MicroRNAs as Possible Biomarkers for Diagnosis and Therapy of Alzheimer's Disease by Regulating the Abnormal Expression of Genes Related to Tau

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Abstract: Alzheimer's disease (AD), the most common dementia with the symptom of deterioration of memory and cognitive functions, becomes one of the prevalent threats globally. Tau hyperphosphorylation is one of major risk factors of AD. At present, there is no effective treatment or quick diagnostic methods for the pre-clinical stage of this disease. MicroRNAs (miRNAs) are small (20-25bp) non-coding, double stranded RNA molecule. They mainly regulate gene expression during the post-transcription by binding to the 3-'UTR of the mRNA then stop translation. Unlike other biomarkers using for Alzheimer's disease, miRNAs are stable and widely found in body fluids such as serum, tissues, and Cerebrospinal fluid (CSF) and one miRNA can regulate multiple genes. As a result, they are potentially used as diagnosis or therapeutic biomarkers for many diseases including Alzheimer's disease. Currently, this field that using miRNAs as biomarkers for diagnosis and treatment has been rapidly developed. To examine the mechanism and function of miRNAs and potential in AD, this review summarizes current diagnostic and therapeutic techniques and comparing several microRNAs that especially regulating tau toxicity as practicable diagnostic biomarkers and treatment agents. It is likely to detect different stages of Alzheimer's disease and reduce Tau hyperphosphorylation if this field of miRNA as biomarkers carries forward.

1 INTRODUCTION

According to epidemiological data prediction, due to the worldwide increase in life expectancy, the prevalence of dementia is expected to double every 20 years. By 2050 there will be 131 million people with dementia (Miya Shaik, 2018), and Alzheimer's disease will account for 35 million people (Femminella, 2015). Alzheimer's disease is a neurodegenerative disease in which memory and cognition deteriorate with age. Age is one of the most significant risk factors. Inheritance of the ε4 allele of apolipoprotein E (APOE) on chromosome 19 also increases the risk of Alzheimer's disease (Femminella, 2015). Besides, cardiovascular stress caused by other diseases such as obesity and heart disease can also trigger Alzheimer's disease (Miya Shaik, 2018). Diet also plays a role in Alzheimer's disease. As a progressive neurodegenerative disease, there is no obvious sign during the pre-clinical stage of AD. However, when this disease develops to the mild stage, it is already too late to be cured. Thus, scientists expect to figure out a better way to detect and treat the AD during the early stage.

The difficulty is to find a suitable biomarker on the nervous system. More and more research give attention to miRNAs, the small non-coding RNA molecules that exist in CNS.

According to Chen (2018), unlike other small nuclear RNAs (snRNAs), binding of microRNA (miRNAs) requires only partial complementarity between miRNA and target mRNA. Six to eight base pairs are enough (Kehl, 2017). It has been estimated that miRNAs can directly control one-third or more of gene expression in the genome, basically in stem maintenance, differentiation, proliferation, apoptosis, metabolism (Chen, 2018). Furthermore, the various functions of other miRNAs give a possibility for scientists to study whether they can be a diagnostic marker for different stages of Alzheimer's Disease predictions, also, based on the prospective idea that is using the dysregulation of the miRNAs expression to target the AD's pathological condition, miRNAs can be used as a therapeutic agent (Angelucci, 2019). This paper focuses on the possible factors for AD and summarized the current diagnostic and treatment strategies. The the diagnostic miRNA biomarkers and treatment agents for regulating Tau toxicity in AD will be discussed. Finally, the future investigations are discussed and more possibilities of the field of miRNA involved in Alzheimer's disease are predicted.

2 miRNAs BIOGENESIS AND FUNCTION

MicroRNAs (miRNAs) are widely found in the serum, blood, tissues, and fine-needle aspirate specimens (Walayat, 2018), as a result, they are potentially used as a diagnosis or therapeutic biomarker for many diseases.

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miRNAs are usually a strand of 20-25bp nucleotides that form small non-coding RNA molecules to regulate gene expression during the post-transcription. In the nucleus, miRNA genes are generally transcribed by RNA polymerase II. The transcription product pri-miRNA is a long precursor with a hairpin structure. Under the action of the RNaseIII enzyme Drosha and its cofactor DGCR8, the hairpin loop structure was processed into pre-miRNA composed of about 70 nucleotides, which transported into the cytoplasm. Most of the miRNAs are generated from the hairpin structure of single-stranded RNA precursors after Dicer processing, another RNase III enzyme. The mature miRNA is a double-stranded RNA with a length of about 21-25bp nucleotides (Walayat, 2018). Subsequently, the mature miRNA is incorporated into a protein complex called Argonaute protein (Ago), which leads one strand of the miRNA to degrade. In contrast, the other strand (guide strand) is bound to form an RNA-interfering silencing complex (RISC). RISC can recruit other proteins to degrade the target mRNA or recruits p-bodies to store the mRNA when it binds to the 3'-untranslated region (3'-UTR) of the mRNAs with the complementary sequence. This ensures the mRNA will no longer be translated into protein.

Unlike other small nuclear RNAs (snRNAs), miRNA silencing requires only partial complementarity between miRNA and mRNA. Six to eight base pairs are enough (Kehl, 2017). Therefore, a single miRNA can silence hundreds of genes, and multiple miRNAs can silence only one gene.

In addition, the expression of the same miRNA varies in different tissues and at different developmental stages. For example, a miRNA can be expressed early in development but not later in development. Thus, multiple copies of a miRNA can simultaneously regulate different genes or a single gene as required. When numerous other miRNAs bind to the same target, they cooperate to reduce the level of target mRNA, and the effect is greater than the sum of the effects of individual miRNAs. This property of miRNA is based on target recognition.

3 FEATURES AND DIAGNOSE OF ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the most common form of dementia. Every three seconds, there is an additional case of Alzheimer's disease worldwide. According to epidemiological data prediction, due to the worldwide increase in life expectancy, the prevalence of dementia is expected to double every 20 years. By 2050 there will be 131 million people with dementia (Miya Shaik, 2018), and Alzheimer's disease will account for 35 million people (Femminella, 2015).

Alzheimer's disease is a neurodegenerative disease in which memory and cognition deteriorate with age. 5% of people who age over 65 have the disease; At the age of 80's, the prevalence goes up to 10%; By age 90, the incidence reaches a staggering 50 percent. Age is one of the most significant risk factors. Inheritance of the ε4 allele of apolipoprotein E (APOE) on chromosome 19 also increases the risk of Alzheimer's disease (Femminella, 2015). Besides, cardiovascular stress caused by other diseases such as obesity and heart disease can also trigger Alzheimer's disease (Miya Shaik, 2018). Most diseases, for instance, obesity and diabetes, are caused by diet. As a result, diet also plays a role in Alzheimer's disease.

There are many features of Alzheimer's disease at the cellular and molecular levels. At the cellular level, AD is characterized by the progressive loss of neurons in the brain cortex and several other subcortical areas. Also, reduced synapses can be observed in these areas. In addition, the function of the synapses will be blocked, and the atrophy of the brain will be led by the dead neurons (Miya Shaik, 2018).

At the molecular level, Alzheimer's disease will be mainly characterized by three changes. Firstly, accumulations of the ß-amyloid (Aß) lead to the amyloid plaques. Synaptic activities of the brain and neuronal pathway are regulated by the Aß. As a result, the form of the neurotic plaques will influence the regulation of the normal neuronal activities and cause neuronal damage (Kehl, 2017). Tau protein belongs to the family of microtubule-associated proteins (MAP). It processes the ability to bind and stabilize the microtubules, so they are involved in neuronal migration, supporting dendrite elongation, and regulating microtubules during mitotic metaphase. Tau is also a phosphoprotein with more than 80 sites for phosphorylation. If increasing phosphorylation at the pathological sites of tau happens, tau will no longer bind to microtubules, and this hyperphosphorylation is also recognized as one of the features of Alzheimer's disease (Chen, 2018). Additionally, tau is involved in the neurofibrillary tangle (NFTs) formation in Alzheimer's disease and other dementias. The aggregation of misfolded proteins such as ß-amyloid plaques, tau, and NFTs has triggered inflammation in AD patients' brains (Kehl, 2017). Neuroinflammation is often used as a symbol to tell the progression of Alzheimer's disease.

4 CURRENT DIAGNOSIS AND THERAPY STRATEGIES IN AD

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Clinical diagnosis and therapeutic strategies are always the challenging part of Alzheimer's Disease. There is no very effective diagnosis method or treatment (Folch, 2016) for Alzheimer's disease currently.

The number of cures by serval drugs used for the clinic has been developed in the last 30 years is almost zero. According to the US National Institute on Aging (NIA) and the Alzheimer's Association (AA) suggests there are three stages of Alzheimer's Disease progress: 1) Preclinical AD; 2) Mild cognitive impairment (MCI); 3) real dementia (Miya Shaik, 2018; Femminella, 2015; Folch, 2016). The easiest way for a neurologist or a trained geriatrician will first examine a patient's history, medication history, and symptoms to rule out other conditions such as stroke, Parkinson's disease, or depression that might affect the patient's behavior and mental state. Besides, the doctor will evaluate the following aspects: Whether the patient's memory or thinking skills were impaired, the changes in the patient's personality and behavior, and the extent to which the patient's memory and thinking changes. In addition, the doctor may have the patient undergo brain imaging tests to provide information that may aid the diagnosis. Using brain imaging can help doctors determine whether the brain is bleeding, brain tumors or strokes, and various levels of brain disorders. Most of the time, the diagnosis of the cognitive disease is not a single examination. It takes few months sometimes to figure out the development of the brain. In addition, brain imaging can track specific biomarkers. Two toxic proteins are commonly found in the brains and cerebrospinal fluid of people with Alzheimer's disease, beta-amyloid (which forms plaques) and tau (which includes tangles) (McKhann, 2011). Alzheimer's can be diagnosed by testing the brain and cerebrospinal fluid to show abnormal development of these two toxic proteins.

Standard brain imaging techniques include the following:

- Magnetic resonance imaging (MRI): structural MRI is the most widely used neuroimaging technique to study the structural and neural changes of the brain. With the development of the disease, AD patients will have significant brain atrophy, atrophy of the temporal cortex, hippocampus, etc.
- Computed tomography (CT): CT scan uses X-rays to obtain a cross-sectional image of the brain, which can be used to analyze whether the brain has lesions according to the changes in brain volume atrophy.
- Positron emission tomography (PET): PET scans use radioactive substances called tracers to detect substances in the body. There are different types of PET scans: The most used PET scan is a fluorodeoxyglucose (FDG) PET scan, which identifies areas of the brain where glucose metabolism is reduced. Patterns of metabolic changes can distinguish between different types of degenerative brain disease. It can also detect clumps of amyloid protein (plaques) associated with Alzheimer's disease, but this PET scan usually needs under some specific conditions (Schwarz, 2021; Dubois, 2021).

Researchers worldwide are developing new diagnostic methods that will allow people to predict cognitive symptoms before they are mild or even present, such as blood, genes, gene-related proteins, and brain imaging tests (Hampel, 2018). Recent studies show that most therapeutic strategies build around the inhibition of the Aß and tau aggregation and some active immunotherapy. However, lack of early treatment can only relieve the cognition and memory loss, still, no help preventing the development of the neurogenerative disease (Angelucci, 2019; Folch, 2016).

5 miRNA AS A DIAGNOSTIC MARKER IN AD BY REGULATING TAU PROTEIN

The diagnostic marker used for Alzheimer's Disease is related to the Aß plaques and Tau proteins. There are many limitations with those biomarkers, such as the selection of the cutoff, although they are found in blood and Cerebrospinal Fluid CSF (Hampel, 2018). Unlike those biomarkers, miRNAs are stable enough in body fluids, blood, and CSF. They can directly target the genes in AD patients' brains, and they can be amplified by PCR easily. In the study of Shaik (2018), they also found the difference of miRNAs in healthy and AD patients' brains, which is related to Tau proteins. They are much simpler to be analyzed. As a result, those miRNAs have the possibilities to be diagnostic biomarkers (see table 1).

Table 1. Alternated miRNAs in Alzheimer's disease and have the potentials to down or upregulate tau toxicity.

Regulation	miRNA	change	target	Reference
Downregulation of tau toxicity	miR-106b	↑	Fyn	[23]
	miR-124-3p	↑	CAPN1	[22]
	miR-137	↓	CACNA1C	[24]
	miR-219	↓	MAPT	[14]
Upregulation of tau toxicity	miR-125b	↑	CDK5/P35/25	[17]
	miR-132	↓	tau mRNA	[21]
	miR-138	↑	RARA/GSK-3ß signal pathway	[20]
	miR-146a	↑	ROCK1/ PTEN signal pathway	[20]
	miR-219-5p	↓	GSK-3ß	[16]
	miR-322	↑	BDNF-TrkB	[19]
	miR-128	↑	NF-kB pathway	[25]

Tau is the most abundant expressed MAP in neurons in human brains. Tau phosphorylation is essential to maintain the normal function of the microtubules. miRNAs can directly control the Tau production, such as miR-219, by targeting MAPT (Santa-Maria, 2015; Wei, 2020). MiRNAs such as miR-125b, miR-219-5p (Li, 2019) can also regulate the tau phosphorylation by targeting enzymes that tau related to, for example, CDK5/P35/25, GSK-3ß. MiR-322 plays an essential role in regulating the brain-derived neurotrophic factor, then upregulated the tau hyperphosphorylation (Zhang, 2018). Besides, increasing miR-138, miR146a levels leads to upregulation of tau hyperphosphorylation. These two miRNAs are targeting the signaling pathway RARA and ROCK1 relatively (Wei, 2020). miRNA 132 regulates the tau mRNA, and miR-124-3p regulates CAPN1 mRNA, then upregulated and downregulated tau protein toxicity (Smith, 2015). MiR-106b inhibits tau hyperphosphorylation by targeting Fyn, which then suppresses the expression of Aß42 (Liu, 2016). Similarly, miR-137 inhibits the CACNA1C (Jiang, 2018), miR-128 inhibited the NF-kB pathway (Geng, 2018). Analysis on different alternated miRNAs, which target other genes to regulate Tau proteins, will give a general idea that miRNAs can be used as a diagnostic biomarker by observing the changes of their levels.

6 miRNA AS A THERAPEUTIC TARGET IN AD BY REGULATING TAU PROTEIN

As described in table 1, some classes of miRNAs have the potential of downregulation of Tau toxicity. For example, increasing miR-106b, miR-124-3p leads to less tau hyperphosphorylation, decreasing miR-137, miR-219 leads to less tau hyperphosphorylation; however, other classes of miRNAs upregulated the tau toxicity, such as by increasing miR-125b, miR-138, miR-146a, miR-322, miR-128, or decreasing miR-132, miR-219-5p. Therapeutic regulation of miRNA can be accomplished in two ways.

- To inhibit miRNAs' function, a complementary antisense oligonucleotide (ASO) can be used to react with the single-stranded miRNAs.
- 2In contrast, miRNA expression can be increased or restored to its normal or higher level by applying compounds that stimulate its production or synthesis of oligonucleotide so that miRNAs will have the same function as the mimic miRNAs (Angelucci, 2019). As shown in table 2, in order to keep the Tau phosphorylation on the normal track, miR-106b, miR-124-3p, miR-132, miR-219-5p needs to be increased or restored. On the contrary, it is necessary to inhibit the level of miR-137, miR-219, miR-125b, miR-138, miR-146a, miR-322, miR-128.

Table 2. Different treatments for miRNAs to regulate the Tau phosphorylation.

Methods	miRNA	Aim	target
Apply compounds that stimulate its production or synthesis of oligonucleotide	miR-106b	Increase	Fyn
	miR-124-3p	Increase	CAPN1
	miR-132	Increase	tau mRNA
	miR-219-5p	Increase	GSK-3ß
Add complementary antisense oligonucleotide (ASO)	miR-137	Decrease	CACNA1C
	miR-219	Decrease	MAPT
	miR-125b	Decrease	CDK5/P35/25
	miR-138	Decrease	RARA/GSK-3ß signal pathway
	miR-146a	Decrease	ROCK1/ PTEN signal pathway
	miR-322	Decrease	BDNF-TrkB
	miR-128	Decrease	NF-kB pathway

7 CONCLUSION

MiRNAs play an essential role in the post-transcriptional regulation of gene expression. One of the most vital benefits is that one miRNA can regulate groups of proteins. MiRNA is very stable in the body fluid, also it can participate in almost all physiological processes. An individual miRNA can be expressed early in development but not later in development. Thus, multiple copies of a miRNA can simultaneously regulate different genes, or a single gene as required. When numerous other miRNAs bind to the same target, they cooperate to reduce the level of target mRNA, and the effect is greater than the sum of the effects of individual miRNAs.

In many preclinical studies, MiRNAs as therapeutic targets and biomarkers were shown to be effective and are awaiting clinical development. Current studies suggest that many other miRNAs are already discovered for Aß accumulation, Neuroinflammation, cell death, synaptic dysfunction. By considering the regulation of the miRNAs in Tau toxicity, it indicates the possibility of the miRNAs for clinical use. However, miRNA still has many limitations, for example, the expression of the same miRNA varies in different tissues and at different developmental stages, it is hard to confirm the specific miRNAs to use. Besides, Alzheimer's patients may associate with other brain diseases, whether those diseases influence the level of the miRNAs that we found is unknown. Also, some studies are inconsistent with the function of the specific miRNAs that have the potential used in AD. Further investigations are needed to obtain mature results.

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