better understanding of normal and diseased brain aging and cognition will have a significant public health impact, given that the oldest-old persons older than 85 years of age represent the fastest-growing segment in the population in developed countries, with more than 30 million new cases of dementia predicted to occur worldwide each year by 2040. Dysregulation of gene expression and, more generally, genome organization and function are thought to contribute to age-related declines in cognition. Remarkably, nearly all neuronal nuclei that reside in an aged brain had permanently exited from the cell cycle during prenatal development, and DNA methylation and histone modifications and other molecular constituents of the epigenome are likely to play a critical role in the maintenance of neuronal health and function throughout the entire lifespan. Here, we provide an overview of age-related changes in the brain's chromatin structures, highlight potential epigenetic drug targets for cognitive decline and age-related neurodegenerative disease, and discuss opportunities and challenges when studying epigenetic biomarkers in aging research.

The 20th century saw an unprecedented gain of 30 years in life expectancy in the Western world, and data from authoritative sources, including the US Census Bureau, show that the oldest old (OO; persons older than age 85 years) represent the fastest-growing segment of the population of the United States and other developed countries. Dementia is highly prevalent in this population, with a projected worldwide incidence of more than 30 million by 2040. The OO are not only at the highest risk for dementia (40%), but dementia is a stronger predictor of mortality in the OO than cardiovascular disease, cancer, or male sex. In addition, psychiatric disorders, such as depression, anxiety, and psychotic disorders, are relatively common among elderly individuals, affecting approximately 20% of persons older than age 65 years.

It is likely that both genetic and environmental factors contribute to the relationships between age, dementia, cognitive function, neuropathology, and risk factor markers. It has commonly been argued that genetic influences on longevity account for 25% of the variance and environmental and lifestyle factors govern the remaining 75%. However, it is unlikely that a simple 2-factor gene × environment mechanism will address these complex interactions fully. In this review, we argue that the advent of advanced approaches in neuroepigenetics hold significant promise for uncovering some of the mechanisms associated with compromised aging and the promotion of successful aging. After a brief primer on the basic principles of epigenetic regulation, we provide a concise update on our knowledge about some of the epigenetic mechanisms implicated in normal brain development and aging, as well as describe how studies in preclinical model systems and human brain tissue point to novel drug targets such as histone deacetylases (HDACs) and other chromatin modifiers to achieve improved cognition and neuroprotection for the aging brain. As brain

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tissue itself cannot be studied in the context of clinical studies (with living subjects), we complete this review with a brief discussion on opportunities and challenges when studying epigenetic biomarkers related to cognitive functions using surrogate tissues from the periphery.

**WHAT IS IN THE TERM EPIGENETICS? A PRIMER FOR THE NEUROLOGIST**

Most scholars in the field ascribe the term epigenetics (epi- being Greek for over or above) to Conrad Waddington, an Edinburgh-based geneticist in the first half of the last century who was interested in the mechanisms governing the unfolding of the genetic program during development. While the more traditional definition of epigenetics is often equated with heritable changes in gene expression and function in the absence of DNA sequence alterations, the term is broadly applied to dividing and postmitotic cells alike to describe the regulation and organization of chromatin structures, including changes during development or disease. Obviously, the role of the human genome in health and disease cannot be fully captured by a sole analysis of its linear sequence of 3 billion base pairs; therefore, epigenetic approaches quickly moved center stage in many areas of medicine once the human genome had been sequenced.

Therefore, it is best to start with a brief discussion on chromatin, with the nucleosome as the elementary unit, consisting of 146 bp of DNA wrapped in 2.5 loops around an octamer of small proteins, the nucleosome core histones H2A, H2B, H3, and H4. Chromatin fibers are essentially nucleosomal arrays configured as beads on a string, interconnected by linker DNA and linker histones (Figure 1). However, it is important to emphasize that while nucleosomal organization leads to a 7-fold increase in packaging density of the genetic material compared with naked DNA, the actual level of compaction in the vertebrate nucleus is about 3 orders of magnitude higher. Other than for some of the more general principles of genome organization—for example, actively expressed genes typically are associated with more loosely packed euchromatin, while telomeric and pericentric repeat DNA at the extreme ends of chromatin are organized as compact heterochromatin—much remains to be learned about the regulation of higher-order chromatin in the nervous system. Presently, the bulk of epigenetics literature, as it pertains to human and animal brains, is focused on chemical modifications of the genomic DNA and the nucleosome core histones, including methylation and hydroxymethylation of DNA cytosine residues, and posttranslational histone modifications, such as lysine acetylation and methylation that are primarily positioned at the N-terminal histone tails protruding from the nucleosome core (Figure 1). However, to date, only a very small fraction of the more than 100 histone modifications that are encountered in a typical vertebrate cell have been explored in the brain. Nonetheless, it is generally accepted that the various DNA methylation and histone modification markings, in combination, define the functional architecture of brain chromatin along the same lines as previously established for peripheral cells. Thus, there are specific chromatin signatures differentiating between transcribed and repressed genes, as well as between euchromatin vs heterochromatin and so on. Furthermore, promoter and enhancer sequences—the latter are regulatory DNA elements that in a linear genome are further removed from a gene promoter—and gene bodies each are defined with their own specific set of epigenetic decorations (Figure 1) and, as in the case of DNA cytosine hydroxymethylation, even with tissue-
specific regulation of exon/intron boundaries. Thus, for example, the promoter of an actively expressed gene typically shows high levels of histone acetylation and residue-specific methylation such as trimethylated histone H3 lysine 4 (H3K4me3) or monomethylated H4 lysine 20 (H4K20me1) (the side chain of lysines can carry up to 3 methyl groups). In contrast, the promoter of a repressed gene is rich in DNA methylation, which primarily (but not exclusively) occurs in the context of CpG dinucleotides and certain types of histone methylation, including trimethylated H3 lysine 9 (H3K9me3), H3 lysine 27 (H3K27me3), or H4 lysine 20 (H4K20me3) (Figure 1). These few examples should provide a glimpse of the complexities in the epigenetic code, especially of histone methylation, where not only different lysine residues show differential enrichment at active vs repressed genes but, as in the case of histone H4K20, even the monomethylated and trimethylated forms of the same residue are associated with a different chromatin state.

Perhaps unsurprisingly then, the human genome encodes a complex set of DNA-modifying and histone-modifying enzymes. To provide just a few examples, there are at least 4 separate genes each encoding a DNA methyltransferase (DNMT1, DNMT3A, DNMT3B, and DNMT3L, with the latter viewed as axillary and noncatalytic) and at least 18 genes each encoding a different HDAC, as well as probably more than 100 genes encoding histone methyltransferases and demethylases for the various histone methyllysine and methylarginine residues in the human epigenome. Finally, in addition to these writers or erasers of the epigenetic code, each chromatin marking serves as a potential docking site for scores, if not hundreds, of reader proteins that are defined by domains (protein sequence motifs) that recognize specific epigenetic markings. Thus, there are proteins with a methyl-DNA-binding domain, or with bromodomains that bind to acetylated histones, and chromo, Tudor, MBT, WD40 repeat, and PHD finger domains targeting methylated lysines or arginines in a residue-specific manner. These readers are often integral components of large, multiprotein complexes involved in the regulation of gene expression, repressive chromatin remodeling, and other functions.

Chromatin remodeling and proper assignment of epigenetic marks to specific genomic loci is of fundamental importance during ontogenesis, including neuronal and glial differentiation and migration as key steps during brain development.

Unsurprisingly then, various neurodevelopmental syndromes have been linked to single gene mutations in DNA methyltransferase DNMT3B and histone-modifying enzymes, or their reader proteins. For example, hypomorphic (partial loss of function) mutations in DNA methyltransferase DNMT3B are responsible for a multiorgan syndrome (immunodeficiency-centromeric instability–facial anomalies syndrome 1; OMIM 242860), which is associated with mental retardation, seizures, motor delay, and recurrent infections. Mutations and microdeletions encompassing chromosome 9q34.3 that affect lysine methyltransferase 1D (KMT1D/EHMT1), a regulator of histone H3K9 methylation, were initially linked to mental retardation (Kleefstra syndrome, OMIM 610253), but subsequently they were deemed the causative mutation in some cases of schizophrenia and various nonspecific psychiatric phenotypes associated with neurodevelopmental disease in the postadolescence period. The fact that mutations or DNA structural variants in these chromatin modifier-encoding genes result in adult-onset neurodevelopmental disease leaves little doubt that fine tuning of the epigenetic machinery indeed is pivotal for neuronal health and function even long after early brain development is complete.

AGE-ASSOCIATED CHANGES IN BRAIN CHROMATIN

In simple model organisms, such as the worm Caenorhabditis elegans, mutations in a select set of histone-modification enzymes, including some of the class III HDACs (sirtuins) or the trithorax chromatin remodeling complex-regulating histone H3K4 methylation, are thought to have a strong impact on the animal’s longevity and aging process. Furthermore, mammalian aging is accompanied by distinct changes in epigenetic promoter architectures of cell cycle–regulatory genes, in addition to more generalized alterations including senescence-associated heterochromatic foci. While most of these aging-related epigenetic phenotypes were obtained from the study of peripheral cells, there can be little doubt that chromatin of brain cells too will undergo important changes during aging. Some of these are potentially irreversible, such as the accumulation of somatic mutations in promoters that could compromise proper expression of neuronal genes in the old brain. However, most or perhaps all epigenetic markings studied to date are now deemed to be reversible, and there is no a priori reason for unidirectional accumulation of a specific epigenetic mark in aging brain chromatin. Some of the underlying molecular machineries are often complex and may be prone to become inefficient during the later stages of life. For example, consider that the activity of the 3 DNA methyltransferases, DNMT1, DNMT3A, and DNMT3B, that establish and maintain DNA cytosine methylation is counterbalanced by active demethylation pathways involving ten-eleven translocation dioxygenases and/or deamination of methylcytosines and hydroxymethylcytosines by AID/APOBEC proteins, which some cases of hereditary sensory and autonomy neuropathy type 1 (a rare neurodegenerative condition characterized by various neuropathies and early-onset dementia in the third or fourth decade) were linked to mutations in the protein-coding sequence of the gene encoding DNMT1 gene. These mutations occur within the targeting sequence domain that is important for nuclear localization and preferential enrichment of this enzyme at pericentric and other repeat DNA. Very similar types of DNMT1 mutations recently emerged in some kindreds with autosomal-dominant cerebellar ataxia, deafness, and narcolepsy. Furthermore, mutations and microdeletions encompassing chromosome 9q34.3 that affect lysine methyltransferase 1D (KMT1D/EHMT1), a regulator of histone H3K9 methylation, were initially linked to mental retardation (Kleefstra syndrome, OMIM 610253), but subsequently they were deemed the causative mutation in some cases of schizophrenia and various nonspecific psychiatric phenotypes associated with neurodevelopmental disease in the postadolescence period. The fact that mutations or DNA structural variants in these chromatin modifier-encoding genes result in adult-onset neurodevelopmental disease leaves little doubt that fine tuning of the epigenetic machinery indeed is pivotal for neuronal health and function even long after early brain development is complete.
is followed by a complicated base excision repair-mediated replacement with unmethylated cytosines to restore the nascent (non-DNA methylated) state.31,32 While entirely speculative at this point, it is reasonable to assume that such a complicated process of writing and erasing the DNA methylation mark, with so many different proteins involved, may become less efficient as the organism ages. Therefore, it is interesting that in DNA methylation studies across the lifespan of the human cerebral cortex, DNA cytosine methylation levels tend to increase at a substantial portion of gene promoters, which in turn could negatively affect gene expression for synaptic signaling and other functions in some of the older brains.33-35 Changes in promoter chromatin during the process of normal aging go beyond the level of DNA methylation and promoter-associated DNA methylation. These changes may lead to an overall compaction of the surrounding chromatin and decreased presence of phosphoactivated RNA polymerase (pol) II, thereby decreasing transcriptional activity.

**Figure 2.** Epigenetic determinants of decreased neuronal gene expression in the aging brain. Hypothetical, simplified scheme for age-related decline in neuronal gene expression, which is accompanied by a shift from open chromatin-associated histone acetylation, including histone (H) 3 lysine (K) 27 acetyl (ac) and H4K12ac, and methylation (trimethylated H3K36 [H3K36me3] to repressive [H3K9me2/3 and H3K27me3]) chromatin-associated histone methylation and promoter-associated DNA methylation. These changes may affect gene expression profiles of the brains of older persons meeting clinical criteria, such as Alzheimer disease (AD), Lewy body dementia, and frontotemporal dementia, often associated with the loss of transcriptional activity.

**EPIGENETIC TARGETS IN THE TREATMENT OF AGE-ASSOCIATED COGNITIVE DECLINE**

Prior to the discussion on the preclinical literature on cognition and dementia, it is important to emphasize that dementia is not a unitary entity. Among the dementing diseases, AD, cerebrovascular-associated dementia, Lewy body dementia, and frontotemporal dementia are the most common with estimated prevalence rates of 60% to 80%, 20% to 40%, 5% to 20%, and 5% to 20%, respectively.48,49 Importantly, even within a well-defined disease category such as AD, recent evidence from postmortem and some biomarker studies suggests that the mechanisms underlying dementia in young-old (YO) persons (<85 years old) may not necessarily be the same as those associated with dementia in the OO. For example, separation in the density of AD-associated lesions (including neurofibrillary tangles and neural plaques) between nondemented and demented persons diminishes in the OO such that approximately 50% of nondemented OO persons meet neuropathologic criteria for AD.50-51 One recent study demonstrated that the gene expression profiles of the brains of OO persons with dementia were profoundly different than those of YO persons with dementia, with less than 30% overlap at any level of dementia severity.52 Furthermore, psychiatric disorders that occur during the course of dementia (including anxiety, depression, or psychosis) often are defined by a trajectory that does not necessarily follow the severity of dementia, suggesting a potentially independent etiology from the cooccurring dementing condition.53 Therefore, care must be taken when extrapolating findings from the preclinical animal models to a patient population because diagnosis is ultimately defined by neuropathology and there is presently very little information about epigenetic alterations specific to a neuropathology rather than global degeneration.

To date, the regulation of histone acetylation is in many areas of medicine, including neurology, perhaps the best-
studied epigenetic process. The activity of histone acetyltransferases is counterbalanced by HDACs, and it is the latter group of molecules that has received the most attention in the field. Histone deacetylases are commonly divided into class I/II (and IV) that are different from class III (also known as sirtuins) based on their homology to their counterparts in yeast and requirement for different cofactors for catalytic activity (eg, zinc ion for class I/II and nicotinamide NAD+ for class III). Studies in genetically engineered mice with increased expression or gain of function of HDAC1 ascribed this particular HDAC with a strong neuroprotective effect in AD and ischemia/stroke models, while ablation of a related gene, HDAC2, resulted in improved learning and memory and increased synaptic plasticity. Histone deacetylase 5 strongly modulates motivational behaviors in the neural circuitry regulating reward and mood-related functions and so forth. Thus, even closely related chromatin-modifier genes potentially elicit a very different central nervous system phenotype, which underscores the prevailing view that despite (as in the case of class I/II HDACs) multiple members of a gene family sharing the same catalytic domains, their roles in the actual organism are largely nonredundant and nonoverlapping.

On the other hand, in terms of target selectivity, all HDACs are considered highly promiscuous, and they deacetylate in addition to the nucleosome core or linker histones various nonhistone proteins located in the nucleus or even cytoplasm. The case of HDAC1, which in the brain is located predominantly but not exclusively in the nucleus, could serve as a representative example because this enzyme deacetylates, in addition to its classical substrate, the nucleosomal core histones, also adenosine monophosphate-activated protein kinase as a key regulator for cell signaling and metabolism, as well as multiple proteins of the pre-messenger RNA 3’ end processing machinery and several transcriptional activators or repressors including p53, NFκB, MyoD, and E2F. Therefore, neuroprotective effects of HDAC1 in mouse models for chronic neurodegeneration and stroke/ischemia could be due to epigenetic remodeling at gene promoters, alterations in transcription factor activity, differential regulation of polyadenylation and other pre-messenger RNA processing, or adenosine monophosphate-activated protein kinase–mediated shifts in energy homeostasis of neurons or glia and a combination thereof.

The HDAC catalytic sites can be readily targeted by various compounds, which include the recently US Food and Drug Administration–approved HDAC inhibitor and antineurodegenerative therapy, valproic acid, and derived prodrug BIX-01294 experimental drug for clinical use. In addition, a number of cyclic hydroxamic acids (Vorinostat and romidepsin (Istodax), and older drugs, such as the short-chain fatty acid (valproic acid and phenylbutyrate). Whether the therapeutic range of the anticonvulsant and mood stabilizer valproate and of phenylbutyrate (which is used for treating congenital urea cycle disorders) suffices to inhibit HDAC activity in the brain is questionable. In any case, one of the most surprising findings from the preclinical field is the wide range of neurologic conditions with a therapeutic response to HDAC inhibitors. These range from acute brain injury and stroke paradigms to various neurodegenerative conditions such as Parkinson disease, AD, and triplet-repeat disorders, including Huntington chorea and spinocerebellar ataxias, as well as motor neuron disease, depression, and other psychiatric illnesses. Importantly, the beneficial effects of HDAC inhibitors are not limited to the degenerating or injured brain, but some of these compounds dramatically improve hippocampal-dependent learning and memory in normally aged animals, in conjunction with an upregulation of the aforementioned transcriptional mark, acetyl-H4K12 (Figure 1), which is of special interest in brain aging research because it tends to decline in older brains.

Because virtually all HDAC inhibitors tested thus far are pleiotropic by inhibiting multiple members of a particular HDAC class, each of which could regulate the acetylation of a different set of protein targets, the link between neuroprotection and drug-induced histone hyperacetylation remains speculative. In any case and whichever of the HDAC inhibitor–mediated actions turn out to be important for brain function and cognition, given their broad therapeutic profile and lack of prominent cytotoxicity and other prohibitive adverse effects, HDAC inhibitors are likely to be explored in the nearby future in a clinical setting for the treatment of diseases affecting elderly individuals.

Without a particular focus on the aged brain, various epigenetic drug targets other than HDACs also bear promise for the treatment and prevention of dementias or the attenuation and prevention of age-related cognitive decline. One interesting candidate is the histone H3K4-specific histone lysine methyltransferase KMT2A/MLL1, a member of the mixed-lineage leukemia family of molecules and the aforementioned trithorax chromatin-remodeling complex that recently emerged as a key regulator for aging and longevity in an invertebrate model system. Mice heterozygous for a loss-of-function Mll1 mutation show distinct abnormalities in hippocampal plasticity and signaling in conjunction with defects of learning and memory and, moreover, mixed-lineage leukemia 1 has been implicated in cortical (gamma-aminobutyric acidergic) interneuron dysfunction in schizophrenia. Regulation of histone H3K9 methylation, either by the BIX-01294 experimental drug or ablation, or transgene-mediated overexpression of H3K9-specific methyltransferase enzymes, including G9A/GLP or KMT1E/ESET/SETDB1, could also emerge as a promising approach to modulate the brain’s cognitive and motivational states.

**EPIGENETIC BIOMARKERS TO STUDY COGNITION AND DEMENTIA IN ELDERLY INDIVIDUALS?**

Presently, we are far from understanding the causes and neurobiological substrates of dementia in elderly individuals. Factors leading to longevity and successful aging are complex; their study is only at the very beginning stages and many of the findings reported to date are paradoxical and complex. Recent cross-sectional or short-term longitudinal studies shed light into potential biomarkers for dementia and their age-related contributions. For example, poor cardiac functions, such as low ejec tion fraction, are associated with poor cognitive function in adults and YO persons, but the reverse is true in the
OO. Other cardiovascular and systemic illness risk and biomarkers have shown similar relationships. Similarly, high blood levels of C-reactive protein, in addition to serving as biomarkers for systemic inflammation, have been associated with poor cognitive function in YO persons. However, this relationship is not only absent in the OO, but it predicts cognitive health and good performance on tests of cognition in their siblings. Some of these paradoxical and counterintuitive relationships between risk factors, cognitive function, age, and longevity have been attributed to the potential buffering influence of other genes and to antagonistic pleiotropy. The complexity of disentangling these relationships is aggravated further by the scarcity of large-scale longitudinal studies that follow individuals over the course of decades into old and very old age, thus they have the potential to uncover life-long protective, or risk-associated, determinants for normal and diseased brain aging. For example, it is not obvious whether the paradoxical findings alluded to previously in cognitively intact successfully aging OO persons are life-long characteristics or whether they develop through unknown adaptive or protective mechanisms. Even if some of these characteristic features of successful aging are life long, it is not clear whether these are genetic or life-style modifiable, or turn out to be of practical and actionable significance.

It remains to be determined whether chromatin-based biomarkers will make a useful contribution to the field of aging research, as it pertains to cognition and dementia. The list of candidate markers worth being explored in blood and other peripheral tissues includes acetyl-histone markings in bulk chromatin that broadly inform about transcriptional regulation and activity in the aged and/or degenerating brain. Because age-related neurodegenerative disease, including AD, is multifactorial with a complex etiology and because blood-based and other peripheral tissue-based molecular profiling would only have limited proximity to the target tissue, the brain, the investigation of gene expression profiles as an early detection biomarker, rather than expression of particular genes, may be more pertinent to the neurobiology of disease (AD). Likewise, a recent DNA methylation study reported that genes subject to downregulated expression in AD also showed age-associated DNA cytosine methylation changes both in the brain and blood, suggesting that for epigenetic analyses, blood could be a surrogate for related changes in the brain, at least for some genomic loci. It will also be interesting to explore chromatin status and gene expression of well-established AD genes. For example, one study reported that probable AD cases in the OO had a 60% reduction in APOE expression in lymphocytes relative to control subjects, with an inverse correlation between APOE expression and β-amyloid levels; the changes in APOE expression could be related to a single nucleotide polymorphism in the promoter, which further supports the role of transcriptional regulation. With the exploration of epigenetic determinants of aging and cognition barely beginning, we expect that this avenue of research will provide valuable insights into age-associated human health and disease in the not too distant future.
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