIN AND BEHAVIOR DNA methylation Histone methylation Histone acetylation DNA Nucleosome Glucose TCA ATP ETC One key point is that the staggering numbers of writers, erasers, and readers provide unlimited potential for diagnostic and therapeutic pharmacologic discovery. Throughout this chapter, we focus on histone acetylation and methylation, the most abundant and the most well-studied hPTMs (Fig. 497-1), although a wealth of additional modifications, such as

serine/threonine/tyrosine phosphorylation, lysine ubiquitination, lysine SUMOylation, and lysine ADP-ribosylation, among others, play important roles in transcriptional and chromatin regulation. For instance, histone phosphorylation targets histone H2A at Ser139 (γH2A.X), which marks DNA double-strand breaks immediately fol lowing DNA damage and is critical for the recruitment of the DNA repair machinery. Histone mono-ubiquitination functions similarly to other hPTMs, in signaling and marking the chromatin template, in particular serving to mark the initiation region or elongation of transcribed genes for future rounds of transcription, whereas histone

SUMOylation plays a role in transcriptional repression. Polyubiq uitination serves to tag proteins for degradation by the proteasome, and dysfunction in this system may play a role in the pathogenesis of neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's. ADP-ribosylation involves a class of enzymes, the polyADP-ribose polymerases (PARPs), which transfer ADP-ribose units from NAD+ to a variety of nuclear proteins. This PARylation alters the chromatin environment through the recruitment and modification of chromatin-associated proteins. In general, future studies of the profuse types and functions of hPTMs will enhance our understanding of these chromatin-based mechanisms and processes and will illuminate new opportunities and targets for therapies. In contrast, there is extensive understanding of histone lysine acetyl transferases (KATs) and methyltransferases (KMTs). KATs, previously known as HATs, were among the first identified histone modification enzymes. They attach acetyl groups on the lysine residues of histone tails and other proteins, resulting in both a novel side chain (acetyllysine) and an increase in negative charge (from positive charged lysine to neutral acetyl-lysine). This alteration results in loosening of chromatin structure to become more permissive to the binding of tran scription factors, and acetylation also creates a novel binding surface for the association of reader proteins. Acetylation on core histones, such as lysine 9 on histone H3 (H3K9ac) or lysine 27 (H3K27ac), is typically associated with transcriptional activation. Acetylation is very dynamic and can be rapidly removed by histone deacetylases (HDACs), of which there are multiple classes, including HDACs and sirtuins (NAD-dependent deacetylases), acting to return the lysine to unmodified ground state. Methylation of histone tails by KMTs provides more nuanced regulation, in that particular methylated lysines are associated with transcriptional activation (e.g., H3K4me3, H3K36me3, H3K79me3), transcriptional repression (e.g., H3K27me3), or DNA repeat and cen tromeric silencing (e.g., H3K9me3). The output is strictly determined by effector protein binding, as methylation of lysine does not alter side chain electrostatic charge. Lysine methylation is also a more stable chemical modification than is acetylation and turns over more slowly. Lysine demethylases have been identified for several of the specific methylated sites (H3K4, H3K9, H3K36, H3K27, H3K79). In addition to their impacts upon local chromatin structure through electrostatic alterations and through recruitment of reader effector proteins, some histone modifications can influence other epigenetic processes. For example, H3K36me3 is involved in a variety of tran scriptional processes including elongation and splicing. However, through its recruitment and interaction with other methyltransferases, such as DNMT3B and METTL14, H3K36me3 impacts both DNA and RNA methylation, respectively. Frequently coordinating with histone modification enzymes are nucleosome remodeling enzymes, which use the energy derived from the hydrolysis of ATP to reposition and remove nucleosomes along the DNA template and to exchange core histones and variant histones (including variants that are located at the transcriptional initiation sites [H2AZ] and over the transcribed genes [H3.3]). The nucleosome remodeling complexes can activate or repress transcription. The SWI/ SNF family creates nucleosome-free regions for transcriptional activa tion, the ISWI family evenly spaces nucleosomes

to repress transcrip tion, and the INO80 family exchanges H2A with H2AZ at transcription start sites to poise transcriptional activation. Other remodeling com plexes play key roles in the DNA damage response and apoptosis, among additional genomic processes. As alluded to above, RNA can also be methylated, and “RNA epi genetics” is now an emerging area of gene regulation beyond the direct methylation of DNA and hPTMs. Methylation of RNA, such as mes senger RNAs (mRNAs), has been known to exist for over half a century. However, in the last decade, the discovery of enzymes that perform reversible methylation of RNAs led to an explosion of this new field, called epitranscriptomics. Indeed, RNA methylation leads to mRNA degradation or facilitates translation. However, mRNA methylation itself occurs co-transcriptionally. Notably, the writer methyltransferase enzymes (METTL3, METTL14) and the demethylases (ALKBH5, FTO)

have important roles in a variety of disease pathologies, and drugs target ing their clinical activities are currently in human clinical trials.

Because multiple enzymes redundantly and synergistically write, erase, and recognize these modifications on DNA, RNA, and histones, there is great complexity and the potential for fine-tuning of gene regulation. While extensive knowledge gaps remain to fully explicate these mechanisms of gene regulation, epigenetics has become a fully established discipline within biomedical research. In the coming years, it is likely that the basic understanding of these processes will be fur ther harnessed for further betterment of human health. ■ ■TOOLS FOR THE STUDY OF EPIGENETICS Central to the rapid pace of epigenetic discovery has been the continual development of new cutting-edge epigenetic technologies. Chromatin immunoprecipitation (ChIP), developed over three decades ago, has been a mainstay across epigenetics and molecular biology research more broadly (Fig. 497-2). ChIP involves using formaldehyde to cross link proteins to DNA and then fragmenting the DNA and reversing of the crosslinks in order to analyze the DNA. The linking of ChIP to next-generation sequencing (ChIP-seq) provided a major leap forward in allowing researchers to probe the entire genome-wide landscape of histone modifications and DNA-binding transcription factors and chromatin-modifying enzymes. This has led to fundamental discov eries regarding the role of the epigenome in the regulation of gene expression and cellular phenotypes in development and disease. More recent refinements in these methods have expanded the appli cability of these methods. Studying chromatin accessibility has become possible through the assay for transposase-accessible chromatin using sequencing (ATAC-seq) (Fig. 497-2). Through ATAC-seq, a Tn5 trans posase can be utilized to insert sequencing adapters into open regions of chromatin, allowing for the identification of DNA regulatory elements such as promoters and enhancers even at the single-cell level. Building upon both ChIP-seq and ATAC-seq, the tethering of an antibody of interest to micrococcal nuclease (MNase) allows for the cleavage of the DNA on either side of the target, sidestepping the need for any formal dehyde fixation step, significantly scaling down the signal-to-noise ratio, and reducing the number of cells and DNA required in comparison to standard ChIP-seq. This method, referred to as CUT&RUN (cleavage under targets and release using nuclease), offers the ability to obtain his tone and chromatin-binding information in systems and models where cell numbers were previously rate limiting. A further modification of the CUT&RUN protocol replaces the MNase with a Tn5 transposase fused to sequencing adapters (CUT&Tag), offering the ability to profile histone modifications at the single-cell level. CHAPTER 497 The Role of Epigenetics in Disease and Treatment While ATAC- and ChIP-seq and their derivatives have provided tremendous insights into how chromatin accessibility and histone modi fications play a role in gene regulation, they did not provide information on how the physical organization and

folding of the genome might con tribute to gene expression. This was only able to begin to be understood by techniques that could elucidate the three-dimensional architecture and structure of chromatin. Here, techniques such as HiC (Hi-C is a high-throughput form of 3C (chromosome conformation capture) technology to study 3D genome organization) and Hi-ChIP (Hi-ChIP combines Hi-C with ChIP-sequencing to study the relationship of DNA-binding proteins to 3D genome organization) have emerged to reveal nuclear architecture and how it can either inhibit or facilitate gene expression. Collectively, these studies have revealed a model whereby enhancer state drives gene regulation. Once enhancer-promoter loops have formed, these topologically associated domains (TADs) are rein forced and can ultimately help to constrain motion of the genome and in turn increase the likelihood of further promoter-enhancer connections forming to facilitate transcription. More recently, the latest frontier in biomedical research is spatial technologies that allow the capture of molecular data at subcellular resolution within their native tissue context. While techniques such as spatial CUT&Tag are still in development, as they continue to advance in resolution, throughput, and accessibility, they are certain to offer unprecedented insights into how tissue and disease pathology corre lates with alterations in the transcriptome, epigenome, and proteome.

ChIP–seq ATAC-seq Epigenome editing UV Tn5 transposase Peaks (kb) Sequencing peaks corresponding to open chromatin Purified DNA Adapter Data collection Sequencing library PART 20 Emerging Topics in Clinical Medicine Reads: …GTTCCTTCAGCATTTGCAGCGT… Reads: Reference Genome Peak identification NGS Sequencing Motif 1 Motif 2 FIGURE 497-2 Core experimental techniques for the study of epigenetics. The explosion of interest and research in the past few decades has been fueled by fundamental advances in the experimental approaches and ability to profile the epigenome. Chromatin immunoprecipitation–next-generation sequencing (ChIP-seq) allows for the ability determine the genome-wide binding of a histone modification or DNA-binding protein of interest. In contrast, assay for transposase-accessible chromatin using sequencing (ATAC-seq) provides a method for determining chromatin accessibility genome-wide even down to the single-cell level. More recently, the development of CRISPR-based epigenome-editing technologies has offered a way to directly deposit histone modification in order to activate or repress specific genes. NGS, next-generation sequencing. Finally, another major technological breakthrough, and one with tremendous therapeutic potential, is development of CRISPR-based epigenome editing approaches (Fig. 497-2). By fusing a nucleasedeactivated Cas protein to an epigenetic modifying enzyme, one can use guide RNAs to precisely target gene regulatory effectors to turn on and turn off specific genes by changing the gene’s histone acetylation or methylation levels. For example, using CRISPR to guide the mRNA of an epigenetic repressor to the oncogene MYC is one strategy currently being tested for cancer treatment. These advances promise to not only elucidate new knowledge regarding principles of gene regulation but also to offer new therapeutic opportunities for disease. ■ ■EPIGENETICS IN DEVELOPMENT

AND DIFFERENTIATION Epigenetic processes are critical to organismal development and to cellular differentiation and reprogramming of cell fate (Fig. 497-1). Transcription factors establish the epigenomic landscape that enables and stabilizes cell-type-specific gene expression while simultane ously ensuring stable repression of alternative cell fates. This results in chromatin profiles that display remarkable cell-type specificity in

dCas9 Effector domain Epigenetic modifications Repressed locus dCas9 Activated locus Epigenetic editing differentiated cells, particularly at the key regulatory nodes of gene enhancers, which are gene-distal DNA elements that control transcrip tion. In fact, epigenome profiling of the chromatin landscape in tumors of unknown cell origin can provide a better index of the origin tissue than does DNA sequencing of gene mutations within the tumor. The cell-type-specific epigenetic program is first derived from the template of embryonic stem cells, where numerous genes required for differentiation exist in a “bivalent” state, marked by both the activat ing histone modification, H3K4me3, and the repressive modification, H3K27me3. Due to this unstable epigenetic state, the genes are “poised” for activation or for repression, depending on their subsequent cell fate. Critical genes directing toward a specific cell fate will be turned on, with maintained H3K4me3 and erased H3K27me3, whereas genes leading toward alternative fates will be repressed, with maintained H3K27me3 and removed H3K4me3. Once differentiated, an epigenetic barrier will prevent the cells from returning to the stem cell state. For example, con stitutive heterochromatin in the form of H3K9me3 can serve as a barrier to cellular reprogramming when attempting to create induced pluripo tent stem cells, and inhibiting the enzymes that catalyze H3K9me3, such as SUV39H1, can enhance reprogramming efficiency.

DNA methylation contributes to the specification of cell fate and to other developmental pathways. DNA methylation alterations are involved in critical processes ranging from sex chromosome dosage compensation to coordinating expression of imprinted genes. Disrup tion of this latter process can lead to imprinting disorders including Prader-Willi syndrome, Angelman syndrome, and Beckwith-Wie demann syndrome. Recent discoveries have served to highlight the tremendous amount of interplay between epigenetic modifications and, in particular, between DNA methylation and various histone modifications. Beyond embryonic development, epigenetics can provide the neces sary coordination and balance between adult stem cell self-renewal compared to cell differentiation. This epigenetic control is critical, as impaired self-renewal can lead to stem cell exhaustion and premature aging, while excessive self-renewal may promote cancer. Key epigenetic regulators tend to play conserved roles across diverse tissue types. For instance, BMI1, a component of the polycomb repressive complex 1 (PRC1), is required for stem cell proliferation and self-renewal, and its ablation leads to stem cell depletion in hematopoietic, epidermal, muscle, intestinal, and mammary stem cells. Similarly, the DNA meth yltransferase DNMT1 is required for stem cell self-renewal in hemato poietic, epidermal, and mammary stem cells. HDACs 1 and 2 possess some overlapping functions and are required for normal epidermal differentiation. Likewise, a loss of these HDAC enzymes in hematopoi etic stem cells can lead to failure of differentiation and severe anemia. In a similar fashion, inhibition or loss of histone lysine demethylase 1 (LSD1), a repressor of transcription, is known to promote differ entiation across multiple cellular contexts. These factors represent repressive chromatin regulation, leading to the general concept that restraining specific transcription pathways related to differentiation is crucial to maintaining undifferentiated self-renewing stem cell pools. The epigenetic regulation of the tumor suppressor p16 (CDKN2A) locus during differentiation provides a prime example of this finely tuned system. For example, as mentioned above, DNMT1 is necessary for self-renewal in human epidermal stem cells. Levels of DNMT1 are high in the basal undifferentiated layer of the epidermis, decreasing progressively with epidermal stratification, leading to de-repression of the tumor suppressors p16 and p15, thereby promoting cell cycle arrest and full differentiation. BMI1 displays a similar phenotype in both hematopoietic and epidermal stem cells, repressing key genes that promote differentiation, such as p16 and p19ARF. Consistently, a loss of BMI1 leads to premature

differentiation and defective self-renewal. In addition to the repression provided by DNMT1 and BMI1, the p16 locus is highly decorated with the repressive H3K27me3 catalyzed by EZH2 in epidermal stem cells. Then, during epidermal differentiation, H3K27me3 is removed by the KDM6B (JMJD3) histone demethyl ase. Loss of this control over programmed p16 expression occurs in epithelial cancers, such as squamous cell carcinoma (SCC), where EZH2 is overexpressed and KDM6B expression is lost. Breast cancer is another example where progesterone can increase levels of EZH2 to promote mammary epithelial cell proliferation, and excessive EZH2 expression can occur in cancer. This exemplifies how epigenetics can integrate environmental signals and have a profound influence on the fine balance between stem cell maintenance and overt carcinogenesis. In general, a recurrent theme in cancer is loss of key chromatin regula tion that promotes cell differentiation, combined with gain of activities that promote stemness. Chromatin-modifying enzymes also play a major role in influenc ing cell-type specificity. High levels of EZH2 that modify H3K27me3 promote adipogenesis while simultaneously inhibiting osteogenesis. In contrast, the H3K27me3 demethylases, KDM6A (UTX) and KDM6B, derepress those same genes, driving stem cells toward osteogenesis. Through interactions with tissue-specific master regulators, epigenetic modifiers also shape cell-type specificity. In the epidermis, p63, the p53 family member that is a master regulator of the epidermal compart ment, interacts with several chromatin regulators including HDAC1 and HDAC2, SATB1, MLL4 (KMT2D), and BRG1 to orchestrate epidermal differentiation. Similarly, the gene-activating H3K4 histone methyltransferases, MLL3 (KMT2C) and MLL4, are required for

adipogenesis by forming a complex with the transcriptional activator ASC2 and the transcription factor PPARγ to induce adipogenic genes. Overall, loss of epigenetic regulation can reduce cell differentiation and increase stem cell specification to drive diseases encompassing devel opment, cancer, and, broadly, diseases associated with aging.

■ ■EPIGENETICS OF METABOLISM One of the fascinating aspects of epigenetics is that it represents a mechanism for direct connection between the environment and gene expression. Numerous studies in the field of metabolism have identi fied a complex interplay between diet, metabolism, and the epigenome (Fig. 497-1). Seminal findings in Drosophila and mice have shown that changes in diet, particularly the paternal diet, and other environmental factors, can influence the metabolism of offspring, ultimately promot ing obesity in later generations. Epidemiologic studies in humans have supported these results, as the nutritional status of grandparents has been correlated with phenotypic effects in grandchildren. In fact, diet can directly affect the levels and activity of chromatin modifiers. For instance, high-fat diets reduce histone acetylation through their ability to inhibit the enzymes ACLY and ACSS2, which produce acetylCoA. Levels of acetyl-CoA, in comparison to all measured metabolites, are indeed the best predictor of histone acetylation levels. Consistent with this, increased acetyl-CoA correlates with rising levels of total histone acetylation, including at the promoters of growth-associated genes. This increase in nuclear acetylation is associated with cell cycle progression and proliferation, and it can have clinically relevant down stream effects. For example, high levels of acetyl-CoA can delay stem cell differentiation and suppress autophagy. The oncogenes MYC and AKT can both hijack metabolic networks to enhance nutrient uptake by cancer cells, thus promoting acetyl-CoA production and resulting in both the initiation and progression of tumorigenesis. Additional evidence suggests that dietary intake of alcohol can directly contribute to acetate levels and therefore histone acetylation in the brain, with effects on the transcription of genes involved in learning and memory. CHAPTER

497 The Role of Epigenetics in Disease and Treatment Contrary to convention that metabolic enzymes are strictly mito chondrial or cytosolic, certain metabolic enzymes can be present in the nucleus and can thereby directly regulate histone acetylation enzymes. This is the case for several enzymes that generate acetyl-CoA, includ ing ACLY, PDH, and ACSS2, which generate acetyl-CoA from citrate, pyruvate, and acetate, respectively. Further, ACSS2 can be chromatinbound to regulate gene expression, leading to physiologic responses such as autophagy in the liver and mammalian hippocampal func tion in learning. This direct metabolic-epigenetic enzyme cross-talk illuminates a crucial local role of the acetyl-CoA metabolite to effect rapid gene transcription and represents a fertile intersection for future therapeutics. Methylation is also altered by metabolism. S-Adenosylmethionine (SAM) is the key metabolic cofactor for histone and DNA methyla tion. Dietary factors are estimated to explain 30% of the variation in human serum methionine concentration and hence can alter SAM levels and histone methylation. For example, dietary methionine avail ability and intracellular production of SAM affect the levels of histone H3K4me3 associated with transcriptional activation. Furthermore, these fluctuations can have critical physiologic consequences: DNA methylation levels in rectal mucosa and colonic polyps are increased by higher levels of dietary folate, and a diet low in methyl donors reduces the formation of gastrointestinal cancers in mice predisposed to these tumors. Methionine metabolism and the availability of SAM regulate stem cell differentiation and contribute to carcinogenesis. For instance, cancers with mutations in metabolic regulatory genes such as IDH1/2, FH, and SDH lead to the accumulation of by-products (2-hydroxy glutarate, fumarate, and succinate, respectively), which all inhibit α-ketoglutarate (α-KG)–dependent histone demethylases and thus pro mote hypermethylation and lead to impaired cellular differentiation. Notably, some of the α-KG–dependent demethylases, which are highly mutated in numerous cancers (i.e., KDM5A, KDM6A), also serve as cellular oxygen sensors, thus linking environmental oxygen levels to epigenetic control of methylation levels. In contrast to hypermethyl ated states, loss of the MTAP gene, which is part of the 9p21 locus

containing p16 and one of the most frequent events in human cancer, disrupts normal methionine metabolism. This both lowers methyla tion levels, and, interestingly, also sensitizes cancer cells to inhibitors of the PRMT5 methyltransferase, therefore opening a therapeutic opportunity. These observations illustrate how connections between epigenetics and metabolism can generate unanticipated advances in medicine. Furthermore, these data highlight the tight interconnections between environmental inputs, metabolism, and epigenetics.

■ ■CANCER EPIGENETICS Cancer is now understood to be a mixed genetic and epigenetic disease, as epigenetic dysregulation is pervasive in human cancers (Fig. 497-1). Beyond simple activation of oncogenes or reduced expression of tumor suppressors, epigenetic mechanisms can contribute to chemotherapy resistance and to failure of antitumor immunity. Accordingly, the development of drugs targeting epigenetic pathways is one of the most active areas of clinical and pharmaceutical development, with several compounds already approved for human use and shown to be effec tive in a variety of cancers. Epigenetic perturbations in cancer largely affect chromatin-regulating enzymes, which represent robust targets for development of novel small-molecule inhibitors, especially as com pared with canonical oncogenic transcription factors (e.g., MYC) and tumor suppressors (e.g., p53). Epigenetics can contribute to carcinogenesis in a variety of ways. First, on a global scale, chromatin organization is the single most influ ential factor in determining local mutation rate across the genome. Analysis of abundant tumor sequencing data has demonstrated

that heterochromatic regions of the genome contain a higher frequency of mutations compared with more open euchromatic regions. This differ ence is due to the improved accessibility of the DNA repair machinery to less compact, more open regions of chromatin. PART 20 Emerging Topics in Clinical Medicine The first discovery of an epigenetic mutation was found in 1998 when the chromatin remodeler SMARCB1 was shown to drive the for mation of malignant rhabdoid tumors. Extensive sequencing of human tumors from the majority of cancer types has been performed by The Cancer Genome Atlas (TCGA) consortium, and remarkably, 25–30% of identified cancer driver mutations occur in chromatin regulatory proteins. Similar to SMARCB1, numerous other chromatin modifi ers (e.g., methyltransferases MLL3 and MLL4, and acetyltransferases EP300 and CBP) and nucleosome remodeling enzymes and associated complex components (e.g., SMARCA4, SMARCA2, ARID1A) are heav ily mutated and inactivated in many cancers. The majority of these mutations are loss-of-function mutations, and indeed, enzymes like MLL4 and demethylase KDM6A possess tumor-suppressive activ ity across a variety of tissues and cellular contexts. In contrast, the H3K27me3 histone methyltransferase EZH2 is an oncogene, and accordingly, it is overexpressed in many advanced-stage or metastatic solid tumors such as breast cancer, prostate cancer, and melanoma. Mechanistically, EZH2 represses the p16 tumor suppressor and other cell cycle genes required for cell cycle exit via H3K27me3 deposition. Consistent with a broad growth regulatory role, EZH2 inhibitors are therapeutically successful for a number of cancers in preclinical models and are being actively studied for B-cell lymphoma, melanoma, and other solid tumors. In addition, provocative evidence has emerged for a direct tumori genic role of histones based on the discovery of causative mutations, such as histone H3 mutations identified in pediatric high-grade glio mas. Specifically, the majority of these mutations are in the H3 variant H3.3, where lysine 27 is replaced by methionine (K27M). Similarly,

> 90% of chondroblastomas replace lysine 36 with methionine (K36M) in histone H3.3. These effects appear to be dominant negative because (1) in H3.3, these are heterozygous mutations, and (2) the mutations also occur in the canonical H3, which exists in ~30 orthologous genes in the human genome. Thus, a minority of H3/H3.3 mutant protein leads to global defects in the associated histone modifications (K27 or K36 methylation), possibly via irreversible inhibition of the cognate enzymes by the mutant histones. These “oncohistone” mutations pro mote resistance to apoptosis and failure of normal differentiation in a number of pediatric and adult cancers.

Beyond mutations, genetic translocations involving chromatin modifiers also implicate chromatin pathways as direct drivers in cancer. MLL1 (KMT2A), the H3K4 histone methyltransferase, is a frequent translocation partner occurring in adult and pediatric acute myeloid leukemia (AML) and in ~80% of infant acute lymphoid leuke mia (ALL) cases. MLL1 can fuse with >70 translocation partners, and these mutant proteins prevent normal hematopoietic differentiation. Consistent with a causative role of MLL1 in these gene fusions, drugs inhibiting the catalytic activity of MLL1 are effective in preclinical models of AML and are currently being evaluated in human clinical trials. Given the abundance of epigenetic abnormalities in cancer com bined with the inherent reversibility of epigenetic changes, extensive efforts are underway to develop epigenetic drugs. The first epigenetic therapeutic involved the use of DNA methylation inhibitors (DNMTi) to

reactivate tumor-suppressor genes. Interestingly, the mechanism of traditional chemotherapeutics, such as azacitidine and decitabine, is to inhibit DNMT1, thereby promoting global hypomethylation; these are currently in clinical use for myelodysplastic syndrome (MDS) and AML. In a second broad mechanism, loss of acetylation occurs in many cancers, and thus, HDAC inhibitors (HDACi) are under inten sive development. HDACi are effective and approved for treatment in cutaneous T-cell lymphoma and multiple myeloma. Bromodomain (BRD)-containing proteins bind to lysine acetylated target proteins, including histones, and rationally designed BET inhibitors (BETi) block their binding. BETi reduce the amplified expression of oncogenes such as MYC in hematologic cancers. Current studies are now focused on optimizing combinatorial epigenetic therapies with conventional chemotherapies and immunotherapies, particularly given the ability of epigenetic therapeutics to promote re-expression of tumor antigens and interferon (IFN)-mediated antitumor immunity. Indeed, the development of a new generation of more specific epigenome-targeted inhibitors, combined with our increased knowledge of the underlying epigenetic mechanisms contributing to tumorigenesis, has enabled a precision medicine–based approach to harnessing the potential of these drugs. This may be particularly valuable in the context of improv ing patient responses to a variety of therapies beyond chemotherapies and immunotherapies, such as radiation and hormone therapies. There are several hundred chromatin enzymes and binding proteins in the human genome, and the current focus is to identify more specific inhibitors. Indeed, targeted inhibitors of numerous mutated chroma tin regulators have been developed, with >30 compounds currently in various stages of development and preclinical trials. Some notable examples showing early clinical success include EZH2 inhibitors for lymphomas, sarcomas, and melanoma; IDH inhibitors for AML and gliomas carrying mutant IDH1 or IDH2 genes; LSD1 inhibitors for AML and small-cell lung cancer; and DOT1L and MLL1 inhibitors for leukemias with activated MLL1. Given the broad potential effects of epigenetic regulators, it is perhaps not surprising that there have been some dose-limiting toxicities, particularly among those that are less target-specific. Collectively, the emerging picture is that the most effec tive and robust use of epigenetic drugs in cancer will be fine-tuning and potentiating the effects of other therapies that are either incom pletely effective or marked by widespread resistance. ■ ■EPIGENETICS OF AGING Like many diseases of aging, human aging itself results from the complex interplay between genes and the environment. Evidence that the epigenome may be the key link between these processes derives from observations that numerous environmental stimuli and stressors—ranging from diet and exercise to hormones and circadian rhythms—contribute to both aging and epigenetic alterations (Fig. 497-1). Thus, a lifetime of exposures progressively disrupts the chro matin landscape. These age-dependent changes in chromatin organi zation increase the susceptibility of the genome to mutations and also reduce transcriptional fidelity. Further, provocative findings in model systems demonstrate that stress-induced epigenetic changes can be transmitted over several generations and can even affect the life span of later generations. Among these global epigenetic alterations, there

is dysregulation of histone modifications and a general loss of histone proteins with aging across taxa. Amazingly, experimental increases in histone levels, particularly histones H3 and H4, but not H2A or H2B, can reverse these age-related changes in mammalian cells and in the yeast Saccharomyces cerevisiae model. Thus, the sum of current evidence suggests a model of aging via a general increase in activating epigenetic modifications along with a loss of repressive modifications. Together these changes create a state of transcriptional instability and “noise” that inhibits accurate transcrip tion. Cells from patients with Hutchinson-Gilford progeria syndrome

(HGPS), the most severe form of human premature aging, display reduced levels of both H3K9me3 and H3K27me3 repressive chromatin. In another premature aging disease, Werner syndrome, DNA damage induces global loss of H3K9me3 and H3K27me3 due to the inherent absence of the Werner syndrome ATP-dependent DNA helicase, which is critical for DNA repair. Such heterochromatin loss is not limited to premature aging conditions, as aged cells derived from healthy older humans display age-dependent loss of H3K9me3 leading to aberrant expression of normally repressed transposable elements. Activation of these mobile elements correlates with neurodegenerative disorders and may also promote other aging-related phenotypes such as cancer. Human fibroblasts undergoing cellular senescence (exit from cell cycle due to replicative or other stress) undergo destabilization of compact heterochromatin adjacent to the nuclear periphery, in so-called laminassociated domains (LADs). At LADs, in addition to a reduction of repressive histone modifications as discussed above, there are broad new regions of the euchromatic histone modification H3K4me3. This general loss of heterochromatin can promote the activation of cytosolic DNA and RNA sensing pathways that promote innate immune signal ing and "inflammaging." In addition to age-associated alterations of histone modifications, direct manipulation of chromatin-modifying enzymes that control these marks affects the balance between heterochromatic and euchro matic regions, and it alters the lifespan of model organisms. Inhibiting the H3K27me3 histone demethylase KDM6A results in increased repressive H3K27me3 and extended lifespan in Caenorhabditis elegans. Consistent with this, genetic reduction of enzymes (ash-2, set-2, wdr-5) that add the activating H3K4me3 histone modification also extends lifespan in C. elegans. The consequences of these genetic manipulations nicely correspond to the observed changes in histone modifications as described above. Beyond histone-modifying enzymes, dysregulation of the levels or function of chromatin remodelers can also affect lifespan in model organisms. This dysregulation occurs in humans as well, as in the nucleosome remodeling deacetylase complex (NuRD), which is reduced in HGPS fibroblasts and in aged healthy donors. In addition to age-related changes in histone methylation, histone acetylation also contributes to aging phenotypes. Dysregulation of his tone acetyltransferases (HATs) and HDACs is associated with reduced longevity across model organisms. Further, sirtuin deacetylases (class III NAD+-dependent HDACs) promote health span and lifespan across species as key mediators of pro-longevity effects of caloric restriction. Indeed, loss of Sirt6 results in premature aging in mice, while caloric restriction–induced increases of Sirt1 and Sirt6 expression can delay aging. As discussed previously, metabolism and acetylation are intri cately linked, and the sirtuins, via NAD+ levels, and other HDACs may play key roles connecting the environment, gene expression, and physiologic output. For instance, exercise in humans reduces activity of HDACs 4 and 5, leading to increased H3K36ac in skeletal muscle, which likely promotes beneficial gene expression. Epigenetic alterations with aging are not limited to histone modifi cations and extend to DNA methylation. Consistent with the histone patterns, DNA methylation data support the model described above—that is, general decompaction of the epigenome with aging. Specifically, levels of 5-mC are reduced in senescent human cells, and global DNA hypomethylation occurs across the human genome with aging. Con current with this overall hypomethylated state, there are local regions of hypermethylation focused near CpGs at gene promoters, particu larly at genes that maintain cellular differentiation and cell identity. This epigenetic disruption during aging thus leads to profound changes

in transcription. For example, in hematopoietic stem cells, DNA hyper methylation blocks proper binding of transcription factors, resulting in dysregulation of normal gene expression with aging.

Importantly, these patterns are not merely stochastic alterations in response to environmental stressors throughout aging. Indeed, the methylation status of a defined number of CpG sites is a highly accurate predictor of chronologic age in human tissues. This work reveals that DNA meth ylation status with aging outperforms previous standard biomarkers of aging, such as p16 expression levels and telomere length, and will be highly valuable in the near future to gauge effects of treatment aiming to ameliorate diseases of aging.

■ ■EPIGENETICS OF THE BRAIN AND BEHAVIOR Brain disorders are among the greatest clinical challenges to under stand and to treat. Most neurologic and psychiatric disorders result from complex dysregulation of numerous genes and pathways. In this interplay between underlying genetic predisposition and external environmental factors, aberrant epigenetic regulation is increasingly recognized as a potentially key modulator (Fig. 497-1). More directly, however, several progressive neurodevelopmental disorders are caused by germline mutations in chromatin regulators. Mutations in methyl CpG binding protein 2 (MECP2), a protein important for binding to methylated DNA and contributing to gene repression, are the major cause of Rett syndrome. MeCP2 loss leads to overactive gene transcription in neurons and impaired presynaptic excitatory functions. Similarly, Kabuki syndrome, another progres sive neurodevelopmental disorder, is caused by germline mutations in either the H3K4me1 histone methyltransferase, MLL4 (KMT2D), or the H3K27me3 demethylase, UTX (KDM6A). This disorder may derive from dysregulation of transcriptional enhancers, a major class of gene regulatory elements, as both MLL4 and UTX play a key role in activa tion of enhancers. Finally, the acetyltransferase CBP (CREBBP) also is important for gene enhancer function and, when mutated, can lead to Rubinstein-Taybi syndrome, a cause of intellectual disability. CHAPTER 497 The Role of Epigenetics in Disease and Treatment Beyond germline mutations, altered methylation dynamics can drive disorders of neural development and of neurodegeneration. Fragile X syndrome, characterized by learning disabilities and cogni tive impairment, is caused by mutations in the FMR1 or FMR2 gene or by hypermethylation of the transcriptional promoters regulating FMR1 or FMR2. Similarly, Prader-Willi syndrome and Angelman syndrome, neurodevelopmental conditions caused by abnormal imprinting of the paternal or maternal chromosomal region (15q11-13), respectively, are frequently caused by aberrant DNA methylation. Further, DNA hypomethylation is implicated in some neurodegenerative condi tions. For instance, in Parkinson’s disease, several genes involved in pathogenesis are hypomethylated due to DNMT1 depletion, includ ing the α-synuclein gene (SCNA). In Alzheimer’s disease (AD), DNA hypomethylation occurs at promoters of key pathogenic genes such as amyloid precursor protein (APP). Indeed, APP promoter methylation is responsive to environmental factors, including aging, a major risk factor for AD. Likewise, presenilin-1 (PSEN1) is implicated in AD and displays altered DNA methylation in response to variations in metabolic stimuli. Recent evidence from human AD brains demon strated significant enrichment of H3K9 and H3K27 acetylation and provided evidence that this dysregulation of the epigenome promotes gene transcription pathways involved in AD pathogenesis. Studies of Huntington’s disease (HD) have demonstrated DNA hypomethylation and decreased histone acetylation, in part due to altered function of the acetyl transferase CBP, leading to transcriptional dysregulation. Together, these observations underscore altered epigenetic regulation as a crucial feature of neurodegeneration. Additional gene regulatory proteins in the nervous system interact with and are regulated by chromatin modifiers. REST (repressor ele ment 1–silencing transcription factor) is important in neuronal homeo stasis through its ability to recruit chromatin regulatory enzymes, such as histone deacetylases and histone methyltransferases, and via its control over gene expression. REST levels increase with aging and serve a protective

function in neurons against age-associated stressors and loss of cognitive function associated with AD. Similar to REST,

brain-derived neurotrophic factor (BDNF), another important media tor of neural development and homeostasis, is implicated in a variety of neurologic and psychiatric disorders including HD, depression, schizophrenia, bipolar disorder, and autism. Knockdown of BDNF in the dentate gyrus leads to depression-like behavior in mouse models, and BDNF mediates effects of antidepressant therapies. Chromatin pathways, including DNA methylation/MeCP2 and H3K27me3, play a key role in BDNF regulation as observed in brains from patients with schizophrenia.

Finally, addiction medicine is another frontier where epigenetics holds great promise to reveal connections between environmental exposure and phenotypes. Although still in its early stages in terms of mechanistic understanding, emerging evidence demonstrates disrup tion of epigenetic homeostasis as a consequence of addictive substances ranging from alcohol to cocaine. For example, the acetylation of regula tory elements in the FOSB gene by the histone acetyltransferase CBP is associated with behavioral effects of cocaine. Opioid exposure appears to promote a generally more open and permissive state of chromatin marked by increases in histone acetylation and reductions in histone methylation, which may allow for a more hyperresponsive state and reinforce reward-seeking behaviors. Ethanol also induces histone acetylation and a decompacted chromatin structure with direct effects on learning and memory function. ■ ■EPIGENETIC INFLUENCES ON INFECTION, IMMUNITY, AND INFLAMMATION Alterations in gene expression patterns are important determinants of immune-mediated disease, and in turn, epigenetics regulates infection, immunity, and inflammation (Fig. 497-1). Treatment with immunestimulating agents such as lipopolysaccharide (LPS) and tumor necrosis factor α activates expression of numerous inflammatory genes within hours, with precise gene pathways and activation kinetics determined by the cellular epigenetic state. HATs and HDACs are critical components of this response, coordinating with proinflammatory transcription fac tors, such as AP-1 and NF-κB, to either activate (in the case of HATs) or repress (in the case of HDACs) inflammatory genes. For example, cor ticosteroids recruit HDAC2 to promoters of NF-κB–stimulated inflam matory genes to prevent activation during asthma treatment. PART 20 Emerging Topics in Clinical Medicine Type 1 IFN responses are exceptional examples of regulatory com plexity governed by epigenetic control. In an unstimulated state, the H3K9 methyltransferases G9a (EHMT2) and EHMT1 suppress expres sion of IFN and IFN-induced genes. Upon induction of IFN-stimulated genes, STAT transcription factors recruit chromatin remodeling com plexes, such as BAF (SMARCA4), and recruit HATs including p300, CBP, and GCN5 (KAT2A). In turn, chromatin remodeling and acetyla tion recruit chromatin binding proteins including the bromodomain protein, BRD4, which promotes transcriptional elongation and full activation. Beyond the DNA level, METTL3-mediated m6A methyla tion on mRNAs also is a critical regulator of IFN signaling in a variety of distinct cellular contexts. Major regulators of adaptive immunity pathways are similarly epige netically regulated. CD4+ and CD8+ T cells undergo extensive changes in histone modification profiles during differentiation to distinct subsets of effector T cells. For example, genes associated with effector T-cell functions in CD8+ memory T cells (e.g., PRDM1, KLRG1, IFNG) display enrichment of H3K4me3 and low levels of H3K27me3 com pared with those genes in naïve T cells. DNA methylation also plays an important regulatory role and may contribute to disease. For example, CD4+ T cells from individuals with rheumatoid arthritis (RA), sys temic scleroderma, and latent autoimmune diabetes in adults display hypermethylation of the FOXP3 gene, which activates regulatory T cells that

dampen immune responses. In addition, hypermethylation of the CTLA4 locus occurs in regulatory T cells from RA patients, impair ing their immunosuppressive abilities. During infection, epigenetic processes can play critical roles in both the immune response and defense against pathogens, as well strategies exploited by microorganisms to co-opt the host cellular machinery to advantage of the pathogen. Respiratory syncytial virus (RSV) infection promotes the expression of the histone demethylase KDM5B, which

removes H3K4 methyl groups from antiviral genes such as type 1 IFNs, driving a switch from T helper 1– to T helper 2–type immune responses, thereby contributing to chronic infection. Similarly, influ enza upregulates the repressive H3K9me3 methyltransferase SETDB2 to block expression of CXCL1 and a variety of NF-κB target genes involved in attracting neutrophils and host defense, both serving to lengthen the infection and contributing to bacterial superinfection. Regarding the host response to infection, studies have revealed that differences in host tissue-, age-, and sex-biased epigenetic profiles might shape susceptibility and responses to infection. For example, differential DNA methylation at the ACE2 gene may impact expression levels of this key cellular receptor and ultimately the ability of SARSCoV-2 to infect hosts, while alterations in antiviral IFN signaling may lead to more severe COVID-19 infection and disease. These findings are all supported by new discoveries demonstrating that epigenetics is a key component for the inflammatory memory that has been observed now across a wide variety of contexts. Numerous perturbations ranging from infections and vaccination to skin wounding and Western diets have now been shown to elicit an epigenetic memory that is maintained and propagated. This epigenetic memory extends beyond just the immune system to the involved tissues. These findings have suggested a potential for epigenome-modifying drugs for the treatment of inflammatory and immune-related condi tions. For example, the DNA methylation inhibitors azacitidine and decitabine have immunosuppressive effects possibly mediated by enhanced expression of FOXP3, which generally suppresses immune responses. HDACi upregulate and downregulate immune genes, and they inhibit cytokine production in macrophages from patients with RA. Further, the HDACi vorinostat and panobinostat inhibit primary B-cell responses and antibody production in vitro and in vivo. Given these broad effects, it is not surprising that the HDACi trichostatin A (TSA) has efficacy in various model systems for treatment of RA, systemic lupus erythematosus (SLE), asthma, acute kidney injury, sepsis-induced lung and cardiac damage, and acute pancreatitis. Simi larly, BETi also display broad effects in blocking antigen presentation and T- and B-cell activation and thus beneficial protective effects in a variety of inflammatory settings including autoimmunity, sepsis, atherosclerosis, psoriasis, periodontitis, and arthritis. Beyond these “broad-spectrum” epigenetic inhibitors, GSK-J4, which is a specific inhibitor of the H3K27me3 demethylases KDM6A and KDM6B, has anti-inflammatory activity, presumably by preventing loss of H3K27me3 repression over inflammatory genes. Similarly, inhibition of the H3K4me3 histone methyltransferase MLL1 blocks the induction of proinflammatory cytokine gene expression in a variety of contexts. CONCLUSION Due to the enormity and complexity of the chromatin and epigenetics fields and their reach into all areas of biology and medicine, it is not possible to cover such a broad scope in a single chapter. Thus, here we provide a concise snapshot highlighting key areas of development in medicine. We hope to have conveyed the tremendous excitement and promise that pervades the discipline. Indeed, given the expo nential growth in uncovering the interface between the epigenome and epigenetic therapies with the environment and disease, there is little doubt that the coming years will bring important additions to this field. ■ ■FURTHER READING Bates SE: Epigenetic therapies for cancer. N Engl J Med 383:650, 2020. Carter B, Zhao K: The epigenetic basis of cellular heterogeneity. Nat Rev Genet 22:235, 2021. Dai Z et al: The

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Revision #1
Created 2026-01-06 16:36:18 UTC by Omar Ayman
Updated 2026-01-06 16:36:18 UTC by Omar Ayman