

Evaluation of the Application Value of m6A in the Treatment of Alzheimer's Disease

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Abstract. This paper focuses on the pathogenic mechanisms of m6A modification in Alzheimer's disease (AD), potential therapeutics and current application status. The mechanisms of neuroinflammation, neurotoxicity and cognitive impairment are shown. Besides, some related treatments such as small molecule activators and inhibitors, DNA and RNA therapy are also included in this paper. Through the analysis of this paper, people can have a general understanding of the relationship between m6A and AD, some inherent treatments and current research progress. The author believes that studies on enzymes such as Mettl3 and Mettl14, as well as proteins like tau and A β , will be crucial for the treatment of AD. The analysis of this paper can be a reference for future research on the mechanism of AD and the development of m6A-based therapeutics. However, there are still limitations. For example, the methods of drug delivery have not been developed, which means that there are still some problems in transporting drugs to target areas in brains. In addition, some risks of the potential treatments are not discussed in this paper. Therefore, more researches are needed. It is hoped that more details of the pathogenic mechanisms of m6A in AD can be found in the future. The therapeutic methods will also be completed, which means the challenges will be overcome. They might provide new ideas or reference to the development of treatments for other difficult miscellaneous diseases.

Keywords: m6A, Alzheimer's disease, mechanism, therapeutic methods.

1. Introduction

Alzheimer's disease (AD) is one of the causes of dementia that accounting for 60–80% of dementia cases [1]. It leads to severe global health problems. Most of the patients are elder people. This disease leads to their memory loss and difficulty in moving and taking care of themselves in their daily life. As the aging problem becomes more and more serious, widespread AD turns out to be a big challenge to society. However, the development of therapeutic methods still dose not have been much progress. The mechanism of AD is very complex. It relates to a variety of reasons. The characters of AD include plaques formed by amyloid- β 1–42 (A β 1-42) aggregates and neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein (p-Tau) aggregates [1].

Currently, m6A modification is found to be one of the main reasons for AD. It is a post-transcriptional modification and mainly modifies mRNAs. According to research, in eukaryotes, especially mammals, the level of m6A RNA methylation in the brain is high and it also plays an important role in neurodevelopment [2]. m6A is involved in the regulation of the development, differentiation, activation, migration, and polarization of immune cells, pro-inflammatory and anti-inflammatory responses are consequently controlled. Methyltransferase, demethylase and binding protein are also reported to be engaged in m6A modification. Some research showed that the sites of m6A primarily locate near the 3' untranslated region (3' UTR) and the stop codon of mRNA [3]. In the brain of AD patients, m6A is found to be exceptionally expressed [4]. Thus, m6A is a popular direction in the research of methods for treating AD. In addition, various proteins are also involved in this process such as Methyltransferase-like (Mettl3), Mettl14, human insulin-like growth factor 2 (IGF2), etc [2,5]. In the development of therapeutic methods, these proteins also gain some attention. Identifying the relationship between m6A and related proteins and AD and developing effective treatment methods has become one of the hot topics in this field.

The aim of this paper is to introduce the pathological mechanism of m6A, its relationship with AD, potential treatment methods, and some current research status. It is significant to find the treatment of AD. This disease not only affects the self-care ability of patients, but also causes a great loss of money. By recovering the elder people who suffered from AD, the emotional and financial burdens of many families will be solved. It is also beneficial to the sustainable development of the social economy. Additionally, the development of therapeutic methods of AD is important to the searching of treatment of other diseases.

2. The relationship between m6A and the pathogenic mechanism of AD

2.1. The neuroinflammation which is mediated by m6A modification in AD

Recent reports show that there is a high possibility of a strong connection between m6A modification and AD. M6A is a kind of RNA modification that widely exists in eukaryotes. It is composed of three groups of proteins: methyltransferases, demethylases and m6A-binding proteins. METTL3, which is one of the important proteins of the m6A-METTL-complex (MAC), is reported to regulate neuroinflammation [6]. According to the research, the over-expressed METTL3 will promote the expression of inflammatory cytokines and inflammatory proteins, which will further facilitate microglial inflammatory responses [4,7]. In addition, Mettl14 is a significant methyltransferases of m6A modification. It can support the apoptosis of spinal cord neurons. In the substantia nigra region, microglia and astrocytes will be activated by the silent Mettl14 [2]. Therefore, Mettl3 and Mettl14 proteins are highly related to the regulation of microglia and astrocytes. In this way, both of them can facilitate the neuroinflammation. In some specific conditions, intracellular signaling pathways of astrocyte will be activated, pro-inflammatory factors will then be secreted and finally lead to inflammation [8]. In the same way, microglia is response to the formation of synapse connections, elimination of synapse connections, and cells phagocytosis. It can be activated and turn into M1 phenotype, which contributes to inflammatory factors and neurotoxicity, regulates cellular inflammation and results in numerous neurodegenerative diseases [4].

2.2. The neurotoxicity which is triggered by m6A in AD

Hyperphosphorylation and division of the microtubule-associated protein tau will drive synaptic impairment, neuronal dysfunction, and neurofibrillary tangles. This is also one of the main pathogenic traits of AD that will finally cause neuritic plaques, neuroinflammation, and cognitive disorder [2]. The tau protein plays an important role in many pathways that are involved in decision of cellular functions and survival. However, this protein will pathologically deposit and form NFTs because of distraction in post-translational processes. This will damage the activity, structure and function of nerve cells. It is shown that reduction of synapses, synaptic vesicles and negative impacts on morphology and connectivity of newborn granule neurons of dental gyrus occurred when tau proteins were injected into hippocampus. In addition, tau proteins can also lead to stress reactions of mitochondria and result in severe metabolic disorders and reduction of energy production [9]. Therefore, the NFT which is caused by tau proteins, is one of the main reasons of AD. The Fat Mass and Obesity Associated gene (FTO) is a RNA demethylase of m6A. In neurons, the overexpression of this protein will facilitate the phosphorylation of tau proteins through activating mTOR and its downstream signaling pathways. These genes are responsible for regulation of Regulate autophagy, neuronal function, and inflammation. Besides, it is reported that the Mettl3 is also involved in deposition of HNRNPA2B1, which plays the role of an m6A reader. An m5C RNA methyltransferase called NSun2 also functions as an m6A RNA methyltransferase and reduces the level of tau proteins phosphorylation, recovers the activity of nerve cells and remits neurotoxicity by modifying microRNA in the m6A way. It means that NSun2 is a protein that is used to protect nerve cells [4].

2.3. The cognitive impairment that is related to m6A in AD

Cognitive impairment is thought to be an omen of AD. The hippocampal neurons are widely proven to be highly related to memory and cognition. Therefore, one of the main traits of AD is the injury of hippocampal neurons and synapses. A β is a kind of protein that has high toxicity to nerve cells. It can break synapse plasticity and thus injure memory and cognition in many ways. For example, it can recognize and damage synaptic membrane receptors so that structure and adaptability of synapses will be negatively affected. A β can also destroy the glutamate cycle and the glutamate will massively deposit outside the synapses. Neurotoxicity will thus be caused. Besides, it can destroy functions of synapses not only by disturbing synapse-related signaling pathways and molecular targets, but also by damaging mitochondria and processes of energy metabolism [10]. Therefore, A β can severely lead to synaptic damage and result in cognitive impairment. It is demonstrated that the amount of Mettl3 reduces in AD patients' hippocampus. This leads to abnormal methylation and down-regulation of synaptic-related genes and finally cause the loss of synapses. According to the research, the mice whose Mettl3 is knocked out showed memory loss. And the overexpression of Mettl3 can recover synaptic damage and cognitive impairment caused by A β . However, A β will also inhibit the expression of Mettl3 [4]. It is shown that Mettl3 is the antagonist of toxic A β . In conclusion, the reduction of Mettl3 leads to an increase in A β and thus results in synaptic dysfunction.

3. The therapy of AD

3.1. Small molecule activators and inhibitors of m6A

Small molecule activators are a group of small molecules that can bind with specific proteins and promote their activity. As mentioned in the section 2, m6A modification is involved in protection of synapses, and it also plays an important role in brain development and synaptic regeneration. Mettl3 and Mettl14 are two of the core methyltransferases involved in m6A [4]. Therefore, employing small molecule to activate the activity of Mettl3 and Mettl14 becomes a possible therapeutic method for AD. Through triggering these two methyltransferases, the level of m6A can be raised so that neuronal regeneration can be facilitated [5]. According to the research, 4 activators are found. For example, the activator C4 is a good one. It can bind with Mettl3 and Mettl14 and enhance the binding of the methyl donor SAM to the complex and further promote the m6A modification [11]. In addition, an important mRNA demethylase of m6A called FTO can also be a target of AD treatment. The level of phosphorylation of tau proteins can be reduced by inhibiting the FTO and thus nerve cells can be protected. According to the research, there are two small molecules that can stably bind to FTO and inhibit its activity. These two molecules can also be easily transported to the target in brain so they might be useful in the therapy of AD in the future [12].

3.2. Genetic therapy of AD

Nowadays, using genetic engineering to deal with diseases is popular point in this field. Some diseases can be effectively treated by employing genetic engineering methods to directly change genes in patients. Changing specific genes in patients' cells is helpful to regulate the production of proteins and thus control the cells. CRISPR/Cas9, as a technique that is widely used to edit genes, can also be used to edit specific genes that are involved in AD in order to treat it. It is reported that the mutation of APP is one of the reasons for abnormal production of A β . By restoring the mutation of this gene, the amount of A β can be reduced. In addition, CRISPR/Cas9 can also be used to silence β -site APP Cleaving Enzyme 1 (BACE1) gene and production of A β will be significantly decreased. As stated by the research, after the mice were treated in this way, the amount of A β in hippocampus effectively reduced [13]. Besides, this technique is also useful in increasing the amount of Mettl3 and Mettl14 by overexpressing the relevant genes such as enhancing the promoter or changing upstream regulating genes. This can not only help restore the damage caused by A β , but also facilitate the regeneration of synapses [5]. However, there are still some challenges when using this method. For example, the off-target effect can lead to unexpected results in patients' cells. Therefore, more researches are needed before employing it as a treatment of AD.

3.3. RNA therapeutic method of AD

Therapeutic methods that are based on RNA are novel treatment technologies of various diseases including central nervous system (CNS) disorders such as AD. Antisense oligonucleotides (ASOs) are one of the RNA therapeutic methods. They can bind with the target mRNA involved in A β production or tau proteins phosphorylation and then disturb the expression by two different mechanisms. The first mechanism is to recruit RNase H and decompose the mRNA. The second one is to inhibit the further translation without RNase H [14]. Besides, siRNA can also be a kind of treatments of AD. siRNAs will bind with TAR RNA-binding protein (TRBP) and the argonaut 2 enzyme (AGO2) and form RNA-Induced Silencing Complex (RISC) in cells. Then the RISC will

match with the target mRNA of A β or tau proteins phosphorylation enzymes by using siRNAs. Once they bind together and form a complex, the mRNA will be decomposed by AGO2. This is an effective method because siRNAs have high degree of specificity [15]. Therefore, these two RNA therapeutics can be used to disturb the expression of mRNA of AD and thus deal with it.

4. Current application status of m6A in treating AD

4.1. The research about m6A application in vivo models

There are many studies that are done by using vivo models such as mice. There are three main reasons why mice are one of the appropriate models in researching m6A in AD. The first reason is that the level of m6A modification rising in brains of mice is similar to human beings. It is also found that the sites of m6A of mice highly overlap with human beings. In addition, the structure of tau proteins of mice is similar with human beings. Besides, because zebrafish are genetically similar to humans and their brain structure is similar to that of mammals, it is also a good vivo model [4]. Therefore, using mice and zebrafish as in vivo models is helpful in understanding the mechanism of m6A in AD and finding effective therapeutics. Through doing various researches on them, there are many new targets of m6A modification involved in AD found such as Mettl3.

4.2. The research about m6A application in vitro models

It is significant to directly use in vitro models to do experiments because they are almost the same with cells of human cells. Nowadays, using human pluripotent stem cells to form organoid models is an effective way. There are many ways to make human pluripotent stem cells. For example, it is shown that some small molecules can be used to directly change human fibroblasts into neuronal cells. According to the research, the compulsory expression of some neuronal transcription factors is also useful to convert somatic cells into neuronal cells. The structures and functions of the transformed neuron cells are very similar to ordinary neuron cells [16]. These two techniques are helpful in forming in vitro models, which are crucial to studying the m6A in AD. Besides, this can also show the challenges and limitations of treatments of AD.

5. Conclusion

AD is a serious neurodegenerative disease that leads to hypomnesia, cognitive dysfunction, behavioral abnormalities and loss of daily living skills. It not only afflicts elderly people who get this disease, but also causes huge problems for the economy and well-being of society. Nowadays, it is found that m6A modification is involved in this disease. Various proteins offer targets to the treatment of AD. However, there are still some challenges in drug delivery so more researches are needed. This paper analyses research on the relationship between m6A and the pathogenic mechanism of AD, including neuroinflammation, neurotoxicity and cognitive impairment that are related to m6A. In addition, some potential therapeutics are involved in this paper. For example, it is available to design small molecule activators and inhibitors. DNA and RNA drugs can also work in recovering AD. Finally, this essay shows current application status. Vivo and vitro models can be successfully used in finding treatment. This paper provides a reference for future research on the m6A mechanism and treatment plans. One of the limitations that have not been solved is that it is difficult to deliver drugs into the target area in brains. Therefore, the future research can focus on development of a drug delivery system.

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