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Age-related epigenetic regulation in the brain and its role in neuronal diseases.

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Abstract

Accumulating evidence indicates many brain functions are mediated by epigenetic regulation of neural genes, and their dysregulations result in neuronal disorders. Experiences such as learning and recall, and physical exercise induce neuronal activation through epigenetic modifications and by changing noncoding RNA profiles. Animal models, brain samples from patients, and the development of diverse analytical methods have broadened our understanding of epigenetic regulation in the brain. Diverse and specific epigenetic changes are being suggested to correlate with neuronal development, learning and memory, aging and age-related neuronal diseases. Although there are some discrepancies among the results, a careful comparison of the data, including methods, regions and conditions examined, would clarify the problems confronted in understanding epigenetic regulation in the brain.

Keywords:

Epigenetics, neurodegenerative diseases, learning and memory

Introduction

The term ‘epigenetics’ was used initially in the context of ‘beyond genetics’ in describing a phenomenon in development that could not be explained by genetics; however, it is currently used to describe gene expression changes that occur without changes in the DNA sequence (1). We now understand many components underpinning the epigenetic regulatory system, and this knowledge is widely applied in cancer studies to understand its mechanism of regulation (2). The field of epigenetic research has been expanded, and for the last two decades there has been great progress in the field of neuroscience, as well (3). It is not surprising that epigenetic regulation constitutes a large portion of the regulatory mechanism for brain function, as *de novo* transcription is required for the consolidation and reconsolidation of memory, one of the main functions of the brain (4,5). Structural changes in neurons to establish synaptic plasticity and long-term memory storage also require mRNA transcription and protein synthesis upon receiving training stimuli (6). In regulating transcription and translation, chromatin, DNA, and RNA modifications, which constitute the majority of epigenetic regulation, play important roles. Indeed, activity-dependent neuronal histone modifications have been found to regulate the learning and memory process (7). Mutations in lysine acetyltransferases (KATs) result in memory impairments both in mice and humans, whereas reduction of histone deacetylases (HDACs) by HDAC inhibitors can enhance memory (8). The increasing relevance of epigenetics to neuroscience encompasses brain development, neuronal differentiation, cognition, neurodevelopmental disorders, psychiatric disorders, and neurodegenerative diseases (9,10). However, due to space limitations, our main focus in this review will be the aging-dependent epigenetic mechanisms in the brain and in neurodegenerative diseases. Because epigenetic regulations are most extensive at the level of histones, DNA modifications, and non-coding RNAs, we will focus on these types of modifications during age-related physiological changes in the brain.

1. Epigenetic regulation for normal brain function

For proper homeostasis and functioning of the adult brain, a balanced control of neural stem/progenitor cell self-renewal, differentiation, production of neurons and glia (known as “adult neurogenesis”), repair, learning, and memory, are important (11). These neuronal processes accompany dynamic patterns of gene expression and epigenetic control has emerged to have critical roles in generating specific gene expression patterns (Fig. 1). Generally, DNA methylation silences gene expression by preventing the formation of transcriptional machinery at the target sites. DNA methylation is catalyzed by DNA methyltransferases (DNMTs), and deletion of both *Dnmt1* and *Dnmt3a*, two major DNMTs, in mouse forebrain excitatory neurons caused deficits in synaptic plasticity, learning, and memory (12). There is additional direct evidence for neural activity-dependent DNA methylation changes. Transient activation of neuronal circuits that trigger adult neurogenesis induced significant hypomethylation at CpG sites in the regulatory regions of *brain-derived neurotrophic factor* (*Bdnf*) and *fibroblast growth factor-1* (*Fgf-1)*. The immediately induced *growth arrest and DNA damage inducible beta* (*Gadd45b),* which is required for adult hippocampal neurogenesis, may promote the demethylation of DNAs at these sites (13). In addition, extensive analysis of the neuronal DNA methylome in adult mouse confirmed that neuronal activity can modify the DNA methylation landscape: about 1.4% of 219,991 CpGs measured showed rapid active demethylation or *de novo* methylation in the hippocampus upon electro-convulsive treatment (14). These modified CpGs were significantly enriched in brain-specific genes related to neuronal plasticity. These results indicate that activity-induced DNA methylation changes are involved in neuronal function.

Chromatin can exist in either highly condensed or loosely packed states, which are associated with gene silencing or with active gene expression, respectively. More than 100 histone post-translational modifications have been identified so far, and the number continues to increase (15). Among them, methylation, acetylation, and phosphorylation are the most abundantly studied. Several types of neuronal activity can modulate histones: facilitation of motor neuron synapses or memory training can enhance histone H3 and H4 acetylation and phosphorylation (16), and potassium chloride-mediated neuronal depolarization leads to H2B acetylation (17). BDNF is a well-known neurotrophin and it can also trigger histone modification by regulating the dissociation of HDACs from chromatin; BDNF induces S-nitrosylation of HDAC2, which results in the release of HDAC2 from the chromatin, thus inducing the hyperacetylation of histones at neurotrophin-dependent gene promoters (18). A number of amino acids in the histone tail are targets of post-translational modification. Acetylation at histone H3 lysine 9 (H3K9), H3K14, H4K5, H4K8, and H4K12, methylation at H3K4, H3K9 and H3K36, and phosphorylation at H3S10 are examples of epigenetic modifications mediating synaptic plasticity, learning, and memory. Additional evidence of epigenetic regulation in learning-dependent synaptic plasticity is accumulating, and epigenetic modifications can also be detected in a highly interwoven network in the brain: co-occurrence of H3K14 acetylation with H3S10 phosphorylation and H3K36 trimethylation, and H3 acetylation with DNA methylation can be observed (19,20). The co-occurrence patterns of epigenetic modifications often result from crosstalk among epigenetic modifying enzymes that have differential preferences toward preexisting histone modification patterns at their binding sites. Noncoding RNAs such as microRNAs, snoRNA, siRNAs, piRNAs, and lncRNAs also function as

epigenetic regulators by regulating mRNA transcription and translation. Noncoding RNAs are more enriched in the brain than in other tissues, and among microRNAs, the most extensively studied noncoding RNAs, miR-7, miR-9, miR-23, miR-124, miR-125a-b, miR-128, miR-132, miR-137, miR-139, miR-184, and miR-195 have been reported as being highly enriched in brain (21,22). Neuronal function can be regulated by microRNAs, and miR-124 is a well-known example. When the level of miR-124 increases, it promotes neuronal differentiation by downregulating a repressor of the neuron-specific splicing regulator *polypyrimidine track binding protein 1* (*PTBP1*), a component of the RE-1 silencing transcription factor (REST)/neuron-restrictive silencing factor (NRSF) transcription repressor complex *synaptonemal complex protein 1* (*SCP1*), and the glial cell specification factor *SRY box 9* (*Sox9*) (22,23). The epigenetically regulated targets of microRNAs during adult neurogenesis include a number of transcription factors, indicating the importance of microRNA-mediated regulation in gene expression (24).

2. Epigenetic changes in brain during normal aging process

Aging is characterized by a progressive decrease in physiological capacity, reduced ability to respond adaptively to environmental stimuli, and increased susceptibility to diseases, and it is a complex process influenced by multiple factors including environmental conditions and genetic factors. Thus, a large degree of fluctuation can be detected among individuals, which makes it difficult to understand the mechanism underlying the aging process. Model organisms from the same genetic background and culture conditions would be beneficial in defining this process, and an investigation of specific regions of the body, such as the brain, could identify specific genes vulnerable to aging (25,26). In particular, with the recent development of next-generation sequencing technology, studies have been conducted to investigate gene expression changes in the human brain. These studies all indicate that an increase in epigenetic variation, in addition to changes in the transcriptome profile, has a strong correlation with the aging process (27). However, more recent studies revealed an interesting phenomenon: the gene expression profile and epigenetic modifications of brain regions become similar among aged individuals in much later stages of life (older than 75) despite genetic and lifestyle heterogeneity in humans, and this phenomenon appears to be conserved even across species (28).

Aging induces diverse epigenetic changes in the brain, but DNA methylation appears to play a prominent regulatory role. Global and gradual depletion of DNA methylation has been suggested initially to correlate with aging (29), but age-related increases in the levels of 5-methylcytosine (5-mC), 5-hydroxymehtylcytosine (5-hmC), and even DNA methyltransferase Dnmt3a have been reported in brain tissue (30). Indeed, methylation status seems to change bidirectionally with aging: whereas DNA regions with repetitive elements tend to become hypomethylated, many developmental genes are hypermethylated as a function of age (31). Recently, a large-scale age-related gene expression study was performed using human whole blood cells in 14,983 individuals of European ancestry (32). Of the 1,497 genes that were found to be differentially expressed with chronological age, 19 to 26% were also identified to change concordantly in the cerebellum and frontal cortex of brain tissue. Analysis of CpG sites in or near these age-associated genes revealed that changes in gene

expression with chronological age were correlated with changes in DNA methylation levels at the regulatory regions of these genes.

Age-associated memory impairment can also be found with altered hippocampal chromatin plasticity. Deregulation of histone H4K12 acetylation and failure in the expression of learning-induced genes has been found to be linked to age-related memory impairment (33). Age-related increase of HDAC2, which is known to decrease acetylation, has been reported in the mouse hippocampus. HDAC2 knockdown or HDAC inhibitors exhibit protective effects against age-related cognitive impairment (33). There seems to be an interplay between DNA methylation and histone modification, because co-localization and positive correlation of 5-mC with HDAC2 could be detected in the CA3 and CA1-2 regions of the hippocampus (34). Methylation on memory consolidation genes and its association with methyl-CpG binding proteins appear to recruit HDAC, which ultimately leads to chromatin remodeling and silencing of gene expression.

Along with changes in histone modifications and DNA methylation, noncoding RNAs appear to play some role in neuronal development. Although the role of noncoding RNAs in senescence is relatively poorly understood, microRNAs have been reported to contribute to loss of brain function during normal aging, based on studies in mice, rats, chimpanzees, and humans (35,36). Although a general decline in microRNA abundance has been observed in peripheral blood mononuclear cells of aging individuals (37), recent studies by Yin *et al.* (38), did not find an overall decrease in microRNA expression with advancing age when the rat whole brain was used. However, profiling of microRNAs according to specific regions of the brain identified differentially regulated microRNAs in aged brains. In general, microRNA levels remained relatively stable in the cortex but showed an overall decline in the cerebellum during aging. However, specific microRNAs such as miR-144 were consistently upregulated in the cortex and cerebellum of aging chimpanzees and humans (35), whereas the level of miR-186 gradually decreased in the mouse brain cortex during aging (39). Response elements for miR-144 and miR-186 are found in a proapoptotic gene *programmed cell death 4* (*PDCD4*) and a β-secretase encoding gene *beta site APP-cleaving enzyme 1* (*BACE1*), respectively. The upregulation of miR-144 may promote tumorigenesis, while the downregulation of miR-186 contributes to the development of Alzheimer's disease (AD) by the overproduction of amyloid beta (Aβ) protein. Therefore, diverse types of age-related pathogenesis in the brain may result from dysregulation of epigenetic regulations.

3. Epigenetic changes in aging-related neurodegenerative diseases

Alzheimer's disease (AD)

AD is characterized by progressive loss of memory and cognitive ability, aggregation including extracellular deposition of the Aβ peptide, and intracellular aggregation of phosphorylated tau protein. Early onset of AD is associated with genetic mutations in *amyloid beta precursor protein* (*APP*), *presenilin 1* (*PSEN1*) and *PSEN2*, while late onset AD is known to be associated with various factors such as lifestyle, diet, stroke, and environmental factors, etc. As most AD cases are late-onset, sporadic, and highly related to aging, it has been suggested that epigenetic regulations may play

important role in increasing the risk of AD development. Based on the observation of decreased levels of 5-mC and DNA methyltransferase in AD patients, it has been suggested that global DNA hypomethylation is associated with AD (40). Accordingly, the levels of essential methylation substrates such as the universal methyl donor S-adenosylmethionine (SAM), S-adenosylhomocystein (SAH), and folate, were also decreased in the brain of AD patients (41). However, other studies report no change or even a global gain in DNA methylation in AD patients (42). Similar contradicting results were also observed in the analysis of hydroxymethylation of DNA, which may represent the demethylation process, in AD patients. These variations in observed AD-associated epigenetic changes could result from differential changes in DNA methylation in different regions of the genome in AD patients, which may involve global as well as local dysregulation of DNA methylation (43). Although the occurrence of familial AD is much rare than sporadic AD, methylation changes have been investigated in genes that are known to be associated with familial AD. *APP*, *PSEN1,* and *microtubule associated protein tau* (*MAPT*)*,* which encode precursors of Aβ, γ-secretase, and tau, respectively, have been predominantly used in the investigation of epigenetic alterations and in generating AD animal models. Some studies reported hypomethylation of the *APP* promoter in AD patients and in normal aging (44); however, Barrachina and Ferrer (45) reported no alteration in DNA methylation in the *APP*, *PSEN1,* and *MAPT* promoters when the frontal cortex and hippocampal area of various stage AD patients were examined. Wang et al. (46), also reported there was no alteration in DNA methylation in the promoters of *APP*, *PSEN1*, *BACE1*, and *DNMT1*. However, studies on *sorbin and SH3 domain containing 3* (*SORBS3*), *ankyrin 1* (*ANK1*)*,* and *neutral endopeptidase* (*NEP*)*,* which encodes the Aβ clearing enzyme neprilysin, showed consistent hypermethylation in both AD animal models and AD patients (47,48). Inter-individual variation seems to occur in AD patients, rendering it somewhat challenging to judge the role of DNA methylation in the pathogenesis of AD. Therefore, the importance of DNA methylation in AD pathology requires further investigation of specific loci or their regulatory relationship with the development of AD.

In addition to the involvement of DNA methylation in the development of AD, histone deacetylation could partially explain AD etiology. The impairment in learning and memory observed in the AD mouse model was reversible and could be improved by treatment with HDAC inhibitors (9,49,50). An actual increase in HDAC2 level, and a decrease in histone acetylation, specifically at promoters of genes found to be involved in learning, memory, and synaptic plasticity, were detected in late-stage AD mouse models and AD patients (9,49,50). Knockdown of HDAC2 abolished neurodegeneration-associated memory impairment (50). Enrichment of hypoacetylation and HDAC2 was found to be negatively correlated with RNA polymerase binding and mRNA expression at the genes required for memory and learning. However, AD-related histone hypoacetylation appears to be enriched at specific histones: H4 acetylation was generally decreased in transgenic *APP/PS1* mice, whereas global H3 acetylation remained unchanged. H3 acetylation even increased at specific promoters such as *BACE1* upon the administration of Aβ in both AD mice and AD patients (51).

Contrary to the general decrease in histone acetylation, histone methylation is reported to be upregulated in AD patients (52). Upregulation of phosphorylation in H3 was also observed in the

hippocampus of AD patients in some studies but could not be confirmed by others (52,53). Brain region-, gene-, or disease stage-specificity might affect epigenetic modification patterns. Therefore, further investigation with comparable samples and genetic loci would reveal histone modification changes that are associated with AD.

MicroRNAs also regulate gene expression epigenetically, and the potential role of microRNA as a biomarker has been studied in AD. Upregulation of miR-34a and miR-181b, and downregulation of miR-9, miR-29a/b, miR-137, and miR-181c were observed in blood mononuclear cells from AD patients (54). Serum can also serve as a source of cell-free microRNA; miR-23a, miR-26b and miR-125b were found to be downregulated in serum (55). Changes in the microRNA profile were also observed in cerebrospinal fluid and the plasma exosome. In a review by Kumar and Reddy (56), which summarized 12 studies covering diverse biofluids from 1268 participants, 100 microRNAs were identified as differentially expressed in AD patients; 54 microRNAs were upregulated and 46 microRNAs were downregulated. Although inconsistencies remain in microRNA profiling data between different studies, some microRNAs, such as miR-34a, which was overexpressed in blood cells of AD but down regulated in neurotoxic Aβ overexpressing mouse model, provide insight into the triggering mechanism of cortical neuronal apoptosis in AD patients (57).

Parkinson's Disease (PD)

PD is the second most common neurodegenerative disorder that lacks an effective treatment modality. It is characterized by the loss of dopaminergic neurons in the midbrain substantia nigra, a critical region for the initiation of motor events. The occurrence of cytoplasmic Lewy bodies in the midbrain and cortex, where α-synuclein is localized, and the malfunctioning of neurotransmitter systems, are linked to cognitive impairments and other PD symptoms. A genome-wide association study of familial forms of PD, although rarer than sporadic forms, identified *synuclein α* (*SNCA*) and *leucine-rich repeat kinase 2* (*LRRK2*) as the main risk factors for PD (58). Most PD is sporadic, and emerging evidence identifies aberrant epigenetic modification as a possible mediator of environmental inputs (toxin as a major factor) that drive PD development. However, compared to AD cases, relatively little is known regarding epigenetic alterations in PD, with only a limited number of studies based on *in vitro* or animal PD models. So far, α-Synuclein is the best known target of abnormal epigenetic regulation in PD.

α-Synuclein is a natively soluble protein, but mutant protein produced as a result of missense (A53T, A30P, E46, H50Q, and G51D) mutations, or its overexpression due to *SNCA* gene amplification, often results in abnormal aggregation of α-Synuclein in Lewy bodies in familial PD patients (59). Hundreds of SNPs of *SNCA* are associated with sporadic PD. In addition, hypomethylation at the promoter and introns of *SNCA* appeared to increase *SNCA* expression in PD patients (60,61). Overexpressed α-Synuclein can also sequester DNMT1 to the cytoplasm; this protein would otherwise be mainly located in the nuclei of neurons in the healthy brain. Reduction in nuclear DNMT1 can explain the global DNA hypomethylation observed in PD model mice and patients (62).

MAPT, a major risk factor for AD, was also found to be a risk factor for sporadic PD. Tau protein is

associated with microtubules and helps stabilize the axonal cytoskeleton. The methylation level of *MAPT* varies along different regions of the normal brain, but the methylation levels are consistently in negative correlation with *MAPT* expression levels, indicating their regulatory role in *MAPT* expression. Indeed, when the methylation level of *MATP* was examined in the leukocytes of PD patients, the age at PD onset was positively correlated with the *MAPT* methylation level (63). Therefore, *MAPT* methylation appears to have a protective function against PD. Consistently, *MAPT* methylation was significantly higher in females compared to males, correlating with the lower incidence of PD in women. Intriguingly, *MAPT* methylation levels are different in different regions of brain, and these regional differences correlate with the pathological severity of PD: the cerebellum was more effective in inducing compensatory *MAPT* hypermethylation against PD progress than the putamen, thus more severe pathological PD development was obvious in the putamen area (63).

Histone modifications are also involved in PD pathogenesis. Nuclear accumulation of α-Synuclein appears to play an important role in PD development: a significant amount of α-Synuclein is found in PD animal models and patients, and injection of herbicide induces the translocation of α-Synuclein into the nucleus of nigral neurons (64). The nuclear function of α-Synuclein appears in the regulation of histone modification. α-Synuclein was shown to bind directly to histone proteins, which resulted in the reduction of H3 acetylation. In addition, the toxicity of α-Synuclein in PD models was rescued by the administration of HDAC inhibitors (65). In contrast to the pathological correlation of histone deacetylation in PD, the detrimental effect of histone acetylation in dopaminergic neuronal cells has also been reported (66). Pesticide-induced neuronal loss comprises a large fraction of PD development; thus the effect of Dieldrin, a widely used insecticide originally developed as an alternative to DDT, on histone modification in dopaminergic neuronal degeneration was examined. Intriguingly, Dieldrin was able to induce H3 and H4 acetylation in a time-dependent manner along with reduced HDAC activity accordingly (66). In addition, these Dieldrin-induced effects were attenuated effectively by KAT inhibitors. Therefore, further experiments appear to be required to clarify the functional association of histone acetylation status with PD.

In addition to epigenetic regulation via histone modification and DNA methylation, key risk factor genes for PD, such as *SNCA*, are also targeted by microRNAs aberrantly in PD. miR-7 and miR-153 are highly expressed in the brain and can synergistically repress *SNCA* translation (67). Overexpression of *SNCA* has been shown to impair dopaminergic neuron function in PD, and a decrease in miR-7 expression was observed in PD model mice (68). Many microRNAs involved in neuronal function and survival were also shown to be dysregulated in PD. miR-124 and miR-133b play important roles in adult neurogenesis and dopaminergic neuronal functions, and they are significantly deficient in the brains of PD patients or mouse models (69,70). On the other hand, miR-132 which targets a key regulator of neurite outgrowth, *nuclear receptor related 1* (*Nurr1*), is significantly increased in affected PD model rats and suppresses NURR1 expression (71). MicroRNA profiles from blood of PD patients revealed downregulation of miR-1, miR-22p, and miR-29a, along with upregulation of miR-125a-3p, miR-137, miRmiR-181c, miR-193a-5p, miR-196b, miR-331-5p, and miR-454 (72,73). In addition, extensive investigation of microRNA alterations in the prefrontal cortex region of postmortem patients identified 125 microRNAs that are differentially expressed in PD.

Although only a few were matched to microRNAs known to be dysregulated in PD, the abnormal expression of 36 microRNAs appeared to correlate with dementia in PD, and miR-10b-5p expression level was negatively correlated with PD onset (74), indicating their functional relevance in PD development. Therefore, a more thorough investigation into these microRNAs would help reveal epigenetic regulatory mechanisms mediated by microRNAs to influence PD pathogenesis.

Huntington's Disease (HD)

HD is primarily a genetic disease initiated by the expansion of polyglutamine repeats in the *huntingtin* (*HTT*) coding region. Chorea, cognitive deterioration, and psychiatric disturbances are symptoms of HD due to impairment of the basal ganglia and cerebral cortex. Although *HTT* has been identified as the main gene causing HD, there is a variability in disease onset and severity, indicating that environmental factors and the epigenetic alterations caused by them are additional factors that influence disease symptoms.

Several lines of evidence suggest the involvement of epigenetic modulation by *HTT* in HD patients. The polyglutamine-containing domain of HTT was shown to bind histone acetyltransferase, CREB binding protein (CBP) and P300/CBP-Associated Factor (P/CAF), and accumulation of CBP in the intracellular inclusion body was often found in the brains of HD model animals and patients (75,76). In addition, overexpression of mutant HTT induced the global hypoacetylation of histone H3 and H4. Epigenetic alterations induced by HTT appear to play important role in HD pathogenesis, as evidenced by the fact that HDAC inhibitor treatment arrested the progress of neuronal degeneration, in addition to reversing of aberrant histone acetylation pattern[76]. These lines of evidence suggest that HTT with expanded polyglutamine acts through epigenetic modifications in the affected region to downregulate gene expression. However, in other studies, global hypoacetylation of histone could not be found, although HDAC inhibitor treatment had a similar beneficial effect on disease symptoms (77). Instead, hypoacetylated H3 was found at the *Drd2* gene locus encoding the dopamine D2 receptor, specifically in the striatum, but not in other regions of brain, such as the cortex, hippocampus and cerebellum. These results indicate that gene-specific epigenetic regulation is more important than global alterations in histone modifications, but further experiments are needed to clarify this discrepancy. H2A histone family member Y (H2AFY) is a histone variant that is known to modulate transcription factor binding and transcription repression, and overexpression of H2AFY was found in the blood and frontal cortex from a number of HD patients. Because H2AFY levels were in correlation with therapeutic effect of HDAC inhibitors against HD in clinical trials, chromatin regulation appears to be an important pathogenic mechanism used in HD development (78). Histone methylation is also involved in the pathogenesis of HD. ERG-associated protein with SET domain (ESET), a histone H3K9 methyltransferase, mediates gene silencing and its expression is markedly increased in HD animals and patients (79). The downregulation of ESET by a pharmacological method not only suppressed the hypertrimethylation of H3K9, but also significantly ameliorated HD symptoms. Therefore, modifications in histone acetylation and methylation appear to play a significant role in the etiology of HD.

Although DNA methylation is often associated with disease, alterations in DNA methylation appear to

be minimal in HD. Only recently, Ng *et al.* (80) raised the possibility of DNA methylation as one of the epigenetic modifications involved in HD, by showing that regions with low CpG content, where methylation changes occur in response to neuronal activity, undergo methylation changes with the overexpression of polyglutamine expanded HTT. When DNA methylation profiling in HD patients was examined by De Souza *et al.* (81), some evidence of HD-associated DNA methylation was found in the cortex region alone.

Changes in the expression profiles of microRNAs are also detected in HD. Of the 752 human mature microRNAs, 168 microRNAs were found to be altered in circulating plasma samples from symptomatic patients (82). Many other research groups also identified different altered microRNA profiles in HD models or patients. Some of these alterations are due to changes in the interaction between the mutant form of HTT and its partners to which wild-type HTT could bind under normal conditions. HTT interacts with REST and maintains it in the cytoplasm in normal conditions, but its association is disrupted in HD. A group of microRNAs can be downregulated by REST, which has a repressor activity for many neuronal coding and non-coding genes. REST which becomes translocated into the nucleus represses the transcription of BDNF and several microRNAs, including miR-9, miR-29a, miR-29b, miR-124a, miR-132, and miR-330 (83,84). Ago2, a component of the RISC complex, is another protein known to interact with HTT, and its dissociation from HTT leads to an increase in microRNA-mediated gene silencing in HD (85). Thus, the maintenance of selected microRNA expression could be a default protective function in the brain, but its deregulation may contribute to neurodegenerative disease. The three major epigenetic modifications mentioned above, to control gene expression in the brain during aging and the three neurodegenerative processes are summarized in Fig. 2.

4. Epigenetic modulation of the blood milieu and its reflection of neuronal function

As neurodegenerative diseases affect neuronal cells in the brain, examination of gene expression changes is not easily achieved prior to mortality. Detection of microRNAs in circulatory biofluids such as blood, urine, and saliva, and their stability in extracellular fluids (86) opened up the possibility of, and provided accessibility, for the detection of gene expression changes occurring inside tissues. MicroRNAs encapsulated with exosomes can even cross the blood-brain barrier and be secreted in the cerebrospinal fluid or blood (87) and reflect the microRNA profile inside the brain. The identification of biomarkers in blood or blood components facilitates less invasive procedures and would allow the assessment of neurodegenerative disease at the early stages. In addition to microRNA profiles, many studies searching for epigenetic modifications in the brain were performed using peripheral blood mononuclear cells, and disease-associated epigenetic alterations were successfully identified. Peters *et al.* (32) examined whole-blood gene expression in 14,983 individuals and identified 1,497 genes that are differentially expressed with chronological age. Those genes whose expression level changed in accordance with age were enriched with CpG methylation sites at locations that are tied to gene expression regulation, such as promoters, enhancers, and insulators. They even found that transcriptomic age and epigenetic age were positively correlated. Indeed, many epigenetic alterations associated with AD, PD, and HD were also identified using blood samples. The

use of blood samples renders the study of aging and neurodegenerative diseases applicable beyond animal models and extended to humans.

5. Perspectives for drug development

Neurodegenerative diseases are among the most feared disorders especially in aging or aged societies. When PD patients are diagnosed based on typical motor symptoms, about 50% of the dopaminergic neurons already have been irreversibly lost, and the same applies for other neurodegenerative diseases. Diagnosis before the loss of intellectual or cognitive impairment would be beneficial; therefore, the development of specific diagnostic tests or effective treatments is in demand. Although research has focused on identifying biomarkers that can detect diseases early in their pathogenesis, no biomarkers and no drugs that can completely delay or prevent the progression of AD, PD and HD are developed yet. Because histone acetylation affects the regulation of gene transcription, acetylation or deacetylation at specific histone sites can represent biomarkers for the disease state. HDAC inhibitors showed great potential not only in treating cognitive impairment in neurodegenerative disorders, but also in enhancing cognitive function in healthy people (8). Therefore, the use of inhibitors for HDAC or DNMT could be considered for the treatment of neurodegenerative diseases. MicroRNAs are secreted into the blood, and exosome-associated microRNAs are known to be stable. Several upregulated or downregulated microRNAs have been identified in all the three neurodegenerative diseases mentioned in this review. Therefore, manifestation of disease can be easily measured based on microRNAs, and they could also be a promising components in developing drugs for neurodegenerative diseases. Because epigenetic regulations are reversible, drugs targeted to epigenetic alterations would be easier to access and they can also be used to measure the effectiveness of the treatment. Diseases would be manageable if the onset of diseases could be diagnosed at the very early stages. Therefore, more efforts have to be made to select biomarkers to detect disease onset as soon as possible so that they can be utilized for preventive treatment.

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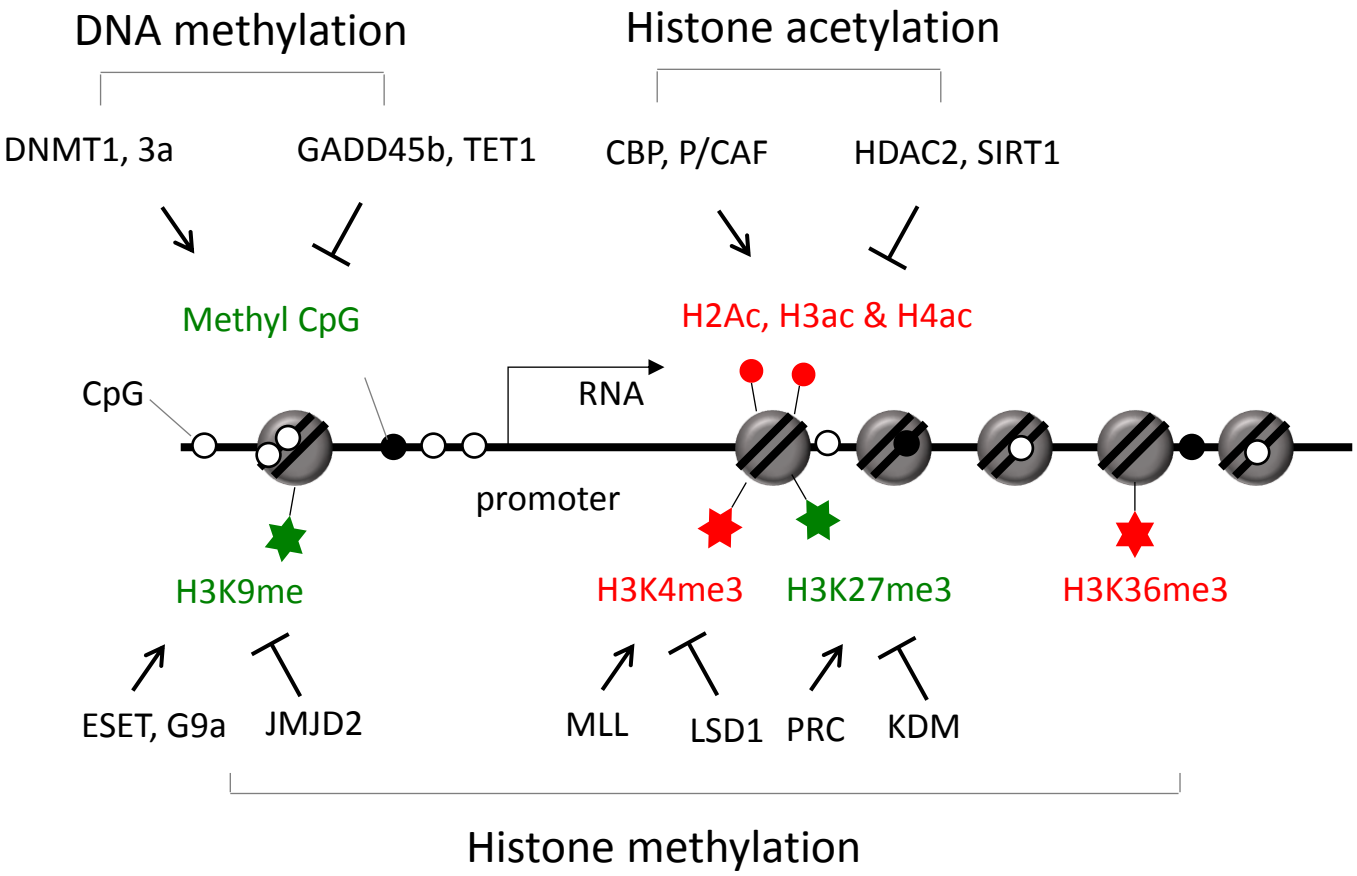
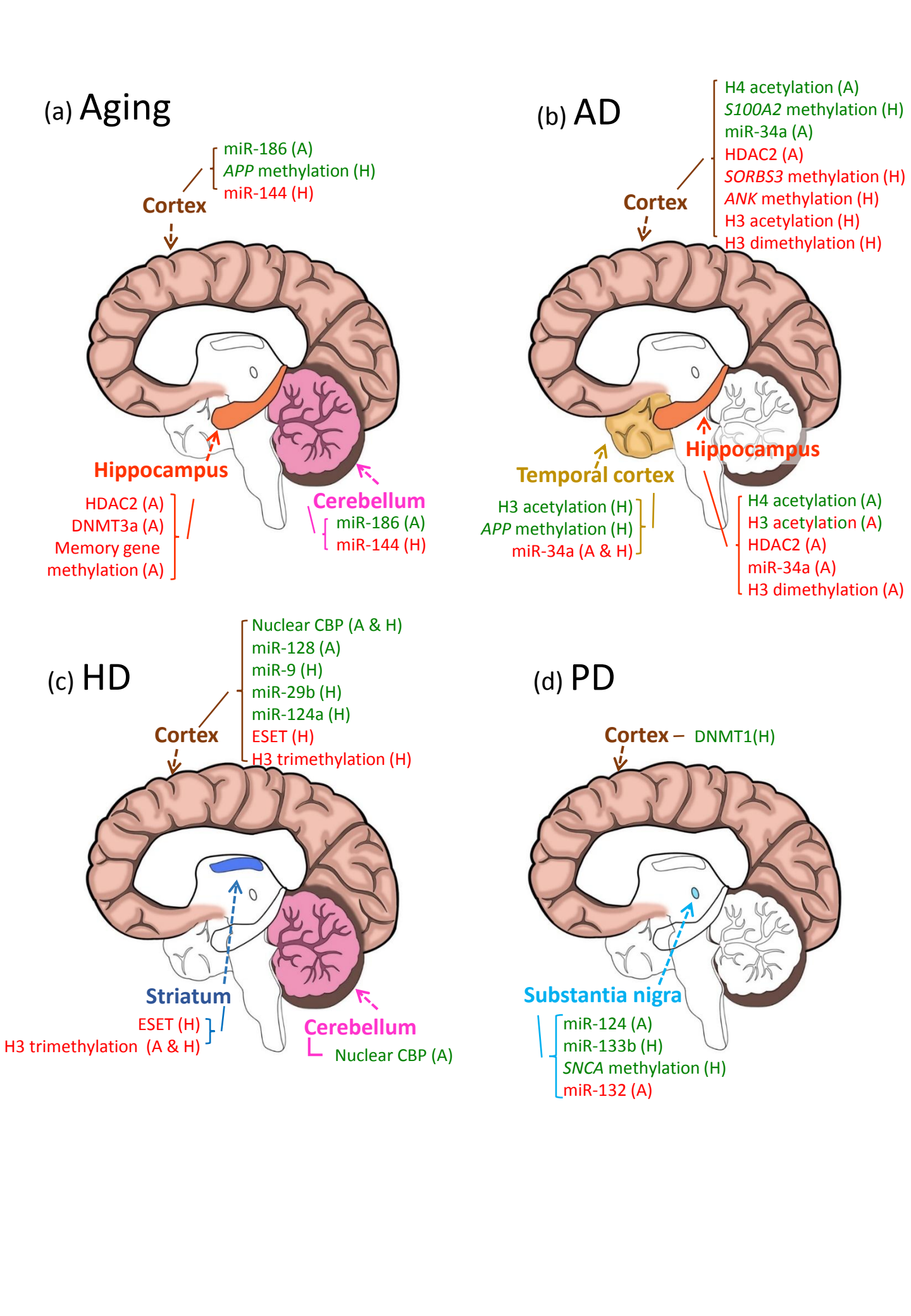
Figure legend

Figure 1. Epigenetic modifications and their roles in gene expression. Major epigenetic modifications involved in neuronal gene expression around promoter regions are shown. Epigenetic markers stimulating transcription are shown in red, while those of inhibitory ones are shown in green. Enzymes involved in the generation (↑) or removal (т) of the epigenetic marks are indicated.

Figure 2. Epigenetic modifications observed in specific brain areas of aged or neurodegenerative disease-affected animal models or patients. Modifications in DNA and histones, and changes in microRNA abundance associated with aging (a) or the three neurodegenerative diseases, AD (b), HD (c), and PD (d) are shown. Brain regions studied in each disease model are marked with specific colors: cerebral cortex (brown), temporal cortex (golden rod), hippocampus (orange), cerebellum (hot pink), striatum (royal blue), and substantia nigra (sky blue). Up (red) and down (green) regulated epigenetic markers in animal models (A) or in human patients (H) were mapped to the region where they were examined. H3 acetylation in the hippocampus of AD is written in both colors to indicate conflicting results reported. Results that did not show any change are not mentioned in this figure, but are mentioned in the main text.


DNA methylation
Histone acetylation
DNMT1, 3a
GADD45b, TET1
CBP, P/CAF
HDAC2, SIRT1
Methyl CpG
H2Ac, H3ac & H4ac
CpG
RNA
promoter
H3K9me
H3K4me3
H3K27me3
H3K36me3
ESET, G9a
JMJD2
MLL
LSD1
PRC
KDM
Histone methylation


(a) Aging
Cortex
miR-186 (A)
APP methylation (H)
miR-144 (H)
Hippocampus
HDAC2 (A)
DNMT3a (A)
Memory gene methylation (A)
Cerebellum
miR-186 (A)
miR-144 (H)
(b) AD
Cortex
H4 acetylation (A)
S100A2 methylation (H)
miR-34a (A)
HDAC2 (A)
SORBS3 methylation (H)
ANK methylation (H)
H3 acetylation (H)
H3 dimethylation (H)
Hippocampus
Temporal cortex
H3 acetylation (H)
APP methylation (H)
miR-34a (A & H)
H4 acetylation (A)
H3 acetylation (A)
HDAC2 (A)
miR-34a (A)
H3 dimethylation (A)
(c) HD
Cortex
Nuclear CBP (A & H)
miR-128 (A)
miR-9 (H)
miR-29b (H)
miR-124a (H)
ESET (H)
H3 trimethylation (H)
Striatum
ESET (H)
H3 trimethylation (A & H)
Cerebellum
Nuclear CBP (A)
(d) PD
Cortex – DNMT1(H)
Substantia nigra
miR-124 (A)
miR-133b (H)
SNCA methylation (H)
miR-132 (A)