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A Critical Evaluation of Neuroprotective and Neurodegenerative MicroRNAs in Alzheimer's Disease

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Abstract

Currently, 5.4 million Americans suffer from AD, and these numbers are expected to increase up to 16 million by 2050. Despite tremendous research efforts, we still do not have drugs or agents that can delay, or prevent AD and its progression, and we still do not have early detectable biomarkers for AD. Multiple cellular changes have been implicated in AD, including synaptic damage, mitochondrial damage, production and accumulation of A β and phosphorylated tau, inflammatory response, deficits in neurotransmitters, deregulation of the cell cycle, and hormonal imbalance. Research into AD has revealed that miRNAs are involved in each of these cellular changes and interfere with gene regulation and translation. Recent discoveries in molecular biology have also revealed that microRNAs play a major role in post-translational regulation of

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gene expression. The purpose of this article is to review research that has assessed neuroprotective and neurodegenerative characteristics of microRNAs in brain samples from AD transgenic mouse models and patients with AD.

Keywords

microRNAs; Alzheimer's disease; Phosphorylated tau; Synaptic damage; Mitochondrial dysfunction

Introduction

Alzheimer's disease (AD) is an age-related, multifactorial, progressive neurodegenerative disease, characterized by memory loss, multiple cognitive impairments, and changes in personality and behavior. Currently, 5.4 million Americans suffer from AD, and this number is expected to increase up to 16 million by 2050. With a growing aging population not only in the United States but worldwide, AD has become a major health concern. AD has had a huge economic impact, with dementia health care costs alone reaching an estimated total of \$818 billion worldwide in 2015 (World Alzheimer Report 2015). Despite extensive research into AD, we still do not have drugs or agents that can delay or prevent AD progression, and we do not have detectable biomarkers for early AD diagnosis.

AD is associated with synaptic loss, mitochondrial dysfunction, amyloid beta (A β) production and accumulation, inflammatory responses, phosphorylated tau formation and accumulation, cell cycle deregulation, impaired cholinesterase transmission, deficits in neurotransmission, calcium dyshomeostasis and hormonal imbalance, neuronal loss, and an accumulation of senile plaques and neurofibrillary tangles in learning and memory regions of the brain [1–4]. Synaptic pathology and mitochondrial damage have been identified as early events in AD pathogenesis [5]. An accumulation of A β and mislocalization of phosphorylated tau in synapses cause synaptic starvation and degeneration, and cognitive decline in AD patients [6]. The precise cause underlying AD pathogenesis are not completely known or understood.

Anatomical studies of AD brains and the brains from several lines of AD transgenic mice have revealed that AD begins in brain regions that are responsible for learning and memory, including layer 2 of the entorhinal cortex. The AD process spreads to the hippocampus, temporal cortex, frontoparietal cortex and, finally, to subcortical nuclei [4,7].

AD occurs in two forms, early-onset familial and late-onset sporadic. Early-onset AD is caused by genetic mutations in 3 loci: the amyloid precursor protein (APP), Presenilin 1 (PS1), and Presenilin 2 (PS2). However, genetic mutations in these 3 loci are responsible for only a small proportion (1–2%) of all AD cases [1–3]. The remaining AD cases are late-onset sporadic AD, but unlike early-onset AD, the cause of late-onset AD is not known. However, several risk factors have been identified in late-onset AD, including apolipoprotein E (ApoE) genotype 4 and genetic polymorphisms in sortilin-related receptor 1, clusterin, complement component receptor 1, CD2AP, CD33, EPHA1, and MS4A4/MS4A6E genes [1]. Multiple risk factors, including lifestyle, diet, environmental factors such as stress,

vascular diseases, depression, stroke, hypertension, traumatic brain injury, and type 2 diabetes are other contributing factors to late-onset AD [8].

In early-onset AD, the pathological cellular changes occur early in a person's life (typically less than 30 years old) because of genetic mutation(s), whereas in late-onset AD, cellular and pathological changes occur later in life due to aging processes that take time to induce AD [1,2]. At cellular and pathological levels, there are no marked differences between early-onset AD and late-onset AD.

Research into AD has revealed that micro RNAs (miRNAs) play a large role in the regulation of genes, protein expressions, and phenotypic changes in human diseases. Well-studied miRNAs in human diseases include cancer [9,10], cardiovascular diseases [11], hypertension [12], nephropathy [13], stroke [14], and neurodegenerative diseases such as schizophrenia [15], Huntington's [16], Parkinson's [17], and AD [18–20].

miRNAs regulate genes that are responsible for A β production and phosphorylated tau, including APP, PS1, and PS2 [Table 1]. miRNAs also regulate cellular changes via the ApoE4 genotype and other polymorphisms, including sortilin-related receptor 1, clusterin, complement component receptor 1, CD2AP, CD33, EPHA1, and MS4A4/MS4A6E genes that are involved in AD pathogenesis [21].

This article reviews research on protective and toxic miRNAs in AD. Also reviewed is research that focuses on miRNAs that may be responsible for early-onset familial AD and late-onset sporadic AD.

Biogenesis of microRNAs

miRNAs are a large family of conserved small (20–22 nucleotides), non-coding RNAs. miRNAs play a central role in the post-transcriptional regulation of gene expression [20,22]. In mammals, miRNAs are believed to control about 50% of all protein-coding genes [23]. At present, over 2000 miRNAs have been identified (see details at www.mirbase.org). One-third of these miRNAs are found in the coding part of genes, and the remaining are in the intronic regions.

As shown in Figure 1, miRNA biogenesis is initiated in the nucleus with transcription of primary miRNA transcripts from miRNA coding genes. Pri-miRNAs are converted to hair-pin loop pre-miRNAs by enzymatic digestion with Drosha and DGCR8 proteins. Pre-miRNAs are transported to the cytoplasm by Exportin-5/Ran/GTP proteins where they are again digested by the cytoplasmic proteins Dicer and TRBP, resulting in the generation of the miRNA duplex. Duplex structure is unwinded by helicase, resulting in the generation of mature miRNA strands. Mature miRNAs form the RISC complex with the Ago2 protein and target the 3' UTR of mRNA, and they modulate gene activity either by translation suppression or mRNA cleavage [24,25]. miRNAs can be found as a group or a family, harboring the same initial sequence and targeting a single gene. In some cases, a single miRNA can affect a large number of genes that are involved in the regulation of multiple cellular events and pathways [26].

Research has revealed that miRNAs are differentially expressed in different cell types and tissues in mammals, including humans. miRNAs are believed to alter multiple cellular processes, including development, cell proliferation, replicative senescence, and aging [27,28]. Over 70% of reported miRNAs are expressed in the human brain. miRNAs found in the human brain include: miR-9, miR-7, miR-128, miR-125 a-b, miR-23, miR-132, miR-137, and miR-139. A recent deep RNA sequencing analysis has revealed a large number of miRNAs that are brain-specific, including: miR-134, miR-135, let-7g, miR-101, miR-181a-b, miR-191, miR-124, miR-let-7c, let-7a, miR-29a, and miR-107 [29,30]. Most of these miRNAs are responsible for synaptic functions, neurotransmitter release, synapse formation and neurite outgrowth. Expression levels of several miRNAs are altered in a diseased state, such as AD.

The AD brain and microRNAs

The progressive loss of synapses and neurons, reduced volume of the hippocampus, and reduction in size and weight are typical features of the AD brain. Similar to brains from humans with AD, brains from AD mice have most of these same features. The loss of synapses and synaptic damage correlate the closest with cognitive decline and memory loss in AD patients and AD mice [5]. Studies revealed a reduction in miRNA expression in the AD brain, which in turn appears to correlate with a reduction and in A β production and reduced phosphorylated tau (Table 1). In contrast, several miRNAs are known to increase levels of A β , phosphorylated tau, and inflammation not only in the brains of humans with AD but also in the brains of mice with AD (Figure 2). Interestingly, the brains from Dicer knockout mice exhibited similar features found in the brains from humans and mice with AD, such as reduced brain size, enlarged ventricles, inflammation of brain, loss of synaptic branching and connectivity, and spine length [31–33]. Dicer knockout mice also showed oxidative stress, phosphorylated tau, and memory loss, and reduced levels of a large number of miRNAs [31–33], conditions also found in the brains of humans and mice with AD. The similarities between the brains of humans with AD and Dicer knockout mice suggest that Dicer may play a large role in memory and cognition and that a progressive loss of Dicer may be linked to reduced learning and memory in persons with AD. Research is needed to investigate whether Dicer is linked to cognitive decline in AD.

Amyloid beta and microRNAs

A β production and A β deposits in the brains of humans with AD are accompanied by alterations in the levels of many miRNAs from distinct miRNA classes. These miRNAs may be involved in AD pathogenesis, in particular the generation of A β [18,34–37]. Several miRNA classes, such as miR-101 and miR-106, target APP, resulting in an elevated generation of A β [18,36,38,39]. Interestingly, several nucleotide polymorphisms associated with AD are located in the miRNA-binding region of the APP mRNA, which can modulate protein expressions [40].

Another class of miRNAs that are downregulated in AD is the neuron-specific miR-124 [41]. The downregulated miR-124 leads to an over-expression of its targeted mRNA, polypyrimidine-tract binding protein 1 (a pre-RNA splicing regulator), in turn leading to the

altered splicing of APP [41]. miR-124 also targets the A β cleaving enzyme 1 BACE1, and the downregulation of miR-124 promotes the transformation of APP into A β , probably by activating BACE1 [42]. It is not known whether there are reduced levels of other miRNAs in the brains from humans and mice with AD. Reduced levels of miR-9, miR-29a, miR-29b, and miR-107 may result in elevated BACE1 expression and an over-production of A β known to characterize brains from humans and mice with AD [37,38,].

Studies in mutant AD mice suggest that miR-298 and miR-328 have similar roles [44]. Loss of miR-9, miR-29a, and miR-29b in AD, together with the loss of miR-137 and miR-181c disinhibits serine palmitoyltransferase, the rate-limiting enzyme of ceramide synthesis, leading to the mislocation of BACE1 in lipid rafts and augmenting the excessive processing of APP into A β [43].

Increasing evidence suggests that miRNAs affect A β production. As shown in Table 1, several miRNAs increase A β levels and others reduce A β production. On the other hand, A β itself reciprocally impacts the production of miRNAs, including miR-9, miR-106b, and let7 [43,45,46]. Despite evidence in support of a role for reduced levels of miRNAs in AD, it remains unclear whether reduced miRNAs play a primary role in AD induction.

Kim and colleagues (2014) studied the effects of elevated levels of miRNA126 in dopamine neuronal cell survival in models of Parkinson's disease [47]. They showed that elevated levels of miR-126 increase the vulnerability of neurons to ubiquitous toxicity that is mediated by staurosporine or A β 42. The neuroprotective factors IGF-1, nerve growth factor, brain-derived neurotrophic factor, and soluble APP α could diminish, but not abrogate, the toxic effects of miR-126. MiR-126 overexpressing neurons from a Tg6799 familial mouse model of AD exhibited an increase in A β 42 toxicity, but surprisingly, both A β 42 and miR-126 promoted neurite sprouting. Pathway analysis revealed that the overexpression of miR-126 downregulated elements in the GF/PI3K/AKT and ERK signaling cascades, including AKT, GSK-3 β , and ERK; the phosphorylation of tau; and the miR-126 targets IRS-1 and PIK3R2. In this same study, the inhibition of miR-126 was found to be neuroprotective against both STS and A β 42 toxicity. Although the Kim study focused on Parkinson's disease, it provides evidence for a novel miR-126 mechanism in a neurodegenerative disease that is capable of regulating GF/PI3K signaling in neurons, suggesting that miR-126 may be an important mechanistic link between metabolic dysfunction and neurotoxicity in another neurodegenerative disease, namely AD.

BACE1 and microRNAs

Altered levels of miRNAs increase the production of A β and BACE1 activity. Yang and colleagues (2015) studied the expression levels of the miR-29 family in peripheral blood samples from patients with AD and age-matched controls [48]. They found a comparatively marked decrease in miR-29c expression and a significant increase in BACE1 expression in the samples from the AD patients. Correlation analysis revealed that miR-29c expression negatively correlated with the protein expression of BACE1 in the samples from the AD patients. Yang and colleagues also investigated the role of miR-29 on hippocampal neurons in vitro and in vivo. They found miR-29c upregulation promoted learning and memory

behaviors in SAMP8 mice by increasing the activity of the protein kinase A/cAMP response element-binding protein, which is involved in neuroprotection, suggesting that miR-29c may be a possible therapeutic target against AD [48].

Zhang and colleagues (2014) studied the role of miR-188-3p that targets BACE1 in humans and mice with AD [49]. They found miR-188-3p to be significantly downregulated in the brains of AD humans and the AD TG mice, an APP mouse model of AD. The downregulated miR-188-3p was restored by the inhibition of MAGL. Overexpression of miR-188-3p in the hippocampus of the TG mice reduced BACE1, A β , and neuroinflammation, and prevented deterioration in hippocampal basal synaptic transmission, long-term potentiation, spatial learning, and memory. Loss of miR-188-3p function correlated with 2-AG-induced suppression of BACE1. Moreover, miR-188-3p expression was upregulated by 2-AG or peroxisome proliferator-activated receptor- γ (PPAR γ) agonists and suppressed by PPAR γ antagonism or NF- κ B activation. Reduction of A β and neuroinflammation by MAGL inhibition was occluded by PPAR γ antagonism. In addition, BACE1 suppression by 2-AG and PPAR γ activation was eliminated by the knockdown of NF- κ B. The Zhang study revealed a novel molecular mechanism underlying improved synaptic and cognitive function in TG mice by 2-AG signaling – a mechanism that appears to upregulate miR-188-3p expression through the PPAR γ and NF- κ B signaling pathways, resulting in suppression of BACE1 expression and the consequent formation of A β [49].

Long and colleagues (2014) identified miR-339-5p, a known miRNA, as a key contributor to the regulatory network [50]. Two distinct miR-339-5p target sites were predicted in the BACE1 3'-UTR by in silico analyses, and both were found to be linked to BACE1. Co-transfection of miR-339-5p with a BACE1 3'-UTR reporter construct resulted in significant reduction in reporter expression [50]. Mutation of both target sites eliminated this effect. Delivery of the miR-339-5p mimic also significantly inhibited expression of the BACE1 protein in human glioblastoma cells and human primary brain cultures. Delivery of target protectors designed against the miR-339-5p BACE1 3'-UTR target sites in primary human brain cultures significantly elevated BACE1 expression. In addition, miR-339-5p levels were significantly reduced in brain specimens from AD patients compared to those from age-matched controls. Therefore, miR-339-5p appears to regulate BACE1 expression in human brain cells and to be dysregulated in at least a subset of AD patients, warranting the study of miR-339-5p as a novel drug target for patients with AD [50].

Using AD mouse models, Boissonneault and colleagues studied the roles of miR-298 and miR-328 in A β production in mice With AD [44]. They observed a loss in correlation between BACE1 mRNA and protein levels in the hippocampus of the AD mouse model. These findings prompted an investigation of the regulatory role of the BACE1 3'-UTR element in AD progression and the possible involvement of specific miRNAs in cultured neuronal cells and fibroblastic cells from humans with AD. Using such experimental approaches, these researchers demonstrated that miR-298 and miR-328 recognize specific binding sites in the 3'-UTR of the BACE1 mRNA and exert regulatory effects on ACE1 protein expression in cultured neuronal cells [44]. These results may point to a molecular basis underlying BACE1 deregulation in AD and may offer new perspectives on AD.

Galimberti et al. (2014) studied the profiles of circulating miRNAs in serum and cerebrospinal fluids (CSF) from humans with AD and correlated them with profiles of AD patients [51]. Using a two-step analysis – micro-array analysis followed by validation via real-time PCR – they found miR-23a to be down-regulated in the serum from 22 AD patients compared to 18 non-inflammatory controls (NINDCs), 8 inflammatory neurological controls (INDCs), and 10 patients with frontotemporal dementia. Significant down-regulation of miR-125b and of miR-26b was also confirmed in the CSF from AD patients. These researchers found that cell-free miR-125b serum levels from AD patients are less than levels from INDCs and NINDCs with an accuracy of 82%.

Alexandrov and colleagues (2013) studied the effects of miRNA-34a on TREM2 mRNA 3'-untranslated region of TREM2 and found that miRNA-34a significantly down-regulated TREM2 expression [52]. Mutations in TREM2 are known to cause rare, autosomal recessive forms of early onset dementia that present with and without bone cysts and fractures. Aluminum-induced miRNA-34a up-regulation and TREM2 down-regulation were effectively quenched with the natural phenolic compound and the NF- κ B inhibitor CAPE (2-phenylethyl-(2E)-3-(3,4-dihydroxyphenyl) acrylate; caffeic-acid phenethyl ester). These results suggest that an epigenetic mechanism in AD involving an aluminum-triggered, NF- κ B-sensitive, miRNA-34a-mediated down-regulation of TREM2 expression may impair phagocytic responses that may ultimately contribute to an accumulation and aggregation of the A β 42 peptide, amyloidogenesis, and inflammatory degeneration in the AD brain [52].

Alexandrov et al. (2012) analyzed the relative amount of A β and miRNA in CSF from the neocortices of patients with AD and of age-matched controls, in short post-mortem intervals (PMI <2.1 hr) [53]. They found a decreased but nonsignificant abundance of A β 42 in the CSF and extracellular fluid of AD patients. The most abundant nucleic acids in the CSF and ECF from AD patients were miRNAs. This result led to additional studies of the speciation and inducibility. Fluorescent miRNA-array-based analysis indicated significant increases in miRNA-9, miRNA-125b, miRNA-146a, and miRNA-155 in the CSF and ECF of AD patients [53]. Primary human neuronal-glial cell co-cultures stressed with AD-derived ECF also displayed an up-regulation of these four miRNAs, an effect that was quenched using the anti-NF- κ B agent caffeic acid phenethyl ester. Increases in miRNAs were confirmed independently, using a highly sensitive LED-Northern dot blot assay. Several of these NF- κ B-sensitive miRNAs are known to be up-regulated in AD brain and are associated with the progressive spreading of inflammatory neurodegeneration. Results from these confirmation studies indicated that miRNA-9, miRNA-125b, miRNA-146a, and miRNA-155 are CSF- and ECF-abundant. NF- κ B-sensitive pro-inflammatory miRNAs, and their enrichment in circulating CSF and ECF, suggest that miRNAs may be involved in the modulation or proliferation of miRNA-triggered pathogenic signaling throughout the central nervous system [53].

Using SAMP8 mice and BALb/c mice, Liu et al. (2012) examined the post-transcriptional regulation mechanism of APP mediated by micro ribonucleic acids [54]. They found miR-16 to be one of the post-transcriptional regulators of APP in the SAMP8 mice. Overexpression of miR-16, both in vitro and in vivo, led to reduced APP expression. Further, miR-16 and APP displayed complementary expression patterns in the SAMP8 mice and BALb/c

embryos. Taken together, these findings indicate that an abnormally low expression of miR-16 could lead to an accumulation of APP in AD mice and that APP may be a target for miR-16 [54].

Pogue and colleagues (2009) studied the effects of complement factor H on metal-sulfate-stressed human brain cells when miR146a was modulated [55]. They found an NF-kappaB-sensitive, miRNA-146a-mediated modulation of CFH gene expression in AD neurons that may contribute to inflammatory responses in aluminum-stressed HN cells. This finding underscores the potential of just a nanomolar of aluminum that may be necessary to drive genotoxic mechanisms characteristic of neurodegenerative disease processes.

Alpha secretase and microRNAs

A β secretase is an enzyme in AD that cleaves the amyloid domain of APP and reduces the production of the A β peptide in neurons. There are numerous miRNAs that are involved in the increased activity of alpha secretase in neurons, and there are other miRNAs responsible for reduced alpha secretase activity, the latter group of which results in an increase in alpha secretase production and a cascade of cellular changes in AD progression.

Interestingly, the loss of miR-107 disinhibits the alpha-secretase ADAM10 and favors the non-amyloidogenic pathway of APP processing. Compensatory effects from this disinhibition shunts the cleavage of APP away from the generation of A β plaque toward the generation of soluble APP [56].

Cerebrospinal fluids and microRNAs

Using open-array technology, Denk and colleagues (2015) studied the CSF of AD patients (n = 22) and controls (n = 28) to profile the expression of 1178 different miRNAs [57]. Using a Cq of 34 as cut-off, they identified positive signals from 441 miRNAs, but could not identify positive signals from 729 other miRNAs indicating that at least 37% of all miRNAs in the body are present in the brain. They found 74 down-regulated miRNAs and 74 up-regulated miRNAs with a 1.5 fold change threshold. By applying the new explorative “measure of relevance” method, they identified 6 reliable and 9 informative biomarkers. Confirmatory MANCOVA revealed reliable miR-100, miR-146a, and miR-1274a as differentially expressed in AD, an analysis that reached Bonferroni-corrected significance. MANCOVA also confirmed the differential expression of informative miR-103, miR-375, miR-505, miR-708, miR-4467, miR-219, miR-296, miR-766, and miR-3622b-3p. Discrimination analysis using a combination of miR-100, miR-103, and miR-375 detected AD in the CSF by positively classifying controls and AD patients with 96.4% and 95.5% accuracy, respectively. Using the ingenuity database, Denk et al. identified a set of AD-associated genes that these miRNAs targeted [57]. These targets included genes involved in the regulation of tau and amyloid pathways in AD, such as MAPT, BACE1, and mTOR.

Gamma secretase complex and microRNAs

γ -secretases are a group of widely expressed, intramembrane-cleaving proteases involved in many physiological processes associated with AD. Mutations in PS1 and PS2 lead to

increased γ -secretase activity, which has been associated with the formation of A β in AD. In addition to PS1 and PS2, two other subunits – nicastrin and anterior-pharynx defective-1 – were identified as essential co-factors. These four γ -secretase enzymes together generate an active and stable complex that cleaves APP at the end of the A β domain in APP. Inhibition of these enzymes redirects the amyloidogenic pathway towards the non-amyloidogenic pathway by reducing A β production. Similar to miRNAs that activate BACE1, several MiRNAs are believed to be involved in the increased production of γ -secretase.

Loss of presenilin function has been proposed to underlie memory impairment and neurodegeneration in AD pathogenesis [58]. Using brain tissue from the PS1 knockout mouse, Krichevsky and colleagues (2003) studied the miRNA profiles [59]. They found that the down-regulation of miR-9 coincides with neurodegeneration in PS1 knockout mice. Other studies using zebrafish and mice found miR-9 to be an important regulator of neurogenesis [60,61]. Based on these results, miR-9, which is downregulated in the AD brain, may actively participate in maintaining neurons and in sustaining A β production. Further research is needed to determine the role of γ -secretase-linked miRNAs in AD.

Tau and microRNAs

The detrimental effects of miRNA changes in AD neurons might not be restricted to A β formation. For example, the loss of miR-15a favors the hyperphosphorylation of tau by disinhibiting ERK1 [62]. Increased levels of miR-128 lead to decreased activity of Bcl2-associated athanogene, leading to a reduction in the removal of sarkosyl-insoluble tau, a reduction that is known to favor the formation of toxic tau inclusions [63]. Based on studies in an AD TG mouse model, putative increases in levels of miR-34a were found to suppress Bcl2 expression, which exacerbated neuronal loss by the recruitment of caspase-3 and apoptosis [64]. Increases in miR-206, in the temporal cortex of AD brains, led to the suppression of BDNF, which contributed to compromises in morphological and functional synaptic plasticity [65].

Li and colleagues (2011) examined miRNA-146a levels in several human primary brain and retinal cell lines from the neocortex and hippocampus of patients in early-, moderate-, and late-stage AD, and of 5 different TG mouse models of AD (Tg2576, TgCRND8, PSAPP, 3xTg-AD, and 5xFAD) [66]. Inducible expression of miRNA-146a was significantly up-regulated in a primary co-culture of human neuronal-glial cells that were stressed using interleukin1-beta. This up-regulation was quenched using specific NF- κ B inhibitors that include curcumin. Expression of miRNA-146a correlated with senile plaque density and synaptic pathology in the Tg2576 and 5xFAD TG mouse models [66].

ApoE4 and microRNAs

The ApoE4 genotype is a major risk factor for late-onset sporadic AD. Alterations in microRNAs and cognitive decline are expected in humans with the ApoE4 genotype—mainly because the ApoE4 status increases the production of A β [1]. Several lines of evidence support this notion. However, there are no published studies that have linked miRNAs and the ApoE4 genotype in AD patients.

Kim et al. (2014) studied miR-33 and its relationship to ABCA1 and A β levels in the brain [67]. Overexpression of miR-33 impaired cellular cholesterol efflux and dramatically increased extracellular A β levels by promoting the secretion of A β and impairing the clearance of A β in neurons. In contrast, genetic deletion of mir-33 in mice dramatically increased ABCA1 levels and ApoE lipidation, but decreased endogenous A β levels in the cortex. Most importantly, pharmacological inhibition of miR-33 via antisense oligonucleotide specifically in the brain markedly decreased A β levels in the cortex of APP/PS1 mice, suggesting that miR33 is a potential therapeutic strategy for AD. Additional research is needed to determine how ApoE4-linked miRNAs alter cellular changes in the AD brain, such as altering the production and accumulation of A β , the phosphorylation of tau, and the triggering of synaptic damage. Further research is needed to understand precise links between miRNAs and ApoE4 genotype association with A β levels in patients with AD and mouse models of AD.

Inflammation and microRNAs

Inflammatory responses are strongly associated with altered miRNA expressions in the AD brain. miRNA-155 is involved in diverse physiological and pathological mechanisms, such as inflammation and immunity. Recent studies indicate that miR-155 regulates T-cell functions during inflammation. Song and Lee (2015) investigated the role of miRNA-155 in AD, finding miRNA-155 to be a multifunctional miRNA in AD pathogenesis, with a distinct expression profile and links to T-cell functions [68].

In addition, in studies of miR-125b and miR-146 in the human AD brain, levels of these miRNA were found to be elevated, which might aggravate neuroinflammation [69,70] and reduce complement factor H, which is associated with the neuronal release of mR-146a and miR-155 and inflammatory spreading in the AD brain [70,71]. Altered miR-106b levels impact the expression of transforming growth factor beta178.

In investigating the significance of miRNA release in the AD brain, Lehmann et al. (2012) focused on the miRNA let-7b [72]. They found that, following its release, let-7b activates the toll-like receptor 7, resulting in neuronal degeneration. They also found that loss of miR-29a disrupts the activity of another target gene, neuronal navigator 3, a protein that is involved in axonal guidance and is enriched in degenerating pyramidal neurons in AD [73].

Mitochondrial microRNAs and AD

Dysfunction of mitochondria and oxidative stress have been found to be involved in neurodegenerative diseases, including AD. Mitochondria are cytoplasmic organelles, and control/regulate cell survival and cell death. Mitochondria perform several key cellular functions, including ATP production, regulation of intracellular calcium, apoptotic cell death, sites of free radical production, and scavenging and activation of caspase family of proteases. Mitochondria are synthesized in cell soma, travel along axons and dendrites, and supply ATP for several synaptic functions, including synapse formation and outgrowth, neurotransmitter release and vesicle fusion. Mitochondria move from cell soma to nerve

terminals via kinesin-based anterograde fashion and travel back to cell soma via dynein-based retrograde manner.

The human mitochondria carries 37 polypeptide genes in a 16.5 kb circular genome. The DNA of mitochondria has 2 strands: an outer strand enriched with guanine (heavy strand) and an inner strand enriched with cytosine (light strand). It also has a non-coding segment comprised of a displacement loop, a region of 1121 base pairs.

Studies have identified several mitochondrial miRNAs in the human mitochondria [74–81]. More recently, Shinde and Bhandra (2015) have identified six pr-miRNAs and miRNAs from mitochondria genome, indicating miRNAs in the mitochondrial genome [82]. Berray et al. (2011) identified 169 miRNAs in the mitochondrial genome that are believed to regulate polypeptide genes in the mitochondrial genome, oxidative phosphorylation, and ATP synthesis [81].

miRNAs regulate mitochondrial structure. A recent study of miR-761 found that it is responsible for the downregulation of the mitochondrial fission factor (Mff) and the suppression of mitochondrial fission machinery [83]. In studies of miR-30, Goud and Hua (2015) found miR-30a, -30b, and -30d to be highly expressed in myocardial cells that are exposed to hydrogen peroxide [84]. The gene p53, a target of the miR-30 family, promotes Drp1 transcription while triggering apoptosis. Goud and Hua concluded that miR-30 regulates mitochondrial fission and apoptosis via the targeting of P53 and Drp1 [84].

miR-30 and miR499 have been found to be involved in regulating mitochondrial dynamics via Drp1 through p53 and calcineurin [85,86]. Li et al. (2010) found that miR-30 family members inhibit mitochondrial fission and target p53, which is known to induce mitochondrial fission by transcriptionally upregulating Drp1 expression. miR-30 inhibits mitochondrial fission by suppressing the expression of p53 and its downstream target Drp1 [85]. Regarding miR499, Wang and colleagues (2011) found that it directly targets α and β isoforms of the calcineurin and inhibits cardiomyocyte apoptosis by suppressing calcineurin-mediated dephosphorylation of Drp1, which in turn decreases the translocation of Drp1 to mitochondria and Drp1-mediated activation of mitochondrial fission. Findings from these studies revealed that Drp1 is regulated by p53 [86,87]. However, there are no published studies characterizing the role of miRNAs in mitochondrial dynamics either for fission activity or fusion in the AD. Additional research is needed to understand the role of miRNAs, particularly mitochondrial miRNAs, in mitochondrial dynamics and mitochondrial biogenesis in the disease process of AD.

Aging, cellular senescence, and microRNAs

With aging the number one risk factor for AD and other neurodegenerative diseases, it is important to assess miRNAs that affect aging and cellular senescence in order to determine the roles of miRNAs in aging. Multiple tissues, including skin, skeletal muscle, and the brain, are the best indicators of aging and senescence. Skin, as the largest organ in the human body, performs important functions and protects internal organs from external aggression and environmental factors, heat, trauma and microbial pathogens. Culturing skin,

skeletal muscle, and brain cells, and studying them for mRNA and miRNAs, may yield new insights into the role of miRNAs in aging.

Increasing evidence suggests that miRNAs are largely implicated in aging and cellular changes associated with aging. Some of these cellular changes are: telomerase shortening (miR155, miR138, miR34abc), inflammaging (miR155, miR21, miR146a), oxidative stress and mitochondrial dysfunction (miR34a, miR335, miR146a, miR145), DNA damage response (miR34abc, miR192, miR194, miR215, miR421, miR24, miR504, miR125b, miR106b, miR21, miR210, miR373), protein misfolding (miR320, miR1, miR26b, miR106b, miR301b), stem cell impairment (miR369, miR371, miR29c, miR499, let7, miR290, miR291, miR292, miR293, miR294, miR295) and altered nutrient sensing (miR17, miR19b, miR126, miR190b, miR496, miR20a, miR106a, miR1, miR320, miR206) [88]. Altered miRNA expression is associated with aging, which in turn implicates AD. Further research is needed to better understand the involvement of age-related miRNAs in AD pathogenesis and in other neurodegenerative diseases.

Circulatory microRNAs as peripheral biomarkers for AD

A research field of peripheral miRNAs is rapidly emerging in the study of neurodegenerative diseases. Recent molecular biology discoveries (Kumar and Reddy 2016) have revealed a notable number of miRNAs in peripheral tissues, such as serum, plasma, ECF and CSF [20]. The levels of miRNAs in these fluids change with age, body physiology, diet, and disease state.

Recently, Kumar and Reddy (2016) assessed miRNAs in the blood and CSF from patients with AD in order to inform the development of miRNAs as possible peripheral biomarkers for AD [20]. They found a small number of changes in miRNAs associated with cognition in non-demented healthy humans and in humans with mild cognitive impairment and with AD.

Kumar and Reddy hypothesize that miRNA analysis of a blood sample should be able to diagnose whether a person has or will have cognitive impairment due to AD. In addition to a blood-based miRNA analysis, imaging of the brain should provide data on synaptic and neuronal connectivity, hippocampal volume and brain size that should be able to indicate the onset of AD. These two types of tests in conjunction should provide an accurate diagnosis of AD-related dementia.

Conclusions and future directions

AD continues to be a growing health concern that affects millions of persons worldwide. Although progress that has been made in AD research in terms of understanding the molecular basis of early onset familial AD and late-onset sporadic AD, we still do not have drugs or agents that can delay or prevent AD progression, and we still have not identified early detectable biomarkers for AD. A major breakthrough in developing such biomarkers is the discovery that miRNAs connect missing link between cellular changes and disease progression.

There are many questions about miRNAs in the AD brain that need to be answered, including which specific miRNAs are involved in cellular changes associated with AD pathogenesis and progression; which specific miRNAs are involved in cellular changes associated with other neurodegenerative diseases and aging; whether AD can be prevented, delayed, or stopped via strategic alterations of miRNA expression; and whether and how blood and imaging tests can be developed that focus on identifying miRNAs changes that correspond with AD onset and progression. Comprehensive epidemiological-based miRNA studies are urgently needed to inform the development of miRNA-based diagnostic tools.

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Abbreviations

AD	Alzheimer's Disease
miRNA	microRNA
Aβ	Amyloid β
APP	Amyloid Precursor Protein
PS1	Presenilin 1
PS2	Presenilin 2
ApoE	Apolipoprotein E
CD2AP	Cluster of Differentiation 2 Associated Protein
mRNA	messenger RNA
BACE1	Beta-site Amyloid precursor protein Cleaving Enzyme 1
IGF1	Insulin-like growth factor 1
GSK-3β	Glycogen synthase kinase 3
PI3K	Phosphatidylinositol-3-kinase
GF	Growth Factor
ERK	extracellular signal-regulated kinase
IRS1	Insulin receptor substrate 1
PIK3R2	Phosphoinositide-3-Kinase Regulatory Subunit 2
TG	Transgenic
MAGL	Monoacylglycerol lipase

PPARγ	peroxisome proliferator-activated receptor γ
NF-κB	Nuclear Factor Kappa B Subunit
INDCs	Inflammatory Neurological Controls
NINDCs	Non-inflammatory controls
TREM2	Triggering Receptor Expressed On Myeloid Cells 2
CAPE	Caffeic-Acid Phenethyl Ester
CSF	Cerebrospinal Fluid
ECF	Extra Cellular Fluid
SAMP8	Senescence Accelerated Mouse Prone 8
ADAM10	A Disintegrin and metalloproteinase domain-containing protein 10
MANCOVA	Multivariate analysis of covariance
MAPT	Microtubule Associated Protein Tau
mTOR	Mechanistic Target of Rapamycin
ABCA1	Adenosine Triphosphate Binding; Cassette Subfamily A Member 1
Drp1	Dynamin-related protein 1
PTEN	Phosphatase and Tensin Homolog
FOXO3a	Forkhead Box O3
Sirt1	silent mating type information regulation 2 homolog
SORL1	Sortilin-Related Receptor 1
CDK5R1	Cyclin-Dependent Kinase 5, Regulatory Subunit 1
LRPAP1	Low Density Lipoprotein Receptor Related Protein Associated Protein 1
Rb1	Retinoblastoma 1
DUSP6	Dual Specificity Phosphatase 6
PPP1CA	Protein Phosphatase 1 Catalytic Subunit Alpha
VAMP2	Vesicle Associated Membrane Protein 2
SYT1	Synaptotagmin 1
HCN1	Hyperpolarization-activated Cyclic Nucleotide-gated channel 1
NR2A	N-methyl-D-aspartate receptor subunit 2A
GluR1	Glutamate Receptor 1

NDUFC2 Nicotinamide Adenine Dinucleotide Ubiquinone Oxidoreductase subunit C2**References**

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Highlights

1. Multiple cellular changes have been implicated in Alzheimer's disease.
2. MicroRNAs interfere with gene regulation and translation.
3. Differential expression of MicroRNAs modulate genes related to Alzheimer's disease.
4. MicroRNAs affect the levels of amyloid beta, phosphorylated tau and synaptic damage.
5. MicroRNAs affect aging, cellular senescence, inflammation and mitochondrial dysfunction in Alzheimer's disease.

Figure 1. Hypothesized miRNA biogenesis pathway

miRNA biogenesis initiates in the nucleus with transcription of primary miRNA transcripts from miRNA coding genes by RNA Polymerase II or III. pri-miRNA converted to hair-pin loop pre-miRNA by enzymatic digestion with Drosha and DGCR8 proteins. Pre-miRNA is transported to the cytoplasm by Exportin-5/Ran/GTP proteins and is digested by the cytoplasmic proteins Dicer/TRBP and generates miRNA duplex. The duplex structure is unwound by helicase, and a mature miRNA strand is generated. Mature miRNAs form the RISC complex with the Ago2 protein. The mature miRNAs target the 3' UTR of mRNA and modulate gene activity either by suppressing mRNA cleavage or the translation.

Figure 2. AD risk factors and associated miRNAs

Deregulation of miRNAs expression modulates the expression of certain key AD related genes and promote disease progression. Beside miRNAs also involved in several cellular process that are critical for AD.

Table 1

Neuroprotective and neurodegenerative miRNAs in Alzheimer's diseases

A. Neuroprotective miRNAs in Alzheimer's disease				
miRNAs	Status in AD	Target gene	Effect on target genes	Reference
miR-29a/b-1 cluster	Brain	<i>BACE1/β-secretase</i>	Upregulation	Hébert et al., 2008 [89]
miR-101	-	<i>APP</i>	Downregulation	Vilardo et al., 2010 [90]
miR-124	↓ brain	<i>BACE1</i>	Downregulation	Fang et al., 2012 [41]
miR-132/212	↓ brain	<i>PTEN, FOXO3a and P300</i>	Downregulation	Wong et al., 2013 [91]
miR-34	-	<i>tau</i>	Downregulation	Dikson et al., 2013 [92]
miR-193b	↓ hippocampus	<i>APP</i>	Downregulation	Liu et al., 2014 [93]
miR-188-3p	↓ brain	<i>BACE1</i>	Downregulation	Zhang et al., 2014 [49]
miR-339-5p	↓ brain	<i>BACE1</i>	Downregulation	Long et al., 2014 [50]
miR-212/132 & miR23a/b	↓ frontal cortex	<i>sirt1</i>	Upregulation	Weinberg et al., 2015 [94]
miR-219	↓ brain	<i>tau</i>	Downregulation	Santa-Maria et al., 2015 [95]
miR-16	↓ neuronal cells	<i>APP</i>	Downregulation	Zhang et al., 2015 [96]
Mir-29c	↓ peripheral blood	<i>BACE1</i>	Downregulation	Yang et al., 2015 [48]
miR-135b	↓ peripheral blood	<i>BACE1</i>	Downregulation	Zhang et al., 2016 [97]
miR-1229-3p	-	<i>SORL1</i>	Downregulation	Ghanbari et al., 2016 [98]
miR-15/107 family	↓ brain	<i>CDK5R1</i>	Downregulation	Moncini et al., 2016 [99]
miR-603	↑ hippocampus	<i>LRPAP1</i>	Downregulation	Zhang et al., 2016 [100]
miR-29	↓ brain	<i>hBACE1</i>	Downregulation	Pereira et al., 2016 [101]

B. Neurodegenerative miRNAs in Alzheimer's disease				
miR-26b	↑ brain cortex	<i>Rb1</i>	Upregulation	Absalon et al., 2013 [102]
miR-30a-5p	-	<i>BDNF</i>	Downregulation	Croce et al., 2013 [103]
miR-206	↑ brain	<i>BDNF</i>	Downregulation	Tian et al., 2014 [104]
miR-125	↑ brain	<i>DUSP6, PPP1CA & Bcl-W</i>	Downregulation	Banzhaf-Strathmann et al., 2014 [105]
miR-33	-	<i>ABCA1</i>	Downregulation	Kim et al., 2015 [67]
miR-34a	↑ brain	<i>VAMP2, SYT1, HCN1, NR2A, GLUR1, NDUFC2,</i>	Downregulation	Sarkar et al., 2016 [106]
miR-126	↑ brain	<i>IRS-1 and PIK3R2</i>	Downregulation	Kim et al., 2016 [107]