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MicroRNAs as a Diagnostic Marker and a Therapeutic Target for Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a progressive, neurodegenerative disease and is the most common cause of dementia. The disease pathology of AD is marked by the accumulation of Beta-amyloid (Aβ) plaques and deposition of phosphorylated tau NFTs in the brain. Currently, the therapeutic approaches to AD are solely palliative in nature and thus can do little to stop or reverse the disease pathology once it has begun. MicroRNAs (miRNAs) are a class of noncoding nucleotides that are critically involved in the regulation of various post-transcription mRNA processes, such as synapse plasticity and neuron differentiation. Owing to the multifactorial nature of AD, miRNAs have garnered much attention in the research field due to their concomitant dysregulation and appearance in several AD pathophysiological processes. This review paper offers insight into the potential use of miRNAs as a therapeutic target and diagnostic marker in AD.

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1. Introduction

Alzheimer's disease (AD) is the leading cause of dementia in the world, with a disease burden exceeding more than 5.5 million individuals in the United States and up to 80% of all dementia diagnoses overall[1]. The two clinical hallmark pathologies of the disease are the Beta-amyloid (Aβ) plaque and deposition of neurofibrillary tangles (NFTs) of hyperphosphorylated tau hypotheses. The Beta-amyloid hypothesis suggests that an accumulation of amyloid plaques in the brain triggers a cascade of pathological events that ultimately lead to the clinical presentation of the disease[1]. Isoforms of the Aβ peptide are implicated in the beta-amyloid hypothesis, and Aβ38, Aβ40, and Aβ42 exhibit the greatest tendency to cluster and produce the neuronal oligomers that trigger the amyloid cascade[1]. Within this cascade, various biological stressors, such as localized inflammation, glutamate production, and oxidation, are involved. Conversely, the Tau hypothesis entails the process of hyperphosphorylated tau decoupling from their normal microtubule function to form NFTs that disrupt normal neuronal function and transport. While the Beta-amyloid and Tau hypothesis were initially regarded as two distinct pathophysiological pathways, recent evidence has given credence to the notion that both pathways act interdependently, resulting in the clinical presentation of AD[1].

Symptoms of cognitive impairment and memory loss are the main assessment criteria for AD patients, and differential diagnosis can be made through cerebrospinal fluid (CSF) and positron emission tomography (PET) analysis[1]. These methods, however, suffer from issues such as the high cost of application, invasiveness, and low specificity that combine to limit their accessibility and usage in routine clinical scenarios[2]. As such, a definitive diagnosis of AD can only be made through post-mortem tissue analysis of suspected AD patients.

The current treatment landscape for AD primarily involves palliative therapeutic approaches, such as cholinesterase inhibitors and N-methyl D-aspartate (NMDA) antagonists like memantine. Cholinesterase inhibitors include drug classes like donepezil and galantamine and work by stopping the degradation of acetylcholine molecules in the brain[3]. Cholinesterase inhibitors are typically prescribed for AD patients in the mild, moderate, or

severe dementia stages based on the mini-mental state examination score (MMSE)[4]. NMDA antagonists function by regulating glutamate action in the brain[3]. While the current pharmacological approaches for AD can enhance patient quality of life, they are unable to alter disease progression or overall life expectancy for individuals with AD. As such, there exists a great need to develop a non-invasive, cost-effective approach to provide robust early diagnosis and treatment options for this condition in the medical community.

MicroRNAs (miRNAs) are a class of small, non-coding RNA molecules that have the potential to act as therapeutic agents and biomarkers in AD. Ranging approximately 22 nucleotides in length, miRNAs work on the post-transcriptional level of mRNAs and are involved in the regulation of nearly every cell and protein-coding process in the body[5]. A few of the regulatory facets that miRNAs act on include cellular differentiation, proliferation, as well as programmed cell-death mechanisms like apoptosis[6]. A single miRNA can alter up to 200 mRNAs and multiple genes, thus making them increasingly valuable targets for research into how changes in their expression levels contribute to disease states such as AD or cancer[5]. MiRNAs specifically function by attaching and binding to the 3' untranslated region (UTR) of their associated mRNAs, in which the complementarity between the miRNA and its target eventually results in the degradation of the mRNA targets[7]. Current research shows that approximately 70% of identified miRNAs have expression patterns in the brain, which further highlights the dynamic potential of miRNA processes in healthy brain function and diseased states[7]. Within the brain and nervous system environment, miRNAs are particularly involved in regulating synapse plasticity, dendritic spine morphology, and neurite outgrowth, thus contributing to the modulation of the cognitive and memory functions typically lost in AD pathology through acting upon protein synthesis in synapses[8].

MiRNAs are predominantly found in circulatory biofluids, such as blood serum and plasma[9]. Due to its proliferation in the blood serum, miRNA has wide potential to be a cost-effective and less invasive diagnostic approach for AD. In this review, we focus on exploring the most recent advances in the

research and development of miRNAs involvement in AD, which has broad therapeutic and diagnostic potential that remain to be unlocked.

2. Pathophysiology of Alzheimer's Disease

2.1. Amyloid plaques

Amyloid pathogenesis starts with altered cleavage of Aβ peptide from the internal transmembrane protein amyloid precursor protein (APP) by the alpha, beta, and gamma secretase[10]. Two main types of Aβ polymers are involved in plaque formation and induced neurotoxicity. Aβ40 is more abundant and less neurotoxic than Aβ42, which is highly insoluble and neurotoxic. Aβ40/Aβ42 aggregation blocks ion channels, alters calcium homeostasis, increases mitochondrial oxidative stress, and diminishes energy metabolism and glucose regulation, which promotes neuronal cell death[11]. Under normal conditions, proteolysis of APP by either alpha or β-secretases (BACE1) cleaves off small nontoxic fragments. However, altered cleavage of APP by β followed by γ-secretases results in 42 amino acid peptides (Aβ42) which then aggregate to form oligomers that diffuse into synaptic clefts and interfere with synaptic signaling. Increased level of Aβ42 leads to the polymerization of insoluble amyloid fibrils that causes neuronal toxicity[10,11,12]. Accumulation of these amyloid plaques initially occurs in the basal, temporal, and orbitofrontal neocortex region of the brain and later progresses throughout the hippocampus, amygdala, and cerebral cortex[12].

Sustained elevation and continuous aggregation of dense amyloid plaques can stimulate a chronic response of the innate immune system by inducing astrocytes and microglia recruitment surrounding the plaques. This results in the increased level of local inflammatory response due to the elevated release of inflammation related mediators, such as complement factors, eicosanoids, chemokines, and proinflammatory cytokines, which can disrupt microglial clearance of amyloid plaques, while also increasing microglia mediated neuronal death, damage to axons and dendrites, synaptic loss, and neurotoxicity[10,11]. Consequently, these neuronal dysfunction inhibits cell synaptic communication, contributing greatly to AD pathogenesis.

2.2. Neurofibrillary Tangles (NFTs)

Tau protein, encoded by the microtubule associated protein tau (*MAPT*) gene, is a microtubule-associated protein that can form insoluble filaments that accumulate as NFTs. Tau protein facilitates the maintenance of neuronal structure and function. In Alzheimer's, the abundance of Aβ plaques in the cell causes the Tau protein to become hyperphosphorylated, leading to oligomerization. Consequently, the loss of its affinity for tubulin leads to dissociation of tubule subunits that eventually fall apart and form large fragments of tau filaments that can aggregate into NFTs. NFTs are straight, fibrillary, and highly insoluble patches in the neuronal cytoplasm that are composed of paired helical fragments (PHFs) of tau fibrils approximately 20 nm in diameter. They spread throughout the brain as AD progresses, beginning near the entorhinal cortex, leading to abnormal loss of communication between neurons and signal processing and finally apoptosis, cell death, in neurons. In addition, the transfer of hyperphosphorylated tau proteins diffuse to surrounding cells and cause damage to neuronal function, contributing to the onset of AD. Genomic studies of brain tissues of AD patients have found significant downregulation of miRNA-124 and miR-425-5p, which under normal conditions inhibit the abnormal hyperphosphorylation of Tau protein. On the other hand, a significant upregulation of miR-132 and miR-125b in AD patients has been found to induce Tau protein phosphorylation and neuronal apoptosis.

Moreover, miRNAs could promote the progression of AD by promoting phosphorylation of the Tau protein. miR-483–5p, miR-125b-5p and miR-23b indirectly regulates Tau phosphorylation by targeting extracellular signal-regulated kinases 1 and 2(ERK1/2), protein phosphatase methylesterase-1 (PME-1) and N-acetylglucosaminyltransferase III (GNT-III) signaling pathways, respectively[13]. Amyloid pathology appears to precede that of tau, with NFTs only being found in regions where amyloid was already present, but both Aβ plaques and neurofibrillary tau tangles are significant factors of the pathogenesis of AD that lead to synapse loss and neuronal atrophy, especially throughout the hippocampus and cerebral cortex[11, 12].

2.3. Microglial Activation

Microglia are macrophages that reside in the CNS and play a significant role in maintaining neuronal plasticity, synapse remodeling, phagocytosis, and clearance of Aβ plaques in healthy individuals. The binding of microglia to extracellular Aβ plaques via cell-surface receptors such as SCARA1, CD36, CD14, α6β1 integrin, CD47, and Toll-like receptors (TLRs), a class of pattern recognition receptors (PRRs) that ignites innate immune response, initiates the endocytosis of Aβ oligomers and NFTs for degradation by microglial proteases such as neurolysin and insulin-degrading enzymes[11]. However, under pathological conditions, the phagocytotic functioning of the Aβ oligomers by the microglia is weakened, and overexpressed Aβ plaques and NFTs can lead to microglial activation around the plaque areas and release inflammatory factors such as IL-1β and TNF-α, which aggravate neuronal damage and exacerbate AD pathology by giving rise to increased synaptic damage, oxidative stress, and neuroinflammation[7]. Furthermore, insufficient Aβ plaque clearance is observed due to elevated levels of localized cytokine concentrations that downregulate the expression of Aβ phagocytosis receptors, thus decreasing Aβ clearance[13]. Moreover, proliferation and activation of microglia in the brain can affect insulin receptor substrate 1 (IRS-1) and block intracellular insulin signaling, which has an important role in neural health[11].

3. Genetic basis

The role of genetics in AD pathogenesis accounts for about 70% of the AD cases. Most cases of early onset AD (EOAD) are known to be inherited in an autosomal dominant pattern and mutations in the dominant genes including Amyloid precursor protein (APP), Presenilin-1 (PSEN-1), Presenilin-2 (PSEN-2)[14], apolipoprotein E (ApoE), Clusterin (CLU), Bridging Integrator 1 (BIN1), and TREM2[15].

3.1. APP

APP is a transmembrane protein cleaved by α-, β-, and γ-secretase that releases Aβ and other proteins and is encoded by the APP gene on chromosome 21. Twenty-five out of thirty mutations have been found in the APP gene to be related to AD, along with the elevated level of insoluble Aβ aggregates. KM670/671NL mutation in mouse models has shown an increasing level of

amyloid plaques in the hippocampus and cortex. A673V, D678H, D678N, E682K, and K687N mutations have shown cortical atrophy, and E682K has shown hippocampal atrophy[15]. Moreover, A673V mutations have shown a presence of NFTs and Aβ, activation of microglia and astrocytes, and neuronal loss. Other mutations such as T714I, V715A, V715M, V717I, V717L, L723P, K724N, and I716V affect the γ-secretase cleavage site, increasing the Aβ42/Aβ40 ratio. E693G, E693K, D694N, and A692G mutations affect the α-secretase cleavage site, causing polymorphic aggregates that can disrupt bilayer integrity. Meanwhile, one protective mutation (A673T) has been identified, which protects against AD by decreasing Aβ, Aβ40, and Aβ42 secretion[15].

3.2. PSEN1 and PSEN2

Presenilin genes (PSEN1 and PSEN2) are part of the γ-secretase family and were also found to be mutated in AD[14]. It has been found that AD patients may be predisposed to PSEN1 mutation leading to familial AD at a young age. Mutation in the PSEN1 gene is more common, with more than 200 mutations identified. On the other hand, a rare form with less than 40 mutations was identified in the PSEN2 gene, causing an increased level of toxic forms of amyloid Aβ42 as opposed to non-toxic Aβ40, aggravating AD pathology. PSEN1 plays an important role in the production of Aβ from APP by activating γ-secretase. Knockout studies of PSEN1 in mice models showed synaptic dysfunction and memory impairment. On the other hand, PSEN-2 mutations might have a critical effect on the Aβ 42/40 ratio, causing familial AD in the presence of normal PSEN-1 alleles, but some of the PSEN-2 mutations are rare polymorphisms and are not pathogenic mutations and plays a relatively minor role in Aβ production[15].

3.3. APOE4

ApoE protein is a glycoprotein expressed highly in the liver and brain astrocytes and some microglia. It serves as a receptor-mediated endocytosis ligand for lipoprotein particles like cholesterol, which is essential for myelin production and normal brain function. The ApoE gene located on chromosome 19 has three isoforms, ApoE2, ApoE3, and ApoE4, which cause changes in the coding sequence[15]. The APOE4 variant is the most significant genetic risk factor for sporadic Alzheimer's disease (sAD). APOE4

has a multidimensional impact on the pathogenesis of AD, including dysregulation of lipids and lipoproteins, such as APOE plasma levels[13]. The ApoEε4 allele is a strong risk factor for both early onset AD (EOAD) and late onset AD (LOAD) compared to ApoEε2 and ApoEε3 alleles, which are associated with lower risk and protective effect, respectively. ApoEε4 plays an important role in Aβ deposition as a senile plaque and causes cerebral amyloid angiopathy (CAA), which is known as a marker for AD. ApoEε4 was also shown to be associated with vascular damage in the brain, which leads to AD pathogenesis[15].

3.4. CLU and BIN1

Clusterin (CLU) and Bridging Integrator 1 (BIN1) genes are novel risk factors for LOAD. CLU gene is reported to be located on chromosome 8. Under normal conditions, CLU plays a protective role by interacting with Aβ and promoting its clearance or a neurotoxic role by reducing Aβ clearance. However, in AD patients, CLU is upregulated in the cortex and hippocampus. The Aβ ratio values determine whether the CLU role is neuroprotective or neurotoxic. BIN1 is a Bin-Amphiphysin-Rvs (BAR) adaptor protein that is involved in the production of membrane curvature and other endocytosis cellular functions. BIN1 has several isoforms—some are found in the brain, where they interact with different proteins such as clathrin, synaptojanin, and amphiphysin 1, and others in which they regulate synaptic vesicle endocytosis. Recently, BIN1 was recognized as the second most important risk factor for LOAD after ApoE, where it plays a role in Aβ production and as a tau and NFT pathology modulator[15].

3.5. TREM2

TREM2 is a transmembrane receptor expressed in cells of the myeloid lineage. It facilitates mediating phagocytic clearance of neuronal debris and binds anionic carbohydrates, bacterial products, and phospholipids to transmit intracellular signals through the associated transmembrane adaptor DAP1255 and further phosphorylation of downstream mediators. In AD, a rare mutation of TREM2 (R47H) has been reported that plays a potent role in aggravating the risk of developing AD. This mutation leads to an inability of the receptors to clear Aβ from the CNS, contributing to Aβ accumulation and further intensification of pathogenesis in AD patients[16].

4. miRNA usage in the treatment of AD

As a result of their ability to post-transcriptionally regulate many of the genes involved in AD, miRNAs have emerged as a valuable potential therapeutic agent for the treatment of AD. Various miRNAs have been shown to be integral to the production and regulation of amyloid-beta, and thus modification of miRNA expression levels could attenuate facets of AD pathology[16]. Therapeutic approaches to AD utilizing miRNAs generally function through two pathways. MiRNAs expression levels may be restored to their normal baseline magnitude or be upregulated through the *in vivo* transfection of miRNA oligonucleotide that mimics endogenous miRNA functions. Alternatively, harmful miRNAs in AD can be downregulated through the administration of complementary antisense oligonucleotides[17].

Subsequently, the most recent studies in the field have demonstrated promising results of miRNAs facilitating neuroprotectant and ameliorating cognitive deficits through investigations using *in vivo* AD animal models and *in vitro* cell cultures[17]. In one study, upregulation of the miR-23b-3p molecule via *in vitro* transfection reduced hyperphosphorylated tau clusters and inhibited levels of neuronal apoptosis in Swedish mutant amyloid-precursor protein (APPswe) cells via inhibiting the upstream expression of glycogen synthase kinase-3 (GSK-3) apoptotic pathway[18]. This is significant since miR-23-b-3p has clinical diagnostic potential due to it being greatly downregulated in the blood plasma of AD patients when compared to healthy controls, and further investigations revealed a positive correlation between increased miR-23b-3p and improved measures of cognitive function for individuals with AD[18].

The miRNA-200 family has also been shown to play an innate defensive role against Aβ-induced neurotoxicity. A study conducted by Higaki et. al. demonstrated that administration of members of the miRNA-200 family – such as miR-200b/c – showcased reduced Aβ in cellular mediums and *in vivo* amelioration of Aβ-induced cognitive impairments in Tg2576 mouse models, thus leading to restoration of spatial memory performance in the Barnes maze test[19]. Aβ has been shown to degrade the neuronal memory centers and synapses that act upon the hippocampus and frontal cortex tissue, making the miR-200 family a potential therapeutic target due to their

ability to alter S6k1 levels and modulate extracellular insulin signaling in the brain[19]. Similarly, another study conducted by An et. al demonstrated the differential expression of miR-124 in AD compared to healthy control populations[17]. miR-124 was revealed to interact with the Beta-site Amyloid precursor protein Cleaving Enzyme 1 (BACE1) network in AD, which is prominently involved in AD via functioning in the production of Aβ from APP[20]. It was found that miR-124 expression was downregulated in AD models while BACE1 was simultaneously overexpressed[20]. Subsequently, miR-124 is a potential novel therapeutic target as its administration via transfected mimics could be utilized to regulate and inhibit expression of BACE1 mRNAs and proteins in affected AD tissues, resulting in lowered Aβ and NFT burden[20].

Many other miRNAs are upregulated in AD pathogenesis and contribute to neurotoxicity, and thus therapeutic approaches that leverage under-expressing these classes of miRNAs could potentially alleviate the negative effects on neurons posed by AD. Through an analysis study on SH-SY5Y cell cultures, it was shown that the pathogenesis of AD triggers dysregulation of miRNA-146a, causing significant overexpression of these miRNAs in the brain that results in the disruption of the phosphatase PTEN, thus causing increased hyperphosphorylation of tau protein and NFT cluster formation[21]. The effects of miRNA-146a overexpression were most pronounced in the brain regions that are generally most susceptible to tau aggregation in AD and other related dementias – most notably in the hippocampus and temporal cortices, which are fundamental to learning and memory formation[21]. In this same study, the 5xFAD transgenic mice model reacted positively to the downregulation of miRNA-146a via a series of miRNA-146a antagomir injections, ultimately showing a restoration of endogenous frequencies of phosphatase PTEN and a rescue of function in hippocampal memory and neural pathways[21]. While miRNAs have traditionally been downregulated in AD pathology, the overexpression of miR-146a has been experimentally shown to be intricately involved in the tau pathology of AD, thus making the selective inhibition of this miRNA molecule a viable target for an *in vivo* therapeutic of the disease. A more extensive list of identified miRNAs and their pathophysiologic targets in AD are displayed below in Table 1.

The clinical application of miRNA in AD can be achieved through diverse pathways and delivery methods. Studies have shown that naturally occurring compounds like resveratrol can act upon and regulate specific miRNAs in the body and, therefore, can be attuned to modulate miRNA expression levels *in vivo*[22]. Conversely, certain anti-inflammatory therapeutics can exhibit regulatory-like control on the expression of certain miRNA molecules, thus providing a treatment option for AD that functions via miRNA modulation[23]. Other innovative and emerging approaches involve using exosome vesicles as a targeted vehicle to effectively cross the blood-brain barrier and deliver miRNA into localized regions for the greatest benefit in the diseased brain[23].

miRNA	Target/Pathologic Process
miR-126	Involved in BACE1 expression; overexpression suppresses BACE1 while under expression increases BACE1 proliferation
miR-146	Observed to have higher levels of expression in MCI patients that later manifested with AD; is also associated with APOE4 protein expression
miR-26b	Shown to increase tau phosphorylation and neuronal apoptosis
miR-181a	Associated with an increased Aβ concentration in cerebrospinal fluid
miR-200b	Under expressed in experiments with APP/PS1 transgenic mice & positively reduced APP expression
miR-339-5p	Targets & inhibits BACE1; expression levels were shown to be reduced in AD patients
miR-16-5p & miR-708-5p	Differentially expressed molecule in the cerebrospinal fluid cells of AD patients; potential biomarker

miR-93-5p	Differentially expressed molecule in the cerebrospinal fluid cells of AD patients; potential biomarker

Table 1: List of the relevant miRNAs & the corresponding nervous system targets

5. MiRNAs functioning as predictive biomarkers of AD

5.1. Amyloid Cascade Hypothesis

There is evidence that Aβ and its imbalance between production and destruction is a leading cause of AD, as it causes a cascade of events in the bodily systems[24,25]. APP is cleaved by the BACE-1 gene first to produce the Aβ protein[25, 26]. Some proteases are meant to clear the Aβ in the system through proteolytic degradation and receptor-modulated endocytosis[24]. When this process is not done at an adequate rate, the Aβ protein can build up in the blood plasma or CSF. Some forms of this protein are toxic and are known to cause neurotoxicity, neuron apoptosis, inflammation, and synaptic loss, which all play a role in AD[24,25,27]. The buildup of Aβ protein is deposited as plaque in the systems of the brain and CNS, which in turn causes increased production of proinflammatory cytokines. These cause the degeneration of glial cells and their tissue, which causes and progresses Alzheimer's[25].

5.2. Other miRNA in pathogenesis of AD

Astrocytes regulate ion homeostasis in the brain, and therefore, support neuronal function. Additionally, astrocytes have been shown to release cytokines which play a crucial role in disease progression and creation[25]. miRNA is an important immunoregulator, and an imbalance in the expression of specific miRNAs can cause certain proteins or cytokines to be over or under-produced. For instance, an excess of inflammatory cytokines causes the nearby neural cells and neurons to die[25]. This process, along with the Amyloid Cascade Hypothesis, has shown that although AD cannot be pinpointed to one specific trigger or cause, the levels of particular miRNA play a role in the existence, pathogenesis, and pathophysiology of AD.

5.3. Specific miRNAs as biomarkers

There are many studies that show patterns in the levels of expression of specific miRNA segments that relate to AD[26]. In the serum of AD patients, segments such as miR-149, miR-34a-5p, miR-125b-5p, miR-15b, miR-16,

miR-124, miR-29c and miR-374b-5p were all found in reduced quantities when compared to healthy individuals[27]. Out of these, miR-149, miR-34a-5p, miR-16, miR-29c and miR-374b-5p were found to directly target BACE-1[27]. This relates to the upregulation of BACE-1 found in AD patients, which in turn cleaves the APP and promotes the production of Aβ proteins. There were other miRNAs, such as miR-146a and miR-181a, which were found in higher concentrations in AD patients, more specifically in patients who initially had mild cognitive impairment (MCI) and later progressed into AD[21]. In different parts of the body, the expression levels of different miRNAs were shown to have similar patterns. The downregulation of miR-132/212 and miR-335–5p in the brain tissues were found in AD patients, which caused a higher production of Aβ and more plaque buildup in the brain, causing symptoms consistent with the disease[26]. Generally, miRNA has shown to be strongly related to the pathophysiology of Alzheimer's and plays a direct role in many of the genes of interest.

6. Genes/factors of interest

6.1. APP

Studies have indicated that an increased APP level due to genomic locus duplication or mutation in the *APP* regulatory sequences can result in the development of early-onset dementias, including AD. APP regulation is of special interest as it provides valuable insight into the genetic basis of AD and its novel therapeutics. A study that tested the regulatory effect of miRNAs on the level of APP gene expression demonstrated that complementary binding between miRNAs hsa-mir-106a and hsa-mir-520c and their predicted target sequences within the 3′ UTR of APP genes results in repression of reporter gene expression, and that over-expression of these miRNAs resulted in translational repression of *APP* mRNA and significantly reduced APP protein levels in human cell lines. These results were the first to experimentally demonstrate the post-transcriptional regulatory role of miRNA in the levels of human APP[28]. On the other hand, another study has demonstrated the activity of miR-346, which requires the chelation of an intracellular iron Fe, that targets the APP mRNA 5′-UTR to upregulate APP translation and Aβ production. It has also been found that miR-346 levels are altered in late-Braak stage AD. As a result, miR-346 leads to the upregulation of APP in the CNS, and participate in maintaining APP

regulation of Fe, which is disrupted in the late stages of AD[29]. Substantial progress towards understanding the role of miRNA-induced alterations in the APP gene expression and Aβ production levels and the multifaceted biological involvement of miRNAs in APP metabolism is of interest to current research underlying the pathophysiology of AD[28,29].

6.2. Hyperphosphorylated Tau Peptide (p-tau)

Several functional *in vivo* studies have demonstrated the potential of microRNA-23b-3p (miR-23b-3p) in various neurologic disorders[30]. Study results support the potent involvement of miR-23b-3p as a neuroprotectant in AD. Functional *in vivo* studies demonstrated that intracerebroventricular delivery of miR-23b-3p in APP/PS1 mice reduced cognitive impairment and p-tau levels, while upregulation of miR-23b-3p resulted in an increased level of p-tau, Aβ production, and neuronal apoptosis. These results thereby identify miR-23b-3p as a promising therapeutic target for AD[30]. Several studies have speculated the functional role of tau in promoting microtubule assembly, stabilization, and spacing necessary for axonal transport. Moreover, 6 isoforms of tau are present in the central nervous system due to the inclusion and exclusion of exons 2,3, and 10. Another study aimed to uncover the role of miRNAs in tau metabolism identified miR-16 and miR-132 as putative endogenous regulators of neuronal p-tau and exon 10 splicing, respectively, and mutations in tau exon 10 have been associated with neurodegeneration and dementia in the adulthood[31]. Overall, altered levels of miR-16 and miR-132 were associated with tau pathology in human neurodegenerative disorders[31].

6.3. Amyloid-β (Aβ) peptides and BACE-1

AD has been associated with increased production of amyloid-β (Aβ) peptides and impaired function of its clearance. miRNAs targeting the key proteins of the amyloidogenic pathway are of interest to current research on AD therapeutics. A study has shown that miR-31, previously found to be decreased in AD patients, suggests that miR-31-mediated modulation of APP and BACE1 can become a therapeutic option in the treatment of AD. miR-31 upregulation in 17-month-old AD triple-transgenic (3xTg-AD) female mice simultaneously reduced APP and BACE-1 mRNA levels in the hippocampus, significantly ameliorated deficits in short and long-term

memory, and reduced anxiety and cognitive inflexibility. In addition, lentiviral-mediated miR-31 expression significantly reduced AD neuropathology in the mouse mode with a reduced Aβ accumulation in both the hippocampus and subiculum[26]. MiRNAs typically inhibit protein expression by binding to its complement mRNAs' 3′-untranslated regions (3′-UTR) in a cell-specific manner. However, the mechanisms of the variation of miRNA activity remain unknown. Another study demonstrated the differing effects of treatment of miR-298 reduced native APP and BACE-1 translation levels in astrocytic but not in a neuron-like cell line. The variation in its effects on APP-3'-UTR activity and native protein levels are according to cell type and 3'-UTR specificity. Researchers postulated that naturally occurring variations in the length of APP 3' UTR could account for miR-298 cell-specificity. Such novel miR-298 involvement in AD provides valuable insight into the clinically relevant research on tailoring miRNA's effect to a specific cell type in the treatment of AD[32].

6.4. Apolipoprotein E (ApoE4)

A study identified the involvement of miR-195 in ApoE4-associated pathology in AD disease progression. Results demonstrated that reduced levels of miR-195 in the AD brain correlated with early disease progression but not advanced stages of AD. Moreover, it has been noted that the ApoE4 genotype accelerated miR-195 reduction, leading to increased tau pathology and cognitive decline[33]. In addition, miR-195 overexpression reduced the expression of its target, synaptojanin1 (synj 1), a brain PIP2-degrading enzyme that plays an important functional role in AD pathogenesis in mice models. For example, reduced synj 1 expression is associated with faster Aβ clearance via the lysosomal degradation pathway, ameliorating elevated tau hyperphosphorylation levels and rescuing ApoE4-associated neuronal impairment[33]. Ongoing research aims to uncover specific mechanisms of miR-195 involvement targeted at ApoE4 for future therapeutic purposes.

7. Practical/Clinical Applications and Feasibility

7.1. miRNA Benefits

This novel therapeutic has shown promising results in research and laboratory environments. But how are these principles and tools translated and applied to the real world? The basis of the therapeutic is the molecule

miRNA. Compared to most nucleic acids, miRNA is a highly stable molecule, therefore "surviving" in bodily fluids, such as the CSF, when observing brain diseases such as AD[34]. This stability allows for an easier analysis, making it ideal for clinical settings.

Furthermore, it has been shown that miRNA is better amplified in PCR treatments which is a very commonly used method in the scientific and medical field[34]. Another benefit of the miRNA is that they constrain sequences that create molecules that have been proven to be directly related to AD. More specifically, miRNAs are known to regulate the production of genes like APP and the BACE-1, which affects the production of Aβ[32,34]. For example, miR-340 can be used to alleviate AD by targeting the Aβ and miR-342–3p was shown to be in higher concentrations in AD patients, which increased the stress kinase c-Jun N-terminal kinase, therefore increased the accumulation of Aβ[34, 36]. The treatment is relatively simple and noninvasive, making it feasible for common use in clinical settings[35]. Lastly, it has proven to be more cost-effective than the current or competing biomarkers for AD[34].

7.2. miRNA Uncertainty

A drawback or point of uncertainty in the research of miRNA as therapeutics for Alzheimer's is the discrepancy between the different trials and projects, starting from the beginning at the root of the collection[34]. Sometimes the miRNAs are extracted from the plasma in the patient's blood, and other times in the CSF. The best example is miR-342-3p, a better biomarker in blood, and miR-127-3p, a better biomarker in CSF[34]. This discrepancy, along with different measurements and amplifications or testing methods, has caused different results from various studies. However, it is unlikely that only one specific approach would be used. It is instead important to standardize each type of treatment or biomarker. Some approaches are not limited to difficulties in delivering and targeting these miRNA treatments, safety issues, and off-target effects of these treatments[37]. These miRNAs play a role in Aβ production through cascades of different molecules. Similarly, they have control over other molecules and proteins. So, by changing the levels of specific miRNAs apart from the Aβ pathway being changed, other molecules and pathways could have residual effects.

7.3. Current Use

The idea of using miRNA has already been adapted into some clinical aspects. There are patents in place using the technique of miRNA as biomarkers and therapies. One patent that has been filed and granted claims to be an invention that is able to measure the level of one neurite miRNAs, specific miRNAs that relate to MCI, the starting stage of AD. These specific miRNAs include miR-7, miR-125b, miR-128, miR-132, miR-874, miR-134, miR-323-3p, and miR-382[38]. Another patent in China has published a similar invention in which miRNA is used as a biomarker to diagnose Alzheimer's in patients. This invention is said to compare miRNAs such as miR-191, miR-15b, and miR-142-3p with healthy controls to determine if the given sample has the precursors for AD[39].

8. Future directions

8.1. miRNA delivery

One step for the future of this novel treatment is enhancing the delivery method of the miRNA therapies. miRNA therapy is very targeted; therefore, Alzheimer's patients would need the miRNA injected directly into the CNS[40,41]. MiRNA cannot pass through the blood-brain barrier, so conventional techniques would not be adequate[40]. The system by which the treatment is delivered is important as it affects how feasible and applicable it is in the clinical world as well as its effectiveness.

8.2. miRNA studies

Currently, there is promise in miRNA studies; however, there are limited clinical trials that are ongoing that study this potential therapy[41]. Having more of these studies in the future will help the understanding of this therapy and has the potential to make it a more conventional practice in the medical field for diagnosing and treating AD. There are also other issues that need to be addressed, such as the tolerance of the patient's miRNA supplementation or inhibition[41]. The lag of miRNA therapies, compared to treatments of other common or deadly illnesses, decreases the potential of issues like this from being resolved. More research needs to be done to garner a holistic view of miRNA therapy.

9. Conclusion

Alzheimer's disease (AD) is a progressive neurodegenerative disease and is the leading cause of dementia across the globe. AD is frequently characterized by the deposition of Aβ plaques and the formation of NFTs in the brain. Despite continued research into the disease, AD continues to be one of the leading causes of mortality in elderly populations globally, thus highlighting the need for novel and advanced therapies that can produce robust anti-AD effects. Current AD treatments are typically invasive, costly, and non-curative, thus highlighting microRNAs as an attractive alternative for the care of AD patients. MiRNAs are associated with several neurodegenerative diseases and play an outsized role in the pathogenesis of AD. Several classes and families of miRNAs are shown to be either upregulated or under-expressed in amyloid-beta and AD models and cell cultures, therefore making the targeting of the relationship between miRNAs and the protein signaling an important agent for the diagnostic and treatment potential of the disease.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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