REVIEW ARTICLE

RNA m6A Methylation and Alzheimer's Disease: Current Evidence and Future Perspectives

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ABSTRACT

Background: Alzheimer's disease (AD) is the most common neurodegenerative disease characterized by the pathological accumulation of b-amyloid and neurofibrillary tangles. Despite substantial progress in both basic and clinical research on AD, the detailed mechanism of AD pathogenesis is still elusive. RNA N6-methyladenosine methylation (m6A) is the most predominant post-transcriptional modification on eukaryotic mRNA, prominently enriched in the mammalian brain. Notably, m6A-modified RNA showed significant changes during the development of AD, indicating an important role of this modification in AD pathogenesis.
Aim: In this study, we aim to provide a summary of recent advances highlighting the indispensable role of m6A in AD pathogenesis.
Result: From the perspective of m6A modification, we review our current understanding of the association between RNA m6A machinery and the risk factors of AD, as well as its involvement in various pathophysiological hallmarks of AD. We also discuss the main obstacles in current studies about m6A in AD pathogenesis and the corresponding caveats and solutions to them.
Conclusion: This review emphasizes the significance of investigating m6A in the context of AD and highlights the considerable potential for m6A to emerge as a novel therapeutic target for AD.
Keywords: Alzheimer's disease; RNA m6A modification; Pathological protein aggregates; Disruptions of proteostasis; Neuroinflammation; Synaptic dysfunction; Mitochondrial dysfunction.

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INTRODUCTION

The elderly makes up a great proportion of our population and this proportion is still increasing. Consequently, this will bring about a series of problems, such as the burden of age-related diseases and a crippling public health crisis. Alzheimer's disease (AD) is the most common neurodegenerative disease characterized by cognitive impairment (1). According to the China Alzheimer Report 2022, China has 9.83 million individuals aged 60 years old and over with AD (2). In regards to its etiology, only in less than 1 % of cases, AD is caused by specific genetic changes, while in most cases the pathogenesis of AD is complicated and related to a combination of genetic, lifestyle and environmental factors that damage the brain over time (1). Unfortunately, there has been no efficient treatment that cures AD so far (3). Therefore, elucidation of the mechanism of AD pathogenesis is crucial to facilitate the development of efficient diagnostic and therapeutic strategies.

In recent decades, post-transcriptional regulation of gene expression has attracted wide attention due to its prominent role in regulating physiological conditions and cell behavior. Of note, RNA N6-methyladenosine modification (m6A), as the most abundant epitranscriptional mark, modulates almost every step of RNA metabolism in response to various internal or external stimuli (1). Accordingly, maintenance of the dynamic balance of m6A is crucial for various physiological activities in the brain, including neurodevelopment, axon guidance, synaptic plasticity, myelination, stress response, and so forth (4). Alterations in m6A may occur in any type of neural cells, and dysregulated m6A methylation has been detected in many neurological diseases, such as major depression disorder, traumatic brain injury and others (5). Accumulating evidence demonstrates that defective RNA metabolism contributes to the disease onset and/or progression of neurodegenerative diseases (6). Therefore, to improve our understanding of the pathomechanism of AD from the perspective of epitranscriptional regulation, it is necessary to explore the role of m6A in the pathogenesis of AD in a comprehensive and in-depth manner. In this review, we highlight the growing evidence for the correlation between m6A and AD, and the indications that its dysregulation underlies the pathogenesis of AD.

GENERAL OVERVIEW OF m6A AND AD-RELATED RNA DEFECTS

The function of m6A is largely dependent on the RNA m6A machinery, including writer, eraser, and reader proteins. The writer complex, comprising METTL3 and METTL14, is responsible for installing m6A on RNA in conjunction with WTAP, RBM15, and other accessory factors (7). In addition, other methyltransferases, like METTL16 (8) and ZCCHC4 (9), have been reported to mediate m6A on various types of RNAs, such as small nuclear RNA (snRNA) and ribosome RNA (rRNA). On the contrary, only FTO (10) and ALKBH5 (11) are identified as demethylases, responsible for dynamically eliminating m6A modifications. Both the writer and eraser proteins are involved in maintaining the dynamic equilibrium of m6A. Furthermore, m6A-modified RNA is recognized and bound by the reader proteins, which imparts the biological functions to m6A in diverse processes (12). Up to now, the ranks of this category have been steadily growing. In addition to the YTH-domain family (YTHDFs and YTHDCs), insulin-like growth factor-2-binding proteins (IGF2BPs) and heterogeneous nuclear ribonucleoproteins (hnRNPs), new members are constantly being unearthed, such as FMRP (13) and PRRC2A (14).

The interaction between m6A and its regulators ensures the normal metabolism of the target RNA, such as splicing, translocation, translation, degradation, and structural switch (15). Any deviation in the above steps can lead to various diseases, especially neurodegenerative diseases (16, 17). Several studies have reported abnormal RNA metabolism during the development of AD, suggesting a crucial role of m6A in the progression of this disease. For example, m6A-dependent alternative splicing is a well-observed form of RNA regulation (18). Alternative splicing events in the transcripts encoding AD-related proteins, such as MAPT, APP, and BACE, contribute to neuronal hyperexcitability and cognitive deficits associated with AD pathology (19). Hence, there is a plausible hypothesis that m6A plays a regulatory role in the progression of this disease by modulating the alternative splicing of transcripts associated with AD. Next, the regulation of transport and local translation by m6A appears to be particularly crucial in neurons, ensuring the development and maturation of neural circuits and synaptic plasticity (20). Numerous studies have documented the impact of m6A dysregulation on the transport and local translation of synaptic RNAs, ultimately contributing to cognitive and memory impairment which are typical symptoms in AD (21-23). Moreover, m6A can regulate mRNA localization in stress granules (SGs), which is crucial for RNA translation and degradation in response to different stimuli, such as oxidative or heat-shock stress (24, 25). A recent study has shown that m6A-modified RNAs are deposited in Tau oligomer-induced SGs in the brains of AD patients (26). This observation suggests the potential involvement of m6A in the pathological aggregation of stress granules induced by AD-related chronic stimulation. Because of the complexity of AD pathogenesis, we have so far lacked a comprehensive and in-depth understanding of the molecular mechanism of this disease. It has been demonstrated that RNA regulation plays a crucial role in the prevention and treatment of AD (27, 28). Therefore, exploring the relationship between AD and m6A-mediated RNA regulation would contribute to a more comprehensive understanding of the molecular network associated with AD.

m6A AND DYSFUNCTIONAL COGNITION/MEMORY

m6A is highly abundant in the mammalian brain, regulating neurodevelopment, adult neurogenesis, as well as learning and memory (4). A great deal of studies have demonstrated that m6A maintains the self-renewal and differentiation of neural stem cells (NSC) by regulating RNA metabolism. For instance, a deficiency of either *Mettl3* or *Mettl14* in the developing mouse brain could diminish the m6A on transcripts of neurodevelopmental regulators and prolong the cell cycle of radial glia cells to extend cortical neurogenesis (29). Meanwhile, conditional *Ythdf2* knockout mice also exhibited neurodevelopmental delays, confirming that YTHDF2-mediated mRNA degradation plays an important role in NSC proliferation and differentiation (30). Adult neurogenesis supports essential brain functions, and its impairment during aging contributes to cognitive decline in aged individuals and elevated risk of developing AD (31). Chen *et al* found that the deletion of *Mettl3* in adult neural stem cells (aNSCs) reduced m6A levels and inhibited NSC proliferation. Moreover, not only did *Mettl3* deletion inhibit neuronal development and make aNSC differentiation more biased to the glial lineage, but it also affected the morphological maturation of newborn neurons in adult brains (32). Conversely, selective knockout of *Fto* in aNSCs transiently boosted aNSC proliferation and promoted neuronal differentiation, but ultimately inhibited adult neurogenesis. Mechanistically, *Pdgfra* and *Socs5* were identified to be the direct targets of FTO, which further activated the STAT3 pathway to impact adult neurogenesis (33). Neurodevelopment serves as the basis for constructing neural networks, while adult neurogenesis ensures brain plasticity during learning and memory. Consequently, exploring the regulatory role of m6A in these processes will contribute to uncovering the mystery of cognitive memory impairment resulting from abnormal neurodevelopment or adult neurogenesis at the post-transcriptional level.

There is a significant content of m6A on synaptic RNAs, indicating a crucial role of m6A and its regulators in the process of memory formation (21). Zhang Z. *et al.* reported that the abundance of METTL3 in mouse hippocampus is positively correlated with learning efficacy. The specific knockout of *Mettl3* in the forebrain excitatory neurons hampered hippocampal-dependent long-term memory formation while leaving short-term memory and learning abilities unaffected. Mechanistically, they found that METTL3 facilitates the translation of genes vital for long-term memory formation by modulating m6A, thereby enhancing the process of long-term memory formation (34). In response to behavioral experience, increased m6A level was also observed in the medial prefrontal cortex (mPFC), probably resulting from the upregulation of METTL3 and downregulation of FTO. Furthermore, lentivirus-mediated *Fto* knockdown in the mPFC led to a significant increase in cued fear memory in mice (35). A similar phenomenon was also observed in the hippocampus. Concretely, upon stimuli of contextual fear conditioning, FTO expression decreased in the dorsal hippocampal CA1 neurons, particularly in the synapse. Oppositely, artificially decreasing FTO expression in the dorsal hippocampus could enhance contextual fear memory (36). These findings suggest that opposite from METTL3, FTO plays a role in constraining memory formation. In addition to writers and erasers, m6A readers have also been reported for their crucial functions in facilitating learning and memory. For instance, YTHDF1 regulates the translation of m6A-modified neuronal RNAs crucial for learning and memory in response to neuronal stimulation. Hippocampus-specific *Ythdf1* deletion disrupted learning and memory formation due to impaired basal synaptic transmission and long-term potentiation (37). Intriguingly, *Mettl14* deletion in adult mouse striatal neurons also caused the impairment of learning and performance (38). Therefore, beyond traditional memory storage areas, striatal-mediated learning ability is also under the control of m6A.

Memory impairments and cognitive deficits are typical clinical features of AD and are usually caused by substantial synaptic loss and overall neuronal damage. Therefore, identifying the regulatory role of m6A in memory impairment may help to explain its significance in AD.

m6A AND RISK FACTORS FOR ALZHEIMER'S DISEASE

AD is regarded as a multifactorial disease associated with a variety of risk factors. In addition to aging, genetic and environmental factors, several acquired diseases can also elevate the risk of AD, such as depression, Down syndrome (DS), and brain injuries (39). The presence of these risk factors is likely to

trigger the onset or progression of AD, however, the precise relationship between them remains largely unknown. This section will summarize the correlations between m6A and those risk factors reported so far, thus providing new ideas for elucidating the pathomechanism of AD.

AGING

Advancing age is one of the major risk factors for AD (40). The extensive overlap between the nine aging hallmarks and AD pathology implies that the regulatory mechanisms between aging and AD have some qualities in common (41). A significant increase in the m6A levels was observed from adolescent to old in both the cerebral cortex of aged mice and the postmortem human brain (region BA9) (42), suggesting an important role of m6A in the aging process. Concretely, 426 hypermethylated and 102 hypomethylated RNAs were identified in the hippocampus of 20-month-old mice, and these differentially methylated RNAs exhibit a significant correlation with changes in RNA expression during aging. This implies a co-regulatory role of m6A in gene expression during aging. As an example, a key regulator of myelin *Gpr17* exhibits decreased m6A level in its 3'UTR in the aging brain, which participates in the translational control of GPR17 in the hippocampus (43).

The emergence of significant cognitive impairment is thought to be a watershed between normal aging and AD. Compared with young mice, a consistent downregulation of m6A on synapse-associated RNAs was observed in multiple brain regions of 16-month-old mice, the age when initial memory impairment manifests (44). Similarly, decreased m6A level was also detected in the cingulate cortex brain tissue of AD patients when compared with cognitively intact human subjects. This simultaneous loss of m6A with the onset of memory impairment in the aging brain suggests a potential impact of dysregulated m6A on cognitive decline.

APOE

Genetic variation represents another crucial risk factor for AD, with APOE4 as the main susceptibility gene and prevalent in 40-50 % of AD patients (45, 46). The precise mechanism underlying how APOE4 mediates AD pathological manifestations remains incompletely understood, emphasizing the necessity to elucidate the molecular mechanisms for effective prevention and treatment. A prospective population study reveals an interaction between FTO and APOE, showing that individuals carrying both *FTO* AA and *APOE* ε4 face an elevated risk of developing dementia (47). Furthermore, *Fto* knockout significantly increased the m6A level on *Apoe* RNA and stabilized its expression through recognition by IGF2BP2 (48). Beyond that, a transcriptomic analysis of postmortem brain tissue from AD patients identified downregulation of YTHDC2 in the *APOE* $\varepsilon4^+$ group, while METTL3, METTL16, RBMX, and LRPPRC were all upregulated (49). These findings collectively suggest the presence of a complex m6A-mediated regulatory relationship between APOE4 and AD.

DEPRESSION

Depression, particularly late-life depression, poses a risk factor for cognitive decline and AD (50). Meanwhile, depression is one of the most frequent neuropsychiatric complications of AD and affects up to 50 % of patients (51). However, the molecular connection between depression and AD remains elusive. As is known, dysregulated stress response is a significant feature shared by both major depressive disorder (MDD) and AD. Engel M. *et al.* reported that the homeostasis of m6A regulating the stress response was impaired in the blood of MDD patients receiving glucocorticoid treatment (52). In addition, single-nucleus RNA sequencing (snRNA-seq) analysis of the cortex in patients with depression unveils cell-specific alterations in m6A regulators, with a consistent increase of FTO observed across all cell types (53). The association between FTO and depression was discovered several years ago (54), although depression is unlikely originated from the genetic variation of *FTO* (55). However, hippocampus-specific knockdown of *FTO* exacerbated depression-like behaviors in adult mice, while overexpression of FTO rescued this phenotype (56). Besides that, a polymorphism study of MDD patients in the Chinese Han population identifies that another RNA demethylase ALKBH5 shows allelic association and genotypic association with the risk of MDD (57). Furthermore, Huang *et al.* found that overexpression of *circSTAG1*, which was originally downregulated in the hippocampus of depression mouse models, could capture ALKBH5 and reduce its nuclear translocation in astrocytes. This reduction in turn led to increased methylation and degradation of fatty acid amide hydrolase *(FAAH)*

mRNA, ultimately relieving corticosterone-induced depression-like behavior and astrocyte loss in vivo (58).

Additionally, m6A holds the potential for improving the treatment of depression and AD. Hypericin, a major active component in Hypericum perforatum, has shown efficacy in treating various diseases, including AD and depression (59, 60). Intriguingly, treatment of depression mouse models with hypericin was found to maintain m6A homeostasis and regulate the expression of genes associated with antidepression by restoring METTL3 and WTAP levels (60). Taken together, since m6A appears to regulate similar pathologic processes both in AD and depression, exploring this avenue from the perspective of m6A may provide insights into the connection between these two diseases.

DOWN SYNDROME

Down syndrome (DS) is the most common form of intellectual disability that arises from trisomy of chromosome 21 (61). In general, patients with DS have a high risk of early-onset AD, probably due to the presence of three copies of the *APP* gene encoding amyloid precursor protein (62). It has been found that most of the patients with complete trisomy of chromosome 21 develop amyloid plaques and neurofibrillary tangles (NFTs) by the age of 40 (63). However, there also exists a small proportion of individuals with DS, who are protected from the onset of AD. Therefore, understanding the genetic or environmental factors leading to this variation will provide key insights into the pathogenesis of AD.

A recent study found that compared with control, the cerebral cortex tissues of fetuses diagnosed with DS exhibit a reduction in METTL3 expression and lower m6A levels (64). Furthermore, they found that NRIP1, a corepressor in oxidative metabolism and mitochondrial biogenesis, exhibits significantly higher expression but a lower m6A level. Mechanistically, METTL3 regulates the expression of NRIP1 by regulating its mRNA stability in an m6A-dependent manner (64). Therefore, aberrant expression of NRIP1 induced by hypomethylation may be one of the causal factors of dysfunctional mitochondria in DS cells. Notably, decreased expression of NRIP1 is also observed in the postmortem brains of AD patients (65). Together with the fact of widespread mitochondrial dysfunction in AD, it will be interesting to investigate whether m6A of *NRIP1* is also involved in the pathogenesis of AD.

Besides amyloid plaques and NFTs, neuronal accumulation of ubiquitylated and aggregated TDP43 is also observed in AD-DS. Actually, TDP-43 inclusions are found in up to 57 % of AD patients, who tend to have more severe defects in cognitive ability (66). Recently, McMillan M. *et al.* discovered that in the spinal cord of patients with amyotrophic lateral sclerosis, m6A-modified RNAs and the associated YTHDF2 protein are required for TDP43 protein binding and autoregulation (67). Therefore, whether the role of TDP-43 in the progression of AD is also dependent on m6A warrants further exploration.

TRAUMATIC BRAIN INJURY

Traumatic brain injury (TBI) is correlated with an elevated risk of AD. The presence of typical pathological features of AD in the brains of TBI patients implies intricate relationships between the mechanisms of TBI and AD (68). It has been demonstrated that m6A responds to stimulation induced by brain trauma. For example, TBI in the mice caused downregulation of METTL3 in the hippocampus as well as altered m6A levels on hundreds of transcripts (69). In addition, significant changes in m6A levels were detected in the RNAs from the cortex of a TBI rat model, which was probably caused by reduced expression of METTL14 and FTO. Accordingly, treatment with the FTO inhibitor FB23-2 exacerbated TBI-induced neurological impairment (70). Intriguingly, hypothermia, a prevalent treatment for TBI, could rescue the abnormal m6A on some transcripts caused by TBI, implying that the m6A regulatory network could offer a new path for TBI treatment (71). TBI-induced neurovascular injury expedites the production of beta-amyloid (Aβ), neurofibrillary tangles (NFTs), and cerebrovascular inflammation, all of which have been demonstrated as m6A-related pathology in AD (72). Therefore, identifying the alterations of m6A in TBI can aid in elucidating the pathomechanism of TBI-induced AD.

m6A AND PATHOPHYSIOLOGICAL HALLMARKS OF ALZHEIMER'S DISEASE

The pathology of AD is primarily characterized by two hallmarks: accumulation of Aβ plaques and formation of NFTs (73). With the abnormal aggregation and defective degradation of proteins, the accumulated plaques and tangles contribute to

synaptic dysfunction and neuronal cell death, particularly in the brain regions crucial for memory and cognition. Furthermore, the accumulation of toxic proteins and cell death in neurons activate immune cells in the brain and induce neuroinflammation. Besides, dysfunctional mitochondria also contribute to energy metabolism abnormalities and are thought to be the early event in the development of AD. Understanding the intrinsic molecular mechanism behind those pathological hallmarks is crucial for developing targeted therapeutic interventions to alleviate or slow the progression of AD. Here we review recent studies investigating the relationship of m6A and the above pathophysiological hallmarks and discuss the significance of dysregulated m6A in AD pathogenesis.

PATHOLOGICAL PROTEIN AGGREGATES

The accumulation of Aβ plaques and NFTs, as exogenous stimuli, could influence the modulation of m6A. Consequently, dysregulated m6A, via disturbing RNA metabolism of the downstream target genes, may induce aggravated pathological changes.

BETA-AMYLOID PLAQUES

Numerous studies have demonstrated that Aβ serves as the trigger of downstream pathological phenotypes, including neurofibrillary tangle formation, synaptic damage, and neuroinflammation (74). Aβ is generated through sequential cleavage of the APP by β-secretase and γ-secretase. Consequently, comprehending the metabolism and processing of APP is imperative for advancing AD treatment strategies. We previously detected m6A on the transcripts of APP and its metabolism-related enzymes in the cortex of both AD patients and APP/PS1 mice (Unpublished data). Coincidentally, a study by Lee *et al.* revealed that FMRP and hnRNPC competitively influence APP translation in opposing directions. FMRP inhibits translation by recruiting *App* mRNA to processing bodies, while hnRNPC promotes *App* translation by displacing FMRP (75). In addition, the splicing of *Bace1* mRNA encoding β-secretase is mediated by hnRNPA2B1 (76), which is decreased in the cerebral cortex of both AD patients (77) and cholinergic-impaired mice (76). Considering that FMRP, hnRNPC, and hnRNPA2B1 have been identified as m6A reader proteins, we speculate that their regulation on the abovementioned RNAs may be mediated in an m6A-dependent manner. On the other hand, Aβ stimulation impacts on the expression of m6A regulators, while restoring the normal expression of these proteins could rescue Aβ-induced phosphorylation of tau protein, axonal damage, and cognitive impairment. Specifically, treatment with Aβ oligomers (AβOs) led to a substantial reduction of METTL3 expression in rat primary cortical neurons, while METTL3 overexpression mitigated AβOs-induced synaptic damage both in vitro and in vivo (78). Consistently, in another cell line-based study, an Aβ1-42 dose-dependent decrease of METTL3 expression was observed. Overexpression of METTL3 rescued Aβ1-42-iduced abnormal autophagy and phosphorylated Tau (p-Tau) accumulation by stabilizing *Stub1* mRNA via m6A-IGF2BP1 pathway (79). Therefore, understanding the interaction between m6A and Aβ will contribute to dissecting the process of Aβ formation and its impact on AD progression.

m6A AND NEUROFIBRILLARY TANGLES

NFTs are composed of hyperphosphorylated and oligomerized microtubule-associated protein Tau. Compared to Aβ, formation of NFTs is more closely associated with cognitive impairment and can cause dementia even in the absence of β-amyloid plaques (80, 81). Unexpectedly, in the brains of aged PS19 mice overexpressing human P301S Tau, a significant positive correlation between m6A and Tau deposition was observed (26). To uncover the intrinsic mystery, Jiang L. *et al.* took advantage of Tau::Cry2 system and identified a molecular complex containing Tau, HNRNPA2B1, and m6A-modified RNAs. Intriguingly, Tau oligomerization induces cytoplasmic translocation of HNRNPA2B1, which further links oligomerized Tau (oTau) to cytoplasmic m6A-modified RNAs. Furthermore, *Hnrnpa2b1* knockdown could reduce oTau-induced neurodegeneration by preventing oTau from interacting with m6A-modified RNAs (26). Remarkably, another independent study shows that accumulation of METTL3 is detected in the insoluble fractions of the hippocampus of postmortem human AD samples and positively correlated with the levels of insoluble Tau protein (82). Taken together, we deduce that abnormal aggregation of METTL3 may impact downstream m6A modification and further affect gene expression patterns associated with neurodegeneration.

Evidence shows that dysregulation of m6A can also exacerbate Tau toxicity. In cortical neurons, *Mettl3* knockdown-induced reduction of m6A prevented the oTau-induced accumulation of both cytoplasmic m6A-modified RNA and HNRNPA2B1 (26). Meanwhile, METTL3 could stabilize *STUB1* mRNA encoding the E3 ubiquitin-protein ligase in an m6A-IGF2BP1-dependent manner, thus facilitating the autophagic clearance of p-Tau in Aβ1-42-treated cells (79). On the other hand, FTO overexpression in 3×Tg AD mouse models activated Tau protein phosphorylation in an mTOR-dependent manner and further accelerated AD pathology (83). Nevertheless, ALKBH5 exhibits an opposing regulatory effect from FTO. Knockout of *Alkbh5* exacerbated Tau phosphorylation in the cerebral hippocampal neurons of mice following chronic cobalt exposure, thereby fostering the formation of cortical tangles in the cerebrum (84). Additionally, in high-glucose stimulated human hippocampal neurons, overexpression of ALKBH5 significantly reversed Tau hyperphosphorylation via demethylating *Dgkh* RNA and subsequent inhibition of PKC-a (85). In addition to its regulatory role in phosphorylation of Tau protein, m6A is also involved in maintaining the normal expression and function of Tau. The m6A-modified lncRNA *Dubr* binds to the YTHDF1/3 complex in an m6A-dependent manner, protecting YTHDF1/3 from proteolytic degradation and maintaining the normal expression of Tau and Calmodulin (86).

To sum up, a mutual regulatory relationship exists between m6A and pathological protein aggregates. The pathological changes resulting from protein aggregation induce dysregulation in m6A, which further exacerbates the neurotoxicity by influencing the expression or aggregation of pathogenic proteins. Therefore, a more in-depth exploration of the intricate relationship between m6A and these proteins holds potential for early diagnosis and treatment of AD.

m6A AND DISRUPTION OF PROTEOSTASIS

Accumulation of abnormal protein aggregates suggests a dysregulation of protein homeostasis during AD (87). As a key step in the regulation of protein homeostasis, protein degradation is exploited to avoid misfolded or defective protein accumulation. Abnormally deposited proteins in AD are degraded through two major proteolytic pathways, namely, the ubiquitin-proteasome system and the autophagosome-lysosome pathway (88).

Disruption of ubiquitination-proteasome pathways in AD has been proven for several years. Early in 2000, a reduction in E1, E2 enzymes, and ubiquitin-protein conjugates was observed in the brain tissue of AD patients, suggesting a potential link between AD pathogenesis and the dysfunctional ubiquitination system (89). The ubiquitination-proteasome pathway is implicated in the formation and clearance of Aβ. For example, proteins like HRD1 (90) and UBE4B (91) promote APP ubiquitination and degradation, while FBX2 promotes the ubiquitination and degradation of BACE1 (92), all of which can reduce Aβ production. Recent studies have also highlighted the role of the ubiquitin ligase CHIP (93) and deubiquitinase UCH-L1 (94) in modulating Tau ubiquitination. These findings underscore the importance of the ubiquitination-proteasome pathway in AD through the regulation of protein degradation.

It has been reported that m6A can affect the expression of ubiquitin-regulated genes in multiple types of cancer (95-97). Apart from its regulatory function in cancer, a recent study shows that m6A also participates in the progression of AD by indirectly regulating ubiquitination. Cheng *et al.* discovered that METTL3 can regulate m6A levels of *circRIMS2,* which in turn led to *circRIMS2* upregulation. Subsequently, its downstream target UBE2K, a Ub-conjugating enzyme, was up-regulated and in turn led to protein degradation of GluN2B, thus resulting in synaptic dysfunction and memory impairment (98). Our group also found that the m6A level of dozens of ubiquitin-regulated genes in the cerebral cortex differs between health controls and AD patients, and between wild-type (WT) and APP/PS1 mice (Unpublished data), which provides clues for studies on m6A and ubiquitination in AD.

Autophagy is a cellular process that encapsulates protein aggregates and damaged organelles within autophagosomes and transports them to lysosomes for degradation (99). Evidence indicates that a compromised autophagy-lysosomal pathway is involved in AD pathogenesis (100). Besides, key components regulating autophagosome formation, such as BECN1, NRBF2, and ULK1/2, also exhibit reduced expression in the brain of AD patients and AD mouse models (100). Forebrain-specific *Atg7*-deficient mice exhibited age-related neurodegeneration accompanied by the accumulation of autophagy substrates and p-Tau (101). Similarly,

neurons in the *Atg5* neural cell-specific knockout mice exhibited aggregates and inclusions of abnormal intracellular proteins, which subsequently led to neurodegeneration and motor dysfunction (102). Furthermore, the disruption of the interaction between Beclin-1 and Bcl-2 stimulated basal autophagy in *Beclin-1*^{F121A} knock-in mice. Crossing this mouse with 5×FAD mice resulted in a significant reduction in Ab accumulation and prevention of cognitive decline (103). These findings emphasize the crucial role of autophagy in clearing large and insoluble protein aggregates to maintain protein homeostasis in AD.

Interestingly, studies show that these autophagy-related genes are regulated by m6A. In the *Fto* knockout mouse, *Atg5* and *Atg7* RNAs exhibited increased m6A levels and shorter half-lives mediated by YTHDF2 protein, thereby reducing protein expression and mitigating autophagy and adipogenesis (104). In addition to FTO, METTL3, in combination with YTHDF2, also plays a role in inducing RNA decay of *Atg7* in an m6A-dependent manner, thus impacting the formation of autophagosomes and autophagic flux (105). A similar regulatory mechanism also exists with METTL14 and IGF2BP1/2/3 in the translational control of Beclin-1. The upregulated Beclin-1 further enhanced autophagy and significantly increased the osteogenic differentiation capacity of bone marrow stromal cells (BMSCs) (106). Certainly, m6A can also influence the expression of regulatory proteins that can impact the occurrence of autophagy. For instance, the ULK complex plays a crucial role in promoting autolysosome assembly (107). FTO, in particular, eliminates m6A on *Ulk1*, which could inhibit YTHDF2-mediated degradation and further facilitate the initiation of autophagy (108). Additionally, FOXO3, a transcription factor of autophagy genes, was identified to be the target RNAs of YTHDF3 in nutrient-deficient mice. Specifically, YTHDF3 could initiate autophagy by recognizing m6A on *Foxo3* RNA and recruiting eIF3a and eIF4B to promote FOXO3 translation (109). These findings underscore the critical roles of m6A in autophagy-related processes associated with AD pathology.

The augmentation of autophagy to clear protein aggregates is regarded as a promising therapeutic approach for AD. Treatment with rapamycin, a specific inhibitor of mTOR known to enhance autophagy, significantly reduced both intracellular and extracellular amyloid deposition in the brains of AD mice (110). On that basis, a thorough investigation into the impact of m6A on autophagy-related AD holds the potential to unveil novel treatments for this condition.

m6A AND SYNAPTIC DYSFUNCTION

Synaptic loss correlates most strongly with cognitive decline in AD as synaptic function underlies cognitive performance. Given the enrichment of m6A-modified RNAs in synapse-related pathways in the brain (111), it is imperative to explore how m6A is involved in synaptic dysfunction in AD.

m6A could regulate the local translation of mRNA to ensure that synapses can rapidly adjust protein composition and cellular morphology in response to external stimuli (20, 112). As reported, the enrichment of m6A reader proteins at synapses underscores the significant regulatory function of m6A in synapse-distributed RNAs (21). Knockdown of m6A readers, *ythdf1* and *ythdf3,* in hippocampal pyramidal neurons diminished the translation of dendritic-localized mRNA, leading to impaired synaptic transmission and abnormal dendritic morphology (21). Specifically, YTHDF1 binds to m6A-modified axonal guidance receptor *Robo 3.1* mRNA, and its deficiency in spinal commissural neurons resulted in a pre-crossing axon guidance defect (22). In addition, YTHDF1 and YTHDF2 regulate the local translation of key components of the Wnt5a pathway in axons, thus affecting axon growth and synapse formation in cerebellar granule cells (23). Additionally, FTO is also enriched in axons and subject to local translation. Specifically, *Fto* knockdown in axons or axon-specific inhibition of FTO by Rhein increased the methylation of *GAP-43* mRNA, thus impeding the local translation of GAP-43 protein (113). As is known, local translation requires delivery of RNA to the distal region of the neuron, thereby dependent on an elegantly regulated RNA transport apparatus. Transcripts associated with synaptic function and plasticity, such as *Camk2a* and *Map2,* are synthesized in the cell body and subsequently transported to the synapse. The depletion of *Mettl3* in hippocampal neurons resulted in abnormal neurite localization of these m6A-modified RNAs, which underwent local translation in dendrites to promote long-term potentiation and synaptic plasticity (114).

Considering the crucial regulatory role of m6A in synaptic RNAs, the regulation of synaptic function by m6A is self-evident. In the homogenate lysate of the mouse forebrain, hypermethylated

mRNAs identified in the synaptosomes exhibit a strong functional enrichment in synaptic pathways compared to hypomethylated mRNA (21). Meanwhile, Martinez *et al.* observed an increase in the abundance of m6A-modified RNAs during synaptic maturation, indicating time-dependent plasticity in m6A methylation of the synaptic RNAs (115). The m6A-mediated regulation of synaptic gene expression and function requires a proper orchestration of RNA m6A machinery. For instance, a specific knockout of *Mettl3* in mouse hippocampal neurons resulted in a significant downregulation of m6A, accompanied by extensive synaptic loss and neuronal death (34). In addition to that, reduced m6A levels of synaptic plasticity-related transcripts were also noted in *Mettl14*-deficient striatal neurons, correlating with impaired neuronal excitability (38). Additionally, ALKBH5 locates in active synaptic ribosomes and participates in active translation via m6A demethylation during short-term plasticity, while the m6A readers YTHDF1 and YTHDF3 have high colocalization with modified RNAs in late-stage plasticity (115). Evidence showed that dysregulation of the m6A reader seems to have a more profound effect on the synapse. Concretely, *Ythdf1* knockout mice exhibited learning and memory deficits, as well as impaired hippocampal synaptic transmission and long-term potentiation (37). The specific knockout of *Ythdf2* in the dentate gyrus led to mossy fibers (MF) overgrowth and impairment of the MF-CA3 excitatory synapse transmission (116). Apart from the synapse itself, m6A also participates in the regulation of transcripts related to myelin, which is a multilamellar membrane wrapped around axons and essential for the propagation of impulses along axons. It has been found that the decrease of m6A levels in the 3'UTR of *Gpr17*, a myelin regulator gene, caused the downregulation of GPR17 in the aged hippocampus (43). Moreover, significant hypomyelination and cognitive defects were observed in *Prrc2a* central nervous system (CNS)-specific knockout mice. Mechanically, PRRC2A functions as an m6A reader and is involved in oligodendrocyte proliferation and fate determination by stabilizing *Olig2* mRNA in an m6A-dependent manner (14).

The synaptic loss and dysfunction resulting from m6A dysregulation always manifest as learning and memory impairments akin to those observed in AD. Indeed, in the cortex of AD patients, hypermethylated RNA was mostly enriched in synapse-related biological processes, such as *Camk2* and *Glua1*, suggesting an m6A-dependent mechanism governing synaptic protein synthesis in AD (44). Beyond mRNA, differentially methylated circRNAs were also detected in the hippocampus of APP/PS1 mice, whose functionally enriched pathways include axon guidance, long-term potentiation, glutamatergic synapses, cholinergic synapses, and long-term inhibition (117). Consequently, m6A fluctuations in AD seem to expedite disease progression by influencing synaptic function. Investigating changes in m6A regulators at synapses in AD will improve our understanding of the significant impairments inherent in the disease, including learning and memory deficits.

m6A AND NEUROINFLAMMATION

Persistent chronic inflammation in AD, as well as the tight association between AD risk genes and innate immune function, indicate a pivotal role for neuroinflammation in the process of AD (118). The activation of inflammatory processes in the brain, involving immune cells such as microglia, astrocytes, and infiltrating blood cells, leads to the release of pro-inflammatory cytokines (118). So far, the development, differentiation, and activation processes of these immune cells have all been shown to be regulated by m6A, thus affecting the inflammatory response process (119).

Microglia, serving as the primary macrophages within the CNS, play a crucial role in regulating synaptic plasticity, clearing damaged cells, and maintaining the immune microenvironment (120). In response to external or internal stimuli, microglia are activated and polarized into either the M1 pro-inflammatory phenotype or the M2 anti-inflammatory phenotype (121). A transcriptome-wide m6A profiling analysis showed that hundreds of mRNAs and lncRNAs exhibit differential m6A levels among M1, and M2 phenotypes and the resting state primary rat microglia. Subsequent functional enrichment analysis suggests that the dynamic m6A might serve as a potential regulator in the inflammatory response of microglia (122). In support of that, decreased expression of FTO was observed in both the retinal microglia of IRBP-induced uveitis mice model and LPS/IFN-γ-induced pro-inflammatory human microglia 3 (HMC3). Moreover, *Fto* knockdown in HMC3 resulted in elevated expression of inflammatory cytokines and enhanced cell mobility. Mechanistically, *GPC4* was identified to be the target of FTO and YTHDF3, which regulated

microglial inflammation mediated by the TLR4/NF-κB pathway (123). Besides, single-cell analysis of an *Aire*$^{-/-}$ spontaneous uveoretinitis mouse model showed that YTHDC1 was downregulated in inflammatory microglial. Furthermore, they found that the regulatory role of YTHDC1 in microglial inflammatory response was mediated via recognizing and stabilizing m6A-modified *Sirtuin 1* mRNA (124). In LPS-induced microglial, IGF2BP1 expression increased and further promoted the pro-inflammatory factor *Gbp11* and *Cp* mRNA expression via m6A-dependent stabilization (125). Of note, with the ongoing advancement of techniques at the single cell level, the conventional M1-M2 classification model is being challenged, giving rise to the proposal of disease-related microglial in AD (126). Elucidating the action and regulation of m6A in these microglial subtypes would offer a more precise foundation for understanding the status of microglial activation in AD. In addition to microglial, blood-derived myeloid cells can cross the blood-brain barrier in response to CNS damage, and differentiate into fully functional CNS macrophages to be involved in the progression of AD (127). Intriguingly, *Mettl3* knockout in mouse mononuclear myeloid cells enhanced the infiltration of monocyte-derived macrophages and Aβ clearance, thereby mitigating the AD-like symptoms. Further analysis demonstrated that it was attributed to the attenuated methylation on *Dnmt3a* RNA and impaired translation mediated by YTHDF1, which further influenced the expression of downstream ATAT1 and resulted in reduced microtubule acetylation (128).

Dysregulation of neuron-microglial crosstalk can potentially exacerbate the inflammatory response in AD (129), while m6A seems to be implicated in the communication between these two cell types. Evidence shows that exosomes generated from primary mouse NSCs could mediate the transfer of FTO from NSCs to microglia. The resultant demethylation of *Nrf2* RNA in the microglia further led to increased NRF2 expression, a critical mediator of inducible defense systems, thereby fostering M2 polarization (130). Additionally, under hypoxic conditions, glioma stem cells (GSCs) produced more glutamate, triggering the activation of local neurons. Activated neurons, in turn, release exosomes that convey miR-200c-3p to microglia. Targeted by miR-200c-3p, the m6A writer ZC3H13 was downregulated, resulting in impaired m6A of *DUSP9* mRNA. Consequently, this obstruction amplified the activation of the ERK pathway and facilitated M2 polarization in microglia (131). These findings indicate that m6A may contribute to the crosstalk between neurons and microglia in the progression of AD, offering a viable avenue for intervention to counteract the abnormal activation of microglia.

In addition to microglial, astrocyte dysfunction also contributes to neuroinflammation in AD through the release of inflammatory mediators, reactive astrogliosis, and modulation of the blood-brain barrier. In a depression mouse model treated with chronic unpredictable stress, overexpression of *circSTAG1* in the mouse hippocampus notably accelerated astrocyte dysfunction and depressive-like phenotypes, via interaction with ALKBH5 and subsequent demethylation of the transcript of *FAAH* (58). Additionally, in Streptozotocin (STZ)-treated astrocytes, the expressions of FTO and YTHDF1 were markedly upregulated in human astrocytes treated with STZ, while the FTO inhibitor MO-I-500 exhibited robust efficacy in mitigating the adverse effects induced by STZ. Intracerebroventricular administration of STZ in rodents induced similar phenotypes as sporadic AD, such as memory impairment, and defective energy metabolism. Therefore, the above findings suggest that perturbed m6A signaling in astrocytes may also be utilized to promote AD progression (132).

m6A AND MITOCHONDRIAL DYSFUNCTION

Mitochondria play a central role in cellular energy metabolism, supplying substantial energy crucial for the high energy-demanding nervous system. Indeed, energy metabolism defect manifests in the early stages of AD development and escalates with the disease progression (3). Subsequently, mitochondrial dysfunctions heighten the production of reactive oxygen species, and anomalous mitochondrial autophagy appears during the progress of AD (133). Mitochondrial DNA contains 37 genes that encode 13 mRNAs, 22 tRNAs, and 2 rRNAs. Previous studies have demonstrated that these mitochondrial RNAs also undergo post-transcriptional modifications, influencing the regulation of mitochondrial biogenesis and function (134). However, despite substantial studies of m6A on nuclear-encoded mRNAs, it remains unknown whether mitochondrial mRNAs are also under the control of m6A. In this section, we mainly review recent

progress in how m6A modification on nuclear-encoded mRNAs is involved in regulating the functions of mitochondria.

m6A AFFECTS MITOCHONDRIAL FUNCTION

Abnormal mitochondrial energy metabolism is one of the earliest and most prominent features of AD, aligning with a reduction in the expression levels of key components of the electron transfer chain in a patient's brain (133). Given that the majority of mitochondrial proteins are encoded by the nuclear genome, this implies that m6A may regulate mitochondrial function by modulating the expression of these proteins. For instance, downregulation of PGC-1α, a primary regulator of mitochondrial biogenesis, is detected in neurodegenerative diseases, and leading to mitochondrial defects and increased ROS levels (135). It is reported that in monocytes, METTL3 coordinates with YTHDF2 to inhibit the *PGC-1a* RNA expression in an m6A-dependent manner, which in turn leads to the accumulation of cellular and mitochondrial ROS (136). The above findings indicate the possibility that dysregulated m6A is one of the causal factors of mitochondrial defects in AD. There also exists an indirect effect of m6A on the expression of proteins related to mitochondrial energy metabolism. For instance, METTL3 could facilitate m6A on pri-miRNAs and regulate RALY protein-mediated miRNA processing in human colorectal cancer cell lines. In turn, these miRNAs target a series of metabolic-related genes and reprogram mitochondrial metabolism (137). In addition to gene expression control, m6A also affects the transport of nuclear-encoded proteins into mitochondria. As seen in microglia, METTL3-mediated m6A could promote TRAF6 translocation to mitochondria, which further induces mitochondrial oxidative stress in the paraventricular nucleus (138).

Maintaining the equilibrium between mitochondrial fission and fusion is crucial for mitochondrial quality control and normal function. Evidence suggests that m6A is intricately involved in the regulation of fusion and fission processes within the cytoplasm. For example, FTO exerts a protective role against hepatic ischemia-reperfusion injury, which is mediated via demethylation of *Drp1*, a key player in mitochondrial fission, thereby consequently impeding proper mitochondrial fission (139). Additionally, in the mouse neurons exposed to cadmium, *and-Gm10532* expression increases and recruits METTL14 protein to deposit the m6A on *FIS1* mRNA, thus leading to an increased FIS1 protein expression and enhanced mitochondrial fission. Consequently, it results in excessive ROS production, alterations in mitochondrial membrane potential, and a reduction in ATP content (140).

Taken together, the influence of m6A on mitochondria encompasses various aspects, including, but not limited to, mitochondrial function maintenance, nuclear-mitochondrial protein transportation, and the regulation of mitochondrial fission and fusion. Therefore, it is worthy of investigation whether similar mechanism exists contributing to the pathogenesis of AD.

MITOCHONDRIA REGULATES RNA m6A MODIFICATION

Mitochondrial respiration inevitably produces byproduct, such as ROS, which accumulates in AD due to mitochondrial dysfunction (141). The resultant oxidative stress can alter m6A levels by influencing the expression or activity of m6A regulators and prompt adaptive responses to harmful insults. As an example, ROS induction by exogenous H_2O_2 stimulation enhanced METTL3-mediated m6A of the nuclear *PPaRα.* Subsequently, YTHDF2 recognized and stabilized *PPaRα* in an m6A-dependent manner, which ultimately influenced liver lipid metabolism (142). Meanwhile, endogenous ROS in hematopoietic stem/progenitor cells promoted post-translational modification of ALKBH5, which impacted overall m6A levels to maintain genome integrity and survival of mammalian cells (143).

Beyond energy production, mitochondria actively participate in diverse metabolic processes and generate essential substrates and cofactors crucial for epigenetic modifications. Notably, mitochondrial one-carbon cycling contributes to maintaining the S-adenosyl methionine (SAM) level, a key methyl donor for various methylation reactions. The ratio of SAM/s-adenosylhomocysteine was found to be lower in the plasma or cerebrospinal fluid of AD patients compared to normal controls (144). Given that some studies have found decreased m6A levels in AD patients, we suspect that the alteration in the supply source may account for the overall changes in m6A. Concurrently, alpha-ketoglutarate (α-KG), an intermediate metabolite within the mitochondrial tricarboxylic acid cycle, supports the demethylation functions of ALKBH5 and FTO (145). Due to the

reduction of α-KG level observed during the aging process (146), we deduce that this may also impact the dynamic balance of m6A. Therefore, exploring the effects of mitochondrial metabolic fluctuations on m6A would be beneficial in elucidating the intricate relationship between mitochondria and AD.

Together, a robust mitochondrial pool not only sustains neuronal activity by providing a stable energy supply but also safeguards neurons by minimizing mitochondrial-related oxidative damage. In this context, delving into the interactions between m6A and mitochondria in the pathogenesis of AD may unveil new and promising therapeutic targets for the disease.

CONCLUSION AND PERSPECTIVES

In most cases, AD has a complex etiology, involving genetic and environmental factors. Hence, m6A, functioning as an epitranscriptional mark influenced by the environment, holds promise to improve our understanding of the pathomechanism of AD from a new perspective. The regulation of RNA metabolism, encompassing processes such as splicing, translocation, translation, stability, and structure, is influenced by m6A and its regulators, thereby impacting the progression of AD. On the one hand, m6A exhibits robust associations with certain AD-related risk factors, including aging, genetic variations, and diseases linked to AD. The alterations in m6A offer fresh insights into elucidating the intricate relationship between AD and its risk factors. On the other hand, m6A promptly responds to and exacerbates various pathophysiological hallmarks, including pathological protein aggregates, disrupted proteostasis, neuroinflammation as well as dysfunctional synapses and mitochondria (Figure1). Consequently, unveiling the mechanism of action and regulation of RNA m6A methylation in AD will promote our understanding of the pathogenesis and provide some clues for its diagnosis and treatments.

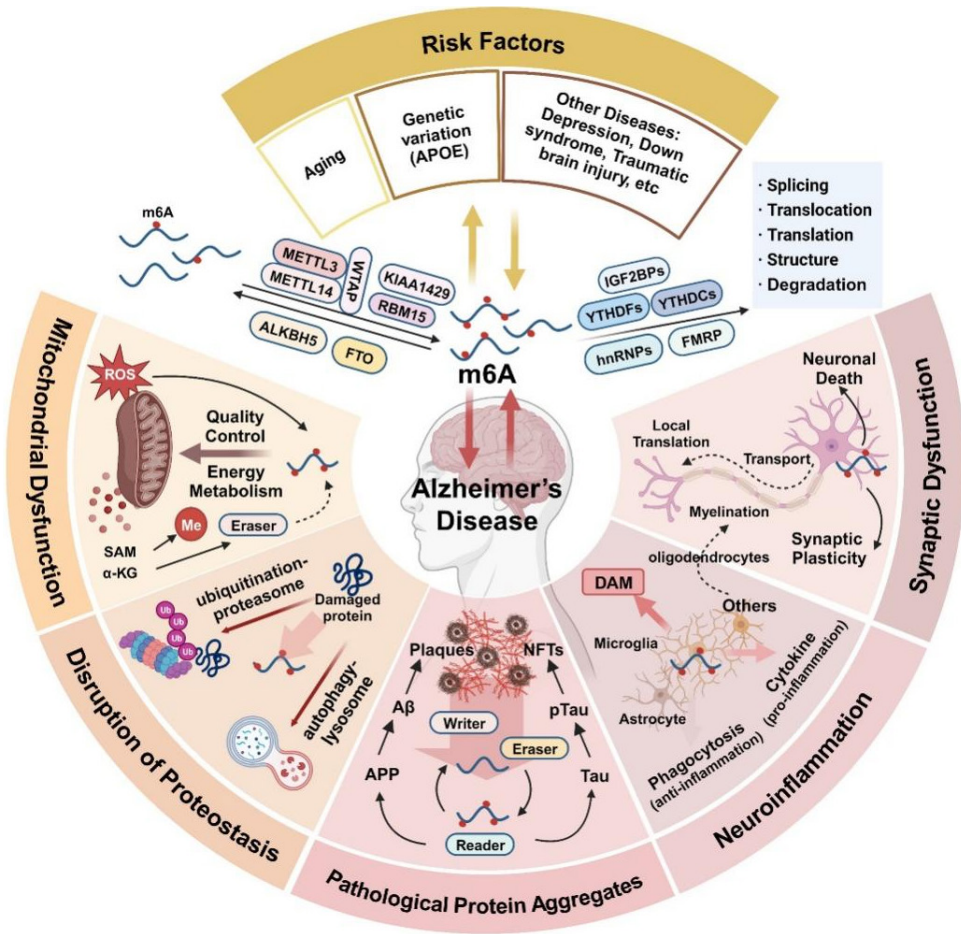

Figure 1. The roles of m6A in Alzheimer's disease.

Schematic representation illustrates the potential roles of RNA m6A machinery in AD. m6A is implicated in the regulation of both the risk factors and pathophysiological hallmarks of AD. AD risk factors, such as aging, genetic variation, and other diseases associated with AD, are influenced by m6A. The pathophysiological hallmarks of AD affected by m6A include the formation of pathological protein aggregates, disruption of proteostasis, neuroinflammation, and dysfunction in synaptic integrity and mitochondrial function. (Created with BioRender.com)

With the aging of the population, the incidence of AD is steadily increasing, imposing a substantial economic burden. Nevertheless, the progress in developing effective AD diagnosis and treatment strategies has been unsatisfactory. Presently endorsed AD therapies, such as acetylcholinesterase inhibitors and memantine, lack efficacy in slowing disease progression (147). The irreversible and severe consequences resulting from the continuous aggregation of Aβ underscore the significance of early AD diagnosis (148). However, existing diagnostic modalities, such as magnetic resonance imaging and positron emission tomography, present challenges for routine clinical application (149). The shared m6A characteristics between AD and its risk factors raise the prospect of m6A serving as an early diagnostic marker for AD. Sequencing data derived from aging mice revealed a concurrent loss of m6A with the onset of memory disorders in the aging brain, potentially contributing to cognitive decline (44). Consequently, m6A levels of specific RNAs in cerebrospinal fluid or blood samples from AD patients may serve as potential staging biomarkers to categorize disease severity. Persistent failures in clinical trials targeting Aβ and Tau underscore the search for novel and comprehensive treatment methods (150). RNA therapy has been paid constant attention and has been approved for application in several neurological diseases recently (151). The pivotal role of m6A and its regulatory mechanisms in various AD pathological phenotypes suggest its potential as an effective adjuvant treatment. Dysregulation of multiple m6A regulatory factors, including METTL3, FTO, and YTHDF1, is implicated in the emergence of AD pathological phenotypes, emphasizing the potential for therapeutic intervention by modulating the expression of these proteins. Notably, the FTO inhibitor MO-I-500 effectively mitigates adverse reactions such as oxidative stress, apoptosis, and mitochondrial dysfunction in astrocytes treated with STZ (132). Notably, advances in nanodrug delivery systems now enable the transportation of proteins-regulating siRNA across the blood-brain barrier. This also offers the potential to reverse the pathological progression of AD through the modulation of m6A factor expression.

However, despite considerable evidence showing the involvement of m6A in AD pathogenesis, there are several issues that need to be raised for attention. First of all, most conclusions reached so far are somewhat descriptive or correlational. Therefore, from now on in-depth studies based on suitable AD models to investigate the causal relationships between m6A and AD pathogenesis is of paramount importance. In addition, we notice that the phenomena reported from different groups are inconsistent. For instance, immunofluorescence results showed elevated overall m6A levels in both the lateral entorhinal cortex of P301S mice and the hippocampal CA3 region of $APP^{NL\text{-}G\text{-}F}$/$MAPT^{P301S}$ mice (26, 152). However, in the cortex of 5×FAD mice, a significantly decreased m6A level was detected by using LC-MS/MS method (42). In addition, data from human sample-based studies are controversial as well. With disease progression, the m6A signal in the temporal cortex of AD patients increased and co-localized with Tau deposition as evidenced by the immunofluorescent staining (26). However, the results of LC-MS/MS from another recent study showed that the global m6A level was unchanged in cortical tissues of AD brains (78). In contrast, a comparative transcriptome-wide analysis of cingulate cortex brain tissue showed decreased m6A levels in AD patients (44). In addition to that, controversial findings also exist regarding the expressions of m6A regulators. For instance, METTL3 was shown to be increased in the cortex of 5×FAD mice as well as the hippocampus of APP/PS1 and $APP^{NL\text{-}G\text{-}F}$/$MAPT^{P301S}$ mice (42, 98, 152), while reduced METTL3 expression was observed in the hippocampus and cortex of AD patients at both RNA and protein levels (78, 82, 153). Importantly, another study based on an independent cohort of postmortem AD brains showed that METTL3 is accumulated in the insoluble hippocampal tissues, in comparison with a decrease in the soluble fractions (82). Furthermore, compared to wild-type mice, elevated expression of FTO was detected in the brain tissue of 3×Tg mice and the cortex of 5×FAD (42, 83). However, it was found to be significantly downregulated in the frontal cortex of the AD brain (78). Besides, although

reduced expression of ALKBH5 was detected in the hippocampus of $APPN^{L\text{-}G\text{-}F}$/$MAPT^{P301S}$ mice (152), it remained unchanged between APP/PS1 and WT mice (98). Additionally, transcriptomic and proteomic analyses of the brain in 5×FAD mice show a mild increase of METTL3 but a decrease in FTO, while all other m6A regulators exhibit no significant changes (42). Intriguingly, even in the same model, decreased m6A level was detected in large pyramidal neurons in the hippocampus and cortex of AD patients, in contrast to a significant increase in the glia cells (78). In agreement with that, m6A was strongly elevated in the astrocytes and microglia in the hippocampus of $APP^{NL\text{-}G\text{-}F}$/$MAPT^{P301S}$ mice (152).

Based on the above issues, significant attention must be devoted to investigating the intrinsic mechanism of how dysregulated m6A associates with disease onset/progression in AD. First, the vast majority of AD cases (>90 %) are late onset and sporadic with complex pathomechanism, while most animal models utilized so far for AD research were established based on less common, familial cases of AD. To address this issue, it would be desirable to incorporate human samples, such as postmortem brain tissues, into the study, which would be a great complement to the findings obtained from animal model-based studies (154). Second, AD has a long lifecycle and typically progresses slowly across multiple stages, during which the molecular and pathological changes in different brain regions are not synchronized. Hence, the clinical samples collected from AD patients may vary significantly from one to another, which accounts for the discrepancies among different studies. Third, many of the results obtained so far are based on the mouse models, which will bring out great variation among different studies. These animal models differ significantly in their design (concerning the target genes to be knocked out or knocked in) and the mouse strains used to generate the mouse model. In addition, both gene expression and RNA m6A methylation exhibit significant tempo-spatial and cellular specificity in the brain. Therefore, in addition to the selection of appropriate mouse models, more concrete information including age, gender, brain regions, and cell types should be taken into consideration to reach an objective and solid conclusion regarding the action and regulation of m6A.

It's also worth noting that the m6A detection techniques widely used in current AD research are still far from achieving our goals. Particularly, the reliability of antibody-based methods, such as MeRIP, dot blot, ELISA, or immunofluorescence analysis, is compromised by the limited binding affinity and specificity of the antibody. What's more, the m6A antibody widely in use can recognize not only RNA N-methyl-adenosine but also DNA modification N6-methyl-deoxyadenosine (6mA) (155). Theoretically, DNA 6mA is located in the nucleus only, while m6A-modified RNAs are distributed in both cytosol and nucleus. Therefore, much attention should be paid to interpreting the results of immunofluorescent analysis. In addition, despite being the most widely used method for transcriptomic analysis of m6A, methylated RNA immunoprecipitation sequencing (MeRIP-seq) is unable to provide a good measure of the occupancy of m6A or exact positional information (156, 157). Hence, there is an urgent need for an improved methodology that allows for precise, quantitative comparisons to analyze the transcriptome-wide m6A sites. In recent years, several new detection methods have been developed, which may enable characterization of m6A at single-cell level or single-base resolution (158-161). It will be fascinating to see whether these techniques can advance the field in translating basic research findings of m6A into clinically applicable diagnostic or therapeutic strategies.

Nowadays, due to the existence of multiple hallmarks in AD, a therapeutic strategy targeting a single hallmark might be insufficient to halt the neurodegenerative process. With the advancement of the techniques detecting m6A and the rational use of animal models and human specimens, our understanding of the role of m6A in multiple pathophysiological processes of AD will be more comprehensive and insightful. On top of that, the development of inhibitors or RNA drugs based on RNA m6A modification will present significant potential in AD treatment.

List of Abbreviations

6mA, N6-methyl-deoxyadenosine; **AD,** Alzheimer's disease; **aNSC,** Adult neural stem cell; **Aβ,** Beta-amyloid; **AβOs,** Aβ oligomers; **BMSCs,** Bone marrow stromal cells; **CNS,** Central nervous system; **Cp,** Ceruloplasmin; **DAM,** Disease-associated microglia; **DS,** Down syndrome; **ELISA,** Enzyme-linked immunosorbent assay; **GSCs,** Glioma stem cells; **HMC3,** Human microglial clone 3; **LC-MS/MS,** Liquid chromatography-mass spectrometry/mass spectrometry; **LPS,**

Lipopolysaccharide; **m6A,** N6-methyladenosine; **MDD,** Major depressive disorder; **Me,** Methyl; **MeRIP-seq,** methylated RNA immunoprecipitation sequencing; **MF,** Mossy fibers; **NFTs,** Neurofibrillary tangles; **oTau,** Oligomerized tau; **p-Tau,** Phosphorylated tau; **ROS,** Reactive oxygen species; **SAM,** S-adenosyl methionine; **scRNA-seq,** Single-nucleus RNA sequencing; **SGs,** Stress granules; **snRNA,** Small nuclear RNA; **STZ,** Streptozotocin; **Tau,** Microtubule-associated protein tau; **TBI,** Traumatic brain injury; **Ub,** Ubiquitin; **WT,** Wild type; **α-KG,** Alpha-ketoglutarate

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Conflict of Interest Disclosure

The authors declare that they have no conflict of interest.

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