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Review

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The role of N6-methyladenosine modification in neurodegenerative diseases

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Abstract

In recent years, transcriptomics has emerged as a key focus in neuroscience research, as transcriptome modifications play a significant role in influencing various biological processes. N6-methyladenosine (m6A) represents a dynamic and reversible form of mRNA modification prevalent in eukaryotes. This modification is involved in virtually every critical stage of RNA metabolism, including mRNA stability, transcription, translation, splicing, nuclear export, and decay, thereby playing a pivotal role in normal brain development. Accumulating evidence suggests that m6A modification plays a substantial role in various neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD), while abnormal m6A modification can lead to neurodevelopmental disorders. This review summarizes the relationship between m6A modification and various neurodegenerative diseases and elucidates its potential pathogenic mechanisms at the molecular level.

Keywords: N6 methyladenosine modification (m6A), Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, frontotemporal dementia, Huntington's disease

INTRODUCTION

Chemical modifications in RNA are considered important mechanisms for regulating gene expression and protein translation. RNA methylation modifications include N6 methyladenosine (m6A), N1 methyladenosine (m1A), N6, 2-O-dimethyladenosine (m6Am), 5-methylcytosine (m5C), 5-hydroxymethylcytosine (5hmC), and 7-methylguanine (m7G), among which m6A is the most common and abundant eukaryotic methylation modification type in RNA[1-3].

M6A methylation modification is a reversible process that involves the participation of methyltransferases (Writers), demethylases (Erasers), and methylated reading proteins (Readers)[4]. Methyltransferases, which include METTL3, METTL14, WTAP, and KIAA1429, catalyze the addition of a methyl group to the sixth nitrogen of adenosine during the methylation process[5]. Demethylases, such as FTO and ALKBH5, can remove this methylation[6]. At the same time, methylated RNA base sites require specific enzymes for recognition, known as methylated reading proteins (Readers). These include YTH domain proteins (e.g., YTHDF1/2/3 and YTHDC1/2), nuclear heterogeneous ribonucleoprotein (hnRNP), and IGF2BP7[7] [Figure 1].

Neurodegenerative disorders, encompassing Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and Huntington's disease (HD), represent a spectrum of neurological conditions characterized by the relentless degeneration of neurons within the central nervous system (CNS) or the peripheral nervous system (PNS)[8,9]. Recent studies have illuminated a pivotal role for m6A RNA methylation modification in the intricate tapestry of brain development. Notably, m6A-associated proteins exhibit ubiquitous expression and profound enrichment within neuronal tissue, intricately modulating fundamental processes such as memory formation and consolidation, adult neurogenesis, axonal regeneration, and cerebellar maturation[10].

Indeed, the exploration of m6A RNA modification stands as a forefront and highly sought-after research avenue within the vast landscape of life sciences. While the spotlight has predominantly shone on its implications in cancer research, encompassing diverse malignancies like hematological tumors, uterine cancer, and breast cancer[11-13], a notable research void persists in the realm of neurodegenerative diseases. This gap underscores the urgency to delve deeper into the role of RNA methylation modification, particularly m6A, in these complex and devastating neurological conditions. In this context, we review the pivotal roles of m6A modification in select neurodegenerative disorders, aiming to stimulate research and enhance understanding of its intricate interplay with neurodegeneration.

AD

AD is a neurodegenerative disorder associated with advancing age, representing the most prevalent underlying cause of dementia[14]. In the United States, about 10% of people aged 65 and above suffer from AD. The ubiquitous neuropathological hallmarks of AD encompass synaptic and neuronal dysregulation, manifested by the presence of intracellular neurofibrillary tangles, coupled with elevated concentrations of neurotoxic amyloid proteins and the extracellular accumulation of neuropathic plaques[15,16]. Multiple studies have demonstrated that dysregulation of RNA methylation is associated with AD, and m6A is the predominant modification type found in eukaryotic RNA[17].

APP/PS1 and 5XFAD mice have been widely used in the study of the pathogenesis and therapeutic effectiveness of AD[18]. Quantitative analysis of RNA m6A methylation levels in APP/PS1 transgenic mice of AD showed an increase in m6A methylation levels in the cortex and hippocampus. Meanwhile, in the APP mouse model, the expression of m6A methyltransferase METTL3 increased while the expression of m6A

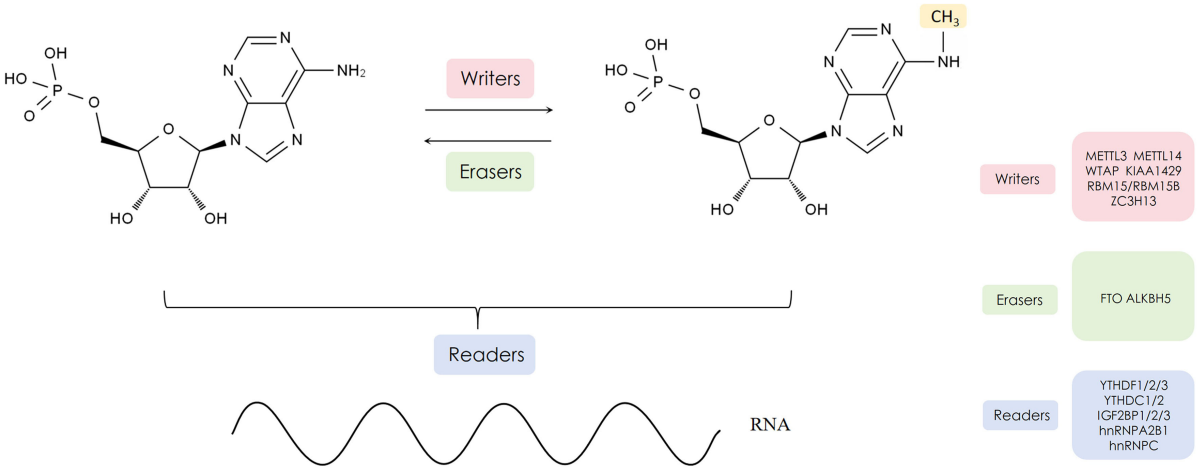

Figure 1. Three kinds of enzymes involved in m6A methylation modification. m6A: N6-methyladenosine.

demethylase FTO decreased[19]. However, in the 6-month-aged 5XFAD mouse model, an increase in FTO expression and a decrease in METTL3 levels were observed through RNA sequencing and proteomic analysis, which is consistent with a reduction in m6A modification levels in these mice[20]. Interestingly, in human AD samples, METTL3 is downregulated in the soluble fraction of the hippocampus but upregulated in the insoluble fraction, mirroring the accumulation of insoluble Tau observed in AD patients[21,22].

AD patients have a significant amount of β-amyloid protein, known as Aβ, in their brains. This protein can lead to the formation of senile plaques and the apoptosis of nerve cells, which are the hallmarks of AD[23]. Recent investigations have uncovered that a scarcity of methyltransferase such as METTL3 in macrophages derived from monocytes can enhance cognitive performance in amyloid-beta-induced AD mouse models. Specifically, the absence of METTL3 triggers the downregulation of α-Tubulin Acetyltransferase 1 (ATAT1), resulting in decreased levels of α-tubulin acetylation. As a consequence, this promotes the migration of monocyte-derived macrophages and facilitates the clearance of Amyloid-beta (Aβ), ultimately mitigating the symptoms of AD[24].

Tau protein hyperphosphorylation is one of the most important causes of AD, which can lead to neurofibrillary tangles, neuronal death, and functional impairment[25]. In a fruit fly model, it was found that the absence of m6A can enhance the toxicity of Tau in the fruit fly AD model, while the absence of METTL3, METTL14, and YTHDF can result in profound motor impairments in fruit flies[26]. Lysine-specific demethylase 1A (KDM1A) plays a protective role in the hippocampus and cortex of mice, preventing neurodegeneration and cognitive decline. However, in its absence, neurodegeneration and cognitive impairment are observed[27]. Recent research indicates that KDM1A promotes the expression of METTL3 through the upregulation of the m6A-dependent pathway. This, in turn, enhances the autophagic clearance of phosphorylated Tau (p-Tau), offering protection against neurodegenerative lesions in AD[28].

Synaptic degeneration arises during the initial phases of AD and is intimately linked to the decline in cognitive abilities[29]. In the AD mouse model with m6A methyltransferase METTL3 knockdown, m6A modification was reduced in hippocampal neurons, resulting in notable memory impairments in mice, accompanied by widespread synaptic loss and neuronal cell death, as well as oxidative stress and abnormal cell cycle events[21]. Circular RNA (circRNA) is abundant in neural tissue, and its abnormal expression precedes AD symptoms, which is strongly associated with the severity of clinical dementia[30,31]. CircRIMS2,

mediated by METTL3-dependent m6A modification, exhibited marked upregulation in 4-month-old APP/PS1 mice. Elevated expression of circRIMS2 led to deficits in synaptic function and memory capacity in 4-month-old C57BL/6 mice, while silencing circRIMS2 significantly rescued synaptic dysfunction in AD mice[32].

The modification of m6A demonstrates temporal and spatial differences during neural development and aging processes. Specifically, in the brains of mice with AD, m6A functions in a distinct spatial and temporal pattern[33]. The m6A level is highest in the brains of 2-week-aged and 52-week-aged (elderly) mice, while the lowest level is detected in the brains of 4-week-aged and 6-week-aged (adolescent) mice[26]. Investigations have demonstrated that m6A assumes a pivotal function in modulating neuronal maturation and exerts a more profound influence during both the initial and terminal phases of development[33].

In essence, investigations utilizing AD mouse models reveal intricate dynamics in m6A methyltransferases and demethylases, intimately linked to disease progression. Specifically, METTL3 depletion in macrophages bolsters Aβ clearance, whereas its aberrant regulation in neurons disrupts synaptic function and memory. Notably, Tau hyperphosphorylation, a process modulated by m6A-associated pathways, contributes significantly to AD pathogenesis. Moreover, circRNA dysregulation, a consequence of METTL3-mediated m6A modifications, precedes AD symptoms and adversely affects synaptic function. The m6A modification displays distinct temporal and spatial patterns during neural development and aging, underscoring its pivotal role in orchestrating neuronal maturation and AD progression.

PD

PD stands as one of the most prevalent age-related neurodegenerative disorders, primarily targeting dopaminergic neurons[34]. The pathological hallmarks of this disease include neural inclusion bodies in the form of Lewis bodies and Lewy neurites, as well as cell loss in the substantia nigra and other brain regions[35,36]. Recent research endeavors have unveiled a compelling association between multiple m6A-methylated proteins and PD, hinting at a potential role of m6A regulatory factors in the intricate pathogenesis of this disorder[37].

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is commonly used to construct PD animal models. This neurotoxin exerts its neurotoxicity by causing a series of injuries, ultimately leading to damage to dopaminergic neurons in the substantia nigra compacta and striatum[38]. In the PD mouse model constructed by MPTP, the m6A modification level decreased and the demethylase FTO significantly increased in the tissues of PD mice. Furthermore, the striatal expression of m6A methyltransferases, METTL3 and RBM15, decreased in PD mice, whereas the expression of the m6A demethylase ALKBH5 increased in the substantia nigra. In parallel, compared to control group mice, the expression level of methylated reading proteins YTHDF1 was downregulated in MPTP-treated mice[39]. Prior research underscores the pivotal role of YTHDF1 in the CNS, as it has been implicated in inducing axonal regeneration and regulating axonal guidance[40,41]. These findings suggest that disruptions in m6A modification pathways may contribute to the pathological processes underlying PD.

Alpha-synuclein (α-Syn), a highly representative biomarker of PD, has been instrumental in the diagnosis of this condition[42]. The abnormal accumulation of α-Syn plays a pivotal role in the onset and progression of PD, impacting various pathological processes including mitochondrial dysfunction, oxidative stress, neuroinflammation, and autophagy[43]. Analysis shows that METTL14 is associated with plasma α-Syn, which is closely related to the severity of PD disease. Further investigation suggests that METTL14 interacts with the m6A motif within the coding sequence (CDS) region of α-Syn, thereby modulating the m6A

modification of α-Syn mRNA through an m6A-YTHDF2-dependent mechanism[44]. Notably, in peripheral blood mononuclear cells of PD patients, the levels of both m6A and its methyltransferase METTL14 are significantly reduced. This reduction leads to impaired m6A modification of α-Syn mRNA, ultimately contributing to the abnormal accumulation of α-Syn and exacerbating PD pathology[45,46].

Glutaredoxin (GLRX), a small protein featuring a single active site cysteine pair, is paramount in sustaining the intracellular reducing environment and safeguarding cells against oxidative stress. Moreover, it plays a pivotal role in the pathogenesis of numerous neurodegenerative disorders, including PD[47]. Specifically, GLRX1 is instrumental in modulating the apoptotic signals in dopaminergic neurons, and its absence exacerbates the demise of these cells in PD patients[48]. NRF1, a vital transcriptional regulatory factor, governs the expression of proteasome genes in neurons. Notably, its expression levels are diminished in PD rat models[49]. It has been reported that NRF1 can alleviate motor impairment and dopamine neuronal deterioration, triggered by MPTP in PD mice, by elevating the transcription level of METTL3 and enhancing the m6A modification process of GLRX mRNA[50].

In general, PD is a neurodegenerative condition primarily impacting dopaminergic neurons, marked by the presence of Lewis bodies and cell loss, and has recently been implicated in the context of m6A-methylated proteins. MPTP-induced models of PD exhibit perturbations in m6A modifications and alterations in the expression of associated enzymes, consequentially impacting dopaminergic neurons. α-Syn, an important biomarker for PD, undergoes abnormal accumulation and interacts with METTL14, modulating its m6A modification status, which is notably diminished in PD patients. GLRX plays a pivotal role in maintaining neuronal health, and NRF1, by regulating METTL3, fortifies the m6A modification of GLRX mRNA, thereby safeguarding neurons. This intricate interplay of m6A modifications underscores the complexity of PD pathogenesis.

ALS/FTD

ALS is a neurodegenerative disease that progresses rapidly and has a high mortality rate[51]. FTD belongs to the second largest family of hereditary cognitive impairment, mainly affecting personality, social behavior, and language function[52]. Up to 50% of ALS patients may experience varying degrees of cognitive impairment, with some developing into FTD, and patients with advanced FTD may also exhibit clinical manifestations of ALS. Moreover, ALS and FTD can occur simultaneously in the same family or even in the same patient[53]. TDP-43 positive aggregates were found in neurons of the vast majority of ALS and FTD patients, and the same FUS positive aggregates were also observed in neurons of ALS patients with FUS mutations and some FTD patients[54,55]. In recent years, it has been discovered that mutations in certain genes can lead to the phenotype of both ALS and FTD, and can even manifest as both ALS and FTD phenotypes simultaneously[56]. Therefore, the overlap in clinical manifestations, pathology, and genetics closely links ALS and FTD, two seemingly dissimilar diseases.

A hexanucleotide repeat expansion (HRE) of a GGGGCC repeat within the first intron of C9orf72 (GGGGCC) is the most prevalent genetic factor of ALS and FTD[57,58]. Based on post-mortem tissue study in patients with C9orf72-ALS/FTD, a conspicuous reduction in the expression levels of methyltransferases METTL3 and METTL14 was discerned, accompanied by a marked downregulation of m6A levels. This finding is in harmony with the observed downregulation of m6A methylation in spinal cord neurons (iPSNs) derived from induced pluripotent stem cells (iPSCs) sourced from ALS/FTD patients[54,59]. Intriguingly, in iPSNs differentiated from iPSCs, the strategic modulation of either methyltransferase or demethylase aimed at reinstating the m6A level exhibits the potential to mitigate the disease-related phenotypes associated with ALS/FTD[59].

The nuclear clearance and cytoplasmic localization errors of TDP-43 are pathological markers of ALS, FTD, and related neurodegenerative diseases, collectively referred to as TDP-43 proteinopathy[60,61]. This mislocalization of TDP-43 from the nucleus to the cytoplasm, along with its clearance from the nucleus, is intimately correlated with RNA splicing errors, translational impairments, and widespread RNA instability in ALS[62]. Furthermore, studies have shown that the upregulation of mRNA expression levels encoding ribosomal proteins and oxidative phosphorylase is associated with TDP-43 deposition in neurons lacking the RNA methyltransferase METTL14[63]. Intriguingly, research has uncovered that not only do most TDP-43 substrates bear m6A modifications, but also YTHDF2 exacerbates TDP-43-related toxicity in both rodent and human neuronal models[64].

Human alphaherpesvirus 1 (HSV-1) commonly establishes a latent infection within the trigeminal nerve, which can persist lifelong and has garnered attention as a potential contributor to the development of ALS and FTD[65]. Recent research has illuminated the mechanisms underlying HSV-1's interaction with cellular RNA methylation pathways. Specifically, during the early stages of infection, HSV-1 enhances the expression of m6A methyltransferases, such as METTL3 and METTL14, as well as m6A reader proteins, including YTHDF1, YTHDF2, and YTHDF3. Subsequently, HSV-1 inhibits the expression of m6A demethylases like FTO and ALKBH5, thereby promoting viral replication by modulating the RNA methylation landscape[66].

The formation of inclusion bodies composed of dipeptide repeat proteins (DPRs), particularly the positively charged and arginine-rich DPR protein poly(GR), is recognized as a pathological hallmark of ALS and FTD[67]. Poly(GR) exhibits considerable toxicity in clinical models of ALS/FTD, manifesting through nucleolar damage, inhibition of protein synthesis, disruption of ribosomal RNA processing and biogenesis, interactions with RNA-binding proteins, and alterations in the liquid-liquid phase separation of membraneless organelles[68]. Remarkably, studies have demonstrated that mRNA modified with m6A modification and the m6A-binding YTHDF proteins not only co-localize with poly(GR) inclusions in the brains of ALS/FTD mouse models and FTD patients but also contribute to the formation of these inclusions. By incorporating RNA into the inclusions, m6A modifications and YTHDF proteins may interrupt essential interactions between poly(GR) and proteins like G3BP1 or YTHDF1, ultimately enhancing poly(GR) aggregation[69].

Translocated in liposarcoma/fused in sarcoma (TLS/FUS) is a nuclear RNA/DNA binding protein that, when mutated, can lead to the development of ALS of the spinal cord. Mutations in the C-terminal nuclear localization signal (NLS) of TLS/FUS have been specifically linked to the onset and severity of ALS symptoms[70,71]. These mutations disrupt the proper localization of TLS/FUS within the cell, causing it to accumulate in the cytoplasm, where it forms cytotoxic aggregates through a process known as liquid-liquid phase separation (LLPS)[72,73]. Notably, TLS/FUS has a preference for binding to mRNA fragments that are modified with m6A modification. However, mutations in the NLS of TLS/FUS can reduce its specificity for binding to these modified RNA fragments. Furthermore, the interplay between TLS/FUS and m6A-modified mRNA segments has been demonstrated to hinder the assembly of cytotoxic agglomerates via LLPS, while the dispersion of these modified RNA fragments throughout cytoplasmic TLS/FUS foci conspicuously augments cellular viability[74]. This observation implies that this specific interaction may serve as a safeguard against the onset and progression of ALS, underscoring its protective function in the context of this neurodegenerative disorder.

ALS and FTD exhibit significant clinical, pathological, and genetic intersections, with TDP-43 mislocalization and alterations in m6A RNA methylation standing as pivotal factors in their pathogenesis.

Mutations in the *C9orf72* gene, resulting in the formation of HREs, contribute to the development of both ALS and FTD, impacting methyltransferases METTL3/14 and modulating m6A levels. Additionally, HSV-1 infection dynamically modulates m6A pathways, enhancing viral replication strategies. In the context of ALS and FTD, DPR proteins such as poly(GR) form characteristic inclusions that co-localize with m6A-modified RNA and YTHDF proteins, underscoring their involvement in disease mechanisms. Meanwhile, mutations in *TLS*/*FUS* genes, prevalent in ALS, disrupt proper nuclear localization, leading to the formation of cytotoxic aggregates. Intriguingly, these mutations' interaction with m6A-modified RNA appears to hinder aggregate formation, suggesting a potential protective mechanism against disease progression.

HD

HD is an autosomal dominant neurodegenerative disease, primarily due to a genetic alteration in the *HTT* gene situated on chromosome 4, leading to the production of a mutated Huntington protein[75,76]. HD patients typically develop symptoms between the ages of 30 and 40, mainly characterized by involuntary dance-like movements, cognitive impairment, and mental abnormalities. The course of the disease ranges from 10 to 20 years, ultimately leading to the patient's death[77]. So far, there is no specific drug that can prevent the pathological process of HD, mainly due to the unclear pathological mechanism of the disease[78,79].

Hdh knockout mice are one of the commonly used animal models for studying HD, and HdhQ111 knockout mice are a group of them with 111 HD polyglutamine fragment residues[80]. High methylation of m6A in synapse-related genes was observed in the hippocampal transcriptome of $Hdh^{+/Q111}$ mice. Research has uncovered that m6A modification in the hippocampus of individuals with HD is abnormally regulated in a manner that is influenced by experiences. This aberrant regulation results in the demethylation of crucial synaptic tissue components, potentially underpinning the cognitive impairments observed in HD[81]. Consistent with these data, knockdown of m6A demethylase FTO in HD mice can improve spatial and recognition memory[82].

In the realm of amplifying CAG repeats, alongside the production of the full-length (FL) HTT mRNA isoform, two smaller transcripts arise due to incomplete splicing. These transcripts, designated HTT1a, encompass exon 1 and intron 1 sequences in their 5' region, a phenomenon attributed to abnormal polyadenylation at an intrinsic polyA site within intron 1[83,84]. Notably, HTT1a encodes not only the well-known highly pathogenic HTT exon 1 protein, which is prone to aggregation, but also contributes to the formation of mRNA nuclear clusters that exhibit resilience against HTT antisense oligonucleotides (ASOs) therapy[85,86]. It is imperative to mention that pharmacological interventions aimed at inhibiting the m6A methyltransferase METTL3 or specifically demethylating HTT intron 1 have been found to significantly reduce the transcription level of HTT1a in HD cells[85]. This suggests that m6A methylation within intron 1 may be influenced by CAG amplification and plays a pivotal role in the production of aberrantly spliced HTT1a transcripts[87].

The pathological hallmark of TDP-43 mislocalization from the nucleus to the cytoplasm has emerged as a consistent feature in patients and mouse models of ALS, FTD, and HD[88,89]. Intriguingly, recent advancements in research have illuminated that TDP-43 deficiency can instigate somatic trinucleotide repeat expansion, exacerbating the neuropathological manifestations observed in HD. Specifically, targeted knockdown of endogenous TDP-43 in the striatum of HD knock-in mice has been shown to accelerate the expansion of CAG repeats[90,91]. Furthermore, recent investigations have highlighted TDP-43 dysfunction and aberrant m6A modification as potential contributors to incorrect splicing in HD, thereby modulating the expression of striatal genes associated with HD[88]. Notably, the reduced binding of TDP-43 is evident

within genes that define the unique characteristics of striatal HD. Analogously, the m6A deposition levels within these same striatal genes are also diminished under HD conditions[92]. In the context of HD, the heightened expression of TDP-43 implicates a reliance on m6A modification for TDP-43 binding. The observed decline in m6A deposition within abnormal HD-associated genes results in a consequential reduction in TDP-43 binding and stability, revealing a novel mechanistic interplay between TDP-43 binding sites and m6A deposition sites[93]. This finding underscores a novel mechanistic correlation between TDP-43 binding sites and m6A deposition sites within the context of HD, suggesting that m6A methylation may serve as a crucial determinant for TDP-43's binding capacity.

In essence, the relationship between HD and m6A methylation is multifaceted and plays a pivotal role in the pathogenesis of HD. Notably, animal models, particularly those with *HdhQ111* gene knockouts, exhibit abnormal m6A methylation patterns in synaptic genes, which subsequently affect cognitive functioning. The generation of HTT1a transcripts, intimately linked to the pathogenesis of HD, is governed by m6A methylation occurring specifically within intron 1. Furthermore, the mislocalization of TDP-43 and its intricate interaction with m6A modifications significantly influence striatal gene expression in HD, uncovering a novel and potentially crucial interplay between TDP-43 binding and m6A deposition that may underlie the fundamental mechanisms of HD pathology.

CONCLUSION

RNA m6A methylation is highly prevalent in the mammalian brain and constitutes a crucial surface transcriptome modification. m6A modulates the function of target genes by influencing mRNA translation, splicing, degradation, and nuclear export, thereby exerting a broad impact on various neurodegenerative diseases, such as AD, PD, ALS/FTD, and HD [Table 1]. Numerous studies have highlighted the pivotal role of m6A in regulating cerebral functions. The absence of m6A methyltransferases in Drosophila insects leads to severe mobility deficits, characterized by disorientation, sluggish walking pace, and decreased activity, all of which can be traced back to impaired neuronal function[94]. Similarly, mice with dysfunctional m6A pathways exhibit a multitude of cerebral abnormalities, ranging from reduced brain size and compromised spatial learning and memory abilities to defects in synaptic transmission, long-term potentiation, and axon regeneration[95]. This emphasizes the significance of the m6A-modulated epitranscriptome in neuronal functioning, yet research on its influence on human neurological or neurodegenerative disorders remains scarce.

In the context of AD, the multifaceted role of m6A modification emerges as a pivotal regulatory mechanism. It intricately modulates macrophage functionality, enhancing the clearance of Amyloid-beta (Aβ) deposits, while simultaneously influencing Tau protein phosphorylation dynamics. In PD, the role of m6A modification revolves around its influence on key molecular players, particularly α-Syn and GLRX. Moreover, in ALS and FTD cases caused by the repetitive expansion of C9orf72, m6A modification and its associated proteins play complex roles in modulating the pathogenesis of ALS/FTD through their interactions with TDP-43, DPRs like poly(GR), and TLS/FUS. Lastly, in HD, heightened m6A modification of synaptic-related genes modulates *HTT* gene expression and splicing, further influencing the cytoplasmic localization of TDP-43 protein [Figure 2]. By elucidating these intricate methylation patterns, we can delve deeper into the fundamental mechanisms underlying neurodegeneration, thereby potentially forging a path for the development of targeted therapeutic interventions. Furthermore, the pivotal proteins intricately involved in m6A modification are anticipated to emerge as promising molecular targets for the diagnosis, treatment, and drug discovery endeavors aimed at combating neurodegenerative diseases.

Table 1. The molecular mechanism between m6A modification and neurodegenerative diseases

Diseases	Pathological features	Biomarker	Related enzymes	m6A modification level	Ref.
AD	β-amyloid protein	ATAT1	METTL3	Elevated methylation levels	[24]
	Hyperphosphorylation of tau protein	KDM1A	METTL3 METTL14 YTHDF	Reduced methylation level	[28]
	Synaptic degeneration	CircRIMS2	METTL3	Reduced methylation level	[32]
PD	Dopaminergic neuron damage	MPTP	METTL3 RBM15 ALKBH5 FTO	Reduced methylation level	[39]
	The abnormal accumulation of α-Syn	α-Syn	METTL14 YTHDF	Reduced methylation level	[44]
	Oxidative stress	GLRX1	METTL3	Reduced methylation level	[48]
ALS/FTD	Deposition of TDP-43 in neurons	TDP-43	METTL14 YTHDF2	Reduced methylation level	[63]
	Human alphaherpesvirus-1 infection	HSV-1	METTL3 METTL14 FTO ALBKH5	Elevated methylation levels	[66]
	Inclusion body composed of DPR proteins	Poly(GR)	G3BP1 YTHDF1	Elevated methylation levels	[69]
	Cytotoxic aggregates through LLPS	TLS/FUS	YTHDF2	Elevated methylation levels	[74]
HD	Highly pathogenic HTT exon1 protein that is prone to aggregation	HTT1a	METTL3	Elevated methylation levels	[87]
	Accelerated expansion of CAG repeat caused by TDP-43 deficiency	TDP-43	METTL3	Reduced methylation level	[90,93]

m6A: N6-methyladenosine; ATAT1: α-Tubulin acetyltransferase-1; AD: Alzheimer's disease; KDM1A: Lysine-specific demethylase 1A; PD: Parkinson's disease; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; α-Syn: alpha-synuclein; GLRX1: glutaredoxin1; ALS: amyotrophic lateral sclerosis; FTD: frontotemporal dementia; LLPS: liquid-liquid phase separation; TLS/FUS: translocated in liposarcoma/fused in sarcoma; HD: Huntington's disease.

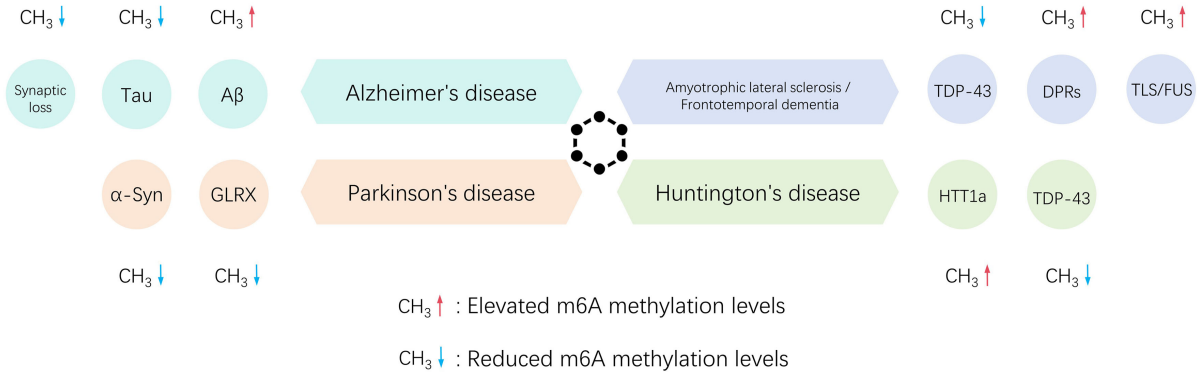

Figure 2. Dynamic changes of m6A modification in four neurodegenerative diseases and pathological features associated with this methylation. m6A: N6-methyladenosine.

The intricate interplay between "Writers", "Erasers", and "Readers" in the m6A methylation modification process further complicates its pathogenic mechanisms. For instance, METTL3 serves as a pivotal component of the m6A methyltransferase "Writers" complex, demonstrating robust methyltransferase

activity. However, there have also been reports suggesting that METTL3 functions as an m6A "reader" in certain contexts. Research indicates that METTL3 directly promotes the translation of several m6A-modified mRNAs by interacting with the translation initiation machinery, independently of its downstream m6A "reader" activity[96]. Such findings underscore the need for a nuanced understanding of the dynamic interplay between these epigenetic regulators and their intricate contributions to neurodegenerative diseases.

Moreover, variations in experimental conditions and the utilization of diverse animal models contribute to discrepancies in research outcomes. A case in point is the study utilizing APP/PS1 mice as models, where contrasting results were obtained. Specifically, while an elevation of m6A methylation levels in the cortex and hippocampus was observed in 9-month-old APP/PS1 mice[19], a study involving 6-month-old APP/PS1 mice reported a downregulation of METTL3 expression, an upregulation of FTO expression, and a consequential decrease in m6A methylation levels[26]. Indeed, the observed disparities in research outcomes likely stem from the intricate compensatory mechanisms that regulate m6A modification during neurodevelopment and aging. These processes, characterized by temporal and spatial differences, contribute to a dynamic landscape of epigenetic modifications. Therefore, a comprehensive experimental strategy that encompasses various developmental stages across a broad spectrum of neurodegenerative diseases is paramount. Such an approach will enable researchers to track the nuanced changes in RNA m6A methylation modification over time and across different disease models. By doing so, we can gain a deeper understanding of the role of m6A in neurodegeneration and how it is affected by disease progression.

Absolutely, the current focus on mouse models in research on m6A methylation modification and neurodegenerative diseases, while valuable, has its limitations. The pathological complexity and age-dependence of neurodegenerative diseases mean that transgenic rodent models often fail to fully recapitulate the selective neurodegenerative changes observed in human brains. This underscores the importance of exploring larger animal models, such as monkeys and pigs, which exhibit greater structural and functional similarities to humans[97,98]. Large animal models offer unique advantages in studying neurodegenerative diseases, including more accurate representation of disease progression, pathology, and response to therapeutic interventions. As such, extending research on the role of m6A methylation modification in neurodegenerative diseases to large-scale animal models has the potential to provide deeper insights into the mechanisms underlying these disorders. With the continued development and maturation of large animal neurodegenerative disease models, we can anticipate a more comprehensive and nuanced understanding of the function of m6A methylation modification in these diseases. This, in turn, will pave the way for the development of more effective and targeted therapeutic strategies aimed at addressing the challenges posed by neurodegenerative disorders.

DECLARATIONS

Authors' contributions

Wrote the review paper: Song J
Revised manuscript, conceived and designed experiments: Yan S
Both authors read and approved the final manuscript.

Availability of data and materials

Not applicable.

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Conflicts of interest

Yan S is a Guest Editor of the journal *Ageing and Neurodegenerative Diseases*, while the other author declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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