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REVIEW ARTICLE

Epigenomics of Alzheimer's Disease

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Alzheimer's disease (AD) is a polygenic/complex disorder in which genomic, epigenomic, and environmental factors are involved. Epigenetic factors have emerged as important mediators of aging, neurodegeneration, and brain disorders. Epigenomic changes underlying the phenotypic expression of AD, represented by deposits of extracellular Aβ aggregates in senile plaques, intracellular neurofibrillary tangles, neuronal loss, dendritic desarborization, and neurochemical alterations are candidate targets for therapeutic intervention. Changes in DNA methylation, histone modifications, chromatin remodeling, and noncoding RNA dysregulation can affect AD-related gene expression, leading to the multistep process of premature neurodegeneration. Epigenetic modifications are reversible and can be potentially targeted by pharmacological and dietary interventions.

1. Introduction

Alzheimer's disease (AD), which is a major health problem in developed countries, is a polygenic/complex disorder in which hundreds of defective genes are involved in close interaction with environmental factors, cerebrovascular dysfunction, and epigenetic changes.[1,2]

Epigenomics/epigenetics refers to phenotypic changes with no apparent alterations in structural DNA. Classical epigenetic mechanisms, including DNA methylation and histone modifications (HMs), and regulation by microRNAs (miRNAs), are among the major regulatory elements that control metabolic pathways at the molecular level, with epigenetic modifications regulating gene expression transcriptionally and miRNAs suppressing gene expression posttranscriptionally.[3]

Epigenetic factors have emerged as important mediators of development and aging, gene–gene and gene–environmental interactions, and the pathophysiology of complex disorders. Major epigenetic mechanisms may also contribute to AD pathology.[4,5]

2. AD-related epigenetics

Epigenetic mechanisms and miRNAs have been shown to closely interact with each other, thereby creating reciprocal regulatory circuits, which appear to be disrupted in AD (Table 1). Brain hypoperfusion-related changes in DNA methylation may also contribute to accelerate neuronal death. Short-term, sublethal hypoxia results in long-lasting changes to genome-wide DNA methylation status, and some of these changes can be highly correlated with transcriptional modulation in many genes involved in functional pathways.[6]

Memory decline is a seminal symptom in dementia. Gene expression is required for long-lasting forms of memory. Epigenetic mechanisms do not only provide complexity in the protein regulatory complexes that control coordinate transcription for specific cell function, but the epigenome encodes critical information that integrates experience and cellular history for specific cell functions as well. Epigenetic mechanisms provide a unique mechanism of gene expression regulation for memory processes. Negative regulators of gene expression, such as histone deacetylases (HDACs), have powerful effects on the formation and persistence of memory. HDAC inhibition transforms a subthreshold learning event into robust long-term memory and generates a form of long-term memory that persists beyond the point at which normal long-term memory fails.[7] Whereas increments in histone acetylation have consistently been shown to favor learning and memory, a lack

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Table 1 Epigenetic mechanisms potentially involved in the pathogenesis of Alzheimer's disease

DNA methylation of pathogenic/susceptibility genes
APP, PSEN1, BACE1, MAPT, APOE, CLU, TBXA2R,
SORBS3, SPTBN4, MTHFR, PP2A, S100A2, CREB5
Histone modifications
Alterations in histone acetylation
Increased levels of HDAC6
Decreased SIRT1
Trimethylation of H3K9
Phosphorylation of H3S10
Phosphorylated H2AX/Ser 139-related DNA damage
Increased EID1
Alterations in Tip60 HAT activity
ncRNA dysregulation
lncRNAs
Sox2OT, 1810014B01Rik, BC200, BACE1-AS, NAT-Rad18, 17A, GDNFOS
miRNAs
miR-34a (1p36.22)
miR-34b/c (11q23.1)
miR-107 (10q23.31)
miR-124 (8p23.1/8p12.3/20q13.33)
miR-125b (11q24.1/21q21.1)
miR-137 (1p21.3)
let-7b (22q13.1)
miR-9 (1q22/5q14.3/15q26.1)
miR-132/212 (17p13.3)
miR-146a (5q34)
miR-148a (7p15.2)
miR-184 (15q25.1)
miR-200 (miR-200b/200a/429, 1p36.33; miR-200c/141, 12p13.31)
Other miRNAs
miRNA-155
miR-214, -23a and −23b, -486-3p, −30e*, −143, −128, −27a and −27b, -324-5p and −422a.
miR-20a family (miRs-20a, −17, and −106b), miRs-106a and 520c, miR-101, miR-16, and miRs-147, -153, -323-3p, −644, and −655
miR-29a/b-1, -29c, −298, −328, and -485-5p
miRNA signatures
hsa-let-7d-5p, hsa-let-7g-5p, hsa-miR-15b-5p, hsa-miR-142-3p, hsa-miR-191-5p, hsa-miR-301a-3p and hsa-miR-545-3p

APP = amyloid precursor protein; HDAC6 = histone deacetylase 6; lncRNA = long noncoding RNAs; miRNAs = microRNAs; PSEN1 = presenilin1.

is causally implicated in cognitive impairments in neurodevelopmental disorders, neurodegeneration, and aging. As histone acetylation and cognitive functions can be pharmacologically restored by HDAC inhibitors, this epigenetic modification might constitute a molecular memory aid on the chromatin and, by extension, a new template for therapeutic interventions against cognitive decline.[8]

Neurons, because of their postmitotic state, high metabolism, and longevity, are particularly prone to the accumulation of DNA lesions. DNA damage is a major contributor to both age-associated neurodegenerative diseases and acute neurological injury. The DNA damage response is a key factor in maintaining genome integrity, relying on highly dynamic posttranslational modifications of the chromatin and DNA repair proteins to allow signaling, access, and repair of the lesion.[9] The repair of DNA lesions, particularly oxidative DNA lesions, might be altered in AD. DNA damage is paralleled by a decrease in DNA repair activities, being inactivated by oxidative-induced posttranslational modifications or degradation. Activating DNA repair pathways might generate death signals ending with neuronal apoptosis. A link between environment-induced epigenetic modification, oxidation, and repair of AD related genes has been proposed.[10] Early life exposure of rodents and primates to xenobiotics may enhance the expression of the gene-associated with AD, repress the expression of others, and increase the burden of oxidative DNA damage in the aged brain. Epigenetic mechanisms that control gene expression and promote the accumulation of oxidative DNA damage are mediated through alterations in the methylation or oxidation of CpG dinucleotides. Environmental influences occurring during brain development inhibit DNA methyltransferases, thus hypomethylating promoters of genes associated with AD, such as *APP*. This early life imprint may sustain and trigger later in life to increase the levels of amyloid beta precursor protein (APP) and Aβ. Increased Aβ levels promote the production of reactive oxygen species, which damage DNA and accelerate neurodegenerative events. These early life perturbations may cause hypomethylation or hypermethylation of genes. The hypermethylated genes are rendered susceptible to Aβ-enhanced oxidative DNA damage because methylcytosines restrict repair of adjacent hydroxyguanosines.[11]

3. DNA methylation of pathogenic genes

Many AD-related genes contain methylated CpG sites in their promoter regions, and a genome-wide decrease in DNA methylation.[5,12] The methylation status of repetitive elements (i.e., Alu, LINE-1, and SAT-α) is a major contributor of global DNA methylation patterns. The study of global DNA methylation levels for long interspersed nuclear element 1 (LINE-1) repetitive sequences in AD patients and controls did not provide clear results. In one study, no differences in LINE-1 methylation levels have been found between patients and controls.[13] But in another study, LINE-1 methylation has been found to be increased in AD patients compared with healthy volunteers.[14] In AD, both hypo- and hypermethylation of specific genes have been reported.[5] DNA methylation of the *APP* promoter has been found to be decreased in the brains of autopsy patients older than 70 years as compared to their younger counterparts.[15] The intracellular domain of APP is a key epigenetic regulator of gene expression controlling many genes, including *APP* itself, the amyloid-degrading enzyme neprilysin, and aquaporin-1.[16] The abnormal processing of neuronal cell membrane APP causes elevated human serum and cerebrospinal fluid levels of 24-hydroxycholesterol, an endogenous ligand of liver X receptor (LXR-α). An epigenomic pathway exists to connect LXR-α activation with genes involved in regulating aberrant Aβ production, leading to the generation of toxic and inflammatory mediators responsible for neuronal death. LXR-α activation by its specific endo- or exogenous ligands results in the overexpression of the *PAR-4* gene and suppression of the *AATF* gene through its inherent capacity to regulate genes coding for SREBP and NF-κB. Overexpression of the *PAR-4* gene causes aberrant Aβ production followed by reactive oxygen species generation and subsequent neuronal death. Aβ-induced heme oxygenase-1 can ensure cholesterol oxidation to provide endogenous ligands for the sustained activation of neuronal LXR-α-dependent epigenomic pathways, leading to neuronal death in AD.[17]

Presenilin1 (PSEN1) is modulated by DNA methylation in neuroblastoma cells and AD mouse models in an experimental model of nutritionally altered one-carbon metabolism. Studies performed on human neuronal cell cultures show that deprivation of folate and other B vitamins from the media resulted in epigenetic modification of the *PSEN1* gene.[18]

Several pathogenic genes (*APP*, *PS1*, *APOE*, *BACE*) and many other AD-related susceptibility genes contain methylated CpG sites. The promoter region where the *APP* gene is hypomethylated can potentially increase Aβ production; however, some authors have reported no relevant changes in APP methylation in AD samples.[19] BACE and PS1 expression is enhanced after folate deprivation-induced hypomethylation, and is restored when folate deficiency is supplemented with *S*-adenosyl methionine. Aβ may induce genome-wide hypomethylation to cause upregulation of genes involved in neuroinflammation (TNF) and apoptosis (caspase 3), contributing to Aβ production in a vicious cycle.[5]

The *APOE* gene exhibits a bimodal structure, with a hypomethylated CpG-poor promoter and a fully methylated 3′-CpG island, containing the sequences for the APOE4 haplotype. According to Wang et al,[5,19] aberrant epigenetic changes in this CpG island may contribute to late-onset form of AD pathology. A hypermethylated CpG island is present within the *APOE* gene. The APOE4 sequence may change the epigenetic function of the methylated 3′-CpG island, because the *APOE4* allele induces a C-to-T transition that is involved in a loss of a methylatable CpG unit.[19] *APOE4* carriers show a dose-dependent risk, and the relative messenger RNA (mRNA) level of *APOE4* is increased in AD compared to controls, indicating that variability in the neuronal expression of *APOE* contributes to disease risk.[20]

Clusterin gene (*CLU*) [apolipoprotein J (ApoJ)], together with *APOE*, influences Aβ aggregation and clearance. CLU levels are increased in AD and may be associated with brain atrophy, disease severity, and clinical progression. The promoter region of *CLU* contains a CpG-rich methylation domain. The demethylating effect of 5-aza-2′-deoxycytidine in prostate cancer cell lines increases the expression of CLU.[21]

Hyperphosphorylated tau is responsible for the formation of intracellular neurofibrillary tangles (NFTs). Changes in methylation status differ among transcription factor binding sites of tau promoter. Binding sites for GCF (granulocyte chemotactic factor), responsible for repression of GC-rich promoters, have been found to be hypomethylated, and binding sites for the transcriptional activator SP1 (specificity factor 1) to be hypermethylated.[22] High levels of Hcy may induce tau hyperphosphorylation, NFT formation, and SP formation through inhibiting methyltransferases and hypomethylation of protein phosphatase 2A (PP2A), a dephosphorylating enzyme of phosphorylated tau.[23] In transgenic APPswe/presenilin (PS) 1 (A246E) mice, PP2A methylation at the L309 site is decreased, in parallel with increased tau phosphorylation at Tau-1 and PHF-1 sites. $A\beta_{25-35}$ induces demethylation and enhances tau phosphorylation.[24] Hypomethylation of PP2A may lead to tau hyperphosphorylation and NFT formation.[5]

Sanchez-Mut et al[25] have identified DNA methylation-associated silencing of three target genes: thromboxane A2 receptor (*TBXA2R*), sorbin and SH3 domain containing 3 (*SORBS3*), and spectrin beta 4 (*SPTBN4*). These hypermethylation targets suggest that the cyclic AMP response element-binding protein (CREB) activation pathway and the axon initial segment might contribute to AD pathology.

Several components of the cell cycle [P16, P21, P27, P53, RB1, cyclin B2, alternate reading frame (ARF) protein product] and apoptosis pathways (caspases 1, 3, 7, 8, 9) are regulated by DNA methylation and appear upregulated in AD neurons. SORBS3 (vinexin, SCAM-1, or SH3D4), encoding a cell adhesion molecule expressed in neurons and glia, is progressively hypermethylated with age. S100A2 (a member of the S100 family of calcium-binding proteins), which has been shown in an age-dependent decrease in DNA methylation later in life, is also hypermethylated in AD.[5]

Chaperones may have a crucial role in AD because of their involvement in protein quality control, folding, and degradation. Silva et al[26] investigated the mRNA and promoter DNA methylation levels of two chaperones, HSPA8 and HSPA9, in postmortem brain tissue (entorhinal and auditory cortices and hippocampus) from healthy elderly and AD patients as well as in the peripheral blood of healthy elderly and AD patients. No changes have been observed in peripheral HSPA8 and HSPA9 expression between elderly controls and AD. A significant downregulation of HSPA8 and HSPA9 has been observed in AD patients across the three brain regions compared to the controls.

In summary, DNA methylation changes are present in AD-related genes; some of these genes are hypermethylated (*MTHFR*, Neprilysin, *MAPT*, *APOE*, *SORB3*), whereas others have been found to be hypomethylated (*APP*, *BACE*, *PSEN1*, *PP2A*, *S100A2*, *CREB5*).[5,27] DNA methylation of CpG units by DNA methyltransferases (DNMTs) disrupts the binding of transcription factors and attracts methyl-CpG-binding domain proteins that are associated with gene silencing and chromatin compaction.[28]

4. Histone modifications

A small bulk of recent information[5,8,29] suggests that HMs are present in AD: (1) histone acetylation is reduced in AD brain tissues[30] and in AD transgenic models[8]; (2) levels of histone deacetylase 6 (HDAC6), a tau-interacting protein and a potential modulator of tau phosphorylation and accumulation, are increased in cortical and hippocampal regions in AD;[31] mice lacking HDAC6 are cognitively normal, but reducing endogenous HDAC6 levels restores learning and memory and α-tubulin acetylation;[32] (3) SIRT1 is decreased in the parietal cortex of AD patients, and the accumulation of Aβ and tau in AD brains might be related to the loss of SIRT1,[33] because SIRT1 may reduce Aβ production, activating the transcription of ADAM10;[34] (4) in the brains of twins discordant for AD, trimethylation of H3K9, a marker of gene silencing, and condensation of heterochromatin structure, are increased in the temporal cortex and hippocampus of the AD twin as compared to the twin without of AD neuropathology;[35] (5) phosphorylation of H3S10, a key regulator in chromatin compaction during cell division, is increased in the cytoplasm of hippocampal neurons in AD cases;[36] (6) evidence of DNA damage, as reflected by phosphorylated H2AX at Ser139, is present in hippocampal astrocytes of AD patients;[37] (7) long-term potentiation (LTP) and memory deficits in APP/PS1 transgenic mice might be mediated in part by decreased H4 acetylation; improving histone acetylation level restores learning after synaptic dysfunction;[38] (8) acetylation of H3 and H4 is increased in 3xTg-AD neurons relative to nontransgenic neurons;[39] (9) nuclear translocation of EP300 interacting inhibitor of differentiation 1 (EID1), a CBP/p300 inhibitory protein, is increased in the cortical neurons of AD patients, and overexpression of EID1 is reported to reduce hippocampal LTP and to impair cognitive function via inhibiting CBP/p300 acetyltransferase activity and disrupting neuronal structure;[40] (10) memory formation leads to a transient increase in acetylation on lysine residues within H2B, H3, and H4;[41,42] (11) inhibition of HDAC induces dendritic sprouting, increases synaptic number, and improves long-term memory;[43] (12) overexpression of neuronal HDAC2 decreases dendritic spine density, synapse number, synaptic plasticity, and memory formation, and HDAC2 deficiency increases synapse number and memory facilitation;[44] (13) HDAC4 is involved in learning and synaptic plasticity, and selective inhibition of HDAC4 activity may deteriorate learning and memory;[45] (14) treatment of hippocampal neurons with HDAC inhibitors facilitates BDNF expression via the hyperacetylation of histones at the BDNF promoters;[46] (15) histone (H3K4) methylation participates in the regulation of BDNF expression and memory formation;[47] (16) histone methylation also facilitates memory consolidation coupled with histone acetylation; inhibition of HDACs with sodium butyrate (NaB) causes an increase in H3K4 trimethylation and a decrease in H3K9 dimethylation in the hippocampus after fear conditioning;[47] and (17) histone H3 acetylation, methylation, and phosphorylation are increased in the prefrontal cortex of Tg2576 mice, and histone H4 acetylation is increased in the hippocampal CA1 neurons of these transgenic mice.[48]

Age-related differences in epigenetic acetylation and methylation of histones are associated with age-related gene regulation. In studies to quantify single cell acetylation and methylation levels across the life span in cultured hippocampal/cortical neurons from the 3xTg-AD mouse model and from nontransgenic mice, Walker et al[39] found that in nontransgenic neurons, H3 acetylation is unchanged with age, and that H4 acetylation is decreased with age of the donor. Compared to nontransgenic neurons, 3xTg-AD neurons

have higher levels of H3 and H4 acetylation beginning at 4 months of age. In contrast to nontransgenic neurons, 3xTg-AD neurons are increased acetylation with age; 3xTg-AD neurons have also responded differently to inhibition of HDACs at an early age. Treatment of nontransgenic neurons with Aβ also can elevate levels of acetylation. The repressive function of histone H3 lysine 9 (H3K9) methylation is increased with age in nontransgenic neurons, being found to be amplified further in 3xTg-AD neurons. The dominant effect of higher H3K9 methylation has been supported by lower BDNF gene expression in nontransgenic and 3xTg-AD mice. The epigenetic states of nontransgenic and 3xTg-AD brain neurons are profoundly different and reversible, beginning at 4 months of age when the first memory deficits are reported.[39]

Nucleosome remodeling is carried out by chromatin remodeling complexes (CRCs) that interact with DNA and histones to physically alter chromatin structure and ultimately regulate gene expression. Human exome sequencing and genome-wide association studies have linked mutations in CRC subunits to intellectual disability disorders, autism spectrum disorder, and schizophrenia. There appear to be both developmental and adult specific roles for the neuron-specific CRC nBAF (neuronal Brg1/hBrm Associated Factor). nBAF regulates gene expression required for dendritic arborization during development, and in the adult, contributes to LTP, a form of synaptic plasticity, and long-term memory. Vogel-Ciernia and Wood[49] proposed that the nBAF complex is a novel epigenetic mechanism for regulating transcription required for long-lasting forms of synaptic plasticity and memory processes, and that impaired nBAF function may result in human cognitive disorders.

The expression of HDAC6 is increased in the hippocampus and other relevant brain regions in both patients with AD and animal models of AD. But when and how HDAC6 expression is increased during the course of AD progression remains unclear. Increased HDAC6 expression contributes to AD-associated neurodegeneration, although beneficial effects have also been identified in some pathogenic mechanisms (axonal growth and transport, synaptic plasticity, oxidative stress, apoptosis, neuroinflammation, and misfolded proteins and aggregates).[50]

Sleep disruption associated with AD is driven by epigenetic changes mediated by the histone acetyltransferase Tip60. Tip60 functionally interacts with the AD-associated amyloid precursor protein (APP) to regulate the axonal growth of *Drosophila* small ventrolateral neuronal (sLNv) pacemaker cells, and their production of neuropeptide pigment dispersing factor (PDF) that stabilizes appropriate sleep—wake patterns in the fly. Loss of Tip60 HAT activity under APP neurodegenerative conditions causes decreased PDF production, retraction of the sLNv synaptic arbor required for PDF release, and disruption of sleep—wake cycles in these flies. Excess Tip60 in conjunction with APP fully rescues these sleep—wake disturbances by inducing overelaboration of the sLNv synaptic terminals and increasing PDF levels, supporting a neuroprotective role for Tip60 in these processes.[51]

Sirtuins are NAD^+-dependent histone/protein deacetylases that are similar to *Saccharomyces cerevisiae* silent information regulator 2 (Sir2). Sirtuins regulate various normal and abnormal cellular and metabolic processes, including tumorigenesis, neurodegeneration, and processes associated with type 2 diabetes and obesity. Several age-related diseases, such as AD, and longevity have also been linked to the functions of sirtuins.[52]

5. Noncoding RNAs

Several lncRNAs are dysregulated in AD (Sox2OT, 1810014B01Rik, BC200, BACE1-AS, NAT-Rad18, 17A, GDNFOS), Parkinson's disease (naPINK1, Sox2OT, 1810014B01Rik, BC200), and Huntington's disease (HAR1F, HTTAS, DGCR5, NEAT1, TUG1).[53] miRNAs belong to the class of noncoding regulatory RNA molecules of ~22 nt length and are now recognized to regulate about 60% of all known genes through posttranscriptional gene silencing (RNA interference). Alterations in epigenetically regulated miRNAs may contribute to the abnormal expression of pathogenic genes in AD.[53,54] Examples of miRNAs directly linked to AD pathogenesis include miR-34a (1p36.22), miR-34b/c (11q23.1), miR-107 (10q23.31), miR-124 (8p23.1/8p12.3/20q13.33), miR-125b (11q24.1/21q21.1), and miR-137 (1p21.3); and examples of epigenetically regulated miRNAs with targets linked to AD pathogenesis are let-7b (22q13.1), miR-9 (1q22/5q14.3/15q26.1), miR-132/212 (17p13.3), miR-146a (5q34), miR-148a (7p15.2), miR-184 (15q25.1), and miR-200 (miR-200b/200a/429, 1p36.33; miR-200c/141, 12p13.31).[54]

miRNAs can be used as biomarkers to discriminate different disease forms, staging, and progression, as well as prognosis.[55] A unique circulating 7-miRNA signature (hsa-let-7d-5p, hsa-let-7g-5p, hsa-miR-15b-5p, hsa-miR-142-3p, hsa-miR-191-5p, hsa-miR-301a-3p, and hsa-miR-545-3p) reported by Kumar et al[55] in plasma, can distinguish AD patients from normal controls with more than 95% accuracy. Leidinger et al[56] have found a novel miRNA-based signature for detecting AD from blood samples. Using this 12-miRNA signature, they differentiated between AD and controls with an accuracy of 93%, a specificity of 95%, and a sensitivity of 92%. The differentiation of AD from other neurological diseases (mild cognitive impairment, multiple sclerosis, Parkinson disease, major depression, bipolar disorder, and schizophrenia) was possible with accuracies between 74% and 78%. Alexandrov et al[57] have found increased levels of miRNA-9, miRNA-125b, miRNA-146a, and miRNA-155 in the cerebrospinal fluid and brain tissue-derived extracellular fluid from patients with AD, suggesting that these miRNAs might be involved in the modulation or proliferation of miRNA-triggered pathogenic signaling in AD brains.

AD-related single-nucleotide polymorphisms (SNPs) interfere with miRNA gene regulation and affect AD susceptibility. The significant interactions include target SNPs present in seven genes related to AD prognosis with the miRNAs miR-214, miR-23a and miR-23b, miR-486-3p, miR-30e*, miR-143, miR-128, miR-27a and miR-27b, miR-324-5p, and miR-422a. The dysregulated miRNA network contributes to the aberrant gene expression in AD.[4,5]

Several miRNAs have been identified *in vitro* to directly regulate the APP mRNA, including miRNA let-7, the miR-20a family (miR-20a, miR-17, and miR-106b), miR-106a and miR-520c, miR-101, miR-16, and miR-147, miR-153, miR-323-3p, miR-644, and miR-655.[5] Inhibition of miR-101 overexpression can reduce APP and Aβ load in hippocampal neurons.[58] MiR-16 targets APP to potentially modulate AD pathogenesis, and miR-16 overexpression may lead to reduced APP expression.[59] Both miR-124 and polypyrimidine tract binding protein 1 (PTBP1) may alter the splicing of APP exons 7 and 8 in neuronal cells.[60] miR-124 also regulates the expression of BACE1.[61] mRNA expression of BACE1 is mediated by both miRNAs (miR-9, miR-29a/b-1, miR-29c, miR-107, miR-298, miR-328, and miR-485-5p) and long ncRNAs (BACE1-antisense) (BACE1-AS), and is repressed by miRs-29a, miR-29b-1, and miR-9 *in vitro*. In transgenic HEK293-APP cells, transient miR-29a/b-1 overexpression decreases BACE1 levels and Aβ production.[62] miR-29c overexpression lowers BACE1 protein levels.[63] miRNAs repress BACE1 through direct binding to sequences in its 3′ untranslated region (3′UTR), whereas miR-485-5p represses BACE1 through binding to its open reading frame in exon 6. miR-107 is downregulated at the intermediate stages (Braak stage 3) of AD pathogenesis and might accelerate AD progression through control of BACE1.[64] miR-298, miR-328, and miR-195 inversely correlate with BACE1 protein, and downregulate Aβ levels by inhibiting the translation of BACE1.[65] miR-125 decreases, whereas BACE1 increases in animal models.[65] Overexpression of miR-485-5p reduces BACE1 protein

levels by 30%, whereas knockdown of miR-485-5p increases BACE1 protein levels.[66] BACE1-AS, an ~2-kb conserved ncRNA transcribed from the opposite strand to BACE1 and coexpressed with BACE, is upregulated in AD, potentially promoting Aβ generation and AD pathogenesis. BACE1-AS may enhance *BACE1* mRNA stability by "masking" the binding site for miR-485-5p, and prevent miRNA-induced translational repression of *BACE1* mRNA.[66,67]

The RNA polymerase III-dependent ncRNA, NDM29, promotes APP amyloidogenesis and Aβ secretion.[68] miR-107 levels are reduced in the AD temporal cortex.[69] Loss of miR-9, 29a/b-1, miR-137, and miR-181c (currently downregulated in AD frontal cortex) increases Aβ production and serine palmitoyltransferase, the first rate-limiting enzyme in ceramide biosynthesis.[70] miRNA-106b (downregulated in anterior temporal cortex) can influence Aβ metabolism either through direct regulation of APP itself, or through modulating APP trafficking, Aβ clearance, and β- and γ-secretase activity through regulation of the ATP-binding cassette transporter A1 (ABCA1), which is elevated in the hippocampus, correlating with cognitive decline.[71] The brain-expressed ncRNA, 17A, is upregulated in the AD cortex, promoting Aβ in response to neuroinflammation injury.[72]

Several miRNAs also regulate tau metabolism. The miR-132/PTBP2 pathway influences MAPT exon 10 splicing in the brain and may contribute to AD pathogenesis. miR-132 has been found to be downregulated in some tauopathies, such as progressive supranuclear palsy (PSP), a major 4R-tau tauopathy, where the protein levels of the neuronal splicing factor PTBP2 are elevated.[73] miR-124, miR-9, miR-132, and miR-137 may regulate the 4R/3R ratio in neuronal cells.[74] Both miR-9 and miR-124 are downregulated in AD and might affect tau. The miR-15/ERK1 pathway mediates tau phosphorylation. miR-15a is downregulated in AD brains.[74] The miR-15 family (miR-15a, miR-16, miR-195, and miR-497) targets extracellular signal-regulated kinase 1 (ERK1) expression; and decreased miR-15 levels may participate in neuronal tau hyperphosphorylation. miR-26a can repress mRNA of the tau kinase GSK-3β, which is involved in Aβ production and NFT formation.[75,76] miR-26a expression is also altered in AD.[77]

In conditional Dicer knockout mice, with reduced brain miRNA production, tau hyperphosphorylation and altered MAPT splicing is observed; and reduced miRNA processing in dicer-1 knockout flies can enhance tau-induced neurodegeneration.[78]

SIRT1 deacetylates tau, and SIRT1 deficiency increases tau acetylation and the accumulation of hyperphosphorylated tau.[33,79] miR-9, miR-34c, and miR-181c repress SIRT1 mRNA.[80] miR-128 modulates the expression of BAG2, and the cochaperone is involved in tau degradation and aggregation.[81] miR-212 is downregulated in AD, being apparently involved in NFT density.[69,77] miR-146a is an inflammation effector associated with immune and inflammation signaling by targeting IRAK1. miR-146a upregulation in AD brain may contribute to neuroinflammation.[82,83] miR-146a interacts with the 3′UTR of complement factor H (CFH), a repressor of the inflammatory response, which is downregulated in AD.[84] miRNA-146a is an inducible, 22-nt, small RNA overexpressed in AD brain. Upregulated miRNA-146a targets several inflammation-related and membrane-associated mRNAs, including those encoding CFH and the interleukin-1 receptor associated kinase-1 (IRAK-1), resulting in significant decreases in their expression. The most significant miRNA-146a-CFH changes are found in HMG cells, the "resident scavenging macrophages" of the brain.[85] miR-101 interacts with cyclooxygenase-2 (COX-2), and downregulation of miR-101 may induce COX-2 upregulation in AD, enhancing the inflammatory response.[58] miR-124, miR-125b, miR-132, miR-134, miR-138, and miR-219 influence synaptic plasticity.

Table 2 Epigenetic targets for therapeutic intervention

- **DNA methylation/demethylation**
  - DNA methyltransferases (DNMTs)
  - DNA demethylases
    - TET family
    - AID/APOBEC family
    - VER glycosylase family
- **Histone modification**
  - Chromatin regulators
    - ATP-dependent chromatin remodeling complexes
      - SWI/SNF (switching defective/sucrose nonfermenting) family
      - ISWI (imitation SWI) family
      - CDH (chromodomain, helicase, DNA binding) family
    - Coactivators
    - Corepressors
  - Histone acetylation
    - Histone lysine acetyltransferases
      - KAT2A/GCN5
      - KAT2B/PCAF
      - KAT6-8
      - CREBBP/CBP
      - EP300
- **Histone deacetylation**
  - Histone deacetylases (HDACs)
    - Class I HDACs
      - HDAC1, 2, 3, 8
    - Class IIa HDACs
      - HDAC4, 5, 7, 9
    - Class IIb HDACs
      - HDAC6, 10
    - Class III HDACs (sirtuins)
      - Nuclear sirtuins
        - SIRT1, 2, 6, 7
      - Mitochondrial sirtuins
        - SIRT3, 4, 5
      - Cytoplasmic sirtuins
        - SIRT1, 2
    - Class IV HDACs
      - HDAC11
  - Histone ubiquitylation
  - Histone sumoylation
    - SUMO E3 ligases
  - Histone phosphorylation
    - Protein kinases
  - Histone dephosphorylation
    - Protein phosphatases
  - Histone deacylation
  - Histone ribosylation
  - Histone methylation
    - Histone lysine methyltransferases
  - Histone demethylation
    - Histone lysine demethylases
- **Regulation of long noncoding RNAs (lncRNAs)S**
  - Small RNAs (<200 nt)
    - Structural RNAs
      - Ribosomal RNA (rRNA)
      - Transfer RNA (tRNA)
      - Small nuclear RNA (snRNA)
    - Regulatory RNAs
      - MicroRNAs (miRNAs)
      - Small interfering RNAs (siRNAs)
      - Small nuclear RNAs (snRNAs)
      - Piwi-interacting RNAs (piRNAs)
      - Splice junction-associated RNAs
  - Long RNAs (>200 nt)
    - Large intergenic noncoding RNAs (lincRNAs)
    - Natural antisense transcripts (NATs)
    - Noncoding RNA expansion repeats
    - Promoter-associated RNAs (PARs)
    - Enhancer RNAs (eRNAs)
    - Small activating RNAs (saRNAs)
  - RNAi
    - miRNAs
    - siRNAs
    - piRNAs

miR-132 is downregulated and miR-125b is upregulated in different AD brain regions, probably affecting miniature excitatory postsynaptic currents (mEPSCs).[86]

The INK4b–ARF–INK4a locus encodes for two cyclin-dependent kinase inhibitors, p15(INK4b) and p16(INK4a), and a regulator of the p53 pathway, ARF. ANRIL, a noncoding RNA, is also transcribed from the locus. ARF, p15(INK4b), and p16(INK4a) are well-established tumor suppressors whose function is frequently disabled in human cancers. SNPs mapping in the vicinity of ANRIL are linked to a wide spectrum of conditions, including cardiovascular disease, ischemic stroke, type 2 diabetes, frailty, and AD. The INK4b–ARF–INK4a locus is regulated by polycomb repressive complexes, and its expression can be invoked by activating signals. Other epigenetic modifiers such as the histone demethylases JMJD3 and JHDM1B, the SWI/SNF (switching defective/sucrose non-fermenting) CRC, and DNA methyltransferases regulate the locus interplaying with polycomb repressive complexes.[87]

6. Conclusion

AD is a polygenic/complex disorder in which hundreds of defective genes distributed across the human genome are involved in close interaction with environmental factors, cerebrovascular dysfunction, and epigenetic changes. Epigenetics involves heritable alterations of gene expression and chromatin organization without changes in the DNA sequence. Epigenetic factors have emerged as important mediators of development and aging, gene–gene and gene–environmental interactions, and the pathophysiology of complex disorders. Epigenetic mechanisms are crucial to stabilize cell type-specific gene-expression programs.

Major epigenetic mechanisms [DNA methylation, HMs (acetylation, methylation, phosphorylation, sumoylation, ubiquitylation, glycosylation, ADP ribosylation, biotinylation) and chromatin remodeling and noncoding RNA regulation] may contribute to AD pathology. Both hypermethylation and hypomethylation of DNA and chromatin changes can affect AD-related gene expression leading to the multistep process of premature neurodegeneration. HMs are essential epigenetic features, with fundamental roles in biological processes such as transcription, DNA repair, and DNA replication. Histone acetylation is achieved by the action of HAT, resulting in chromatin/transcriptional activation; histone deacetylation is produced by HDACs that induce chromatin inactivation and transcriptional repression. Several lncRNAs are dysregulated in AD, and alterations in epigenetically regulated miRNAs may contribute to the abnormal expression of pathogenic genes in AD. AD-related SNPs interfere with miRNA gene regulation and affect AD susceptibility. The dysregulated miRNA network contributes to the aberrant gene expression in AD. miRNAs can be used as biomarkers and as therapeutic weapons in the pathogenic cascade of aberrant events underlying neurodegeneration. Despite the availability of attractive data supporting the role of epigenetic dysregulation in AD pathogenesis, this field is still in a primitive stage, and it is unclear whether epigenetic modifications represent a primary pathogenic mechanism or are the consequence of AD phenotypic expression. As primary or secondary pathogenic events in AD, epigenetic modifications are reversible and can be potentially targeted by pharmacological and dietary interventions. At present, the fragmentary, incomplete information available suggests that the development of novel therapeutic strategies to reverse epigenetic abnormalities might be of use to regulate the aberrant expression of pathogenic genes, to prevent premature neurodegeneration, and/or to halt disease progression.

Table 2 lists the potential epigenetic targets for therapeutic intervention. As another potential candidate drugs for AD,[86] novel strategies with epigenetic drugs are under development, and some compounds of this pharmacological category have been approved by the Food and Drug Administration for the treatment of neoplastic processes.[88]

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