DNA Methylation in Neurodegenerative Disorders

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Abstract Epigenetic mechanisms are essential in a host of biological processes and many different human diseases. Epigenetic modifications include DNA methylation, histone modification, non-coding RNA, and nucleosome positioning. In neuronal systems, extensive studies have revealed important regulatory roles of DNA methylation in brain function, from the embryonic stage through the process of aging. Here we review recent evidence that DNA methylation is involved in aging, learning and memory, and neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and CGG repeat-induced neurodegenerative disorders. We also explore the dynamics of DNA methylation.

Keywords DNA methylation · Neurodegeneration · Cognitive function · Aging · Alzheimer's disease · Parkinson's disease · Amyotrophic lateral sclerosis · DNA demethylation

Introduction

Epigenetics refers to modifications that lead to heritable phenotypes, without affecting the DNA sequence itself [1]. Epigenetic modifications include DNA methylation, histone modifications, the action of small RNAs, and nucleosome positioning [2, 3, 4•]. In mammals, DNA methylation is for

the most part cytosine methylation [5-methylcytosine (5mC)], and is found predominantly within CpG dinucleotides. The DNA methylation machinery in mammals consists of two components: the DNA methyltransferases (DNMTs), which establish and maintain DNA methylation patterns, and the methyl-CpG binding proteins (MBDs), which are involved in “reading” methylation marks [5]. DNA methylation is catalyzed by a family of DNA methyltransferases (DNMTs) that transfer a methyl group from S-adenyl methionine (SAM) to the fifth carbon of a cytosine residue to form 5mC. Five members of the DNMT family have been reported in mammals: DNMT1, DNMT2, DNMT3a, DNMT3b, and DNMT3L [4•]. DNMT3a and DNMT3b can establish a new methylation pattern in unmodified DNA and are thus known as de novo DNMTs. The de novo DNMTs are responsible for establishing the normal pattern of methylation during embryonic development [6]. DNMT3L, despite being catalytically inactive, is required for establishing maternal genomic imprinting [7]. It acts as a general stimulatory factor for DNMT3a and DNMT3b, and interacts and colocalizes with them in the nucleus [8, 9]. DNMT1, the maintenance enzyme of DNA methylation, is the most abundant DNMT and is transcribed primarily during the S-phase of the cell cycle.

In mammalian neuronal systems, DNA methylation has been implicated in the regulation of neural stem cell fate determination, brain development, cognitive function, neurodevelopmental disorders, and neurodegenerative diseases [10–15]. The absence of DNMT1 has a lethal effect in mice, and DNMT3a can regulate neurogenic capability by regulating the neuronal transcriptome. DNA methylation is also associated with the formation of memory. In the context of disease, DNA methylation is involved in neuronal cell death and ischemia-induced injury. Recent studies have shown that DNA methylation plays important roles in a range of human diseases, including neurodegenerative disorders. Here, we will provide an overview of recent findings that

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shed light on the function of DNA methylation in multiple neurodegenerative diseases. We will also discuss the dynamics of DNA methylation.

DNA Methylation and Aging

Aging is defined as a loss of phenotypic plasticity over time. At a cellular level, aging is characterized by oxidative stress, disturbed calcium homeostasis, chromosomal instability, impaired DNA repair, and the accumulation of nuclear and mitochondrial DNA damage [16]. Combined or individually, these factors can lead to neuronal aging, neurodegeneration, and cell death in the central nervous system. DNA methylation is known to play important roles in fundamental cellular processes such as differentiation, proliferation, and senescence by modulating both local and genome-wide gene transcription [5]. The aging cell is found to undergo a DNA methylation drift, with generally an overall decrease in total genomic 5mC content in the brain [17]. Studies in cultured aging human fibroblasts have revealed that DNMT1 expression and activity are gradually reduced with increased population doublings [18].

It is now well established that aberrant DNA methylation processes are often a causal factor in several major neurological disorders [5]. A number of recent studies identified abnormal DNA methylation events in neuronal cells associated with aging and age-related neurodegenerative disorders, such as Alzheimer's disease [19–21]. *Dnmt1* haploinsufficiency (*Dnmt1*^{+/-}) can cause premature aging effects and poor cognitive function without affecting survival or mortality. Further, DNA methylation decreases with normal aging and is also lower in *Dnmt1*^{+/-} mouse brain tissues than in *Dnmt1*^{+/+} mouse brain tissues [22]. A recent study in elderly subjects revealed a gradual age-dependent loss of genomic DNA methylation within the same individual over an eight-year span [23]. The DNA methylation landscape of CD4⁺ T cells from newborn and centenarian individuals showed that DNA obtained from a 103-year-old donor was more unmethylated overall than DNA from the same cell type obtained from a neonate [24•]. Furthermore, the centenarian sample showed a lower correlation in terms of the methylation status of neighboring CpGs versus the newborn sample, which was more homogeneously methylated in nearby located CpGs. The hypomethylated CpGs observed in the centenarian DNA covered all genomic compartments, such as promoters, exonic, intronic, and intergenic regions [24•]. These longitudinal studies provide important *in vivo* evidence that loss of DNA methylation indeed occurs with age under normal physiological conditions in humans.

DNA Methylation and Learning and Memory

Synaptic connectivity among neurons serves as the physical basis for memory formation, which often involves gene products (mRNA or protein) from a vast number of neural activity-related genes. DNMT activity is required for associative memory formation and induction of long-term potentiation (LTP) [25, 26]. Infusion of the DNMT inhibitors 5-aza-deoxycytidine (5-AZA) or zebularine into the hippocampus immediately after contextual fear conditioning reverses the methylation and downregulation of PP1, a memory suppressor gene, impairing the formation of fear memory [25]. Interestingly, methylation levels in the hippocampus return to baseline in 24 hours, although the fear memory is maintained and is still dependent on the hippocampus at that time point [25]. This indicates that, in the hippocampus, DNA methylation might not be a mechanism of contextual fear memory maintenance, but a regulatory mechanism of transient gene expression. Furthermore, cocaine-induced conditioned place preference memory was recently found to require DNA methylation in the hippocampus [27•].

To assess the role of DNA methylation in the mature mouse brain, *Dnmt1* was deleted specifically in the precursors of postnatal excitatory neurons in the dorsal forebrain. The mutant mice showed abnormal development of the somatosensory barrel cortex and impaired thalamocortical long-term potentiation [28]. When *Dnmt1* is deleted under the control of the calmodulin-kinase IIa (CaMKIIa) promoter [29], neither the DNA methylation level of endogenous retroviral repeats nor neuronal survival is affected. Interestingly, double knockout of *Dnmt1* and *Dnmt3a* mediated by the CaMKIIa-cre system results in smaller cell sizes, impaired hippocampal LTP, enhanced LTD, and deficits in spatial and contextual fear memory formation, whereas single knockout lines of each gene display no such abnormalities [30]. In particular, *Stat1*, which is involved in neural plasticity and the interferon pathway, is upregulated at the mRNA level and decreases at the methylation level in neuronal cells. These results indicate that DNA methylation is important for synaptic plasticity and learning, probably by affecting the expression of plasticity-related genes.

Although some genes, including brain-derived neurotrophic factor (BDNF), are demethylated, with a corresponding increase in mRNA after learning, no study has established whether DNA demethylation is necessary for memory consolidation. This is because the demethylation mechanism remains unclear. One recent interesting report described how the activity-induced gene *Gadd45b* is required for activity-dependent upregulation of BDNF [31]. Further studies of the roles of DNA demethylation in the learning and memory paradigm would be of great interest.

A recent report has demonstrated that DNA methylation is required for the maintenance of memory [32•]. Contextual fear memory formation and its initial maintenance depend on the hippocampus, but it is generally believed that memory undergoes systems consolidation over approximately three weeks, so that the remote memory becomes dependent on the prefrontal cortex, including the anterior cingulate cortex (ACC), and independent of the hippocampus [33, 34]. To test whether memory maintenance requires DNA methylation, Miller et al. looked at the ACC region, rather than the hippocampus [32•]. After contextual fear conditioning, hypermethylation of the calcineurin (CaN) gene was maintained for at least 30 days; a correlative decrease of calcineurin mRNA and protein also persisted for at least a month. When a DNMT inhibitor was injected into the ACC 29 days after training, DNA methylation on CaN decreased, and 30-day memory was impaired. These findings suggest that the DNA methylation and demethylation processes are ongoing in the ACC region and that this dynamic balance is required for memory maintenance. Similarly, retrieval of conditioned place preference memory was recently found to depend on DNA methylation in the prelimbic cortex [27••].

The question is how the modification of DNA in the nucleus, which has a cell-wide effect, could be involved in maintaining a specific memory. A neuron has thousands of synapses connecting to a number of other neurons, and there is a high likelihood that it participates in multiple memories through different synapses [15]. It seems quite certain that modification of DNA in the nucleus itself cannot differentially affect each synapse without synapse-specific changes. Therefore, DNA methylation in itself may be insufficient for storing memory, and the exact mechanisms are still open for exploration.

DNA Methylation and Alzheimer's Disease

Alzheimer's disease (AD) is a slow, progressive dementia affecting primarily the cortex and hippocampus, characterized by the deposit of abnormal filaments of hyperphosphorylated Tau protein in neuronal cytoplasm, known as neurofibrillary tangles, accompanied by extracellular aggregates of large amyloid β protein ($A\beta$), known as senile plaques [35]. DNA methylation has been proposed as a mechanism that can lead to aberrant changes in gene expression during the progression of AD. In several AD brain studies, the loss of DNA methylation has important consequences. Furthermore, elevated levels of S-adenosylhomocysteine, a potential inhibitor of methyltransferases, have been detected in AD brains, and are inversely correlated with patient cognition, implicating epigenetic changes during the progression of AD [36]. DNA

hypomethylation in promoter CpGs of AD-associated genes, such as APP, presenilin 1 (PS1), and β -site APP-cleaving enzyme1 (BACE1), is also seen in AD brains [37], and results in abnormal upregulation of these genes, leading to excess accumulation of $A\beta$. In addition, an increased level of methylation at specific loci in the promoter region of certain genes, such as apolipoprotein E and methylenetetrahydrofolate reductase, is also found in the cortex of AD brains versus controls [17]. This suggests that the simultaneous occurrence of hyper/hypomethylation may contribute to the pathogenesis of AD.

As mentioned previously, aging is the major known risk factor for AD, and is associated with multiple epigenetic alterations [38]. Aging-related DNA methylation may represent one of the mechanisms by which environmental and dietary factors can promote AD [39]. Some cytosines in the promoter region of the APP gene are frequently methylated in patients who are under 70 years old, and these methylated cytosines are significantly demethylated in cases over the age of 70. These age-related modifications to DNA methylation alter APP expression and consequently can affect the progressive $A\beta$ deposition with aging in the brain [40].

Age-related epigenetic modifications in AD were further explored in two other studies. The first study presented straightforward evidence of epigenetic involvement in AD pathogenesis, by showing an age-specific epigenetic drift in late-onset AD. PSEN1 and APOE, which participate in $A\beta$ processing, and MTHFR and DNMT1, which are responsible for methylation homeostasis, demonstrated a significant interindividual epigenetic variability in the brain and lymphocytes of these patients, which could contribute to the predisposition to late-onset AD [17]. In the second study, longevity-related genes were investigated with respect to promoter methylation in peripheral blood in relation to gender, age, and AD status. Only one of the genes, HTERT, was shown to be hypermethylated in AD versus aged normal people. This gene, which is transcribed into the mRNA component of telomerase, and unlike the regular effect of methylation on gene expression, is activated by this epigenetic modification. Consequently, these results indicated higher telomerase activity, likely due to telomere and immune dysfunctions involved in AD pathogenesis [20].

DNA Methylation and Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder after AD, with an average age of onset of 60 years, and according to the Parkinson's Disease Foundation, it affects about 1 million people in the United States and more than 4 million people worldwide [41]. Pathologically, PD is characterized by the loss of dopaminergic neurons in the substantia nigra (SN) pars compacta,

and patients frequently have Lewy bodies, eosinophilic intracellular inclusions composed of amyloid-like fibers, and α -synuclein (SNCA) [42].

Thus far, there have been fewer epigenetic studies performed in PD versus AD subjects; however, there is some indication of impaired one-carbon metabolism in PD, as well as altered DNA methylation potential [43, 44]. Epigenetic analyses of PD brains reveal that the SNCA gene could be subjected to epigenetic regulation [45•, 46]. Several lines of evidence, including the identification of families with SNCA locus duplication and triplication and the association of both promoter and 3' UTR polymorphisms with sporadic forms, point to a gene-dosage effect for SNCA in PD pathogenesis [47]. The analysis of SNCA alleles in a PD patient heterozygous for the A53T mutation, the first mutation to be implicated in PD pathogenesis, revealed that SNCA showed monoallelic expression in this patient, with epigenetic silencing of the mutated allele due to histone modifications, but not DNA methylation, and upregulation of the wild-type allele resulting in higher mRNA levels than in matched control subjects [48]. Others have found that the methylation of human SNCA intron 1 decreases gene expression, whereas inhibition of DNA methylation activates SNCA expression. They also show that DNA methylation of SNCA intron 1 is reduced in several brain regions of sporadic PD patients, including in the substantia nigra, putamen, and cortex, pointing towards an epigenetic regulation of SNCA expression in PD [45•]. Another research group identified an SNCA CpG island in which the methylation status is altered, along with increased SNCA expression. Postmortem brain analysis revealed regional nonspecific methylation differences in this CpG region in the anterior cingulate and putamen among controls and PD patients; however, in the substantia nigra of PD individuals, the methylation of this region was significantly decreased [46]. Both findings are consistent with previous reports indicating increased SNCA mRNA levels in PD substantia nigra tissue. A recent paper suggested that α -synuclein sequesters DNMT1 from the nucleus [49•]. These researchers observed a reduction of nuclear DNMT1 levels in human postmortem brain samples from PD patients and from patients with dementia with Lewy bodies (DLBs), as well as in the brains of α -synuclein transgenic mice models. Furthermore, sequestration of DNMT1 in the cytoplasm resulted in global DNA hypomethylation in human and mouse brains, involving CpG islands upstream of SNCA and other genes. The nuclear DNMT1 levels were partially rescued by overexpression of DNMT1 in neuronal cell cultures and in α -synuclein transgenic mouse brains. Therefore, the authors suggested that the association of DNMT1 and α -synuclein might mediate aberrant subcellular localization of DNMT1, resulting in epigenetic modifications in the brain [49•].

Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a fatal, adult-onset disease, and most cases are sporadic, with no known inherited component. Although epidemiological studies implicate some environmental and acquired factors in its pathogenesis, previous genetic studies have been unable to identify bona fide genes responsible for the molecular mechanisms underlying sporadic ALS. DNA methylation in sporadic ALS has been examined in two candidate genes, SOD1 and VEGF [50]; in members of the metallothionein gene family [51]; and in the astroglial EAAT2 gene promoter [52]. None of these studies detected differences in methylation patterns in sporadic ALS cases compared with control cases. Another study using genome-wide analysis of brain DNA methylation in sporadic ALS, which involved chromatin immunoprecipitation followed by CpG microarray hybridization, revealed significant hypermethylation of genes involved in calcium dynamics, oxidative stress, and synapses [53]. Most recently, aberrant regulation of DNA methylation has been linked to the pathobiology of ALS [54•]. In human sporadic ALS, DNMT1 and DNMT3a proteins are upregulated in motor cortex and spinal cord motor neurons. Moreover, DNMT3a protein levels are increased in mitochondrial fractions, which have a particularly prominent pro-apoptotic function in motor neurons.

DNA Methylation and Trinucleotide Repeat Disorders

The expansion of trinucleotide repeats (TNRs) during gametogenesis leads to mutation or silencing of associated genes, with pathological consequences [55]. Although the causes of TNR instability are largely mysterious, DNA polymerase slippage, DNA repair mechanisms, transcription, and nucleosome positioning within the repeat are all candidates [56].

CAG repeat expansion is the underlying cause of at least 12 neurodegenerative diseases [57]. We do not know what effect DNA methylation might have on CAG repeats, which themselves are not targets for DNA methylation since they contain no CG dinucleotides. Nevertheless, three observations point to a connection between DNA methylation and CAG repeat stability. First, in bacteria, CAG repeats in plasmids methylated at CpG sites were mildly stabilized relative to repeats in unmethylated plasmids [58]. Second, treatment of mammalian cells with 5-aza-2'-deoxycytosine (5-aza-CdR), which leads to passive depletion of DNA methylation and destruction of Dnmt1 [59], or with hydralazine, which induces demethylation by inhibiting expression of Dnmt1 [59], both dramatically increased CAG repeat instability [60]. Finally, another study provided a mechanistic link between DNA methylation and repeat stability.

Vincent Dion et al. found that Dnmt1 deficiency in mice promoted intergenerational expansion of CAG repeats at the murine spinocerebellar ataxia type 1 (SCA1) locus. Importantly, Dnmt1^{+/-} SCA1 mice, unlike their Dnmt1^{+/+} SCA1 counterparts, closely reproduced the intergenerational instability patterns. In addition, this group also found aberrant DNA and histone methylation at sites within the CpG islands that abut the expanded repeat tract in Dnmt1-deficient mice [61].

Expansion of methylatable CGG repeats occurs at several sites that are either known or likely to be involved in human disease, and increased DNA methylation is thought to play a role in pathogenesis. The CGG repetitive element as well as an upstream CpG island in the FMR1 promoter is abnormally hypermethylated in most individuals affected with fragile X syndrome. Initially, this methylation was thought to be the primary mediator of epigenetic silencing, with secondary recruitment of histone deacetylases and methyltransferases driving formation of a heterochromatin region over the FMR1 locus. Indeed, this DNA methylation pattern is found to be associated with histone deacetylation and heterochromatin formation across the FMR1 gene in differentiated cells [62, 63].

A connection between DNA methylation and repeat instability has also been noted for the expanded CGG repeats in the 5' end of the FMR1 gene. In somatic tissues, methylated CGG repeat tracts at the FMR1 locus are found to be much more stable than unmethylated ones of comparable length [64], and when vectors carrying CGG repeats were introduced into primate cells, or into bacteria, methylated repeats proved to be more stable than unmethylated ones [58].

DNA Methylation and Demethylation

For decades, DNA methylation was believed to be a stable and irreversible modification that could not be further modified. Although Wilson and Jones implicated DNA methylation dynamics during aging in 1983, the demethylation enzymes had been chased since then. In 2009, the discovery of 5-hydroxymethylcytosine (5hmC) in mammals suggested DNA demethylation in the brain [65, 66]. Oxidation of 5mC to 5hmC by ten-eleven translocation (Tet) enzymes is proposed as an intermediate step in active DNA demethylation. Tet proteins can further oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), and 5caC can be excised further by a thymine-DNA glycosylase (TDG)-mediated base excision repair mechanism [67•, 68]. In mouse preimplant zygotes, an increase in 5hmC is associated with a decrease in 5mC, suggesting that 5mC is actively converted to 5hmC by Tet3, which is the only isoform of Tet protein expressed in zygotes [69]. Moreover, the 5hmC mark

associated with the paternal genome in zygotes is gradually lost in a DNA replication-dependent manner during preimplantation zygote development [70]. It remains to be seen whether such active and passive mechanisms of 5hmC-mediated DNA demethylation could have a specific function in the brain.

Conclusion

DNA methylation plays important roles in common human neurodegenerative diseases, which means modulating DNA methylation could be a potential therapeutic strategy for such diseases. Profiling DNA methylation at a genome-wide level could help us understand the basis of neurodegenerative disorders, including AD and PD. Furthermore, the integration of genome-wide DNA methylation maps with other epigenetic profiling should give us more insight into the function of DNA methylation in human diseases.

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