
The Therapeutic Potential of Epigenetic Modifications in Alzheimer's Disease

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Abstract: Alzheimer's disease is characterized by the formation and deposit of abnormal peptides such as amyloid plaques and neurofibrillary tangles in the brain. Therapeutic strategies aimed at preventing the formation of such deposits have not been successful. Currently, there are no effective treatments for the disease. Since numerous epigenetic changes have been detected in Alzheimer's disease, treatments aimed at reversing these changes by intervening in DNA methylation, histone acetylation, and microRNA expression may constitute promising lines of research in the future. This chapter provides an overview of the epigenetic changes and the potential epigenetic therapies in Alzheimer's disease.

Keywords: Alzheimer's disease; DNA methyltransferase; epigenetic changes; histone acetylation; noncoding RNA

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INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia. It usually occurs in people over 60 years of age and presents with progressive loss of memory and cognitive capacity, language disorders, inability to translate ideas into actions (ideomotor apraxia), impaired planning and judgment, apathy, depression, and, in later stages, psychosis with paranoid delusions. AD is characterized by the presence of abnormal peptide deposits in the brain. The most characteristic lesions are neuritic extracellular plaques of the amyloid β ($A\beta$) peptide, which consists of 33–40 amino acids derived from the proteolysis of the transmembrane protein amyloid precursor protein or APP. These neuritic plaques contain a large number of distorted neuronal expansions, known as dystrophic neurites. Activated microglial cells are observed at their center.

Some evidence suggests that amyloid deposits may be neurotoxic and may cause neuronal dysfunction and even neuronal death. In the normal brain, APP is fragmented into functional segments by the α -, β -, and γ -secretase enzymes. Occasionally, there is an increase in β - and γ -secretase relative to α -secretase, leading to the accumulation of peptides with 40 and 42 amino acids, known as amyloid β_{40} ($A\beta_{40}$) and amyloid β_{42} ($A\beta_{42}$). The $A\beta_{42}$ peptide appears to have greater neurotoxic properties. $A\beta$ oligomers, small aggregates of 2–12 peptides, appear to be especially toxic (1). Diffuse plaques, another kind of plaque, lack a dense center of amyloid and dystrophic neurites. Unlike neuritic plaques, they are not associated with either neuronal destruction or cognitive dysfunction (2).

Neurofibrillary tangles are twisted aggregates of abnormal intraneuronal fibers that have a helical structure, typically paired helical filaments, made up of hyperphosphorylated tau protein. The tau protein is involved in stabilizing microtubules, maintaining the integrity of the cytoskeleton and axoplasmic transport. Neurofibrillary tangles are found in the areas of association of the neocortex, hippocampus, limbic system, substantia nigra, raphe nuclei, locus coeruleus, and the nucleus basalis of Meynert (3). In AD, there is also a significant synaptic loss in certain areas of the neocortex and in the hippocampus, as well as the disappearance of dendritic spines.

AD occurs frequently in humans over 65 years of age. In those aged over 85, the prevalence of AD ranges between 20 and 40% in developed countries (3). In 2010, the prevalence of AD in China among people aged between 85 and 89 was 18.54% (4). In 2006, the number of patients with AD was 26.6 million worldwide. In the United States, the prevalence of AD in people over 70 years of age is 9.51%, and the incidence is 14.26 per 1000 person-years (4). However, AD is not an inevitable consequence of old age. A relatively high number of elderly people show neither cognitive decline nor lesions typical of AD with age. The causes of AD are still not well understood. In a small percentage of cases, AD can be attributed to mutations in genes located on chromosomes 1, 14, and 21. These cases are usually of early onset and are transmitted in an autosomal dominant manner. Most AD cases appear to be caused by the interaction of multiple genetic and environmental factors that are not yet well understood (1).

Areas of association, phylogenetically more recent areas of the human brain, have simpler organization and greater immaturity in the adult than phylogenetically older primary areas. Thus, in the neurons belonging to the areas of

association, myelination occurs very slowly and many neurons belonging to these areas remain incompletely myelinated—that is, immature even in adulthood. Poorly myelinated neurons are chronically subjected to high-energy turnover, which makes them more vulnerable to the influence of oxidative stress. There are, therefore, extensive cortical areas in the human brain that remain structurally immature throughout life (5).

Various studies show that there has been an increase in the expression of genes related to aerobic metabolism and, more importantly, to synaptic plasticity and activity in the human cerebral cortex relative to nonhuman primate brains (6, 7). Learning and memory take place through the formation of new synapses and remodeling of preexisting synapses, suggesting that the increase in the expression of genes related to these functions has occurred in humans, as well as the selection of genes that encode proteins capable of increasing neuroplasticity. The apolipoproteins E (ApoEs) are proteins of 299 amino acids synthesized in the astrocytes of the central nervous system. They influence the transport and reuptake of cholesterol and the stabilization of the neuronal cytoskeleton, contributing to the preservation of synaptic integrity (3).

Humans present a polymorphism for ApoE with three alleles: $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. Possession of allele $\epsilon 4$ of the ApoE is the most important risk factor for the development of AD, after advanced age (3, 6). The most common allele is $\epsilon 3$, whose frequency is 60% or higher in all the populations studied. Possession of the $\epsilon 4$ allele is associated with lower neuroplasticity and lower synaptic repair capacity, and seems to promote the relatively early appearance of brain deposits of neurotoxins, such as A β and neurofibrillary tangles, whose excess is associated to AD.

There has been an increase in the expression of genes associated with neuronal plasticity in the human cerebral cortex, resulting in an increased capacity for learning and memory, neurotransmission, axonal transport, aerobic metabolism, and neuroprotection, all of which are adaptations that promote high neuronal activity over a long life (6, 7). The human brain has a high need for glucose, especially during its development. A child's brain consumes more than 40% of the body's basal energy requirements. Most of the glucose is oxidized to produce ATP. This process is upregulated in anaerobic conditions. Aerobic glycolysis is increased during childhood and is synonymous with high rates of synaptic formation and the growth and remodeling of synapses. Aerobic glycolysis is associated with the persistence of genetic expression associated with childhood, especially genes active in youth, and especially those related to the growth and formation of new synapses (transcriptional neoteny) (8). In the adult human brain, aerobic glycolysis is especially elevated in cortical areas related to cognitive functions that have undergone significant modifications during the evolution of the human species, such as the dorsolateral prefrontal cortex and the brain's default mode network (BDMN), related to the coordination of activity between different cortical areas and to planning and autobiographical memory capacities, which allow "mental travel in time," remembering and planning.

The brain regions where most of the A β deposits are located almost exactly match the regions that make up the BDMN, which suggests that the high synaptic turnover that occurs in these areas predisposes the formation of abnormal peptide deposits characteristic of AD (8). Multiple studies show that AD appears to be associated with oxidative stress (9). Increased aerobic metabolism in neurons that retain juvenile characteristics in adulthood could subject these neurons to high

oxidative stress. It appears that oxidative stress could induce epigenetic changes, reducing the expression of certain genes, including those related to synaptic plasticity.

EPIGENETIC CHANGES AND AD

Epigenetic changes modulate the expression of certain genes without altering the DNA sequence. Epigenetic factors include DNA methylation, histone modification, and the regulation and modification of chromatin by noncoding RNA (ncRNA) (10). DNA methylation modifies cytosine residues by adding methyl groups in regions rich in cytosine-guanine. DNA methyltransferases, such as DNA methyltransferase 1, DNA methyltransferase 2, DNA methyltransferase 3, and DNA methyltransferase 3,6, are involved in the process.

Some cytosines, for example those located in the promotor region of the APP gene, have been found to exhibit methylation with age, which can lead to the formation of A β deposits. Methylation of the gene coding for the microtubule-associated protein tau (MAPT) can lead to the suppression of MAPT, which can end up affecting the level of the tau protein. Further, methylation in the promotor region of the brain-derived neurotrophic factor (BDNF) gene seems to play a significant role in the appearance of mild cognitive impairment (11).

Methylation of certain loci of specific genes, such as sortilin-related receptor 1 (*SORL1*), ATP binding cassette subfamily A member 7 (*ABCA7*), HLA class II histocompatibility antigen DRB5 beta chain (*HLADRB5*), solute carrier family 24 member 4 (*SLC24A4*), and box-dependent-interacting protein 1 (*BINI*), has also been associated with AD (11). The protein encoded by *SORL1* controls the production of A β , so the methylation of the DNA that codifies this protein could lead to increased levels of A β .

Reelin is an extracellular matrix glycoprotein that, together with ApoE, shares the LRP and VLDLR/ApoER2 membrane receptors. During embryonic development, this protein regulates neuronal migration and, in the adult brain, intervenes in synaptic plasticity, interacting with ApoE. Binding of this protein to the membrane receptors activates a series of proteins that constitute the signaling pathway of reelin, inducing changes in the neuronal cytoskeleton. In transgenic mice that have lesions similar to those of AD, reelin counteracts early-phase synaptic dysfunction induced by the A β peptide (12).

In vitro studies have shown that oxidative stress alters the activation of proteins that are part of the reelin signaling pathway, resulting in the hyperphosphorylation of tau, which precedes the formation of neurofibrillary tangles in AD (13). Depletion of brain reelin has been detected in patients with AD prior to the formation of A β deposits (14). Thus, there seems to be a relationship between dysfunction of the reelin signaling pathway and AD.

Some reelin genotypes have been found to be associated with mild cognitive impairment and AD. The reelin single nucleotide polymorphism 2299356 (RELN-rs2299356) guanine-guanine genotype is associated with cognitive decline, while the adenine-adenine genotype triples the risk of developing AD. The reelin single nucleotide polymorphism 528528 (RELN-rs528528) cytosine-cytosine genotype, on the other hand, reduces the probability of mild cognitive

impairment by two thirds. These variations are located in the promoter region of the gene, which seems to play a regulatory role in its expression (15).

Reelin is involved in neuroplasticity, a process that has increased during the evolution of the human brain. Oxidative stress and probably other factors seem to induce epigenetic changes capable of reducing the expression of genes involved in synaptic plasticity. In some cases, such as that of the carriers of certain reelin genotypes, dysfunction of the proteins involved in the reelin signaling pathway caused by a reduction in reelin related to epigenetic changes could increase the probability of having the abnormal peptide deposits that characterize AD (15). It cannot be ruled out that certain alleles are more vulnerable than others to oxidative stress, toxins, inflammation, and other factors possibly related to AD.

Histones are proteins that serve as structural support for the DNA of the cell nucleus. Nuclear DNA associates with histones to form nucleosomes. The distribution and compaction of nucleosomes determines the structure of the chromatin and the accessibility of DNA to factors involved in the transcriptional machinery. Histones are also susceptible to epigenetic changes that can cause an increase or decrease in genetic expression. Nucleosomes are mainly regulated by posttranslational modifications that occur in the N-terminal region of histones.

Both methylation and acetylation can occur in histones through the antagonistic action of histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases, and histone demethylases. The acetylation of histones results in increased genetic activity by reducing the compactness of the nucleosomes and thus facilitating access of the transcriptional machinery to DNA. During senescence, mammalian cell cultures develop highly condensed regions of chromatin that may be associated with transcriptional decline (16).

Various HDAC inhibitors, like valproic acid and sodium butyrate, seem to improve memory in animal models and some neurodegenerative diseases like Parkinson's and even AD (16). Among the epigenetic changes described are the alteration of expression of the ncRNA. ncRNA is involved in genetic silencing as well as other functions, including the regulation of the activity of retrotransposons, genes that are capable of moving from one location in the genome to another. Short fragments of ncRNA, such as microRNA (miRNA), are involved in transcriptional gene regulation. ncRNA is primarily expressed in the brain, where it is involved in neuronal development, control of regions of the genome, which are involved in neuronal migration, homeostasis, and plasticity (17).

Epigenetics has improved our understanding of the evolution of the human brain, synaptic plasticity and neuronal diversity. Several studies have identified DNA methylation, changes in histones and chromatin, and changes in ncRNA expression in various neurological diseases, including AD. A large proportion of the genes that compose our genome are expressed in the central nervous system, where a substantial amount of miRNA is also synthesized. Several factors that have been associated with AD, such as diabetes mellitus, high blood pressure, obesity, diet, excessive sedentary lifestyle, smoking, and even a low educational level, are capable of inducing epigenetic changes (18).

There is currently no effective treatment for AD. However, cholinesterase inhibitors, such as donepezil, rivastigmine, and galantamine, together with *N*-methyl-D-aspartate (NMDA) receptor antagonists, such as memantine, produce moderate and transient symptomatic benefits in the early stages of the disease. Various treatments targeting the supposed causes of the disease are being developed, all still in the

experimental phase. One such treatment, active immunotherapy with A β fragments, which has been effective in transgenic mice (19), has not only been clinically ineffective in human patients but has also caused encephalitis in some cases (20). Passive immunotherapy with antibodies to A β has shown some benefits in transgenic mice and is being tested in humans. These clinical trials have shown that the clearance of A β in humans does not appear to produce significant cognitive improvements, which has led to some researchers questioning the role that A β plays in the cognitive decline associated with AD (20).

Attempts are also being made to develop drugs that prevent hyperphosphorylation or aggregation of the tau protein, although less effort has been made to this end than in inhibiting the formation of A β deposits. Most researchers support the hypothesis that amyloid plaques and the neurofibrillary tangles are neurotoxic. The amyloid cascade hypothesis has led to the development of treatments that promote A β clearance or prevent the formation of plaques. Such treatment has thus far been ineffective. A relatively high number of elderly people develop A β deposits without presenting with cognitive decline, which calls into question the amyloid cascade hypothesis.

Recent studies show that the A β peptide has antimicrobial properties, and that the absence of this peptide leads to an increased vulnerability to infection. Although the immune system has limited access to the central nervous system, it could fight invading pathogens with antimicrobial peptides like A β . The abnormal accumulation of A β observed in AD could be caused by persistent subacute infection or by noninfectious factors, such as trauma, ischemia, toxins, and anesthetics (21). Some researchers defend the hypothesis that A β acts as an antioxidant in response to the oxidative stress that takes place in regions of the brain subjected to high synaptic turnover, like that which occurs in the phenomenon of neuronal neoteny, where certain neurons retain a high synaptic plasticity in adulthood.

The generation of the A β peptide may have an adaptive function in its initial phases, and the same could be assumed about the hyperphosphorylation of tau. This would explain why drugs that reduce A β production have not been effective so far. Attempting to reverse the epigenetic changes that occur in AD could perhaps be of therapeutic value in the future.

EPIGENETIC THERAPIES IN AD

As previously discussed, the treatments currently approved for AD are acetylcholinesterase inhibitors (donepezil, rivastigmine, and galantamine) and the glutamate NMDA receptor antagonist memantine, drugs indicated for the specific treatment of memory disorders. Acetylcholinesterase inhibitors increase the levels of the neurotransmitter acetylcholine, which is decreased in brains with AD, and NMDA receptor antagonists prevent aberrant stimulation (22). These drugs achieve a discrete and transient improvement in cognitive and functional capacities, but do not delay the progression of the disease. Nevertheless, observational studies suggest that the combination of these treatments prolongs the time until patients need to be admitted to a residence (23). As a result, there is a great deal of interest in researching new treatments for the disease.

The main line of research in AD is that of anti-amyloid therapies (24). Despite the serious complications associated with active immunotherapy and the repeated failures of passive immunotherapy, novel anti-amyloid antibodies such as aducanumab and BAN2401 have brought fresh hope in this line of research, since they have been shown to be capable of reducing amyloid load in preliminary clinical trials (25). Other treatments within the amyloid cascade hypothesis have been developed, which promote A β clearance or prevent plaque formation. However, not only have none of these treatments within this line of research been shown to be effective, but some of them have led to clinical worsening (26, 27).

Another line of research is that of anti-tau therapies, drugs that prevent the hyperphosphorylation or aggregation of the tau protein, or antibodies that reduce the levels of the protein in the cerebrospinal fluid. Lastly, other avenues of research that are currently unsuccessful or under investigation are anti-APOE4 drugs, anti-oxidants, anti-inflammatory drugs, cardiovascular drugs, mitochondrial protectors, hormone therapy, and antiviral drugs (28–30).

Due to the difficulty in finding effective drugs for AD, it is crucial that other possible therapeutic avenues are explored, such as that of epigenetic drugs. This line of research is based on the fact that epigenetic changes take place during neurodevelopment and aging, and that epigenetic alterations are common in various neurodevelopmental and neurodegenerative diseases.

In the case of AD, more than 20 epigenetic mechanisms have been identified, most of which involve direct DNA modifications (as in the case of methylation), modifications in chromatin structure (as in the case of histone modifications), or modification of mRNA-related processes, including ncRNA and miRNA.

With regard to changes in methylation in AD, a recent study has established reference maps of the genome-wide distribution of the three possible states of DNA methylation (5mC, 5hmC, and 5fC/caC) in this disease (31). The results of this study, based mainly on cortical neurons obtained from induced pluripotent stem cells, suggest that the changes detected in DNA may precede the appearance of the disease, rather than appear later as a consequence of its progression. These results could mean these markers could be very useful in reaching early molecular diagnosis and therapy.

In addition, it has been detected in AD and frontotemporal dementia that the levels of an important transcriptional repressor—repressor element 1-silencing transcription factor (REST)—do not increase adequately with age (32). Consequently, transcriptional changes occur, and decreases in the expression of neuroprotector genes are found, including forkhead box protein class O (FOXO), which contributes to resistance to oxidative stress. In contrast, increased expression levels of genes that promote AD pathology, such as presenilin 2, are found. Taken together, these changes would increase neuronal fragility in these diseases. Furthermore, in animal models, such as the K-p25 AD mouse model, an increase in the expression of genes associated with the immune response has been detected, along with decreases in the expression of genes involved in synaptic functions and learning (33).

Several changes in the histone acetylation process, which is heavily involved in the consolidation of memory, have been detected in AD. For example, the levels of histone H4 with acetylation at the 16th lysine residue protein (H4K16ac), a histone marker located in enhancers and promoters generally associated with active gene expression, are duplicated in the cerebral cortex in healthy aging but

are barely detectable in the cerebral cortex of people with AD (34). Levels of histone deacetylase 2 (HDAC2), which increase in cultured cells after neurotoxic insults, are also found to be increased in the hippocampus and prefrontal cortex of AD mouse models and in the hippocampus of people with AD (35). Increases in deacetylase lead to worsening of synaptic function. It should be noted that blocking HDAC2 increases synaptic density and alleviates the loss of memory, but does not improve neuronal survival. This means that deficits in AD are caused not only by neuronal loss but also by epigenetic blocking of the functions of neuronal survivors (36). In addition to the reduction in expression of genes important for neuronal function, an increase in aberrant expression of genes that are normally silenced or expressed at low levels has also been observed in AD (37, 38).

Furthermore, the expression of miRNA in the brain is altered in AD. For example, reduced levels of miRNA-29a/b-1 and miRNA-132, and increased levels of miRNA-34c have been detected. The decrease in miRNA-29a/b-1, which is a beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) inhibitor, correlates with an increase in the production of A β (39). A decrease in miRNA-132, which targets the tau protein, HAT-associated protein 300 (EP300), sirtuin deacetylase 1, and FOXO1a, would jeopardize neuronal growth, the integration of newborn neurons, synaptic structure, and plasticity (40). Dysregulation of miRNA expression has also been detected in biofluids, suggesting that these molecules could be used as both biomarkers and therapeutic targets (39, 40).

Finally, an acceleration of epigenetic age has also been observed in AD, especially in the prefrontal cortex. Epigenetic age is estimated from DNA methylation levels in 353 CpG sites, and its acceleration with respect to chronological age is associated with, in addition to AD, higher mortality, cognitive impairment, and other neurodegenerative diseases (41, 42). Epigenetic age could also explain differences in the onset age of AD in members of the same family that share the same gene mutation (43). That is, those who have an accelerated epigenetic age would develop AD symptoms at an earlier age.

The goal of epigenetic therapies is to reverse at least some of the epigenetic changes caused by AD. Such therapies have several advantages. First, specific drugs can be designed because the epigenetic changes are induced by enzymes that act at the DNA or histone level. Second, they act on reversible mechanisms since the epigenetic changes at the DNA and histone level are both regulated by enzymes. Finally, these therapies enable us to unite physiology and pathology, because epigenetics influence gene expression throughout life, and thus epigenetic drugs would be effective in both neurodevelopmental and neurodegenerative diseases. In addition, epigenetic therapies can target any component of the epigenetic machinery.

In the last decade, several epigenetic drugs have been designed for the treatment of neurological diseases. The most promising are DNA-demethylating agents and HDAC inhibitors (HDACis). In fact, there are already drugs of these two therapeutic groups that have been approved by the US Food and Drug Administration for the treatment of hematological cancer. In the former group, there is 5-azacytidine and the 5-aza-2'-deoxycytidine (or decitabine), and in the latter group, suberoylanilide hydroxamic acid (SAHA or vorinostat), romidepsin, belinostat, panobinostat, and chidamide have been approved.

HDAC aims to regulate imbalances in protein acetylation levels and transcription. Their use in neurodegenerative diseases is based on their neuroprotective,

neurotrophic, and anti-inflammatory properties. In the case of AD, HDACs play an important role in memory consolidation and could be useful as therapeutic targets. For example, HDAC2 and HDAC3 have been shown to play a repressive role in memory formation, while HDAC5 has a memory-enhancing effect (44). As a result, HDAC2-inhibiting drugs, such as CI-994, and HDAC5-enhancing drugs could be useful in AD. The therapeutic potential of HDACis has been demonstrated in studies with animals. In APP/PS1 mice, acute treatment with HDACi trichostatin A (TSA), sodium valproic acid, SAHA, sodium butyrate (NaB), butyrate, vorinostat, 4-phenylbutyric acid, MS-275, and crebinostat improved the cognitive performance of these animals (45–49).

Sulforaphane could also be useful in AD. Sulforaphane is an HDACi that decreased HDAC2 levels in the triple-transgenic mouse model of AD (3 × Tg-AD). This was accompanied by an increase in the acetylation of histones H3 and H4 in the BDNF promoter and resulting in an increase in its expression (50).

There are HDACis that affect multiple genes involved in AD, which could be advantageous given the multifactorial etiology of AD. The disadvantage of these compounds is that their wide spectrum theoretically broadens the possibilities of adverse effects with their use. This group includes M344 {4-(dimethylamino)-*n*-[7-(hydroxyamino)-7-oxoheptyl]benzamide}, CM-414 {3-[[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenyl]methyl]-*N*-hydroxycyclobutane-1-carboxamide}, and RGFP-966 {(*E*)-*N*-(2-amino-4-fluorophenyl)-3-[1-[(*E*)-3-phenylprop-2-enyl]pyrazol-4-yl]prop-2-enamide}. Their chronic use in animal models showed cognitive benefits (51–57).

In addition to the HDACis described, there are other HDACis that have been specifically designed. In this way, HDACi W2 was obtained, which features a longer half-life and better penetration of the blood–brain barrier than the HDACis currently available. HDACi W2 has been shown to be capable of significantly reducing Aβ levels in hAPP 3×Tg AD mice by reducing the expression of genes involved in Aβ production and increasing the expression of Aβ degradation enzymes. This HDACi is also capable of decreasing phosphorylation of the tau protein, promoting the formation and growth of dendritic spines, and improving learning and memory in these mice, which makes it a potential candidate for the treatment of AD.

Due to the observed benefits of using HDACis in animal models of AD, there are several ongoing clinical studies with these compounds. One of the compounds under study is valproate, which is a class I HDAC inhibitor. It has already been approved for treating epilepsy, migraine, and bipolar disorder, and since its activity as a HDACi was discovered, it is also being studied to evaluate its effectiveness in neurodegenerative disease. In preclinical studies, it was shown to be effective in reversing cognitive impairment in a mouse model of AD (58, 59).

Another drug under study is vitamin B3 or nicotamide, which is also a class III NAD-dependent sirtuin HDAC inhibitor. This compound can delay aging in mouse oocytes and delay cognitive impairment in a mouse model of AD (60). A phase I clinical trial in patients with AD demonstrated its safety. It is currently in a phase II clinical trial. Another HDACi, vorinostat, is being evaluated in a phase I clinical trial in patients with AD. Finally, RDN-929, which is a CoREST-selective HDAC inhibitor that could reactivate neuronal gene expression, strengthen synaptic function, and promote new synapses, has been studied in two phase I clinical trials as a possible treatment for AD.

Another mechanism for enhancing acetylation is the use of drugs that enhance HATs. The increased expression of an enzyme of this type, Tip60, in *Drosophila* overexpressing human APP was able to restore the benefits of environmental enrichment (61).

With regard to miRNA, utility of the drug gemfibrozil has been studied. Gemfibrozil is capable of modifying miR-107 levels, the reduction of which may accelerate the progression of AD by regulating the expression of BACE1. A phase I clinical trial showed the drug was safe and reduced miR-107 in plasma and in CSF to undetectable levels.

Other possible epigenetic therapeutic targets not yet explored in AD are drugs directed against HATs, ten-eleven-translocation methylcytosine-dioxygenases enzymes (which catalyze the conversion of 5-methylcytosine to 5-hydroxymethylcytosine), DNA demethylation, chromatin remodelers, and other histone modifications.

Finally, it should be noted that there are also nonspecific epigenetic therapies that can be useful in AD. The first is blood plasma therapy from young subjects. In a recent study, in which aged mice were treated with blood plasma from young mice, it was observed that the treatment halved the epigenetic ages of blood, heart, and liver tissue, and also rejuvenated the hypothalamus. The treatment also improved the functioning of these organs as well as cognitive functions (62). The second nonspecific epigenetic therapy is cognitive stimulation, which has been shown to be capable of causing epigenetic changes (63). The third nonspecific epigenetic therapy is physical exercise. In animals, physical exercise is capable of reversing age-related reduction of adult neurogenesis and cognitive function in the aged hippocampus (64). In addition, a recent study showed that the administration of circulating blood factors in the plasma of aged mice subjected to exercise was capable of transferring the beneficial effects to sedentary-aged mice. These investigations led to the discovery that glycosylphosphatidylinositol (GPI)-specific phospholipase D1 (Gpld1), a GPI-degrading enzyme derived from liver, was probably responsible for these effects.

Although epigenetic therapies have a promising future role in AD, there are still problems that must first be addressed. First, we need to bear in mind that perhaps not all epigenetic changes can be reversed with these types of therapy. Second, epigenetic changes are extremely complex, and the therapies could have any number of side effects that are difficult to control. Third, different regions of the same gene can have antagonistic epigenetic changes, so the effect of an epigenetic therapy could be unpredictable. With respect to pharmacological properties, current therapies lack specificity and are not selective for specific brain regions, cell types, or genes. This limitation could be addressed with the use of siRNA or the use of chromatin-modifying enzymes, transcription activation-like effectors or clustered regularly interspaced short palindromic repeats/Cas System, and the use of artificial transcriptional factors of the silencing or promoter type.

The development of new study techniques will also be essential to better understand how epigenetic therapies work and to be able to design future drugs. First, laboratory techniques such as cell-type specific analysis of transcription and DNA methylation in the brain, single cell analysis of DNA-protein interactions, and chromatin 3D structure might be applied in future studies to uncover neuronal cell-type specific chromatin structure and interneuronal variations. Furthermore,

new models for studying the effect of these therapies, such as neuronal cultures or other brain cell types derived from human stem cells or induced pluripotent stem cells, could be better than the animal models used so far. The application of innovative imaging techniques, such as positron emission tomography using radiochemical [^{11}C] martinostat, which binds specifically to certain HDAC isoforms, could help to discover the gene expression patterns regulated by chromatin-modifying enzymes in the live brain. Finally, establishing epigenetic biomarkers for diagnosis, prognosis, and therapy would be important to test the efficacy of epigenetic drugs and to classify patients according to the particular therapy they would most benefit from.

CONCLUSION

At present, there are no treatments for AD. Given that several epigenetic changes occur in this disease, treatments aimed at reversing these changes may constitute promising lines of research in the future.

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