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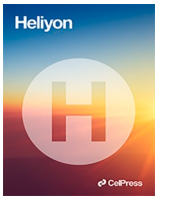

Review article

N^6-methyladenosine modification in ischemic stroke: Functions, regulation, and therapeutic potential

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ABSTRACT

N^6-methyladenosine (m^6A) modification is the most frequently occurring internal modification in eukaryotic RNAs. By modulating various aspects of the RNA life cycle, it has been implicated in a wide range of pathological and physiological processes associated with human diseases. Ischemic stroke is a major cause of death and disability worldwide with few treatment options and a narrow therapeutic window, and accumulating evidence has indicated the involvement of m^6A modifications in the development and progression of this type of stroke. In this review, which provides insights for the prevention and clinical treatment of stroke, we present an overview of the roles played by m^6A modification in ischemic stroke from three main perspectives: (1) the association of m^6A modification with established risk factors for stroke, including hypertension, diabetes mellitus, hyperlipidemia, obesity, and heart disease; (2) the roles of m^6A modification regulators and their functional regulation in the pathophysiological injury mechanisms of stroke, namely oxidative stress, mitochondrial dysfunction, endothelial dysfunction, neuroinflammation, and cell death processes; and (3) the diagnostic and therapeutic potential of m6A regulators in the treatment of stroke.

1. Introduction

Stroke is currently the second leading cause of death and disability worldwide [1]. According to the Global Stroke Fact Sheet 2022 published by the World Stroke Organization, the disease burden of stroke increased substantially in the years between 1990 and 2019 [2]. Strokes can be classified into two primary types, namely hemorrhagic and ischemic stroke. Hemorrhagic stroke, considered the deadliest type, is caused by the rupture of blood vessels within the brain, including intracerebral and subarachnoid hemorrhages [3]. Ischemic stroke, which is the most common type, accounting for approximately 87 % of all stroke cases, is generally attributable to an inadequate supply of blood to the cerebral tissues caused by blockage of the cerebral artery, during which the ischemia may trigger a cascade of events leading to neuronal death [4]. Currently, there are limited options available for the treatment of ischemic stroke,

Abbreviations: m6A, N6-methyladenosine; MCAO, middle cerebral artery occlusion; OGD/R, oxygen-glucose deprivation/reperfusion; METTL3, methyltransferase-like 3; METTL14, methyltransferase-like 14; WTAP, Wilms' tumor 1-associating protein; ZC3H13, zinc finger CCCH-type containing 13; RBM15, RNA binding motif protein 15; FTO, fat mass and obesity-associated; ALKBH5, alkB homolog 5; HNRNP, heterogeneous nuclear ribonucleoprotein; m6A-SNPs, m6A-associated single-nucleotide polymorphisms; DM, diabetes mellitus; T2DM, type 2 DM; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MTCH2, mitochondrial carrier homology 2; I/R, ischemia/reperfusion; ROS, reactive oxygen species; PRDX3, peroxiredoxin 3; Drp1, dynamin-related protein 1; FIS1, fission 1; ECs, endothelial cells; AAV, adeno-associated viral vectors.

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with primary interventions focusing on rapid reperfusion via intravenous thrombolysis and endovascular thrombectomy, albeit within a very narrow therapeutic window [5]. These unfavorable circumstances emphasize the need to identify alternative novel, safe, and effective treatments.

N^6-methyladenosine (m^6A) modification is the most prevalent type of internal modification occurring in eukaryotic RNAs. This dynamic and reversible modification process is modulated by methylated and demethylated transferases, which influence RNA metabolism via interactions with RNA-binding proteins [6] and highlights the prevalent role played by m^6A modification in a range of pathological and physiological processes associated with human diseases and disorders, including strokes [7]. In this context, Chokkalla et al. [8] have found that global m^6A levels are markedly elevated after transient focal ischemia, as revealed by their analysis of the cerebral m^6A epitranscriptome in mice with middle cerebral artery occlusion (MCAO). Cerebral ischemia has been established to be associated with a significant alteration in m^6A-modified transcripts, thereby modulating transcriptional regulation, apoptosis, and poststroke inflammation. On the basis of methylated RNA immunoprecipitation and RNA sequencing analyses, Zhu et al. [9] detected elevated levels of global m^6A in A172 cells subjected to oxygen-glucose deprivation/reperfusion (OGD/R), in the cerebral cortex of mice treated with transient MCAO and reperfusion, and in the peripheral blood of stroke patients. In addition, they observed that a total of 2115 m^6A peaks were modified in patients with ischemic stroke, and that the dysregulated methylated mRNAs were associated with NF-kappa B, Hedgehog, Hippo, MAPK, and calcium signaling pathways, which have been implicated in the development and progression of strokes [10–14]. These findings accordingly provide convincing evidence to indicate the involvement of m^6A methylation and m^6A-mediated regulation in the etiology of ischemic stroke.

In this review, we summarize the findings of recent studies that have examined the involvement of m^6A modification in the development and progression of ischemic stroke and the underlying molecular mechanisms. Additionally, we present an overview of the diagnostic and therapeutic potential of m^6A modifications in the treatment of ischemic stroke.

2. Overview of m^6A modification

As a methylation modification at the N^6-position of adenosine, m^6A was initially discovered in 1974 by Desrosiers et al. during their examination of eukaryotic mRNA derived from Novikoff hepatoma cells [15]. Subsequent studies have confirmed that m^6A is the most abundant and important type of internal modification occurring in eukaryotic RNAs [16,17]. m^6A modification has been established to modulate a wide range of RNA processes, including RNA splicing, processing, translation, localization, stability, and degradation, and has been demonstrated to play prominent roles in multiple pathological and physiological processes associated with human diseases and disorders [18,19], including strokes [7]. As a dynamic and reversible process, m^6A modification is regulated mainly by methyltransferases (also termed “writers”), demethylases (“erasers”), and m^6A-binding proteins (“readers”).

2.1. Writers

N^6A methylation is mediated by a methyltransferase complex, which comprises the core components, namely methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14), and Wilms’ tumor 1-associating protein (WTAP), and certain partner proteins, including zinc finger CCCH-type containing 13 (ZC3H13), virilizer-like m (6)A methyltransferase-associated protein, and RNA binding motif protein 15 (RBM15). METTL3, the first methyltransferase subunit to be identified, interacts with METTL14 to form a heterodimeric complex that synergistically catalyzes the transfer of the methyl groups of *S*-adenosylmethionine to specific RNA adenines, during which, METTL14 assists METTL3 in recognizing substrates [20–22]. Although having no inherent methyltransferase activity, WTAP acts as a regulatory subunit to initiate and direct METTL3–METTL14 heterodimer localization to nuclear speckles, thereby indirectly promoting catalytic activity [23]. Among the partner proteins of the methyltransferase complex, ZC3H13 has been identified as an interactor that promotes m^6A deposition by bridging WTAP with the mRNA-binding factor Nito [24]. Virilizer-like m (6)A methyltransferase-associated protein mediates region-selective m^6A mRNA methylation (preferentially in the 3'-untranslated region and near the stop codon) by recruiting the catalytic core components METTL3/METTL14/WTAP [25]. RBM15 and its paralog RBM15B have also been reported to bind to the methyltransferase complex to mediate m^6A methylation of XIST and cellular mRNAs [26].

2.2. Erasers

Demethylases serve to reverse the m^6A methylation process, and to date, only two demethylases have been identified, namely fat mass and obesity-associated (FTO) and alkB homolog 5 (ALKBH5). ALKBH5 was the first to be identified by Jia et al. [27] in 2011. *In vitro*, FTO has been demonstrated to efficiently remove m^6A under conditions of neutral pH, whereas *in vivo* its oxidative activity influences the intracellular levels of m^6A. The discovery and characterization of the demethylase FTO thus provided evidence to indicate that m^6A methylation is a dynamically regulated reversible process. The second m^6A demethylase ALKBH5 was identified by Zheng et al. [28] in 2013. It was subsequently established to markedly influence RNA metabolism and mRNA export and regulate the assembly/modification of mRNA processing factors via colocalization with nuclear speckles [28]. Both FTO and ALKBH5 are members of the ALKB family of proteins, the demethylation activities of which have been shown to be dependent on Fe^{2+} and alpha-ketoglutarate [27,28]. They can localize to nuclear speckles, although they are characterized by different intracellular localizations and tissue distributions [29,30].

2.3. Readers

m^6A readers are specific m^6A-binding proteins, including YTHDF1/2/3, YTHDC1/2, IGF2BP1/2/3, HNRNPC, HNRNPG, and HNRNPA2B1, that recognize and bind directly or indirectly to m^6A sites, influencing the fate of target RNAs. YTHDF1/2/3 and YTHDC1/2 contain a highly conserved YTH (YT521-B homology) domain that enables these proteins to bind directly to m^6A sites. YTHDF1/2/3 are located primarily in the cytoplasm, and their binding to m^6A sites alters the stability and translation efficiency of the m^6A-modified RNAs [31]. YTHDF1 promotes RNA translation via coordinated interactions with translation initiation factors [32,33], while YTHDF2 regulates mRNA decay by interacting with the CNOT1 subunit [34]. YTHDF3 functions coordinately with YTHDF1 to facilitate protein synthesis and influence the mRNA decay mediated by YTHDF2 [35]. In contrast to other YTH proteins, YTHDC1 is primarily localized in the nucleus, within which it modulates mRNA splicing by recruiting and regulating pre-mRNA splicing factors [36]. Members of the heterogeneous nuclear ribonucleoprotein (HNRNP) family (HNRNPA2B1, HNRNPC, and HNRNPG) are m^6A readers that play key roles in regulating the splicing and maturation of RNAs [37–39], whereas cytoplasmic IGF2BP1/2/3 target m^6A-modified RNAs by recognizing the conserved GG (m^6A)C sequence, thereby promoting the stability and storage of mRNAs [40].

In summary, m^6A modification is a dynamically reversible process that is precisely regulated by multiple interactions among methyltransferases, demethylases, and m^6A-binding proteins (Fig. 1). m^6A modification influences gene expression by regulating almost every aspect of the RNA life cycle, thereby facilitating its involvement in a range of biological processes and in the initiation and development of human diseases.

3. Associations between m^6A modification and the risk factors of strokes

Stroke is a heterogeneous syndrome that is associated with multiple risk factors, the regulation of which plays a key role in reducing stroke burden [41]. Previous studies have demonstrated associations between the levels of m^6A and the risk factors of stroke and pathological changes during its occurrence and development.

3.1. Hypertension

Among the important modifiable risk factor of strokes, hypertension is implicated in more than half of all stroke cases worldwide. It has been reported that the lifetime risk of stroke for 45-year-old men with hypertension is 32.79 %, which is significantly higher than that for those without hypertension (17.21 %). Moreover, the lifetime risk of stroke was found to be significantly higher (48.33 % vs. 20.21 %) in 45-year-old men with a stage of ≤2 hypertension than in those with stage 1 hypertension [42]. Similar lifetime risks of stroke have been reported for women with hypertension. Compared with women without a history of preeclampsia (hypertensive disorder of pregnancy), those with a history are 60 % more likely to develop non-pregnancy-related ischemic stroke [43]. In this

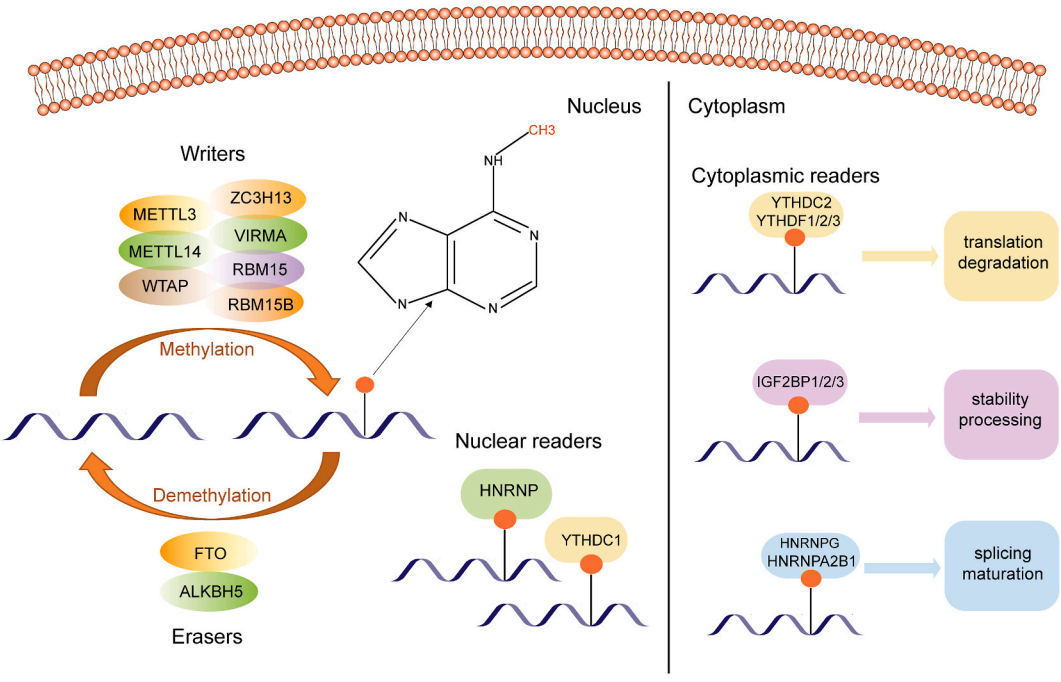

Fig. 1. A schematic diagram illustrating the dynamic regulation of N^6-methyladenosine (m^6A) methylation.
m^6A modification is a dynamically reversible process initiated by methyltransferases ("writers"), reversed by demethylases ("erasers"), and recognized by m^6A-binding proteins ("readers").

regard, it has been reported that a 10 mmHg reduction in systolic blood pressure or a 5 mmHg reduction in diastolic blood pressure is associated with a 41 % reduction in stroke [44], thereby highlighting the preventive effect of blood pressure reduction on the likelihood of stroke occurrence. In addition to the association between hypertension and the risk of stroke, hypertension exacerbates severe stroke outcomes by promoting stroke via enhanced shear stress, endothelial dysfunction, and large artery stiffness [45].

Based on large-scale genome-wide association studies, Mo et al. have observed close associations between m^6A-associated single-nucleotide polymorphisms (m^6A-SNPs) and blood pressure, and among the 1236 blood pressure-associated m^6A-SNPs identified, approximately 10 % were established to be associated with stroke [46]. Among these m^6A-SNPs, two showed to be significantly associated with blood pressure (rs9847953 and rs197922) and were found to modulate the gene expression of ZNF589 and GOSR2 by mediating m^6A methylation, thereby influencing the susceptibility to develop hypertension. Conversely, a C allele rs7398833 genotype at the m^6A site has been found to be associated with reduced levels of systolic and diastolic blood pressure, thereby contributing to a reduction in the risk of ischemic stroke [46]. The demethylase FTO has been established to be highly expressed in the hypothalamus, a brain structure associated with the regulation of blood pressure. In Caucasian populations, individuals with an FTO risk genotype (rs9939609 allele A) have been found to be susceptible to high blood pressure [47], and this genetic variant is negatively associated with diastolic and mean blood pressure in men with hypertension [48]. Pericytes play important roles in the pathogenesis of hypertension by controlling blood-brain barrier permeability and modulating micro-vessel blood flow. Wu et al. have revealed a global reduction in average levels of m^6A in the pericytes of spontaneously hypertensive rats, thus indicating that alterations in the extent of m^6A modification in microvascular pericytes may be involved in the pathogenesis of hypertension [49]. Notably, Paramasivan et al. [50] have proposed targeting m^6A via METTL3 and FTO as a possible therapeutic strategy for hypertension. In addition, the demethylase ALKBH1 has been proposed as a therapeutic target to prevent hypertension via epigenetic programming [51].

3.2. Diabetes mellitus (DM)

A further stroke-associated risk factor is hyperglycemia, which is typically found in patients with DM. It has been reported that DM duration is independently correlated with the risk of ischemic stroke, with a 3 % annual increase in the risk of stroke and a threefold increase in patients with a DM duration greater than 10 years [52]. DM can trigger pathological changes in different micro-vessels and large vessels, which may cause stroke when the cerebral vessels are directly influenced with plausible underlying mechanisms, including systemic inflammation, enhanced early age arterial stiffness, and vascular endothelial dysfunction. For example, compared with individuals having normal blood sugar levels, those with type 2 DM (T2DM) are more likely to experience an increase in arterial stiffness and reduced arterial elasticity, while those with type 1 DM tend to have early structural damage of the common carotid artery [53]. Moreover, DM is associated with poor outcomes after stroke [54]. For example, it has been reported that poststroke hyperglycemia is associated with an increase in infarct volume that occurs during the acute phase of ischemic stroke [55]. Indeed, certain antidiabetic drugs with vascular benefits have been shown to reduce the risk of stroke [56].

Changes in epigenetic modification have also been established to influence the susceptibility to DM [57]. For example, Wu et al. [58] detected significant changes in the expression of seven m^6A-associated genes (increase in IGF2BP2 and IGF2BP3, and decrease in METTL3, YTHDF2/3, ALKBH1, and HNRNPC) in islet samples collected from patients with T2DM. The serum levels of IGF2BP3 were found to show a U-shaped association with the odds of developing T2DM, which gradually increased when the serum levels were $<$ 0.62 ng/mL. The SNPs, rs 4402960 in the m^6A reader IGF2BP2 and rs 9939609 in the m^6A demethylase FTO, have been reported to be significantly associated with T2DM [59]. Moreover, IGF2BP2 rs11705701 has been shown to be significantly associated with prediabetes in women [60]. The findings of these studies accordingly provide evidence to indicate the involvement of m^6A modification in the risk of developing DM.

The findings of further studies by Xie et al. have revealed elevated levels of m^6A methylation and the methyltransferase METTL3 in the liver tissues of patients with T2DM, which were found to be positively correlated with insulin resistance and negatively correlated with the functions of pancreatic β-cells [61]. They also demonstrated that by regulating fatty acid synthase expression via m^6A methylation, METTL3 represses hepatic insulin sensitivity [61]. Similarly, Li et al. found that elevated m^6A levels and METTL3 expression contribute to exacerbating liver metabolic disorders and insulin resistance induced by a high-fat diet. By prolonging the half-life of metabolism-related genes via METTL3 depletion-mediated m^6A loss, hepatocyte-specific knockout of METTL3 was found to markedly enhance insulin sensitivity and ameliorate metabolic disorders [62]. The findings of these two studies thus provide evidence to indicate that METTL3-mediated m^6A methylation plays an important role in the development of hepatogenous diabetes.

A key contributory factor associated with the development of DM is β-cell dysfunction, which can lead to impaired glucose metabolism [63,64]. m^6A modification has been established to be closely associated with the survival and function of β-cells, and m^6A-sequencing analyses have revealed reductions in the overall m^6A methylation of transcripts involved in insulin secretion, cell-cycle progression, and the insulin/IGF1-AKT-PDX1 pathway in human T2DM islets. Furthermore, a depletion of m^6A levels in EndoC-βH1 has been demonstrated to result in a reduction in PDX1 expression and AKT phosphorylation, thereby impairing insulin secretion and inducing cell-cycle arrest [65], whereas specific knock-out of β-cell METTL14 in mice results in a reduction in m^6A levels and early diabetes onset, as well as mortality secondary to a reduction in β-cell proliferation and insulin degranulation [65]. By influencing the expression of transcripts involved in cell death and inflammation, similar manipulation was found to promote glucose intolerance, reductions in β-cell mass and proliferation, and lower levels of insulin secretion [66]. METTL3 and METTL14 have been established to specifically modulate the mass expansion and functional maturation of neonatal β-cells, and their deletion in Ngn^{3+} endocrine progenitors causes hypoinsulinemia and hyperglycemia in 2-week-old mice. Mechanically, METTL3/14 regulates β-cell differentiation by regulating the mRNA stability of MafA in an m^6A-dependent manner [67]. In patients with T2DM and diabetic db/db mice, reductions have been detected in the expression of β-cell METTL3/14, thereby indicating their potential roles in T2DM [67].

METTL3-deficient islet β cells have been found to be associated with islet β-cell failure and hyperglycemia, which could be attributed to a reduction in m^{6}A levels mediated by METTL3 deficiency and reductions in the expression of genes involved in insulin secretion [68]. Furthermore, by modulating the stability and expression of MafA through m^{6}A methylation, the expression of METTL3 has been shown to alleviate the impairment of methylglyoxal-induced insulin in β cells [69]. In addition to METTL3/14, other m^{6}A regulators have also been implicated in DM. For example, under high-glucose conditions, lower levels of m^{6}A methylation and enhanced ALKBH5 mRNA expression have been observed in pancreatic β Min6 cells [70]. Moreover, in mouse and human islets, gene deletion and pharmacological inhibition of FTO have been demonstrated to enhance glucose-stimulated insulin secretion, thereby indicating that FTO may play a negative regulatory role in insulin secretion [70]. In T2DM, chronic inflammation and lipotoxicity have been found to be associated with the downregulated expression of YTHDC1 in islet β cells, and the specific deletion of β-cell YTHDC1 was established to promote reductions in the expression of transcription factors specific to β-cells and genes involved in insulin secretion, thus contributing to β-cell failure and diabetes [71]. Collectively, these findings emphasize the important role played by m^{6}A regulators in the etiology of DM, and accordingly identify these factors as potential targets for the prevention and treatment of DM.

3.3. Hyperlipidemia

It has been established that there is a complex association between dyslipidemia and the risk of stroke. Elevated levels of high-density lipoprotein cholesterol (HDL-C) are associated with a reduced risk of ischemic stroke, whereas high remnant cholesterol levels have been linked to a heightened risk of ischemic stroke, with an adjusted hazard ratio of 1.99 for patients having ≥1.5 mmol/L (58 mg/dL) remnant cholesterol, compared with those with <0.5 mmoL/l (19 mg/dL) [72]. Low-density lipoprotein cholesterol (LDL-C) is considered the most useful serum lipid marker indicative of the risk of stroke and has long been believed to be one of the primary pathogenic risk factors for stroke. LDL-C-lowering therapy or medications, such as statins, can be administered to reduce the risk of stroke, and recent guidelines have emphasized the application of "high-intensity statins" and "low LDL-C target" strategies in patients with stroke [73]. However, although LDL-C-lowering therapy appears to be beneficial in preventing large-artery atherosclerosis, it may have little effect in preventing small-artery occlusion and cardioembolic stroke [74]. Accordingly, the prevention and control of dyslipidemia are advocated to ease the burden of stroke [75].

Based on their large-scale genome-wide association studies, Mo et al. [76] identified 22 m^{6}A-SNPs that are significantly associated with lipid levels. HDL-C and triglycerides, which can serve as indicators of elevated levels of remnant cholesterol particles, were found to be markedly enriched concerning m^{6}A methylation, with m^{6}A-SNP rs6859 in the 3'-untranslated region of PVRL2 being found to be associated with the levels of total cholesterol, HDL-C, LDL-C, and triglycerides [76].

The m^{6}A methylation of genes involved in lipogenesis plays an important role in lipid metabolism. It has been demonstrated that the knockdown of YTHDF2 or METTL3 enhances mRNA stability and the expression of peroxisome proliferator-activator α, which contribute to reductions in the accumulation of lipids [77]. Compared with those in standard-fed mice, elevated levels of m^{6}A methylated RNAs and METTL3 have been detected in mice that were fed a high-fat diet, whereas by promoting reductions in m^{6}A methylation and total mRNA levels of fatty acid synthetase, METTL3 knockout contributed to reducing fatty acid synthesis in these mice [61]. The expression of YTHDC2 has been established to be downregulated in the livers of obese mice and patients with non-alcoholic fatty liver disease, and its dysregulation has been demonstrated to modulate triglyceride accumulation, liver steatosis, and insulin resistance by influencing binding to the m^{6}A-modified transcripts of lipogenic genes (such as stearoyl-CoA desaturase 1, fatty acid synthase, element-binding protein 1c, and acetyl-CoA carboxylase 1), thereby regulating the expression of these lipogenic genes [78]. Moreover, by regulating m^{6}A methylation levels, the demethylase FTO has been shown to modulate the expression of several genes, including PPARγ, CEBPα, stearoyl-CoA desaturase, and sterol regulatory element-binding protein-1, which have been implicated in the syntheses of cholesterol and triacylglycerol [79,80]. For example, Sun et al. have reported that the overexpression of FTO resulted in fat accumulation, elevated levels of triglycerides and total cholesterol, increased expression of PPARγ and CEBPα, and a reduction in m^{6}A levels. Additionally, it is reported that the knockdown of FTO is associated with anti-lipogenic activity, as indicated by reductions in the levels of triglycerides and total cholesterol and the downregulated expression of PPARγ and CEBPα [80].

3.4. Obesity

Obesity is a well-documented risk factor for stroke. Even after adjusting for other cardiovascular risk factors, being overweight, obese, or having abdominal obesity (determined by waist-to-hip ratio), obesity has been identified as an independent risk factor of stroke in young adults [81,82]. Evidence indicates that obesity heightens the risk of an initial ischemic stroke, which is assumed to be dependent on the metabolic abnormalities caused by obesity rather than obesity per se [83]. Conversely, weight loss can substantially reduce the risks of hypertension, DM, and stroke. Obesity is also associated with the outcomes of stroke. Regarding this, an obesity paradox appears to exist, in which patients who are overweight or obese show better outcomes and reduced mortality rates than those with normal-weight or who are underweight, although opinions on this remain divided. Nevertheless, it is speculated that insulin resistance and inflammation might be plausible mechanisms underlying this "obesity paradox" in stroke [84,85]. However, these findings highlight the importance of weight control in patients with stroke.

FTO, the first of the two m^{6}A demethylases to be identified, has been implicated in obesity, with genetic variants of the FTO gene (rs9939609, rs9930333, rs9928094, and rs9935401) being found to be positively associated with the risk of obesity, while rs8061518 is characterized by an inverse association [86]. In addition to obesity risk, the FTO rs9939609 polymorphism has been shown to be associated with a preference for high dietary fat intake [87]. Furthermore, the rs1421085 (T-to-C) SNP in FTO has been found to disrupt the conserved motif of the ARID5B repressor, which in turn promotes the de-repression of a potent preadipocyte enhancer and

causes a twofold increase in the expression of IRX3 and IRX5 during early adipocyte differentiation. Manipulation of these pathways has been found to contribute to significant anti-obesity and pro-obesity activities [88].

As a demethylase that regulates m^{6}A-dependent alternative splicing, FTO has been established to be associated with obesity via the regulation of pre-adipocyte differentiation and adipogenesis [89]. In a 3T3-L1 pre-adipocyte model, FTO knockdown was found to repress pre-adipocyte differentiation, which could be reversed in response to the overexpression of FTO. During this process, the demethylase activity of FTO becomes apparent, as reflected by the impedance of pre-adipocyte differentiation following the overexpression of R96Q, an FTO missense mutant lacking demethylase activity [90]. Similarly, Zhao et al. [91] have indicated that FTO knockdown hampers adipogenesis, and that adipogenesis can be restored only in the presence of catalytically activity FTO. This FTO-mediated differentiation of preadipocytes was achieved by controlling the splicing of RUNX1T1 (a dipogenic regulatory factor) via the modulation of m^{6}A levels in the vicinity of splice sites.

Jiang et al. [92] identified mitochondrial carrier homology 2 (MTCH2) as a key gene involved in adipogenesis, the expression of which was found to be positively associated with m^{6}A levels. Further analyses revealed that the m^{6}A reader YTHDF1 is involved in MTCH2-mediated adipogenesis by regulating MTCH2 expression through specific binding to the m^{6}A-modified transcripts of MTCH2. Furthermore, Wang et al. [93] have found that by regulating adipogenesis, UCP2 and PNPLA2 play important roles in the development of obesity, and that the expression of these two genes is mediated via m^{6}A methylation. Interestingly, the m^{6}A reader YTHDF1 has been established to influence the translation of PNPLA2 by binding to m^{6}A-modified transcripts of PNPLA2, whereas UCP2 was not a target of YTHDF1. The findings of a further study have indicated that the m^{6}A methyltransferase complex (METTL3, METTL14, and WTAP) positively modulates adipogenesis by enhancing cell cycle transition during mitotic clonal expansion [94]. Moreover, the deletion of METTL3 in brown adipose tissue, and consequently the loss of m^{6}A modification, has been demonstrated to perturb the maturation of brown adipose tissue by mediating the transcriptional expression of PRDM16, PPARG, and UCP1, which further promotes high-fat diet-induced obesity and insulin resistance [95]. These findings thus indicate that targeting m^{6}A regulators and their activities in adipogenesis may represent a novel strategy for combating obesity.

3.5. Heart diseases

Heart diseases are recognized as a probable source of a range of embolic materials leading to cerebral infarction, which is regarded as an underlying cause of between 15 % and 30 % ischemic strokes and a putative cause of 30 % of cryptogenic ischemic strokes. Heart disease is the leading cause of strokes in children, accounting for 30 % of pediatric arterial ischemic strokes, and an eightfold higher risk of hemorrhagic strokes than in healthy controls [96]. Cardioembolic stroke is mainly attributed to atrial fibrillation and diseases, such as acute myocardial infarction and rheumatic heart disease, along with the implantation of prosthetic heart valves. Patients with mitral stenosis and atrial fibrillation have been established to have an absolute stroke risk of 4.5 per 100 patient-years [97].

In their study of the pathogenesis of atrial fibrillation, Huang et al. [98] identified 105 m^{6}A-SNPs significantly associated with atrial fibrillation, the associations of two of which (rs1047564 and rs35648226) were found to be linked to their influence on m^{6}A modification. Compared with the expression in normal controls, expression of the m^{6}A regulators YTHDF1, IGFBP2, and IGFBP3 was found

Table 1
The roles of m6A regulators in ischemic strokes.

m6A regulators	Function	Mechanism	Targets	References
FTO	alleviate cerebral I/R injury	Inhibit oxidative stress and cell apoptosis	Nrf2	111
FTO	alleviate neuronal injury in ischemic stroke	decrease mitochondrial dysfunction	Drp1	120
FTO	post-stroke vascular repair	alleviate endothelial dysfunction	phospholipid phosphatase 3	132
FTO	decrease poststroke gray and white matter damage and improve motor function recovery, cognition, and depression-like behavior	/	/	156
METTL3	alleviate excessive sympathetic neural remodeling post-myocardial infarction	Reduce reactive oxygen species		113
METTL3	modulate the fate of damaged neurons in ischemic stroke	formation of stress granule	miR-335	147
METTL3	attenuate OGD/R-induced neuronal cytotoxicity	inhibit neuronal cell death and apoptosis	Lnc-D63785	148
METTL3	neuroprotection in ischemic stroke	inhibit the migration of T lymphocytes to the ischemic brain and reduce neuronal damage		154
METTL14	alleviates atherosclerosis development	macrophage inflammatory responses	Matr3	142
ALKBH5/ FTO	attenuate cerebral ischemia-reperfusion injury	weaken primary neuronal apoptosis	Bcl-2	149
ALKBH5	maintain angiogenesis of endothelial cells after acute ischemic treatment	endothelial cells dysfunction	SPHK1	127
YTHDC1	potentiate neuronal survival	enhance anti-apoptotic protein Bcl-2 and decrease cleaved caspase 3	PTEN	150
YTHDF1	attenuate cerebral stroke	facilitate cell viability and inhibit apoptosis of H/R-treated cells	PTEN	151
YTHDF1	cerebral I/R injury	microglial inflammation	NF-κB	140

I/R, ischemia/reperfusion; OGD/R, oxygen-glucose deprivation/reperfusion.

to be elevated in atrial fibrillation samples, and reductions were detected in the expression of ZC3H13 and HNRNPA2B1. Moreover, a model established based on these five m^{6}A regulators was able to effectively predict the incidence of atrial fibrillation [99]. In further studies using an acute myocardial infarction model, m^{6}A eraser ALKBH5 inhibitor-loaded targeted ferritin nanocages were demonstrated to effectively improve cardiac function and reduce infarct size [100]. By enhancing the m^{6}A modification of RNAs, the downregulation of FTO has also been observed to reduce cardiomyocyte contractile function [101], whereas cardiac-specific conditional depletion of the m^{6}A reader YTHDC1 has been observed to promote dilated cardiomyopathy in mice, as indicated by systolic dysfunction, left ventricular enlargement, reduced cardiomyocyte contractility, and aberrant splicing of titin [102]. Markedly elevated levels of m^{6}A methyltransferase activity and METTL14 expression have also been reported in patients with coronary heart disease, with high levels of METTL14 being significantly correlated with the Gensini score [103]. Furthermore, Zheng et al. [104] established that by modulating Myd88 mRNA stability, a depletion of METTL14 had the effects of reducing the development of atherosclerotic plaques and macrophage inflammation in a mouse model. In addition, during myocardial ischemia/reperfusion (I/R) remodeling, an increase has been detected in the m^{6}A-modified RNAs and METTL14 expression. Cardiac-specific depletion of METTL14 was found to attenuate acute I/R injury and cardiac dysfunction [105].

4. The roles of m^{6}A modification in the pathophysiology of stroke

Poststroke cerebral injury is a consequence of a complex series of pathophysiological events, including oxidative stress, neuroinflammation, mitochondrial dysfunction, endothelial dysfunction, and cell death, in which m^{6}A modification have been established to play a number of important regulatory roles, as summarized in Table 1.

4.1. Oxidative stress

Oxidative stress is closely associated with ischemic stroke, with accumulating evidence indicating that the formation of reactive oxygen species (ROS) increases rapidly after ischemic stroke. Oxidative stress occurs when free radical production exceeds the endogenous scavenging capacity of the body's antioxidant defense system, thereby causing cerebral tissue damage [106–108]. Moreover, by elevating tissue oxygenation levels, a subsequent rapid recovery of blood flow can also lead to a massive generation of ROS, thereby resulting in reperfusion injury [109]. Unsurprisingly, oxidative stress has been proposed as a promising target for the prevention and treatment of ischemic stroke [110].

Numerous studies have demonstrated associations between m^{6}A modification and oxidative stress. For example, Hou et al. [111] observed reductions in the expression of FTO and Nrf2 and an increase in the m^{6}A modification of Nrf2 mRNA in both the OGD/R-induced cell and MCAO/R-induced rat models. In the OGD/R-induced SH-SY5Y cell model, oxidative stress injury was ameliorated in response to FTO blockage mediated via the addition of the methyltransferases METTL3/14. It was also demonstrated that FTO can inhibit oxidative stress and alleviate cerebral I/R injury by enhancing Nrf2 expression via modulation of the m^{6}A demethylation of Nrf2 transcripts [111]. The activities of both METTL3 and METTL14 have been found to promote the generation of ROS [112,113]. For example, Qi et al. established that the ameliorative effects of METTL3 downregulation on excessive sympathetic neural remodeling post-myocardial infarction is associated with a reduction in ROS production [113]. Peroxiredoxin 3 (PRDX3) is a major regulator of mitochondrial oxidative stress, and it has been found that m^{6}A-modified transcripts of PRDX3 can bind to the m^{6}A readers YTHDF1/2/3, although only YTHDF3 directly modulates the expression and translation of PRDX3 in an m^{6}A-dependent manner [114]. ALKBH5 has been established to function as an important modulator that protects cells against DNA damage and apoptosis induced by oxidative stress [115]. The demethylase activity of ALKBH5 can be markedly inhibited under conditions of oxidative stress. Elevated levels of ROS induce the SUMOylation of ALKBH5 and activation of the ERK/JNK pathway. This process efficiently inhibits m^{6}A methylation at the whole-mRNA level, leading to the rapid and effective induction of an array of genes involved in multiple biological processes, including DNA damage repair [115].

4.2. Mitochondrial dysfunction

Mitochondria are not only affected by stroke but also modulate the development of poststroke injury [116]. As a hallmark of ischemic stroke, interrupted blood flow to the brain leads to a cerebral depletion of oxygen and glucose, which eventually lead to mitochondrial dysfunction. In response, this dysfunction triggers cascades of other adverse events, such as neuronal death, disruption of the blood-brain barrier, and neuroinflammation [116,117]. Accordingly, regulation of mitochondrial quality control has been widely proposed as a possible therapeutic target for both hemorrhagic and ischemic strokes [116].

A perturbation of mitochondrial dynamics (such as fusion and fission) may cause mitochondrial dysfunction [118]. Mitochondrial fusion is modulated by mitochondria-associated mitofusins 1 and 2 and optical atrophy 1, whereas mitochondrial fission is regulated by dynamin-related protein 1 (Drp1), mitochondrial fission factor, and fission 1 (FIS1). These mitochondria-associated genes are modulated by m^{6}A regulators, which accordingly play roles in mitochondrial dynamics and dysfunction. Ischemia-induced Drp1 activation can impair the formation of autophagosomes in vascular tissues and induce cell apoptosis, which in turn contributes to a reduction in mitochondrial glutathione levels and ultimately causes an increase in ROS generation, thereby exacerbating mitochondrial dysfunction [119]. The m^{6}A demethylase FTO serves as a key target of exosomal KLF4, which inhibits m^{6}A methylation in Drp1 mRNA, thereby contributing to a reduction mitochondrial dysfunction and alleviating the neuronal injury incurred in ischemic stroke [120]. By regulating Drp1 expression via the demethylation of m^{6}A-modified Drp1 mRNA, FTO has also been demonstrated to mitigate Drp1-mediated mitochondrial fragmentation and oxidative stress in cases of hepatic I/R injury [121]. Additionally, it has been

established that ALKBH5 is involved in mitochondrial fission by modulating m^{6}A demethylation in the 3'-untranslated regions of Drp1 transcripts and promoting the translation of Drp1 in an m^{6}A-YTHDF1-dependent manner [122]. Furthermore, by promoting an increase in m^{6}A levels in the transcripts of FIS1, METTL14 enhances FIS1 expression and mitochondrial fission, and this regulation has been implicated in chronic cadmium-induced mitochondrial dysfunction [123]. In conjunction with YTHDF2, METTL3 modulates mitochondrial dysfunction by synergistically modifying PGC-1α mRNA, thereby reducing the protein expression of PGC-1α, a coactivator that modulates mitochondrial biogenesis and energy metabolism [124].

4.3. Endothelial dysfunction

As a key step in the development of vascular diseases, endothelial dysfunction plays prominent roles associated with the physiopathology, subtypes, clinical severity, and prognosis of stroke [125,126]. In this context, it has been established that the m^{6}A demethylase ALKBH5 is upregulated in endothelial cells (ECs) subjected to ischemic insults and contributes to the maintenance of angiogenesis in ECs after acute ischemic treatment by reducing eNOS phosphorylation and SPHK1 m^{6}A methylation [127]. Under high-glucose conditions, elevated expression of the m^{6}A reader IGF2BP1 is detected in vascular ECs, and by interacting with the m6A-modified mRNA of HMGB1, IGF2BP1 promotes the proliferation and apoptosis of vascular ECs [128]. In T2DM, an established risk factor for stroke, three m^{6}A regulators, namely the m^{6}A writers RBM15B and ZC3H13 and the m^{6}A reader HNRNPC, have been found to be upregulated and associated with vascular EC function. Among these regulators, the activity of HNRNPC can promote endothelial dysfunction and enhance vascular complications by targeting PSEN1-dependent Notch signaling [129]. Pathological tissues of cerebral arteriovenous malformation, a cerebrovascular disease, are characterized by reductions in the expression of both WTAP and METTL3 [130,131], two m6A writers that modulate EC angiogenesis in an m^{6}A-dependent manner, with METTL3 activating the Notch pathway by regulating the expression of DTX3L [130], and WTAP regulating the expression of desmoplakin [131]. Moreover, m^{6}A modification is associated with the functional modulation of vascular repair, a restorative measure that contributes to improving the long-term outcomes postischemic stroke. Similarly, FTO has been shown to be involved in poststroke vascular repair by m^{6}A-dependently enhancing the expression of phospholipid phosphatase 3 in ECs [132].

4.4. Neuroinflammation

Neuroinflammation has been established to play a dual role in the progression of cerebral ischemic injury. Cerebral ischemia triggers strong neuroinflammatory responses to clear damaged tissue, and subsequently promote tissue repair and functional recovery. In contrast, neuroinflammatory cascades in the acute phase are linked to an impaired blood-brain barrier function, neuronal injury, apoptosis, and poor neurological outcomes [133,134]. Stroke-related neuroinflammation is a unique therapeutic target that contributes to the inhibition of secondary cell death and improves patient outcomes [135]. Microglia and infiltrated macrophages have been established to play important roles in the inflammatory processes postischemic stroke [136], which can be modulated by m^{6}A regulators [137].

In their study examining the roles of m^{6}A modification in the regulation of inflammatory responses in microglia, Li et al. [138] observed largescale alterations in m^{6}A modification patterns among different microglial phenotypes (resting, proinflammatory, and anti-inflammatory phenotypes). The differentially m^{6}A-modified transcripts between pro- and anti-inflammatory microglial phenotypes were established to be involved in several pathophysiological processes, including signal transduction, the immune system, and protein degradation, which tends to indicate that these modulate microglia-mediated inflammatory responses by regulating signal transduction and immune system function. Similarly, using a lipopolysaccharide-activated model of microglial inflammation, Ding et al. [139] identified a unique m^{6}A methylation pattern. Among the different m^{6}A regulators, the m^{6}A reader, IGF2BP1, was identified as the most significantly regulated, which, by promoting an increase in the levels of m^{6}A in the mRNAs of guanylate-binding protein 11 and ceruloplasmin, acts as a vital mediator in lipopolysaccharide-induced m^{6}A modification and the inflammatory response in microglia [139]. As an established proinflammatory pathway, the NF-κB pathway plays important regulatory roles in both inflammation and immunity. YTHDF1 is implicated in inflammatory responses associated with cerebral I/R injury by m^{6}A-dependently mediating the protein expression and nuclear translocation of NF-κB p65 in BV2 microglia cells [140]. In a lipopolysaccharide-mediated model of microglial inflammation, which is characterized by the elevated expression of METTL3, inflammatory proteins (TRAF6 and NF-κB), and cytokines (interleukin 1β/6/18 and tumor necrosis factor α), the increase in METTL3 expression has been shown to contribute to microglial inflammation by activating TRAF6-NF-κB signaling in an m^{6}A-dependent manner [141].

In their investigation of the roles of m^{6}A modification in macrophage-mediated inflammatory responses during atherosclerosis, Sun et al. [142] detected an increase in the expression of the methyltransferases METTL3 and METTL14, as well as reduced whole m^{6}A methylation levels in macrophages, in response to oxidized low-density lipoprotein stimulation. In addition to its role in mRNA decay, by repressing the activation of MAPK signaling, the METTL3-METTL14 heterodimer plays a key role in the protective function of matrin-3 against oxidized low-density lipoprotein-mediated macrophage inflammatory responses [142]. Similarly, Zheng et al. [104] observed not only upregulated METTL3 and METTL14 expression but also upregulated METTL16 and WTAP and downregulated ALKBH5 and FTO expression in inflammatory macrophages. Moreover, METTL14 has been identified as a target for the modulation of macrophage inflammation in atherosclerosis mediated via the NF-κB/IL-6 signaling pathway. By influencing the stability of Myd88 mRNA via m^{6}A modification, METTL14 has been established to regulate the expression of Myd88, and the dysregulation Myd88 in turn modulates the transcription of IL-16 by modifying the nuclear distribution of p65. In contrast, by promoting macrophage polarization toward an anti-inflammatory M2 phenotype and inhibiting NF-κB signaling, the knockdown of METTL14 has been demonstrated to

inhibit the inflammatory responses of macrophages [104]. Furthermore, METTL3 has been found to inhibit lipopolysaccharide-induced inflammation by m^{6}A-dependently inhibiting the NF-κB pathway [143] or the NOD1 pathway [144]. By promoting a reduction in the secretion of inflammatory factors that regulate macrophage-mediated inflammatory responses, YTHDF1 has been found to play a key role in the pathogenesis of *Treponema pallidum* infection, which can be attributed to the binding of this reader protein to the m^{6}A-modified transcripts of SOCS3 and subsequent promotion of SOCS3 translation, thereby inhibiting the JAK2/STAT3 pathway [145]. IGF2BP2 modulates macrophage inflammatory responses by promoting the polarization of the proinflammatory M1 phenotype toward a pro-healing M2 phenotype. Moreover, by stabilizing the m^{6}A-methylated mRNAs of TSC1 and PPARγ, the deletion of IGF2BP2 in macrophages has been found to promote and suppress the activation of proinflammatory M1 and pro-healing M2 phenotypes [146].

4.5. Cell death processes

In strokes, damage caused by oxidative stress and mitochondrial dysfunction can induce multiple cell signaling cascades, which can in turn lead to programmed or unprogrammed nerve cell death [117]. A growing body of evidence indicates a close association between m^{6}A regulators and neuronal cell death processes. For example, Si et al. [147] have detected an increase in the levels of apoptosis during stress granule formation, whereas reductions in METTL3 expression and m^{6}A levels were observed during the reperfusion period, following 2 h of ischemia in a MCAO rat model, primary cortical neurons, and PC12 cell OGD/R models. In oxygen- and glucose-deprived PC12 cells, the knockdown or overexpression of METTL3 was found to regulate apoptosis and stress granule formation by mediating the functional maturation of miR-335 in an m^{6}A-dependent manner. Stress granule formation was found to be negatively associated with apoptosis. These findings thus provided evidence to indicate that METTL3-mediated m^{6}A methylation modulates the fate of damaged neurons in ischemic stroke. In this regard, OGD/R stimulation has been found to promote the downregulated expression of lncRNA, lnc-D63785, by inducing METTL3-dependent Lnc-D63785 m^{6}A methylation in neuronal cells, thereby leading to neuronal cell death and apoptosis. METTL3 silencing has been reported to attenuate OGD/R-induced neuronal cytotoxicity [148].

FTO has been established to play an important role in cerebral I/R injury. In OGD/R SH-SY5Y cells, FTO has been demonstrated to modulate apoptosis by mediating the m^{6}A-methylation of Nrf2 mRNA, thereby regulating its expression, whereas in a rat model of MCAO/R, the overexpression of FTO was found to reduce the area of cerebral ischemia infarction and the rate of cell apoptosis [111]. Furthermore, in both a MCAO rat model and OGD/R-treated neurons, Xu et al. observed elevated levels of m^{6}A modification, which were mainly attributable to the activities of the demethylases ALKBH5 and FTO, with ALKBH5 promoting the expression of cleaved-caspase-3 and neuronal apoptosis following OGD/R treatment. The overexpression of FTO was found to attenuate primary neuronal apoptosis. Both ALKBH5 and FTO have been shown to regulate I/R-induced neuronal apoptosis by selectively demethylating Bcl2 mRNA to enhance Bcl2 expression [149]. In ischemic stroke, induction of the binding protein, YTHDC1, in neurons contributes to potentiating neuronal survival by promoting the degradation of PTEN mRNA and enhancing Akt phosphorylation. The silencing of YTHDC1 has been shown to render neurons more susceptible to OGD-induced cell death. In addition, a decrease and an increase in the expression of the antiapoptotic protein Bcl2 and cleaved caspase-3, respectively, have been detected in neurons after the silencing of YTHDC1. Contrastingly, neurons overexpressing YTHDC1 are characterized by elevated levels of Bcl2 expression, a reduction in the expression of cleaved caspase-3, and a more substantial resistance to OGD-induced cell death. This indicates that YTHDC1 plays a protective role against OGD-induced neuronal death [150]. Elevated levels of YTHDF1 expression have been reported in hypoxia/reoxygenation-treated cells, as well as in the brain tissues of MCAO mice. YTHDF1 depletion maintained cell viability and inhibited the apoptosis of H/R-treated cells by binding to PTEN to increase the stability of the PTEN transcript. Moreover, the changes in viability and apoptosis induced by YTHDF1 depletion was reversed in cell overexpressing PTEN [151].

5. Diagnostic and therapeutic potential

The research findings outlined in this review highlight the close association between m^{6}A regulators and their regulatory role in the pathogenesis of stroke. This accordingly emphasizes the importance of identifying potential biomarkers and m^{6}A-based therapeutic strategies for stroke management.

5.1. Diagnostic potential

Zhu et al. [152] discovered 305 m^{6}A-SNPs associated with ischemic stroke, of which 158 had eQTL effects on local genes. A total of 84 local genes were found to be differentially expressed in patients with ischemic stroke and were implicated in biological processes and pathways associated with the pathogenesis of ischemic stroke, thereby indicating that these functional m^{6}A-SNPs could serve as potential genetic biomarkers of ischemic stroke. Furthermore, based on an analysis of the gene expression profiles of peripheral blood from patients with strokes, Jia et al. [153] identified 11 dysregulated m^{6}A regulators, three of which (METTL16, LRPPRC, and RBM15) effectively predicted the occurrence of stroke, with area under the curve values of >0.8.

5.2. Therapeutic potential

Several approaches have been adopted to modulate m^{6}A with a view to investigate its therapeutic potential in stroke, including the use of CRISPR-Cas9 gene-editing system, lentivirus vectors, and adeno-associated viral vectors (AAV).

5.2.1. In vitro investigations

In a study of the effects of demethylases on the regulation of cerebral I/R injury, Xu et al. [149] found that FTO expression was reduced, whereas ALKBH5 expression was elevated, in the brain tissues of a MCAO rat model and in OGD/R-treated primary neurons. To investigate the therapeutic potential of FTO and ALKBH5 in cerebral I/R injury, lentivirus-ALKBH5 shRNA was used to inhibit ALKBH5 expression, and lentivirus-FTO was used to overexpress FTO in primary neurons. Knockdown of ALKBH5 was accordingly found to exacerbate neuronal damage and death, whereas by attenuating neuronal damage and death after OGD/R treatment, the overexpression of FTO conferred a protective effect. Moreover, following OGD/R treatment, an increase in neuronal death was observed following treatment with betaine (a m^6A methylation drug that enhances global m^6A levels), whereas a reduction in cell death was detected following treatment with CL (a demethylation drug that reduces global m^6A levels). Collectively, these findings indicate that ALKBH5 and FTO have potential value as therapeutic targets in the treatment of cerebral I/R injury. Based on an analysis of acute ischemic stroke, Si et al. [147] established that METTL3 expression is elevated during the early stage (reperfusion for 0 h) and reduced during the later stages of acute ischemic stroke injury (reperfusion for 24 h) after 2 h of ischemia. The CRISPR-Cas9 gene editing system was used to knockdown METTL3. Transformation was performed using METTL3 plasmids to facilitate the overexpression of METTL3 in PC12 cells, which were subsequently subjected to OGD. The overexpression of METTL3 in these treated cells was found to promote the formation of stress granules and attenuate apoptosis. Opposite effects were observed following METTL3 knockdown. Similarly, Liu et al. inhibited and overexpressed METTL3 using METTL3-shRNA and METTL3 plasmids, respectively, in OGD/R-treated primary cortical neurons. They found that METTL3 protected against neuronal damage in ischemic stroke [154]. These findings accordingly identify METTL3 as a potential therapeutic target for the treatment of strokes.

5.2.2. In vivo investigations

Using a MCAO rat model, Zhang et al. detected a significant elevation in YTHDC1 expression in the ipsilateral cortex of the rat after 3–12 h of reoxygenation. This elevation subsequently declined when measured at 24 h post-reoxygenation. In response to the stereotaxic injection of lentiviral YTHDC1-shRNA or lentiviral YTHDC1 into the cortex of MCAO rats, a reduction in infarct volume and an amelioration of neurological deficits in rats injected with lentiviral YTHDC1 was detected. Conversely, ischemic brain injury was exacerbated in rats injected with lentiviral YTHDC1-shRNA, thereby indicating that YTHDC1 can contribute to an alleviation of the symptoms of ischemic stroke [150]. Similarly, Li et al. have reported a significant increase in the expression of YTHDF1 in the brain tissues of MCAO rat models. To investigate the potential therapeutic effects of YTHDF1 *in vivo*, lentiviral YTHDF1-shRNA or lentiviral YTHDF1 was injected into the cortex and hippocampus of rats. Reductions in infarct volume, neurological score, and cell apoptosis in the brain tissues of rats following the knockdown of YTHDF1 was detected. Opposite effects were observed in response to overexpression of YTHDF1. These findings thus provided evidence to indicate the therapeutic potential of YTHDF1 in the treatment of strokes [151]. AAV has been established as a powerful tool for delivering therapeutic genes to ischemic stroke lesions [155]. To investigate the potential therapeutic efficacy of FTO in the treatment of strokes, Chokkalla et al. [156] constructed AAV9-FTO plasmids to overexpress FTO in the brains of adult mice. They accordingly found that compared with the observations in sex-matched control mice, FTO AAV9-treated mice were characterized by smaller infarcts, and significantly reduced brain atrophy and depression-like behavior poststroke. Furthermore, both male and female mice receiving this treatment were found to show an enhancement in memory retention. These findings thus indicate that FTO-AAV9 treatment can attenuate motor and cognitive dysfunction as well as depression-like phenotypes in mice poststroke, irrespective of sex.

6. Conclusions and prospects

m^6A modification is the most common internal modification of eukaryotic RNAs. The levels of RNA are regulated by both methylated and demethylated transferases, which further influences RNA metabolism mediated by the action of RNA-binding proteins. In this review, we summarize the established and/or putative roles of m^6A regulators in the development and progression of modifiable risk factors for stroke, including hypertension, DM, hyperlipidemia, and obesity. Targeting the regulators of m^6A modification and their functions, to control the development and progression of these risk factors, may represent an effective primary prevention strategy for the treatment of strokes. We also describe the roles of m^6A modification regulators and their functional regulation with respect to the pathophysiological injury mechanisms associated with strokes, namely oxidative stress, mitochondrial dysfunctions, endothelial dysfunction, neuroinflammation, and cell death processes. The findings of the studies conducted to date highlight the important role of m^6A-modified protein dysregulation, mediated via the modulation of targeted m^6A-modified mRNA levels, in the pathogenesis of stroke. This should provide an incentive for researchers to focus on identifying m^6A-based therapeutic strategies that could be adopted in the treatment of strokes.

However, despite considerable recent progress in elucidating the associations between m^6A modifications and stroke, the current therapeutic exploitation of these molecular processes remains limited. Recently, several techniques for investigating the contribution of individual m^6A modifications have been reported. Among them, programmable dPspCas13b-m6A reader proteins [157] have been assessed for the regulatory effects of specific m6A readers on single transcripts. In addition, CRISPR-Cas9-based engineered m^6A "editing" [158] and CRISPR-Cas13-based targeted RNA methylation systems [159] have been employed to assess the effects and functional roles of individual m^6A modifications. It is anticipated that these and similar novel techniques will make significant contributions to elucidating the functions and underlying mechanisms of specific m^6A regulators in the context of stroke pathophysiology, thereby providing a basis for the development of m^6A-based therapies for the prevention and clinical treatment of strokes.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Fei Han: Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] V. Saini, L. Guada, D.R. Yavagal, Global epidemiology of stroke and access to acute ischemic stroke interventions, Neurology 97 (20 Suppl 2) (2021) S6–s16.
[2] V.L. Feigin, et al., World stroke organization (WSO): global stroke Fact Sheet 2022, Int. J. Stroke 17 (1) (2022) 18–29.
[3] S.N. Ohashi, et al., Role of inflammatory processes in hemorrhagic stroke, Stroke 54 (2) (2023) 605–619.
[4] S.K. Feske, Ischemic stroke, Am. J. Med. 134 (12) (2021) 1457–1464.
[5] B.C.V. Campbell, et al., Ischaemic stroke, Nat. Rev. Dis. Prim. 5 (1) (2019) 70.
[6] H. Zhang, et al., Dynamic landscape and evolution of m6A methylation in human, Nucleic Acids Res. 48 (11) (2020) 6251–6264.
[7] H. Chang, et al., Role of N6-methyladenosine modification in pathogenesis of ischemic stroke, Expert Rev. Mol. Diagn 22 (3) (2022) 295–303.
[8] A.K. Chokkalla, et al., Transient focal ischemia significantly alters the m(6)A epitranscriptomic tagging of RNAs in the brain, Stroke 50 (10) (2019) 2912–2921.
[9] L. Zhu, et al., Comprehensive analysis of blood-based m6A methylation in human ischemic stroke, Mol. Neurobiol. 60 (2) (2023) 431–446.
[10] Y. Zheng, et al., MAPK: a key player in the development and progression of stroke, CNS Neurol. Disord.: Drug Targets 19 (4) (2020) 248–256.
[11] A. Ludhiadch, et al., Role of calcium homeostasis in ischemic stroke: a review, CNS Neurol. Disord.: Drug Targets 21 (1) (2022) 52–61.
[12] L. Liu, et al., The neuroprotective roles of sonic Hedgehog signaling pathway in ischemic stroke, Neurochem. Res. 43 (12) (2018) 2199–2211.
[13] X. Wei, et al., An update on the role of Hippo signaling pathway in ischemia-associated central nervous system diseases, Biomed. Pharmacother. 162 (2023) 114619.
[14] J.A. Howell, G.L. Bidwell 3rd, Targeting the NF-κB pathway for therapy of ischemic stroke, Ther. Deliv. 11 (2) (2020) 113–123.
[15] R. Desrosiers, K. Friderici, F. Rottman, Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells, Proc. Natl. Acad. Sci. U. S. A. 71 (10) (1974) 3971–3975.
[16] S. Garbo, C. Zwergel, C. Battistelli, m6A RNA methylation and beyond - the epigenetic machinery and potential treatment options, Drug Discov. Today 26 (11) (2021) 2559–2574.
[17] I.A. Roundtree, et al., Dynamic RNA modifications in gene expression regulation, Cell 169 (7) (2017) 1187–1200.
[18] H. Lin, et al., Mutual regulation between N6-methyladenosine (m6A) modification and circular RNAs in cancer: impacts on therapeutic resistance, Mol. Cancer 21 (1) (2022) 148.
[19] X. Jiang, et al., The role of m6A modification in the biological functions and diseases, Signal Transduct. Targeted Ther. 6 (1) (2021) 74.
[20] X. Wang, et al., Structural basis of N(6)-adenosine methylation by the METTL3-METTL14 complex, Nature 534 (7608) (2016) 575–578.
[21] X. Wang, et al., Corrigendum: structural basis of N(6)-adenosine methylation by the METTL3-METTL14 complex, Nature 542 (7640) (2017) 260.
[22] J. Liu, et al., A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation, Nat. Chem. Biol. 10 (2) (2014) 93–95.
[23] X.L. Ping, et al., Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase, Cell Res. 24 (2) (2014) 177–189.
[24] P. Knuckles, et al., Zc3h13/Flacc is required for adenosine methylation by bridging the mRNA-binding factor Rbm15/Spenito to the m(6)A machinery component Wtap/Fl(2)d, Genes Dev. 32 (5–6) (2018) 415–429.
[25] Y. Yue, et al., VIRMA mediates preferential m(6)A mRNA methylation in 3'UTR and near stop codon and associates with alternative polyadenylation, Cell Discov 4 (2018) 10.
[26] D.P. Patil, et al., m(6)A RNA methylation promotes XIST-mediated transcriptional repression, Nature 537 (7620) (2016) 369–373.
[27] G. Jia, et al., N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO, Nat. Chem. Biol. 7 (12) (2011) 885–887.
[28] G. Zheng, et al., ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility, Mol. Cell. 49 (1) (2013) 18–29.
[29] H. Shi, J. Wei, C. He, Where, when, and how: context-dependent functions of RNA methylation writers, readers, and erasers, Mol. Cell. 74 (4) (2019) 640–650.
[30] Y. Huang, et al., Meclofenamic acid selectively inhibits FTO demethylation of m6A over ALKBH5, Nucleic Acids Res. 43 (1) (2015) 373–384.
[31] S. Zaccara, S.R. Jaffrey, A unified model for the function of YTHDF proteins in regulating m(6)a-modified mRNA, Cell 181 (7) (2020), 1582-1595.e18.
[32] W.Y. Hao, et al., RNA m6A reader YTHDF1 facilitates inflammation via enhancing NLRP3 translation, Biochem. Biophys. Res. Commun. 616 (2022) 76–81.
[33] H. Chen, et al., YTHDF1 promotes breast cancer progression by facilitating FOXM1 translation in an m6A-dependent manner, Cell Biosci. 12 (1) (2022) 19.
[34] H. Du, et al., YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex, Nat. Commun. 7 (2016) 12626.
[35] H. Shi, et al., YTHDF3 facilitates translation and decay of N(6)-methyladenosine-modified RNA, Cell Res. 27 (3) (2017) 315–328.
[36] W. Xiao, et al., Nuclear m(6)A reader YTHDC1 regulates mRNA splicing, Mol. Cell. 61 (4) (2016) 507–519.
[37] K.I. Zhou, et al., Regulation of Co-transcriptional pre-mRNA splicing by m(6)A through the low-complexity protein hnRNPG, Mol. Cell. 76 (1) (2019), 70-81.e9.
[38] L. Rong, et al., HNRNPA2B1 inhibited SFRP2 and activated Wnt-β/catenin via m6A-mediated miR-106b-5p processing to aggravate stemness in lung adenocarcinoma, Pathol. Res. Pract. 233 (2022) 153794.
[39] J. Wu, et al., Essential m(6)A methylation regulator HNRNPC serves as a targetable biomarker for papillary renal cell carcinoma, JAMA Oncol. 2022 (2022) 9411692.
[40] H. Huang, et al., Recognition of RNA N(6)-methyladenosine by IGF2BP proteins enhances mRNA stability and translation, Nat. Cell Biol. 20 (3) (2018) 285–295.
[41] F.Z. Caprio, F.A. Sorond, Cerebrovascular disease: primary and secondary stroke prevention, Med. Clin. 103 (2) (2019) 295–308.

[42] T.C. Turin, et al., Hypertension and lifetime risk of stroke, J. Hypertens. 34 (1) (2016) 116–122.
[43] D.W. Brown, et al., Preeclampsia and the risk of ischemic stroke among young women: results from the Stroke Prevention in Young Women Study, Stroke 37 (4) (2006) 1055–1059.
[44] M.R. Law, J.K. Morris, N.J. Wald, Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies, Bmj 338 (2009) b1665.
[45] M.J. Cipolla, D.S. Liebeskind, S.L. Chan, The importance of comorbidities in ischemic stroke: impact of hypertension on the cerebral circulation, J. Cerebr. Blood Flow Metabol. 38 (12) (2018) 2129–2149.
[46] X.B. Mo, et al., Examination of the associations between m(6)A-associated single-nucleotide polymorphisms and blood pressure, Hypertens. Res. 42 (10) (2019) 1582–1589.
[47] Z. Pausova, et al., A common variant of the FTO gene is associated with not only increased adiposity but also elevated blood pressure in French Canadians, Circ Cardiovasc Genet 2 (3) (2009) 260–269.
[48] A. Marcadenti, et al., Effects of FTO RS9939906 and MC4R RS17782313 on obesity, type 2 diabetes mellitus and blood pressure in patients with hypertension, Cardiovasc. Diabetol. 12 (2013) 103.
[49] Q. Wu, et al., Epitranscriptomic mechanisms of N6-methyladenosine methylation regulating mammalian hypertension development by determined spontaneously hypertensive rats pericytes, Epigenomics 11 (12) (2019) 1359–1370.
[50] A. Paramasivam, J. Vijayashree Priyadharsini, S. Raghunandhakumar, N6-adenosine methylation (m6A): a promising new molecular target in hypertension and cardiovascular diseases, Hypertens. Res. 43 (2) (2020) 153–154.
[51] M. Chaudhary, Novel methylation mark and essential hypertension, J. Genet. Eng. Biotechnol. 20 (1) (2022) 11.
[52] C. Banerjee, et al., Duration of diabetes and risk of ischemic stroke: the Northern Manhattan Study, Stroke 43 (5) (2012) 1212–1217.
[53] R. Chen, B. Ovbiagele, W. Feng, Diabetes and stroke: epidemiology, pathophysiology, pharmaceuticals and outcomes, Am. J. Med. Sci. 351 (4) (2016) 380–386.
[54] L.H. Lau, et al., Prevalence of diabetes and its effects on stroke outcomes: a meta-analysis and literature review, J Diabetes Investig 10 (3) (2019) 780–792.
[55] T. Shimoyama, et al., Post stroke dysglycemia and acute infarct volume growth: a study using continuous glucose monitoring, Eur. Neurol. 76 (3–4) (2016) 167–174.
[56] B. Fuentes, et al., Stroke prevention in patients with type 2 diabetes or prediabetes. Recommendations from the cerebrovascular diseases study group, Spanish society of neurology, Neurologia 36 (4) (2021) 305–323.
[57] C. Ling, Epigenetic regulation of insulin action and secretion - role in the pathogenesis of type 2 diabetes, J. Intern. Med. 288 (2) (2020) 158–167.
[58] X. Wu, et al., U-shaped association between serum IGF2BP3 and T2DM: a cross-sectional study in Chinese population, J. Diabetes 15 (4) (2023) 349–361.
[59] D.K. Sanghera, et al., Impact of nine common type 2 diabetes risk polymorphisms in Asian Indian Sikhs: PPARG2 (Pro12Ala), IGF2BP2, TCF7L2 and FTO variants confer a significant risk, BMC Med. Genet. 9 (2008) 59.
[60] L. Han, et al., IGF2BP2 rs11705701 polymorphisms are associated with prediabetes in a Chinese population: a population-based case-control study, Exp. Ther. Med. 12 (3) (2016) 1849–1856.
[61] W. Xie, et al., METTL3 inhibits hepatic insulin sensitivity via N6-methyladenosine modification of Fasn mRNA and promoting fatty acid metabolism, Biochem. Biophys. Res. Commun. 518 (1) (2019) 120–126.
[62] Y. Li, et al., m(6)A regulates liver metabolic disorders and hepatogenous diabetes, Dev. Reprod. Biol. 18 (4) (2020) 371–383.
[63] B.O. Roep, et al., Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?), Nat. Rev. Endocrinol. 17 (3) (2021) 150–161.
[64] Y. Miyachi, T. Miyazawa, Y. Ogawa, HNF1A mutations and beta cell dysfunction in diabetes, Int. J. Mol. Sci. 23 (6) (2022) 3222.
[65] D.F. De Jesus, et al., m(6)A mRNA methylation regulates human β-cell biology in physiological states and in type 2 diabetes, Nat. Metab. 1 (8) (2019) 765–774.
[66] J. Liu, et al., METTL14 is essential for β-cell survival and insulin secretion, Biochim. Biophys. Acta, Mol. Basis Dis. 1865 (9) (2019) 2138–2148.
[67] Y. Wang, et al., m(6)A mRNA methylation controls functional maturation in neonatal murine β-cells, Diabetes 69 (8) (2020) 1708–1722.
[68] X. Li, et al., METTL3 is required for maintaining β-cell function, Metabolism 116 (2021) 154702.
[69] Y. Cheng, et al., The m(6)A methyltransferase METTL3 ameliorates methylglyoxal-induced impairment of insulin secretion in pancreatic β cells by regulating MafA expression, Front. Endocrinol. 13 (2022) 910868.
[70] F. Bornaque, et al., Glucose regulates m(6)A methylation of RNA in pancreatic islets, Cells 11 (2) (2022) 291.
[71] X. Li, Y. Yang, Z. Chen, Downregulation of the m(6)A reader protein YTHDC1 leads to islet β-cell failure and diabetes, Metabolism 138 (2023) 155339.
[72] A. Varbo, B.G. Nordestgaard, Remnant cholesterol and risk of ischemic stroke in 112,512 individuals from the general population, Ann. Neurol. 85 (4) (2019) 550–559.
[73] J.S. Kim, Role of blood lipid levels and lipid-lowering therapy in stroke patients with different levels of cerebral artery diseases: reconsidering recent stroke guidelines, J Stroke 23 (2) (2021) 149–161.
[74] G. Hindy, et al., Role of blood lipids in the development of ischemic stroke and its subtypes: a mendelian randomization study, Stroke 49 (4) (2018) 820–827.
[75] A. Alloubani, R. Nimer, R. Samara, Relationship between hyperlipidemia, cardiovascular disease and stroke: a systematic review, Curr. Cardiol. Rev. 17 (6) (2021) e051121189015.
[76] X. Mo, et al., Genome-wide enrichment of m(6)A-associated single-nucleotide polymorphisms in the lipid loci, Pharmacogenomics J. 19 (4) (2019) 347–357.
[77] X. Zhong, et al., Circadian clock regulation of hepatic lipid metabolism by modulation of m(6)A mRNA methylation, Cell Rep. 25 (7) (2018) 1816–1828.e4.
[78] B. Zhou, et al., N(6) -methyladenosine reader protein YT521-B homology domain-containing 2 suppresses liver steatosis by regulation of mRNA stability of lipogenic genes, Hepatology 73 (1) (2021) 91–103.
[79] Y. Hu, et al., GR-mediated FTO transactivation induces lipid accumulation in hepatocytes via demethylation of m(6)A on lipogenic mRNAs, RNA Biol. 17 (7) (2020) 930–942.
[80] L. Sun, et al., Triclosan-induced abnormal expression of miR-30b regulates fto-mediated m(6)A methylation level to cause lipid metabolism disorder in zebrafish, Sci. Total Environ. 770 (2021) 145285.
[81] N. Jaakonmäki, et al., Obesity and the risk of cryptogenic ischemic stroke in young adults, J. Stroke Cerebrovasc. Dis. 31 (5) (2022) 106380.
[82] Y. Guo, et al., Overweight and obesity in young adulthood and the risk of stroke: a meta-analysis, J. Stroke Cerebrovasc. Dis. 25 (12) (2016) 2995–3004.
[83] J.W. Horn, et al., Obesity and risk for first ischemic stroke depends on metabolic syndrome: the HUNT study, Stroke 52 (11) (2021) 3555–3561.
[84] J. Xu, et al., Obesity-stroke paradox exists in insulin-resistant patients but not insulin sensitive patients, Stroke 50 (6) (2019) 1423–1429.
[85] E. Rodríguez-Castro, et al., Obesity paradox in ischemic stroke: clinical and molecular insights, Transl Stroke Res 10 (6) (2019) 639–649.
[86] J. Olza, et al., Influence of FTO variants on obesity, inflammation and cardiovascular disease risk biomarkers in Spanish children: a case-control multicentre study, BMC Med. Genet. 14 (2013) 123.
[87] M. Daya, et al., Obesity risk and preference for high dietary fat intake are determined by FTO rs9939609 gene polymorphism in selected Indonesian adults, Asia Pac. J. Clin. Nutr. 28 (1) (2019) 183–191.
[88] M. Claussnitzer, et al., FTO obesity variant circuitry and adipocyte browning in humans, N. Engl. J. Med. 373 (10) (2015) 895–907.
[89] S.K. Azzam, H. Alsafar, A.A. Sajini, FTO m6A demethylase in obesity and cancer: implications and underlying molecular mechanisms, Int. J. Mol. Sci. 23 (7) (2022) 3800.
[90] M. Zhang, et al., The demethylase activity of FTO (fat mass and obesity associated protein) is required for preadipocyte differentiation, PLoS One 10 (7) (2015) e0133788.
[91] X. Zhao, et al., FTO-dependent demethylation of N6-methyladenosine regulates mRNA splicing and is required for adipogenesis, Cell Res. 24 (12) (2014) 1403–1419.
[92] Q. Jiang, et al., MTCH2 promotes adipogenesis in intramuscular preadipocytes via an m(6)A-YTHDF1-dependent mechanism, Faseb. J. 33 (2) (2019) 2971–2981.
[93] X. Wang, et al., mRNA m(6)A plays opposite role in regulating UCP2 and PNPLA2 protein expression in adipocytes, Int. J. Obes. 42 (11) (2018) 1912–1924.

[94] M. Kobayashi, et al., The RNA methyltransferase complex of WTAP, METTL3, and METTL14 regulates mitotic clonal expansion in adipogenesis, Mol. Cell Biol. 38 (16) (2018).
[95] Y. Wang, et al., METTL3 is essential for postnatal development of brown adipose tissue and energy expenditure in mice, Nat. Commun. 11 (1) (2020) 1648.
[96] K.W. Giang, et al., Long-term risk of hemorrhagic stroke in young patients with congenital heart disease, Stroke 49 (5) (2018) 1155–1162.
[97] G. Karthikeyan, Stroke risk in rheumatic heart disease, Heart 107 (9) (2021) 694–696.
[98] Y. Huang, et al., Genome-wide detection of m6A-associated SNPs in atrial fibrillation pathogenesis, Front Cardiovasc Med 10 (2023) 1152851.
[99] Y. Zhao, et al., Analyses of m6A regulatory genes and subtype classification in atrial fibrillation, Front. Cell. Neurosci. 17 (2023) 1073538.
[100] P. Cheng, et al., Amelioration of acute myocardial infarction injury through targeted ferritin nanocages loaded with an ALKBH5 inhibitor, Acta Biomater. 140 (2022) 481–491.
[101] P. Mathiyalagan, et al., FTO-dependent N(6)-methyladenosine regulates cardiac function during remodeling and repair, Circulation 139 (4) (2019) 518–532.
[102] S. Gao, et al., Depletion of m(6) A reader protein YTHDC1 induces dilated cardiomyopathy by abnormal splicing of Titin, J. Cell Mol. Med. 25 (23) (2021) 10879–10891.
[103] F. Guo, et al., Levels and clinical significance of the m6A methyltransferase METTL14 in patients with coronary heart disease, Front Cardiovasc Med 10 (2023) 1167132.
[104] Y. Zheng, et al., Mettl14 mediates the inflammatory response of macrophages in atherosclerosis through the NF-κB/IL-6 signaling pathway, Cell. Mol. Life Sci. 79 (6) (2022) 311.
[105] L. Wang, et al., METTL14 is required for exercise-induced cardiac hypertrophy and protects against myocardial ischemia-reperfusion injury, Nat. Commun. 13 (1) (2022) 6762.
[106] S. Kim, et al., The antioxidant enzyme Peroxiredoxin-1 controls stroke-associated microglia against acute ischemic stroke, Redox Biol. 54 (2022) 102347.
[107] H.Q. Li, et al., γ-Glutamylcysteine alleviates ischemic stroke-induced neuronal apoptosis by inhibiting ROS-mediated endoplasmic reticulum stress, Oxid. Med. Cell. Longev. 2021 (2021) 2961079.
[108] S. Orellana-Urzúa, et al., Pathophysiology of ischemic stroke: role of oxidative stress, Curr. Pharmaceut. Des. 26 (34) (2020) 4246–4260.
[109] X. Zeng, et al., Activated Drp1 regulates p62-mediated autophagic flux and aggravates inflammation in cerebral ischemia-reperfusion via the ROS-RIP1/RIP3-exosome axis, Mil Med Res 9 (1) (2022) 25.
[110] Z. Li, et al., The role of oxidative stress in acute ischemic stroke-related thrombosis, Oxid. Med. Cell. Longev. 2022 (2022) 8418820.
[111] L. Hou, et al., FTO inhibits oxidative stress by mediating m6A demethylation of Nrf2 to alleviate cerebral ischemia/reperfusion injury, J. Physiol. Biochem. 79 (1) (2023) 133–146.
[112] M. Li, L. Deng, G. Xu, METTL14 promotes glomerular endothelial cell injury and diabetic nephropathy via m6A modification of α-klotho, Mol. Med. 27 (1) (2021) 106.
[113] L. Qi, et al., m(6)A methyltransferase METTL3 participated in sympathetic neural remodeling post-MI via the TRAF6/NF-κB pathway and ROS production, J. Mol. Cell. Cardiol. 170 (2022) 87–99.
[114] R. Sun, et al., The m6A reader YTHDF3-mediated PRDX3 translation alleviates liver fibrosis, Redox Biol. 54 (2022) 102378.
[115] F. Yu, et al., Post-translational modification of RNA m6A demethylase ALKBH5 regulates ROS-induced DNA damage response, Nucleic Acids Res. 49 (10) (2021) 5779–5797.
[116] M. Yang, et al., Mitochondrial quality control: a pathophysiological mechanism and therapeutic target for stroke, Front. Mol. Neurosci. 14 (2022) 786099.
[117] J. Jia, et al., New insights into targeting mitochondria in ischemic injury, Apoptosis 26 (3–4) (2021) 163–183.
[118] B. Haileselassie, et al., Drp1/Fis1 interaction mediates mitochondrial dysfunction in septic cardiomyopathy, J. Mol. Cell. Cardiol. 130 (2019) 160–169.
[119] C. Duan, et al., Drp1 regulates mitochondrial dysfunction and dysregulated metabolism in ischemic injury via Clec16a-, BAX-, and GSH- pathways, Cell Death Dis. 11 (4) (2020) 251.
[120] Q.S. Wang, et al., Bone Marrow Mesenchymal Stem Cell-Derived Exosomal KLF4 Alleviated Ischemic Stroke through Inhibiting N6-Methyladenosine Modification Level of Drp1 by Targeting lncRNA-ZFAS1, Mol Neurobiol, 2023. **Online ahead of print**.
[121] Y.D. Du, et al., N6-methyladenosine demethylase FTO impairs hepatic ischemia-reperfusion injury via inhibiting Drp1-mediated mitochondrial fragmentation, Cell Death Dis. 12 (5) (2021) 442.
[122] J. Wang, et al., ALKBH5 attenuates mitochondrial fission and ameliorates liver fibrosis by reducing Drp1 methylation, Pharmacol. Res. 187 (2023) 106608.
[123] P. Deng, et al., Long-term cadmium exposure impairs cognitive function by activating lnc-Gm10532/m6A/FIS1 axis-mediated mitochondrial fission and dysfunction, Sci. Total Environ. 858 (Pt 3) (2023) 159950.
[124] X. Zhang, et al., The m(6)A methyltransferase METTL3 modifies PGC-1α mRNA promoting mitochondrial dysfunction and oxLDL-induced inflammation in monocytes, J. Biol. Chem. 297 (3) (2021) 101058.
[125] J. Roquer, et al., Endothelial dysfunction, vascular disease and stroke: the ARTICO study, Cerebrovasc. Dis. 27 (Suppl 1) (2009) 25–37.
[126] D.S. Tian, et al., FSAP aggravated endothelial dysfunction and neurological deficits in acute ischemic stroke due to large vessel occlusion, Signal Transduct. Targeted Ther. 7 (1) (2022) 6.
[127] R. Kumari, et al., ALKBH5 regulates SPHK1-dependent endothelial cell angiogenesis following ischemic stress, Front Cardiovasc Med 8 (2022) 817304.
[128] A. Liang, et al., m(6)A reader IGF2BP1 accelerates apoptosis of high glucose-induced vascular endothelial cells in a m(6)A-HMGB1 dependent manner, PeerJ 11 (2023) e14954.
[129] Y. Cai, et al., N6-methylation of RNA-bound adenosine regulator HNRNPC promotes vascular endothelial dysfunction in type 2 diabetes mellitus by activating the PSEN1-mediated Notch pathway, Diabetes Res. Clin. Pract. 197 (2023) 110261.
[130] L.J. Wang, et al., N6-methyladenosine methyltransferase METTL3 affects the phenotype of cerebral arteriovenous malformation via modulating Notch signaling pathway, J. Biomed. Sci. 27 (1) (2020) 62.
[131] L.J. Wang, et al., Wilms' tumour 1-associating protein inhibits endothelial cell angiogenesis by m6A-dependent epigenetic silencing of desmoplakin in brain arteriovenous malformation, J. Cell Mol. Med. 24 (9) (2020) 4981–4991.
[132] B. Li, et al., FTO-dependent m(6)A modification of Plpp3 in circSCMH1-regulated vascular repair and functional recovery following stroke, Nat. Commun. 14 (1) (2023) 489.
[133] E. Candelario-Jalil, R.M. Dijkhuizen, T. Magnus, Neuroinflammation, stroke, blood-brain barrier dysfunction, and imaging modalities, Stroke 53 (5) (2022) 1473–1486.
[134] S.M. Stuckey, et al., Neuroinflammation as a key driver of secondary neurodegeneration following stroke? Int. J. Mol. Sci. 22 (23) (2021).
[135] G. Stoll, B. Nieswandt, Thrombo-inflammation in acute ischaemic stroke - implications for treatment, Nat. Rev. Neurol. 15 (8) (2019) 473–481.
[136] Z.J. Liu, et al., Melatonin protects against ischemic stroke by modulating microglia/macrophage polarization toward anti-inflammatory phenotype through STAT3 pathway, CNS Neurosci. Ther. 25 (12) (2019) 1353–1362.
[137] F. Zhang, et al., Regulation of N6-methyladenosine (m6A) RNA methylation in microglia-mediated inflammation and ischemic stroke, Front. Cell. Neurosci. 16 (2022) 955222.
[138] Q. Li, et al., The potential roles of m(6)A modification in regulating the inflammatory response in microglia, J. Neuroinflammation 18 (1) (2021) 149.
[139] L. Ding, et al., m6A reader Igf2bp1 regulates the inflammatory responses of microglia by stabilizing Gbp11 and cp mRNAs, Front. Immunol. 13 (2022) 872252.
[140] L. Zheng, et al., microRNA-421-3p prevents inflammatory response in cerebral ischemia/reperfusion injury through targeting m6A Reader YTHDF1 to inhibit p65 mRNA translation, Int. Immunopharm. 88 (2020) 106937.
[141] L. Wen, et al., The m6A methyltransferase METTL3 promotes LPS-induced microglia inflammation through TRAF6/NF-κB pathway, Neuroreport 33 (6) (2022) 243–251.
[142] Z. Sun, et al., Matr3 reshapes m6A modification complex to alleviate macrophage inflammation during atherosclerosis, Clin. Immunol. 245 (2022) 109176.
[143] J. Wang, et al., METTL3 Attenuates LPS-Induced Inflammatory Response in Macrophages via NF-Kb Signaling Pathway, vol. 2019, Mediators Inflamm, 2019 3120391.

[144] Y. Cai, et al., METTL3 regulates LPS-induced inflammatory response via the NOD1 signaling pathway, Cell. Signal. 93 (2022) 110283.
[145] Z. Li, et al., YTHDF1 negatively regulates Treponema pallidum-induced inflammation in THP-1 macrophages by promoting SOCS3 translation in an m6A-dependent manner, Front. Immunol. 13 (2022) 857727.
[146] X. Wang, et al., The m6A reader IGF2BP2 regulates macrophage phenotypic activation and inflammatory diseases by stabilizing TSC1 and PPARγ, Adv. Sci. 8 (13) (2021) 2100209.
[147] W. Si, et al., Methyltransferase 3 mediated miRNA m6A methylation promotes stress granule formation in the early stage of acute ischemic stroke, Front. Mol. Neurosci. 13 (2020) 103.
[148] S. Xu, et al., Oxygen glucose deprivation/re-oxygenation-induced neuronal cell death is associated with Lnc-D63785 m6A methylation and miR-422a accumulation, Cell Death Dis. 11 (9) (2020) 816.
[149] K. Xu, et al., N(6)-methyladenosine demethylases Alkbh5/Fto regulate cerebral ischemia-reperfusion injury, Ther Adv Chronic Dis 11 (2020) 2040622320916024.
[150] Z. Zhang, et al., YTHDC1 mitigates ischemic stroke by promoting Akt phosphorylation through destabilizing PTEN mRNA, Cell Death Dis. 11 (11) (2020) 977.
[151] X. Li, et al., Silencing of YTHDF1 attenuates cerebral stroke by inducing PTEN degradation and activating the PTEN/AKT/mTOR pathway, Mol. Biotechnol. 65 (5) (2023) 822–832.
[152] R. Zhu, et al., Genome-wide detection of m(6)a-associated genetic polymorphisms associated with ischemic stroke, J. Mol. Neurosci. 71 (10) (2021) 2107–2115.
[153] K. Jia, et al., RNA methylation pattern and immune microenvironment characteristics mediated by m6A regulator in ischemic stroke, Front. Genet. 14 (2023) 1148510.
[154] B. Liu, et al., METTL3-mediated ANXA2 inhibition confers neuroprotection in ischemic stroke: evidence from in vivo and in vitro studies, Faseb. J. 37 (7) (2023) e22974.
[155] F. Shen, et al., Intravenous delivery of adeno-associated viral vector serotype 9 mediates effective gene expression in ischemic stroke lesion and brain angiogenic foci, Stroke 44 (1) (2013) 252–254.
[156] A.K. Chokkalla, et al., Cerebroprotective role of N(6)-methyladenosine demethylase FTO (fat mass and obesity-associated protein) after experimental stroke, Stroke 54 (1) (2023) 245–254.
[157] S. Rauch, C. He, B.C. Dickinson, Targeted m(6)A reader proteins to study epitranscriptomic regulation of single RNAs, J. Am. Chem. Soc. 140 (38) (2018) 11974–11981.
[158] X.M. Liu, et al., Programmable RNA N(6)-methyladenosine editing by CRISPR-Cas9 conjugates, Nat. Chem. Biol. 15 (9) (2019) 865–871.
[159] C. Wilson, et al., Programmable m(6)A modification of cellular RNAs with a Cas13-directed methyltransferase, Nat. Biotechnol. 38 (12) (2020) 1431–1440.