The Role of MicroRNAs in Alzheimer's Disease: Pathological mechanisms, potential diagnostic and therapeutic values

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Abstract. Alzheimer's disease (AD) is a common and complex neurodegenerative disorder, and the growing prevalence and incidence of AD are expected to place a significant emotional and economic burden on society in the coming decades. MicroRNAs(miRNAs), a type of small-molecule non-coding regulate gene expression by attaching to target mRNAs and preventing translation or encouraging destruction, thereby contributing to a range of biological activities. It has been found that multiple miRNAs are dysregulated in brain tissue and peripheral body fluids of AD patients, and the pathogenic processes that these miRNAs are implicated in include AD-associated β-amyloid metabolism, tau protein phosphorylation, synaptic structural and quantitative changes, neuroinflammation, and organelle dysfunction. Nonetheless, there are ongoing debates over the molecular pathophysiology of AD, and effective diagnostic and therapeutic approaches are desperately needed. In this paper, the role of miRNAs in the pathological mechanisms, diagnosis, and treatment of AD are reviewed, which provide a reference for clinical applications.

Keywords: Alzheimer's disease, microRNA, pathogenesis, Diagnostic, Therapies.

1. Introduction

Alzheimer's disease (AD) is defined as a central nervous system (CNS)-related degenerative disorder, primarily affects older adults and pre-geriatric people and is typified by progressive cognitive dysfunction and behavioral abnormalities. This disease is clinically manifested by memory impairment, attention deficits, impaired language skills, visuospatial impairment, and personality and behavioral changes. With the growing global aging crisis, the incidence of AD continues to rise; approximately Dementia affects 55 million people globally, with 60% to 70% of these cases attributed to AD. According to data published by WHO, the number of people with AD in the world is expected to increase to more than 131.5 million, and the total direct healthcare expenditures and informal care costs of AD and related dementia are projected to reach 2.5 trillion dollars by 2025. AD not only imposes emotional and physical burdens on patients and their families but also has a significant socioeconomic impact. The pathogenesis of AD is a multifactorial, multistep process, with key pathological features including the accumulation of β-amyloid (Aβ) plaques in the brain, abnormal aggregation and hyperphosphorylation of tau proteins to form neurofibrillary tangles, neuroinflammation, and oxidative damage to the intracellular environment. Currently, clinical diagnostic methods for AD rely on clinical cognitive assessments, brain imaging, and CSF testing. The core markers of AD are the levels of Aβ, total tau protein, and phosphorylated tau protein in cerebrospinal fluid (CSF). However, these methods are not only costly and associated with low patient compliance but also have drawbacks such as low sensitivity and specificity and relative invasiveness. The clinical significance of CSF indicators has also been questioned. Only three medications have received approval from the US Food and Drug Administration to treat AD: cholinesterase inhibitors, memantine, and lecanemab. While these drugs show promise for AD treatment, they primarily slow disease progression rather than cure the disease. Therefore, developing new treatment strategies for AD is critical.

MicroRNAs (miRNAs) are a class of small-molecule non-coding RNAs, approximately 22 nucleotides long, that mature from precursor miRNAs through the action of enzymes such as Dicer. Mature miRNAs bind to RNA-induced silencing complexes and inhibit the translation of target mRNAs or facilitate their degradation by binding to the 3' untranslated region of target mRNAs,

thereby finely regulating gene expression. MiRNAs play crucial regulatory roles in a wide range of biological processes, including: (1) cell proliferation and differentiation: miRNAs are crucial for tissue development, stem cell differentiation, and cell cycle regulation. (2) Apoptosis: miRNAs can participate in apoptotic processes by regulating apoptosis-related genes such as Bcl-2 family members. (3) Metabolic regulation: miRNAs are involved in regulating various metabolic pathways, including lipid and glucose metabolism. (4) Immune response: miRNAs have significant roles in both innate and adaptive immune responses, regulating cytokine expression and immune cell differentiation. Numerous miRNAs are differently expressed in AD patients' plasma, according to studies, and that the pathophysiology of AD is significantly influenced by gene expression alternation they control, opening up a novel way to diagnose AD early.

This review aims to outline the role of miRNAs in the pathophysiology of AD and systematically evaluate the scientific development of miRNAs in the early diagnosis and treatment of AD, and focus on the value of specific miRNA biomarkers. Additionally, this review explores the prospects of applying CRISPR technology in miRNA regulation for AD treatment and elaborates on the potential of miRNAs as diagnostic biomarkers and therapeutic targets for AD.

2. Involvement of MiRNAs in AD Pathogenesis

AD is believed to be brought on by a confluence of environmental, age, and genetic variables, such as aberrant accumulation of brain proteins, neuroinflammation, vascular problems, and lifestyle choices. According to recent research, multiple critical roles of miRNAs in the pathogenesis of AD, which involve Aβ production and deposition, tau protein aggregation and phosphorylation, neuroinflammatory regulation, neuronal survival and death, and changes in organelle function.

2.1. Aβ Production and Deposition

Aβ is produced from amyloid precursor protein (APP) through progressive cleavage by β-secretase and γ-secretase. APP undergoes two enzymatic processing pathways: the α-pathway and the β-pathway. Under normal physiological conditions, APP, a transmembrane protein, is mostly transported to the cell membrane surface via vesicles. At the cell membrane, APP is more likely to interact with α-secretase and is subsequently cleaved by γ-secretase (the α-pathway), generating harmless fragments. When cells age or become damaged, APP is more likely to interact with β-secretase before being cleaved by γ-secretase (the β-pathway), producing neurotoxic Aβ fragments—including Aβ40 and Aβ42-that readily self-aggregate into toxic oligomers and eventually form amyloid plaques. Aβ clearance from the brain occurs primarily through enzymatic degradation (e.g., enkephalinase and insulin-degrading enzymes), phagocytosis by microglia, and transport across the blood-brain barrier. When cellular physiology is abnormal, Aβ production exceeds its clearance, leading to Aβ deposition.

MiRNAs are involved in Aβ metabolism by regulating Aβ production in the brain. MiR-106a and miR-520a have been found to target the 3'-UTR of APP mRNA and restrict the production of its protein in human cells. BACE1 (Beta-secretase 1), a protein-coding gene located on chromosome 11q23.3, encodes a beta-secretase that catalyzes the hydrolysis of APP to generate Aβ. MiR-107 and miR-29 both target BACE1 and inhibit BACE1 expression by directly attaching to the 3'-UTR of BACE1 mRNA in neural cells, reducing Aβ production.

MiRNAs are also involved in AD by affecting Aβ clearance. It is found in vitro that overexpression of miR-7a-5p, miR-29a-3p, and miR-146a-5p all markedly decreased phagocytosis by disease-associated microglia, thereby inhibited microglial Aβ clearance. Aerobic exercise significantly increases miR-532-5p levels in neuronal exosomes, improving blood-brain barrier structure and function and encouraging the passage of Aβ through the blood-brain barrier [1]. These studies indicate that miRNAs are directly or indirectly involved in Aβ metabolism, playing a vital part in AD pathogenesis as well.

2.2. Tau Protein Hyperphosphorylation

Tau is a protein associated with microtubules that is mostly present in the axons of neurons. Its main function is to stabilize microtubule structures and support axonal transport systems, ensuring the normal transmission of nerve signals, substance transport within neurons, and cellular structural integrity. Kinases and phosphatases are key regulators of protein function within cells, maintaining protein activity and cellular function through a dynamic balance of phosphorylation and dephosphorylation. Tau hyperphosphorylation occurs in AD patients when tau kinase and phosphatase activity are out of balance. Hyperphosphorylated tau loses function, has reduced solubility, and shows a significantly increased tendency to aggregate into insoluble fibers known as neurofibrillary tangles—primarily found in neuronal cytoplasm and a hallmark of AD brain pathology. Additionally, tau aggregates can be cleared via proteasomal degradation, autophagy, and phagocytosis by neuroglia.

MiRNAs trigger tau hyperphosphorylation and abnormal aggregation. Researchers have found that By attaching itself directly to the 3'-UTR of tau mRNA, miR-219 prevents tau production at the post-transcriptional phase. and affecting the stability and translational function of tau mRNA in mammalian cell models. Its downregulation leads to neuronal damage following tau deposition in neurons. GSK3β is a protein kinase whose overactivation causes tau hyperphosphorylation. It is discovered that AD is associated with dysregulated miR-128 expression; in vitro studies revealed that miR-128 suppress tau hyperphosphorylation by downregulating GSK3β, while in vivo studies revealed that upregulating miR-128 improved learning and memory deficiencies of mice. Additionally, miRNAs indirectly affect tau aggregation by influencing tau degradation. It is reported that miR-9, which targets the UBE4B gene, can impair tau clearance by inhibiting proteasome activity and disrupting autophagy pathways [2]. Overall, miRNAs affect tau synthesis and clearance through multiple mechanisms, leading to its hyperphosphorylation and deposition in neurons.

2.3. Neuroinflammation

Neuroinflammation exacerbates the pathological processes and symptoms of AD, with microglial activation being a key driver. In early inflammation, microglia support neuronal survival and synaptic repair by secreting anti-inflammatory and neurotrophic factors that limit excessive inflammatory responses. However, under persistent stimuli (e.g., chronic infection), microglia become overactivated, shift to a pro-inflammatory phenotype, and release sustained large quantities of pro-inflammatory factors and cytokine mediators, causing further neuronal damage and worsening AD pathogenesis. MiRNAs can influence AD progression by either inhibiting or promoting microglia-associated inflammatory pathways.

A crucial component of the TLR/IL-1R signaling pathway is IRAK1. IRAK1 activation further activates downstream molecules such as TRAF6, ultimately triggering the transcription factors being activated encompassing NF-κB and MAPK, which enter the nucleus and initiate the expression of inflammation-related genes, inducing an inflammatory response. Nitric oxide synthase, encoded by the iNOS gene, induces NO synthesis; NO has dual roles in inflammation, with both excessively high and low concentrations exacerbating inflammation. MiR-146a reduces neuroinflammation by targeting and inhibiting molecules like IRAK1, genes such as iNOS, and decreasing the expression of pro-inflammatory cytokines like IL-1. However, while miR-146a can inhibit excessive inflammatory responses, its continued upregulation and activation may lead to chronic inflammation and further neuronal damage. PU.1 and C/EBP-α, two transcription factors linked to microglial activation, are targeted by miR-124, which encourages the transformation of microglia into a neuroprotective phenotype; it also attenuates neuroinflammation by inhibiting key inflammatory signaling molecules (e.g., p65, JNK). In AD, downregulated miR-124 expression disrupts this anti-inflammatory regulation. ATG16L1 gene is one of the important members of the autophagy-related gene family. ATG16L1 proteins combine with ATG5 and ATG12 proteins to form complexes, guide the localization of autophagy-related proteins, promote the formation of autophagosomes, thereby degrading misfolded proteins and damaged mitochondria accumulated in neurons, and preventing

them from overactivating microglia and inducing inflammation. The researchers found that miR-223 downregulation can inhibit neuroinflammation by increasing the expression of autophagy gene ATG16L1 [3].

2.4. Changes in the Number and Structure of Synapses

Synapses are sites of information transfer between neurons, and changes in their number and structure have a significant impact on the development of AD. Among the primary pathogenic traits of early AD is thought to be synapse loss and cognitive decline; studies show that reduced synapse density strongly correlates with memory loss and other cognitive dysfunctions, and regions of synapse loss often coincide with brain regions showing significant symptoms in AD patients. Synaptic structural changes involve abnormalities in multiple presynaptic, interstitial, and postsynaptic components, collectively disrupting neural signaling and serving as an important pathological basis for cognitive decline in AD patients. MiRNAs are involved in AD pathogenesis through regulating synaptic number, structure, and function.

Dendritic spines are important structures on the postsynaptic membrane that receive signals. MiR-431 has been found to reduce the number of dendritic spines in AD sufferers' brains and cause abnormal morphology (e.g., elongation, reduced branching). These changes decrease the postsynaptic membrane surface area, impairing the ability of postsynaptic neurons to receive neurotransmitter signals, disrupting information transmission, and leading to cognitive impairment. MiR-431 levels are suppressed in AD patients' plasma. Lentivirus-mediated miR-431 overexpression in the CA1 area of the hippocampus reduced memory impairments and synaptic plasticity, partially rescued reductions in the quantity of prominences and thickness of Densities of postsynaptic connections in the CA1 area of the hippocampus as well as increased dendritic spine density, thereby improving cognitive function in APP/PS1 mice [4].

Cholinergic neurons, which use acetylcholine (Ach) as a neurotransmitter, are widely dispersed in the central and peripheral nervous systems. They support higher cognitive functions by regulating synaptic transmission and the activity of extensive neural networks. MiRNAs have been shown to target elements that comprise the cholinergic system or cause functional changes in cholinergic neurons by impacting the pathways linked to the survival and plasticity of cholinergic neurons. Acetylcholinesterase mRNA's 3'-UTR is the target of miR-132 and miR-212, which inhibit acetylcholinesterase-mediated breakdown of acetylcholine in the synaptic cleft and affects cholinergic signaling efficiency [5].

2.5. Mitochondrial Dysfunction

Cells' main energy providers are mitochondria, which make ATP via oxidative phosphorylatio. Abnormal mitochondrial function reduces ATP production, failing to meet the high energy demands of neurons, thereby affecting neurotransmitter synthesis and release, synaptic plasticity, and other key processes, leading to cognitive impairment. Additionally, mitochondrial dysfunction increases oxidative stress, and accumulated reactive oxygen species exacerbate neuronal damage. Studies also show that mitochondrial dysfunction promotes Aβ deposition and tau hyperphosphorylation, collectively causing neuronal dysfunction and AD development. MiRNAs play an important role in regulating mitochondrial dynamics, providing potential targets for AD therapeutic intervention.

Mitochondrial dynamics refer to processes by which mitochondria maintain cellular energy homeostasis and physiological functions through fusion and fission. In AD patients, mitochondrial dynamics are altered, characterized by reduced fusion and increased fission leading to mitochondrial fragmentation; this structural alteration impairs neuronal survival and function. MiR-30 reduces mitochondrial fragmentation and neuronal damage by downregulating p53 and suppressing its downstream target dynamin 1's expression, thus exerting a protective role in AD pathogenesis [6]. Bcl-2 is a key anti-apoptotic gene; its encoded proteins inhibit apoptosis by preventing mitochondrial membrane potential decline, inhibiting the release of pro-apoptotic substances from mitochondria, and reducing reactive oxygen species (ROS) production, thereby maintaining mitochondrial stability

and reducing oxidative stress-induced damage. MiR-34a and miR-181a target and downregulate Bcl-2 to control mitochondrial dynamics, and their overexpression promotes AD development [7].

3. Potential Diagnostic and Therapeutic Value of MiRNAs in AD

3.1. Potential Diagnostic Value of MiRNAs in AD

In AD, miRNAs have several advantages as biomarkers, including stability and the ability to be detected with high sensitivity and specificity using genetic methods or specific biosensors. Certain specific miRNAs show significant expression changes in the plasma, CSF, and serum of AD patients, offering new avenues for early disease diagnosis. Brain-derived extracellular vesicles (EVs) can carry neuron-specific molecules (e.g., miRNAs) across the blood-brain barrier. Reho et al. identified 14 AD-associated miRNAs carried by serum-derived neuronal extracellular vesicles, which showed more pronounced transcriptional changes in clinical AD patients, demonstrating the potential of miRNAs as markers for neurodegenerative diseases [8]. Some studies have also used multi-miRNA combination patterns to differentiate between healthy people and AD patients, improving diagnostic sensitivity and specificity and achieving high accuracy. Kumar et al. found that differential miRNA expression in extracellular vesicles from mild cognitive impairment (MCI), MCI-AD, and AD patients significantly distinguished dementia status from the cognitively normal (CN) group and correlated with greater dementia severity, as indicated by reduced temporal cortical thickness on magnetic resonance imaging (MRI) [9]. However, standardized miRNA detection methods are still lacking. Zhang et al. reported a probe nanocomplex labeled with aggregation-induced emission fluorescence that acts as a fluorescent biosensor to detect miR-125b. This fluorescent biosensor may therefore serve as a pratical technique for future monitoring of miRNAs associated to AD [10].

3.2. Potential Therapies of MiRNAs in AD

MiRNAs can regulate multiple AD-related genes and signaling pathways, influencing processes such as Aβ production and clearance, tau phosphorylation, neuroinflammation, and cellular function. Small RNA molecules known as miRNA mimics imitate the composition and functionality of natural miRNAs; upon entering cells, they bind to target mRNAs and regulate gene expression like natural miRNAs. In AD, miR-146a expression is downregulated. Liang et al. delivered miR-146a mimics to the AD mouse model's hippocampal region via the nasoencephalic pathway to upregulate miR-146a expression, altering microglial phenotype to regulate neuroinflammation and alleviate AD pathogenesis [11]. MiRNA inhibitors are small molecules (e.g., antisense oligonucleotides) that specifically bind to and block endogenous miRNA function; they form stable complexes with target miRNAs through base complementary pairing, preventing miRNAs from binding to target mRNAs and thus inhibiting their impact on target genes in terms of regulation. In AD, miR-429-3p expression is upregulated. Luo et al. delivered a miR-429-3p inhibitor to the hippocampal CA1 region via intranasal administration, inhibiting miR-429-3p-mediated negative regulation of its target gene MKP-1, reducing Aβ generation and deposition, and improving cognitive performance in individuals diagnosed with AD [12]. Antisense inhibitors and sponges represent two additional techniques crafted for research on impairing miRNA function; nevertheless, the stability and specificity of these conventional approaches remain subpar. CRISPR-Cas9 can be used to design sgRNAs targeting miRNA precursor sequences (pre-miRNAs). Guided by sgRNAs, Cas9 proteins bind to and cleave pre-miRNA sequences. After DNA cleavage, cells use non-homologous end-joining (NHEJ) to introduce mutant bases or deletions into the mature region or neck loop of pre-miRNAs, disrupting the overall neck loop structure, blocking pre-miRNA processing, inhibiting mature miRNA production, and consequently reducing miRNA expression. CRISPR-Cas technology provides a new, more efficient approach for miRNA targeting, with higher accuracy and specificity than other gene editing methods.

4. Conclusion

This study provides a systematic summary of the various functions of microRNAs in the pathophysiology, diagnostic utility, and therapeutic uses of AD. Studies show that miRNAs are involved in AD development by regulating key pathological processes such as Aβ metabolism, tau phosphorylation, neuroinflammation, synaptic plasticity, and mitochondrial function. Among these, miR-186 and miR-29 inhibit Aβ production by targeting BACE1; miR-128 reduces tau hyperphosphorylation by downregulating GSK3β; miR-146a regulates microglial phenotype to alleviate neuroinflammation; miR-431 improves memory deficits by increasing synaptic density; and By focusing on and inhibiting Bcl-2, miR-34a and miR-181a control mitochondrial function., inhibiting neuronal apoptosis through effects on mitochondrial dynamics, all playing important regulatory roles in AD pathogenesis.

In terms of diagnosis, differential miRNA expression in the plasma, cerebrospinal fluid, and extracellular vesicles of AD patients provides a new direction for early disease diagnosis. Technologies such as multi-miRNA combination detection and aggregation-induced emission fluorescence biosensors can help improve diagnostic sensitivity and specificity, addressing limitations of existing detection methods. In therapeutics, miRNA mimics and inhibitors have shown potential value: for example, miR-146a mimics can regulate microglial phenotype via the nasoencephalic pathway, and Aβ deposition can be decreased by miR-429-3p inhibitors, thus cognitive performance can be enhanced. Additionally, CRISPR-Cas9 technology provides an efficient tool for precise regulation of miRNA expression, opening new avenues for AD treatment.

Despite significant research progress, multiple challenges remain including lack of standardized, accurate miRNA detection methods, undetermined reference ranges for miRNAs under different physiological conditions, the existence of multi-targeting and off-target effects, and poor stability in the cellular environment. While miRNA-based diagnostic and therapeutic approaches are promising, their application in AD lags behind other disease areas. Future research should further explore miRNA mechanisms of action, establish unified detection standards, and advance targeted delivery technologies to facilitate the clinical translation of miRNAs in AD diagnosis and treatment.

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