Epitranscriptomic regulation by m⁶A RNA methylation in brain development and diseases

Journal of Cerebral Blood Flow & Metabolism
2020, Vol. 40(12) 2331–2349
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/0271678X20960033
journals.sagepub.com/home/jcbfm

Anil K Chokkalla^{1,2} , Suresh L Mehta² and
Raghu Vemuganti^{1,2,3}

Abstract

Cellular RNAs are pervasively tagged with diverse chemical moieties, collectively called epitranscriptomic modifications. The methylation of adenosine at N⁶ position generates N⁶-methyladenosine (m⁶A), which is the most abundant and reversible epitranscriptomic modification in mammals. The m⁶A signaling is mediated by a dedicated set of proteins comprised of writers, erasers, and readers. Contrary to the activation–repression binary view of gene regulation, emerging evidence suggests that the m⁶A methylation controls multiple aspects of mRNA metabolism, such as splicing, export, stability, translation, and degradation, culminating in the fine-tuning of gene expression. Brain shows the highest abundance of m⁶A methylation in the body, which is developmentally altered. Within the brain, m⁶A methylation is biased toward neuronal transcripts and sensitive to neuronal activity. In a healthy brain, m⁶A maintains several developmental and physiological processes such as neurogenesis, axonal growth, synaptic plasticity, circadian rhythm, cognitive function, and stress response. The m⁶A imbalance contributes to the pathogenesis of acute and chronic CNS insults, brain cancer, and neuropsychiatric disorders. This review discussed the molecular mechanisms of m⁶A regulation and its implication in the developmental, physiological, and pathological processes of the brain.

Keywords

Brain development, brain physiology, neurological disorders, N⁶-methyladenosine, post-transcriptional regulation

Received 12 June 2020; Accepted 27 August 2020

Introduction

RNA is not merely a passive carrier of genetic information as it is replete with dynamic and reversible chemical modifications that form an additional layer of post-transcriptional gene regulation. To date, 172 modifications are cataloged for different classes of cellular RNAs, which collectively constitute the “Epitranscriptome”.¹ Of these, 72 are variants of methyl group modifications attached at different positions within RNA bases. Some of the well-studied epitranscriptomic modifications include N⁶-methyladenosine (m⁶A), pseudouridine (ψ), 5-methylcytidine (m⁵C), N¹-methyladenosine (m¹A), ribose 2'-O-methylation (Nm), and N⁴-acetylcytidine (ac4C) (Figure 1).² Among these, m⁶A is the most abundant modification mRNAs undergo and accounts for ~80% of all RNA base methylations.³ In mammals, 0.1–0.4% of total adenines in the transcriptome are m⁶A methylated at any given time.^{4,5}

Although RNA m⁶A methylation was discovered in the early 1970s, its prevalence and distribution was not

assayed due to lack of high throughput techniques.³ Two parallel studies used m⁶A antibody-based next-generation sequencing and found that m⁶A is widespread in the transcriptome, tagging approximately 7000 mRNAs in mammals.^{6,7} Furthermore, m⁶A occurs in the consensus RRACH (R is a purine base, A is adenine that can be modified to m⁶A, and H is a non-guanine base) motif enriched near stop codons and

¹Cellular and Molecular Pathology Graduate Program, University of Wisconsin–Madison, Madison, WI, USA

²Department of Neurological Surgery, University of Wisconsin–Madison, Madison, WI, USA

³William S. Middleton Memorial Veteran Administration Hospital, Madison, WI, USA

Corresponding author:

Raghu Vemuganti, Department of Neurological Surgery, University of Wisconsin–Madison, 600 Highland Ave, Madison, WI 53792, USA.
Email: vemuganti@neurosurgery.wisc.edu

Figure 1. Major epitranscriptomic modifications. Mature RNAs can undergo 172 types of chemical modifications; 72 of those are variants of methyl group modifications. The most studied epitranscriptomic modifications are N⁶-methyladenosine (m⁶A), pseudouridine (ψ), 5-methylcytosine (m⁵C), N¹-methyladenosine (m¹A), ribose 2'-O-methylation (Nm), and N⁴-acetylcytidine (ac4C).

in the 3'-UTRs of mRNAs, indicating its regulatory potential.^{6,7}

Intriguingly, not all RRACH consensus sites are methylated. The fraction of the methylated sites appears to be highly variable, ranging from 6% to 80%.⁸ The m⁶A methylation machinery, comprising of a writer complex, erasers, and readers that deposit, remove, and recognize m⁶A, is well-characterized in yeast, plants, flies, and mammals.⁹ The m⁶A methylation controls multiple post-transcriptional mRNA processing steps, including splicing, export, stability, translation, and degradation.⁹ Additionally, m⁶A methylation mediates partition of the mRNA into diverse subcellular compartments such as P-bodies and stress granules.¹⁰

In mammals, the brain has the highest m⁶A of all organs, which increases from the embryonic stage to adulthood.⁶ In the adult brain, m⁶A is regulated in an activity-dependent manner. For example, neuronal depolarization significantly increases m⁶A levels in plasticity-related genes.¹¹ Within neurons, m⁶A machinery is localized in axons and dendrites and controls the local gene expression.¹² Interestingly, m⁶A methylation displays distinct profiles between different brain regions. For example, m⁶A is enriched in cell cycle-related transcripts in the cerebellum, whereas in synaptic plasticity-associated transcripts in the cerebral cortex.¹³ With these striking features, m⁶A signaling is implicated in

multiple physiological and pathological processes of the brain.

This review delineates the m⁶A machinery, emerging techniques to study m⁶A, molecular mechanisms of m⁶A-mediated mRNA processing, and its role in brain development (Table 1) and physiology (Table 2). The review further highlights the involvement of m⁶A epitranscriptomic regulation in acute CNS injuries, chronic neurodegeneration, brain cancer, and neuropsychiatric diseases (Table 3).

Molecular machinery of m⁶A methylation

The m⁶A methylation is controlled by the coordinated activity of the m⁶A methylase complex (*aka* m⁶A writer complex) and m⁶A demethylases (*aka* m⁶A erasers). Furthermore, m⁶A readers and anti-readers dictate the fate of m⁶A-modified transcripts (Figure 2).

m⁶A writer complex

The m⁶A writer complex contains a catalytic subunit methyltransferase (METTL)-like 3 (METTL3) and two regulatory subunits, METTL14 and Wilms' tumor-associated protein (WTAP).¹⁴ METTL14 mediates RNA substrate binding and WTAP confines the methylase complex to the nucleus.¹⁵ All three subunits (METTL3, METTL14, and WTAP) are indispensable

Table 1. Role of m⁶A methylation in brain development.

Developmental process	m ⁶ A machinery involved	m ⁶ A target transcripts	Functional implications
Cortical neurogenesis	METTL14	Cell cycle (<i>Cdk9</i> and <i>Ccnh</i>); NSC-specific TFs (<i>Pax6</i> and <i>Sox1</i>)	Degradation of stem cell maintenance factors to promote NSC differentiation ¹⁶
	METTL14	Proneural genes (<i>Neurod1</i> and 2)	Transcriptional prepatterning in NSCs ¹⁶
	METTL14	Histone acetyltransferase (<i>CBP/p300</i>)	NSC self-renewal by preventing deposition of H3K27ac at differentiation-related genomic loci ⁸¹
	YTHDF2	Negative regulators of JAK-STAT pathway (<i>Flrt2</i> and <i>Ptprd</i>)	Clearance of negative regulators of neurogenesis ⁸⁰
Cerebellar neurogenesis	METTL3	Apoptosis (<i>Dapk1</i> and <i>Fadd</i>); synaptic plasticity (<i>Grin1</i> and <i>Grm1</i>)	Inhibits apoptosis and promotes synaptogenesis ⁸⁵
Hippocampal neurogenesis	FTO	BDNF signaling (<i>Bdnf</i> , <i>PI3K</i> , <i>Akt1</i> , 2 and 3, <i>S6K1</i>)	Adult neurogenesis by enhancing BDNF signaling ²⁸
	METTL3	Histone methyltransferase (<i>Ezh2</i>)	Adult neurogenesis by modulating the global H3K27me3 levels ⁸²
Oligodendrogenesis	PRRC2A	Oligodendroglial specification factor (<i>Olig2</i>)	Oligodendrocyte maturation by stabilizing <i>Olig2</i> mRNA ⁴²
	METTL14	Nodes of Ranvier component (<i>Nfasc</i>)	<i>Nfasc</i> mRNA splicing and CNS myelination ⁸⁴
Axonogenesis	FTO	Axon elongation factor (<i>Gap43</i>)	Stabilizes <i>Gap43</i> mRNA and promotes axon elongation ²⁵
	YTHDF1	Axon guidance factor (<i>Robo3.1</i>)	Translation of <i>Robo3.1</i> and promotes axon guidance ⁸⁷

METTL14: methyltransferase like-14; NSC: neural stem cell; TF: transcription factor; JAK-STAT: Janus kinase - signal transducers and activators of transcription; METTL3: methyltransferase like-3; FTO: fat mass and obesity-associated; BDNF: brain-derived neurotrophic factor; PRRC2A: proline-rich coiled-coil 2A; YTHDF1: YT521-B homology domain-containing family protein 1.

Table 2. The role of m⁶A methylation in brain physiology.

Physiological process	m ⁶ A machinery involved	m ⁶ A target transcripts	Functional implications
Synaptic plasticity	METTL14 and YTHDF1	Synaptic transcripts (<i>Apc</i> , <i>Homer1</i> , and <i>Cdk5r1</i>)	Synaptic plasticity by inducing local translation of synaptic transcripts ¹²
Circadian clock	METTL3	Clock transcripts (<i>Per2</i> , <i>Bmal1</i> , and <i>CK1δ</i>)	Controls clock speed by promoting nuclear export and translation of clock transcripts ^{90,91}
Behavioral adaptation	METTL14, FTO, and YTHDF1	Synaptic transcript (<i>CamKIIα</i>)	m ⁶ A deficiency causes cognitive dysfunction ^{28,88,89}
	FTO	Dopaminergic signaling (<i>Grin1</i> , <i>Girk2</i> , and <i>Drd3</i>)	Reward learning and addictive behavior ²⁷
	FTO	—	Depression and anxiety-like behavior ⁹⁴
Stress response	METTL3 and FTO	Synaptic transcripts (<i>Homer1</i> and <i>Dusp1</i>)	Stress-induced fear memory consolidation ⁹⁷

METTL14: methyltransferase like-14; YTHDF1: YT521-B homology domain-containing family protein 1; METTL3: methyltransferase like-3; FTO: fat mass and obesity-associated.

as their individual deletion is embryonically lethal in mice.^{16–18} The methylase complex contains several other subunits like vir-like m⁶A methyltransferase associated (VIRMA), zinc finger CCCH-type containing 13, RNA binding motif protein 15/15B, and Cbl proto-oncogene like 1.¹⁹ Specific functions of these

subunits are not yet evaluated in detail. The mechanism of transcript-specific and site-specific m⁶A deposition is also not well understood. However, *de novo* m⁶A methylation of mRNAs is influenced by the interaction between transcription factors and methylase complex, elongation rate of RNA polymerase, and epigenetic

Table 3. The role of m⁶A methylation in brain diseases.

Disease	Model system	m ⁶ A machinery implicated	m ⁶ A target transcripts	Functional significance
Ischemic Stroke	Focal ischemia in mice	FTO	Inflammation and Apoptosis (<i>Tnf-α</i> , <i>Bcl2</i> & <i>Fas</i>)	Neuroprotection ^{54,103}
	Forebrain ischemia in gerbils	FTO	–	Not determined ¹⁰⁴
	H/R injury in hippocampal neurons	–	Cell survival (<i>PTEN</i>)	Inhibition of neuronal survival ¹⁰⁵
	GWAS	–	m ⁶ A SNPs in <i>NDST1</i> & <i>IRF6</i>	Increased disease risk ¹⁰²
Intracerebral Hemorrhage	GWAS	–	m ⁶ A SNP in <i>IRF6</i>	Increased disease risk ¹⁰²
Arteriovenous Malformations	Patient specimen and endothelial cells	WTAP, IGF2BP1 and 3	Cell adhesion (<i>DSP</i> & <i>DTX3L</i>)	Inhibition of angiogenesis ^{106,107}
Peripheral Nerve Injury	Sciatic nerve injury in mice	METTL14 & YTHDF1	Regeneration (<i>Atf3</i> & <i>Sox11</i>)	Promotes axon growth & reinnervation ¹¹⁰
Traumatic Brain Injury	CCI in mice	METTL3	Metabolism (<i>Pde12</i> & <i>Qsox2</i>)	Not determined ¹¹¹
	CCI in rats	METTL14 and FTO	Inflammation and Apoptosis (<i>CD14</i> & <i>Bcl2</i>)	Neurologic dysfunction ¹¹²
Glioblastoma	Tumor-derived GSCs and cell lines	METTL3, FTO and ALKBH5	Oncogenes (<i>FOXMI</i> , <i>c-Myc</i> & <i>ADAM19</i>)	Tumor growth suppression ^{126, 127, 131, 132}
	Tumor-derived GSCs and cell lines	METTL3, WTAP and YTHDC1	RNA editing (<i>ADAR1</i> & <i>APOBEC3A</i>); Splicing factors (<i>SRSF3</i> & <i>6</i>); Reprogramming factor (<i>SOX2</i>)	Oncogenic ^{122, 125}
Parkinson's disease	6-OHDA treatment in rats & PC12 cells	FTO	Dopaminergic signaling (<i>Grin1</i> & <i>Syn1</i>)	Oxidative stress, Mitochondrial dysfunction and Apoptosis ¹²¹
Alzheimer's disease	APP/PS1 double transgenic mice	METTL3 and FTO	Synaptic plasticity (<i>AMPA</i> , <i>NMDA</i> & <i>SEMA</i>)	Synaptic dysfunction ¹¹⁴
	APP/PS1/Tau triple transgenic mice	FTO	–	Cognitive dysfunction ¹¹⁵
Major Depressive Disorder	GWAS	SNPs in FTO	–	Increased disease risk ^{116, 117}
	GWAS	SNPs in FTO and ALKBH5	–	Increased disease risk ^{134, 135}
Attention-Deficit/Hyperactivity Disorder	GWAS	SNPs in FTO	–	Increased disease risk in pre-school children ^{136, 137}
Smith-Magenis Syndrome	Genetic analysis	Deletion in ALKBH5	–	Causative gene ¹⁴⁰
Autism Spectrum Disorder	GWAS	SNPs in YTHDC2	–	Increased disease risk ¹⁴¹
Multiple Sclerosis	GWAS	–	m ⁶ A SNP in <i>METTL21B</i>	Causative gene ¹⁴²

FTO: fat mass and obesity-associated; PTEN: phosphatase and tensin homolog; GWAS: genome-wide association studies; SNP: single nucleotide polymorphism; WTAP: Wilms' tumor-associated protein; IGF2BP1: insulin-like growth factor 2 mRNA binding protein 1; DSP: desmoplakin; METTL14: methyltransferase like-14; YTHDF1: YT521-B homology domain-containing family protein 1; CCI: controlled cortical impact; METTL3: methyltransferase like-3; GSCs: glioma stem-like cells; ALKBH5: alkB homolog 5; YTHDC1: YT521-B homology domain-containing protein 1; ADAR1: adenosine deaminase RNA specific 1; SRSF: serine/arginine-rich splicing factor; APOBEC3A: apolipoprotein B mRNA editing enzyme catalytic subunit 3A; FOXM1: forkhead box M1; 6-OHDA: 6-hydroxydopamine.

modifications such as Histone H3 trimethylation at lysine 36.²⁰ Furthermore, SUMOylation of METTL3 inhibits the *in vivo* m⁶A methyltransferase activity.²⁰ In brain, m⁶A writer complex is predominantly localized

in the neuronal nuclei. Both METTL3 and METTL14 were also detected in the synaptic fractions suggesting the possibility of dynamic m⁶A methylation in the extra-somatic regions.^{12,21}

Figure 2. Molecular machinery of m⁶A methylation. The m⁶A modification predominantly occurs at consensus RRACH (R is a purine base, A is m⁶A-modified adenine, and H is a non-guanine base) motif enriched in the 3'-UTR of mRNAs. The m⁶A is deposited by a multi-subunit methylase complex (*aka* writer complex) primarily composed of a catalytic subunit METTL3 and a regulatory subunit METTL14. The m⁶A methylation is reversible and the methylated RNAs are demethylated by FTO and ALKBH5, with distinct erasure mechanisms. The m⁶A readers bind the methyl group through a characteristic YTH domain to propagate the m⁶A signaling. Additionally, the m⁶A methylation is repelled by m⁶A anti-readers G3BP1/2 that preferentially bind to unmethylated m⁶A motifs in the RNA.

WTAP: Wilms' tumor-associated protein; METTL3: methyltransferase like-3; METTL14: methyltransferase like-14; FTO: fat mass and obesity-associated; YTH: YT521-B homology; G3BP1/2: GTPase-activating protein (SH3 domain) binding protein 1 and 2.

m⁶A erasers

The m⁶A methylation is reversible. In eukaryotes, m⁶A is removed by two demethylases, fat mass and obesity-associated (FTO), and alkB homolog 5 (ALKBH5).^{22,23} Unlike the m⁶A writer complex, FTO shuttles between nucleus and cytoplasm by interacting with exportin 2.²⁴ In the neurons, FTO is also detected in the extra somatic regions, including axons and dendritic shafts, suggesting the dynamic erasure of m⁶A and also certain other epitranscriptomic marks.^{12,25,26} FTO-dependent m⁶A demethylation is implicated in dopaminergic neurotransmission, adult neurogenesis, and axon elongation.^{25,27,28} Due to these wide-ranging roles, FTO deficiency results in postnatal growth retardation, microcephaly, and functional deficits in both humans and mice.^{29,30} FTO catalyzes the removal of m⁶A in steps by oxidizing the methylated base and then fragmenting it.³¹ In contrast, ALKBH5 catalyzes the direct removal of the methyl group in a single step reaction, but with significantly lower catalytic efficiency.²³ ALKBH5 is ubiquitously expressed with the highest expression in the lungs followed by the testes.³² Unlike FTO, ALKBH5 strictly localizes to nuclear speckles, precluding the

dynamic demethylation of cytoplasmic mRNAs.¹² ALKBH5 deficiency in mice results in a highly specific phenotype of compromised spermatogenesis.²³ Although the transcript selectivity for these demethylases is not well understood, a recent study proposed that the interaction of m⁶A-methylated RNAs with FTO or ALKBH5 depends on the sequence context.³¹ Additionally, an RNA-binding protein (RBP), splicing factor proline- and glutamine-rich, confers transcript selectivity by recruiting FTO to promote proximal m⁶A demethylation.³³

m⁶A readers and anti-readers

The downstream effects of m⁶A-methylated RNAs are mediated by specific RBPs called m⁶A readers that influence the post-transcriptional RNA processing. Readers recognize m⁶A by direct binding to m⁶A, by increased accessibility to RNA-binding motifs and by repulsion of RBPs.⁹ The m⁶A readers bind to m⁶A-methylated RNAs directly via a well-conserved YT521-B homology (YTH) domain.³⁴ In mammals, there are five YTH proteins, including YTH domain-containing family proteins (YTHDF) 1, 2, and 3 and

YTH domain-containing proteins (YTHDC) 1 and 2. YTHDC1 recognizes m⁶A in nuclear transcripts, while others recognize m⁶A in cytosolic transcripts. YTH proteins are not site-specific and they bind to m⁶A motifs at a similar ratio.^{35,36} YTH proteins regulate the splicing, export, translation, and degradation of the m⁶A-modified mRNAs.²⁰ m⁶A in the short RNA helices destabilizes and unfolds the RNA and thus increases the binding of RBPs.³⁷ This leads to increased binding of splicing regulatory proteins heterogeneous nuclear ribonucleoprotein (HNRNP) C and G near the m⁶A sites leading to alternative splicing of the target transcripts.³⁸ The m⁶A methylation in the primary microRNAs (Pri-miRNAs) also increases the binding of HNRNPA2B1 leading to Pri-miRNA processing.³⁹

Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs) and certain neuronal-specific RBPs like Fragile X mental retardation protein (FMRP) and proline-rich coiled-coil 2A (PRRC2A) are also potential m⁶A readers.^{40–42} FMRP reads m⁶A and promotes the nuclear export of methylated mRNAs during neural differentiation.⁴³ Recently, stress granule proteins such as GTPase-activating protein (SH3 domain) binding protein 1 and 2 (G3BP1 and G3BP2) were named as anti-readers as they bind to unmethylated m⁶A motif CAACUC in the 3'-UTR of thousands of transcripts and promote their stability.⁴⁰ The functional significance of the anti-readers is not yet fully understood, but a coordinated action of readers and anti-readers define the functionality of m⁶A modifiable transcripts.

Techniques to study m⁶A methylation

As methyl group does not alter the base pairing of adenosine and m⁶A is chemically non-modifiable, it cannot be detected by standard hybridization and sequencing technologies.⁶ Although dot blot and immuno-northern blot using m⁶A antibodies allow faster and easier estimation of global m⁶A levels, these techniques are less sensitive and semi-quantitative.⁴⁴ The m⁶A levels can be quantitated with high sensitivity by liquid chromatography/mass spectrometry, but this technique will not provide the sequence context and localization information,⁴⁵ whereas, m⁶A immunoprecipitation followed by next-generation sequencing (MeRIP-seq) allows analysis of m⁶A methylation with 50–200 nucleotide resolution.⁶ Furthermore, m⁶A individual-nucleotide resolution cross-linking and immunoprecipitation enables identification of specific m⁶A residues at single-nucleotide resolution.⁴⁶ However, m⁶A antibodies cross-react with m⁶A_m; but the abundance of m⁶A_m is <5% of the total methylated RNA residues,⁴⁷ whereas, Site-specific Cleavage and Radioactive labeling followed by Ligation-assisted Extraction and Thin-layer

chromatography (SCARLET) allows the stoichiometric quantification (the fraction of RNAs that are m⁶A modified at a given site) within the total RNA pool. However, this is a tedious technique and hence suitable only to validate the proposed m⁶A changes at a given site.⁸

More recently, RNA digestion via m⁶A-sensitive RNase MazF-sequencing (MAZTER-seq) and deamination adjacent to RNA modification targets-sequencing (DART-seq) allowed single-nucleotide resolution and stoichiometric quantification of transcriptome-wide m⁶A methylation simultaneously. In MAZTER-seq, upstream of unmethylated ACA motifs, but not the methylated m⁶ACA motifs, will be selectively cleaved with a bacterial RNase (MazF) and the ACA read-through will be correlated with m⁶A abundance.⁴⁸ A caveat of this method is the requirement of the ACA motif in single-stranded RNAs, and hence only 16–25% of m⁶A sites in the mammalian system can be captured.⁴⁸ DART-seq employs an engineered fusion protein in which the N-terminus of the m⁶A-binding domain YTH fused to the editing domain of cytosine deaminase, apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1. The YTH domain directs the editing machinery to the m⁶A site facilitating the conversion of proximal C-to-U, and the editing events adjacent to the m⁶A sites can be evaluated by RNA sequencing. Notably, this technique requires only 10 ng total RNA.⁴⁹

Recent studies employed clustered regularly interspaced short palindromic repeats (CRISPR)-based site-specific epitranscriptomic editing to dissect the functional contribution of a specific m⁶A site in a modified transcript. Rauch et al. tethered m⁶A readers YTHDF1 and YTHDF2 to catalytically inactive cas13 protein (dCas13) to specifically target and degrade isomerase B mRNA using a complementary guide RNA.⁵⁰ Liu et al. fused dCas9 protein to methyltransferase domains of METTL3 and METTL14 and demethylases ALKBH5 and FTO.⁵¹ Using this engineered m⁶A writer, they promoted site-specific methylation of heat shock protein 70 (HSP70) mRNA leading to its increased translation upon heat shock stress, recapitulating the context-specific m⁶A regulation.⁵¹ More recently, Li et al. used an engineered ALKBH5-dCas13 fusion protein to decrease the proliferation of cancer cells by site-specific demethylation, and consequent destabilization of the oncogenic transcripts *Egfr* and *Myc*.⁵² These novel tools are indispensable for dissecting the precise roles of m⁶A at particular regions within an RNA (Figure 3).

Cell-type-specific distribution of m⁶A in the brain

Elucidating cell-specific methylation patterns is crucial for understanding the cell-cell communication under

Figure 3. Techniques to study m⁶A methylation. LC-MS/MS can quantitate the m⁶A modification (a). MeRIP-seq can identify the transcriptome-wide m⁶A profiles. MAZTER-seq can show the m⁶A sites along with the stoichiometry of methylation with single-nucleotide resolution (b). CRISPR/Cas9 genomic editing using METTL3 or FTO fused to inactive dCas9 targeted near the vicinity of m⁶A site by sequence-specific gRNA can modulate site-specific m⁶A methylation (c).

physiological and pathological conditions. MeRIP profiling in the brain revealed a higher prevalence of m⁶A in neuronal than glial transcripts.¹³ Moreover, m⁶A effector proteins are more widely expressed in the neurons than in the glial cells, endorsing this bias.⁵³ The m⁶A-modified transcripts and their interactomes are localized in axons and dendritic shafts, indicating their role in activity-dependent gene regulation.^{12,25} Nevertheless, the presence of m⁶A in the non-neuronal cells cannot be overlooked. Astrocytic transcripts, such as *Sparcl1* and *Slc1a2* involved in synapse formation, as well as a microglial transcript *CX₃CR1* involved in the complement cascade, are highly m⁶A modified.¹² The brain microvascular pericytes from the spontaneously hypertensive rats showed ~7500 m⁶A peaks in 5000 mRNAs related to inflammatory response, proximal tubule development, mitogen-activated protein kinase (MAPK) cascade, and cAMP response element binding, indicating the significance of m⁶A methylation in non-neuronal functions.⁵⁴ The m⁶A is also highly prevalent in oligodendrocyte-specific transcripts such as *Olig2*.⁴² Taken together, m⁶A signaling occurs predominantly

in the neurons, but can be seen to some extent in other CNS cell types.

Mechanisms of post-transcriptional regulation by m⁶A methylation

The molecular effects of epigenetic modifications are often singular as they either activate or repress the gene expression, whereas epitranscriptomic modifications like m⁶A methylation influences a multitude of downstream functions, including splicing, export, stability, translation, and degradation of RNAs (Figure 4). The functional outcome of m⁶A RNA methylation is determined by the m⁶A readers, which mobilize specific cellular machinery to the target RNAs. Furthermore, m⁶A regulation is dynamic as the fate of a target RNA is determined by a specific reader depending on the context. For example, heat shock stress increases m⁶A in the 5'-UTR region of *Hsp70* mRNA to promote cap-independent translation.⁵⁵ In contrast, hypoxic stress increases m⁶A in mRNA targets such as *Glut1*, *c-Myc*, and *Jun* to enhance their stability without affecting the translation efficiency.⁵⁶

Figure 4. Post-transcriptional regulation by m⁶A methylation. The m⁶A methylation occurs co-transcriptionally in the nascent pre-mRNAs. The fate of methylated transcripts is dictated by m⁶A readers that bind and recruit diverse cellular machinery. In the nucleus, YTHDC1 binds to exonic m⁶A and recruits splicing regulatory factor SRSF3 to promote exon inclusion and thereby controls alternate splicing (a). The m⁶A writer facilitates the nuclear export of spliced mRNAs by interacting with export machinery TREX complex to guide them through the nuclear pore complex to the cytoplasm (b). In the cytoplasm, the presence of m⁶A repels the binding of stress granule proteins G3BP1/2 that regulates the stability of target RNA (c). Binding of YTHDF1 to methylated RNA recruits translation machinery leading to RNA translation (d). In contrast, YTHDF2 recruits mRNA decay enzymes in the P-bodies leading to degradation of the methylated transcripts (e).

SRSF3: serine/arginine-rich splicing factor 3; YTHDC1: YT521-B homology domain-containing protein 1; G3BP1/2: GTPase activating protein (SH3 domain) binding protein 1/2; YTHDF1: YT521-B homology domain-containing family protein 1; YTHDF2: YT521-B homology domain-containing family protein 2; NPC: nuclear pore complex.

Splicing

In METTL3 knockdown cells, m⁶A abundance correlated with differentially spliced exons indicating its role in alternate splicing.⁷ Colocalization of m⁶A writer complex with pre-mRNA splicing factors in nuclear speckles further supports this notion.⁵⁷ A closer examination of exonic m⁶A peaks from the MeRIP data revealed that m⁶A occurs in the vicinity of 5' and 3' splice sites. Alternate splicing caused by differential splice site selection is primarily controlled by the binding of serine/arginine-rich splicing factors (SRSFs) to cis-regulatory sequences proximal to splice sites to cause exon inclusion or exclusion.⁵⁸ In adipocytes, exonic m⁶A was found to enhance the recruitment of SRSF2 to an RNA promoting exon inclusion.⁵⁹ The nuclear m⁶A reader YTHDC1 was shown to promote exon inclusion by engaging SRSF3 while inhibiting SRSF10 binding to splicing enhancer elements.⁶⁰ Thus, m⁶A controls

alternate splicing by modulating the access of splicing regulatory factors to target RNA.

Export

ALKBH5-deficient cells showed a dramatic increase in cytoplasmic mRNA fraction and overexpression of ALKBH5 normalized it, indicating the role of m⁶A methylation in modulating the mRNA export.²³ Nuclear mRNA export is driven by the multi-subunit protein complex transcription-export (TREX), which interacts with spliced mRNAs to guide them through the nuclear pore complex to the cytoplasm.⁶¹ The m⁶A methyltransferase was shown to recruit TREX to m⁶A-modified RNAs for efficient export. Interestingly, depletion of the m⁶A methyltransferase subunits VIRMA and WTAP inhibits this process.⁶² The m⁶A reader YTHDC1 plays an essential role in facilitating RNA export as its knockdown increases the nuclear retention of m⁶A-modified transcripts.⁶³

Another m⁶A reader FMRP also mediates the nuclear export of m⁶A-containing mRNAs, which was blocked in FMRP-deficient mouse.⁶⁴ FMRP was also shown to regulate neural differentiation by promoting the nuclear export of Notch- and Hedgehog signaling-related mRNAs.⁴³ Mechanistically, FMRP associates with exportin 1 to mediate the export of m⁶A-modified RNAs.⁴³ Thus, m⁶A modulates the nuclear export of selective transcripts by recruiting export factors in a stimulus-dependent manner.

Stability

The mRNA abundance of approximately one-tenth of the neural transcriptome is determined by the mRNA stability.⁶⁵ The mRNA stabilization involves the competitive binding of RBPs to target mRNAs to guard them against degradation. It was shown that RBPs specifically bind to the m⁶A methylated transcripts to control their stability. For example, IGF2BP1, 2 and 3 directly bind to ~80% of m⁶A methylated transcripts and recruit mRNA stabilizer human antigen R to promote mRNA stability.⁴¹ Additionally, certain neuronal-enriched m⁶A readers, including FMRP and PRRC2A, compete with YTHDF2 for binding to methylated transcripts, and thus prevent their degradation.^{42,66}

Translation

The m⁶A methylation was thought to reduce the half-life of mRNAs.⁶⁷ However, the interaction of the m⁶A reader YTHDF1 with the translation initiation factor 3 (eIF3) promotes the translation of the m⁶A-modified transcripts, and the YTHDF1 knockdown curtailed this process.⁶⁸ Furthermore, YTHDF3 binds to YTHDF1 to promote the translation of the m⁶A-modified transcripts.⁶⁹ The translation regulatory mechanisms of m⁶A are site-specific within the mRNA. YTHDF1-directed translation regulation is mainly observed for m⁶A sites in the 3'-UTR region of the mRNAs,⁶⁹ whereas, m⁶A sites present in the 5'-UTR region directly interacts with eIF3 to cause cap-independent translation initiation.⁷⁰ Surprisingly, ~13% of endogenous circular RNAs are also m⁶A methylated and the m⁶A reader YTHDF3 recruits the translation initiation factors eIF4G2 and eIF3A, leading to their translation.⁷¹

Degradation

The m⁶A reader YTHDF2 recruits RNA decay machinery, including CCR4-NOT deadenylase complex and RNase P/MRP to the m⁶A-methylated RNAs, and thus regulates their decay in the P-bodies.^{72–74} YTHDF2 knockdown was shown to increase the half-life of m⁶A modified transcripts by ~30%.⁷²

Transcription

The m⁶A methylation of RNAs is also implicated in transcription regulation. The lncRNA X-inactive specific transcript (XIST) has the highest number of m⁶A sites than any other RNA, and depletion of m⁶A by METTL3 knockdown impairs XIST-mediated transcriptional repression of X-linked genes.³⁶ The m⁶A sites are highly prevalent in RNAs within R-loop structures that regulate transcription termination.^{75,76} METTL3 depletion leads to fewer R-loops near transcription end sites indicating that m⁶A is crucial for efficient transcription termination,⁷⁵ whereas, YTHDF2 binds to m⁶A-modified R-loops to destabilize and prevent their accumulation.⁷⁶ In stem cells, METTL3 deposits m⁶A on chromosome-associated regulatory RNAs and YTHDC1 enable their degradation, resulting in chromatin closure and transcription inactivation.⁷⁷

Role of m⁶A methylation in brain development

Neurogenesis

Epigenetic modifications are known to regulate the proliferation and differentiation of neural stem cells (NSCs).⁷⁸ Several studies showed that m⁶A methylation also controls neurogenesis. During development, METTL3 expression peaks during the early stage, whereas FTO expression peaks during the later stage of neurogenesis.^{16,28} Loss of METTL3 or METTL14 prolongs the cell cycle progression of NSCs, and thereby delays the production of upper-layer neurons in the postnatal mouse cortex.¹⁶ Deletion of METTL14 and YTHDF2 leads to enlarged ventricles and decreased cortical thickness, respectively.^{16,79} Furthermore, in the E13.5 stage mouse forebrain, several transcripts related to cell cycle (*Cdk9*, *Ccnh*, and *Cdkn1C*) and NSC-specific transcription factors (*Pax6*, *Sox1*, and *Emx2*) were shown to be m⁶A tagged and their stability increased in METTL14-deficient NSCs.¹⁶ *Neurod1* and *Neurod2* transcripts were also m⁶A tagged in NSCs, indicating the role of m⁶A methylation in transcriptional prepatternning.¹⁶ Furthermore, YTHDF2-deficient NSCs showed decreased proliferation and differentiation, and neurons derived from YTHDF2-deficient NSCs have shorter neurites and are vulnerable to oxidative stress.⁷⁹ Moreover, transcripts related to the JAK-STAT pathway that negatively regulate the stem cell proliferation like *Flrt2* and *Ptprd* are hypermethylated and stabilized in YTHDF2-deficient NSCs.⁷⁹ In METTL14-deficient NSCs, a significantly increased abundance of histone H3 at lysine 27 acetylation (H3K27ac), lysine 27 trimethylation (H3K27me3), and at lysine 4 trimethylation (H3K4me3) was

observed.⁸⁰ Particularly, loss of m⁶A increased gene-silencing H3K27me3 mark near promoters of proliferation-related genes like *Egr2* and *Egr3*, and gene-activating H3K27ac mark near promoters of differentiation-related genes like *Kif26a*, *Gas7*, and *Pdgfr1b*.⁸⁰ Modulating these changes using inhibitors of histone acetyltransferase *CBP/p300* and histone methyltransferase *Ezh2* promoted proliferation of METTL14-deficient NSCs.⁸⁰ This indicates that m⁶A induces histone alterations to regulate the NSC gene expression. Mechanistically, failure to clear m⁶A-tagged *CBP/p300* impairs neurogenesis in METTL14-deficient embryonic NSCs.⁸⁰ METTL3 knockout decreased m⁶A in the *Ezh2* transcript responsible for global H3K27me3, reduced the NSC proliferation and differentiation, which was rescued by *Ezh2* overexpression.⁸¹ Altogether, these studies indicate the cross-talk between RNA modifications and histone modifications during neurogenesis.

The m⁶A erasers also play a role in neurogenesis. Although depletion of FTO or ALKBH5 did not affect the proliferation of embryonic NSCs,⁸⁰ loss of FTO elevated m⁶A in the brain-derived neurotrophic factor (BDNF) signaling pathway-related transcripts *Bdnf*, *PI3K*, *Akt1*, *Akt2*, *Akt3*, and *S6K1*, which were consequently degraded, leading to reduced proliferation of adult NSCs.²⁸ FTO deficiency also reduced mature BDNF protein levels, and thereby decreased the activation of the prosurvival MAPK signaling pathway leading to impaired adult neurogenesis.⁸²

Gliogenesis

The m⁶A signaling in the context of glial lineage progression is less studied except for the oligodendrogenesis. PRRC2A was observed to compete with YTHDF2 to bind and stabilize m⁶A methylated oligodendroglial lineage determination factor *Olig2* mRNA, and hence PRRC2A-deficient mice fail to develop mature oligodendrocytes and display hypomyelination.⁴² Notably, FTO demethylates and prevents PRRC2A-dependent stabilization of *Olig2*, and hence FTO-overexpressing mice phenocopied PRRC2A-deficient mice.⁴² Moreover, ablation of the m⁶A writer METTL14 in oligodendrocyte lineage cells led to hypomethylation of several transcription factors (*Hey1*, *Klf19*, and *Zeb2*), histone modifiers (*Hdac3*, *Kdm2b*, and *Prdm2*), and growth factors (*Igf-1*, *Fgf*, and *Bmp*) that promote oligodendrocyte maturation and hence reduced the number of mature oligodendrocytes and caused hypomyelination.⁸³ METTL14 deficiency also resulted in decreased oligodendrocytes-specific neurofascin (*Nfasc*) splice isoform, subsequently producing an aberrant node of Ranvier morphology.⁸³ Furthermore, METTL14- and YTHDF2-deficient NSCs failed to differentiate to

astrocytes.^{16,79} Collectively, these studies show that m⁶A promotes the maturation of glial cells.

Cerebellar development

Interestingly, the cerebellum shows two times higher m⁶A methylation than the cerebral cortex.^{13,84} Several apoptotic (*Dapk1*, *Fadd*, and *Ngfr*) and synaptic transcripts (*Grin1*, *Atp2b3*, *Grm1*, and *Lrp8*) were shown to be hypomethylated in the cerebellum of METTL3-deficient mice, leading to severe cerebellar granule cell apoptosis and hypoplasia.⁸⁴ Apart from constitutively methylated mRNAs, a unique set of stage-specific m⁶A-methylated mRNAs were observed in the cerebellum during postnatal development.⁸⁵ This indicates that m⁶A methylation is essential for cerebellar development.

Axonal growth

The m⁶A methylation regulates the translation of mRNAs involved in axon guidance and elongation during development. RNAs in the axons of dorsal root ganglion (DRG) and dorsal spinal cord (DSC) show a high abundance of m⁶A methylation and m⁶A regulatory proteins FTO and YTHDF1.^{25,86} In the axons of DRG neurons, FTO demethylates *Gap43* to promote its translation leading to axon elongation,²⁵ whereas, YTHDF1 promotes translation of methylated axon guidance-related mRNA *Robo3.1* in the DSC.⁸⁶

The role of m⁶A methylation in brain physiology

Synaptic plasticity

The m⁶A methylation is highly prevalent in synaptic transcripts (~76% of the post-synaptic and ~30% of pre-synaptic mRNAs are methylated in mice).¹³ A recent study showed that METTL14, FTO, and YTHDF1, 2, and 3 are localized in the dendritic processes of cortical and hippocampal neurons and > 1200 transcripts related to synapse organization, assembly, maturation, and modulation of synaptic transmission are hypermethylated.¹² Furthermore, FTO-deficient mice displayed increased methylation of synaptic transmission-related mRNAs in the midbrain.²⁷ The loss of the m⁶A reader YTHDF1 in hippocampal neurons caused structural and functional deficits in excitatory synapses, including reduced spine head width, lower levels of post-synaptic density-95, and curtailed excitatory post-synaptic currents.^{12,87} METTL14 deletion in striatal neurons decreased the m⁶A methylation in synaptic plasticity-related transcripts that correlate with impaired neuronal excitability.⁸⁸ Together, these observations highlight the crucial role of m⁶A methylation in synaptic plasticity.

Circadian clock

Inhibition of m⁶A methylation of clock transcripts like *Per1*, *Per3*, *Tef*, *Dbp*, *Nfil3*, *Bhlhe41*, and *Nr1d* with 3-deazaadenosine prolonged the circadian clock oscillation in mice.⁸⁹ Furthermore, METTL3 inhibition caused the nuclear accumulation of unmethylated clock transcripts *Per2* and *Bmal1*.⁸⁹ The *CK1δ* kinase, which is responsible for the degradation of clock proteins, was observed to be heavily m⁶A-methylated, and targeted ablation of the m⁶A motif in the *CK1δ* gene increased its translation in the brain, and those mice showed longer rhythmic locomotor activity.⁹⁰ Thus, m⁶A methylation might play a significant role as the circadian pacesetter.

Behavioral adaptation

By modulating neural plasticity, m⁶A methylation can influence adaptive behavior. Perturbation of m⁶A methylation was shown to alter cognition, addiction, anxiety, and depression-like behavior in mice.² Furthermore, behavioral training by fear conditioning also significantly altered the m⁶A methylome in the mouse brain.^{11,91} Ablation of FTO or METTL14 or YTHDF1 was shown to impair cognition in mice.^{28,87,88} The m⁶A methylation is also thought to be critical for reward mechanisms. Cocaine-induced locomotor and reward-stimulatory actions were observed to be impaired in FTO-deficient mice²⁷ and certain FTO variants strongly correlate with increased alcohol consumption in humans.⁹² FTO deficiency also suppressed depression- and anxiety-like behavior in mice which was observed to be due to decreased inflammation caused by gut microbial dysbiosis.⁹³

Stress response

In non-neuronal cells, various environmental stressors, such as heat shock, hypoxia, oxidative stress, and UV irradiation, alter the m⁶A epitranscriptome.^{55,56,94,95} Acute restraint stress in mice caused m⁶A hypomethylation in the prefrontal cortex and hypermethylation in the amygdala.⁹⁶ All the enzymes of m⁶A machinery harbor glucocorticoid response elements in the 10 kb upstream region of the transcription start site, indicating their putative stress-responsive regulation.⁹⁶ Furthermore, stress-induced m⁶A methylation suppressed translation of synaptic plasticity-related and morphogenesis-related transcripts like *Camk2n1*, *Duspl*, and *Homer1*.⁹⁶ Importantly, neuronal-specific deletion of METTL3 or FTO improved cued/contextual fear memory, mimicking stress.⁹⁶ Two recent studies showed increased m⁶A levels with a concomitant loss of FTO expression in the prefrontal cortex and hippocampus following fear-conditioning.^{11,91} Conversely, FTO inhibition in the hippocampus or prefrontal cortex also enhanced the fear

memory.^{11,91} Collectively, these studies hint the role of m⁶A signaling in stress-related disorders.

The role of m⁶A methylation in brain diseases

Stroke

Secondary brain damage following stroke is synergistically mediated by several pathologic events, including excitotoxicity, edema, apoptosis, mitochondrial dysfunction, oxidative stress, endoplasmic reticulum stress and inflammation.⁹⁷ Recent studies showed that epigenetic changes modulate post-stroke dysfunction.^{98,99} Many studies indicated that epitranscriptomic mechanisms also promote stroke and its consequences. The Mannheim–Heidelberg stroke study showed a genetic association between a FTO variant and transient ischemic attack.¹⁰⁰ Furthermore, 4000 m⁶A-single nucleotide polymorphism (SNPs), including in *IRF6* and *NDST1* genes, were shown to be associated with ischemic stroke.¹⁰¹ Using a rodent model, we and others reported that ischemic stroke down-regulates the cerebral FTO levels, leading to increased m⁶A methylation.^{53,102} We observed that several transcripts that mediate apoptosis (*Fas*, *Trib3*, and *Bcl2a1c*) and inflammation (*Tnf*, *IL-6*, and *Sele*) were hypermethylated in the ischemic brain.⁵³ Additionally, stroke up-regulated the expression of the m⁶A reader YTHDF1, which might bind to m⁶A-methylated inflammatory and apoptotic transcripts to increase their translation.⁵³ Furthermore, FTO overexpression was shown to protect the cortical neurons from apoptosis following oxygen–glucose deprivation by stabilizing anti-apoptotic transcript *Bcl2*.¹⁰² Forebrain ischemia in gerbils was also shown to decrease the hippocampal FTO protein expression.¹⁰³ When hippocampal neurons were subjected to hypoxia, m⁶A levels were elevated in the phosphates and tensin homolog transcript that induced cell death, and over-imposing hypothermia normalized this and enhanced the neuronal survival.¹⁰⁴

A recent study showed reduced expression of the m⁶A writer complex subunit WTAP in the brains of arteriovenous malformation (AVM) patients suffering from congenital vascular dysfunction.¹⁰⁵ Ablation of WTAP decreased the m⁶A levels and expression of the cell adhesion molecule, desmoplakin (DSP), leading to the reduced angiogenic potential of cultured endothelial cells.¹⁰⁵ An inverse correlation was also observed between METTL3 expression and the nidus size of cerebral AVMs.¹⁰⁶ Notch pathway regulator *DTX3L* was implicated in AVM formation, and in METTL3-deficient endothelial cells, *DTX3L* mRNA was observed to be hypomethylated and silenced, indicating that m⁶A is crucial for its stability.¹⁰⁶ Furthermore, m⁶A SNP in

IRF6 transcript correlated with intracerebral hemorrhage in humans.¹⁰¹ Collectively, these studies indicate the role of m⁶A methylation in stroke incidence and post-stroke brain damage.

Peripheral nerve injury

Injury to a peripheral nerve leads to discontinuity of the axon, loss of myelin fibers, and eventual death of the neurons.¹⁰⁷ Epigenetic modifications, including histone acetylation and DNA hydroxymethylation, were shown to promote axon regeneration after peripheral nerve injury by increasing the transcription of regeneration-associated genes (RAGs).¹⁰⁸ A recent study reported that m⁶A RNA methylation increases in many RAGs (*Atf3*, *Sox11*, *Gadd45a*, and *Tet3*) as well as several ribosomal subunits (*Rps14*, *Rps20*, *Rps23*, *Rps28*, *Rps29*, *Eif1a*, and *Eif3b*) in the DRG following sciatic nerve injury in mice.¹⁰⁹ Furthermore, depletion of either METTL14 or YTHDF1 decreased the translation of RAGs in the injured DRGs and significantly reduced the axon growth and reinnervation leading to curtailed functional recovery.¹⁰⁹

Traumatic brain injury

Several studies showed the functional significance of epigenetic modifications in brain damage after traumatic brain injury (TBI).⁹⁸ TBI in adult mice decreases METTL3 expression and global m⁶A abundance in the hippocampus within 6–24 h post-injury.¹¹⁰ Many of the differentially m⁶A-methylated transcripts in the post-TBI hippocampus were observed to control neuronal metabolic processes.¹¹⁰ TBI in rats was also shown to decrease the mRNA expression of METTL14 and FTO in the cerebral cortex and many of the m⁶A-modified transcripts such as *CD14* (inflammation), *Dll4* (Notch signaling), and *Bcl2* (apoptosis) are known promoters of post-TBI pathology.¹¹¹ More importantly, FTO inhibition exacerbated the neurologic dysfunction after TBI but did not affect the cognitive function.¹¹¹

Alzheimer's disease

Epigenetic dysregulation is thought to drive the protein aggregation and neuronal loss in Alzheimer's disease (AD).¹¹² The hippocampus of the double transgenic AD mice showed induction of METTL3 and suppression of FTO, leading to altered global m⁶A methylation of RNAs involved in dendrite development, synaptic growth, pre- and post-synaptic assembly, glutamate receptor signaling, axon guidance, and long-term potentiation.¹¹³ This implies aberrant m⁶A methylation as a promotor of AD pathogenesis. Expression of the m⁶A demethylase FTO was shown to be increased in the brains of triple transgenic

AD mice, and its knockdown decreased the phosphorylated tau without affecting A β accumulation in the neurons.¹¹⁴ This effect was further shown to be mediated by methylation of the tuberous sclerosis complex 1, which is an upstream inhibitor of the mammalian target of rapamycin kinase that regulates tau. Interestingly, FTO-deficient AD mice showed significantly better cognitive function compared to wild-type mice.¹¹⁴ In Caucasians, the presence of FTO AA-genotype was found to increase the risk of AD by 1.6-fold.¹¹⁵ Furthermore, three SNPs in the *FTO* gene leading to decreased FTO expression was shown to be associated with AD risk in Caucasians and Caribbean Hispanics.¹¹⁶ Overall, FTO modulation seems to be a potential strategy to alleviate the AD-associated deficits.

Parkinson's disease

Epigenetic changes such as reduced DNA methylation at the promoter of SNCA (the gene that transcribes α -synuclein) as well as decreased histone acetylation are implicated in α -synuclein aggregation and Parkinson's disease (PD) progression.^{117,118} Interestingly, FTO deficiency increased the m⁶A methylation and decreased the translation of dopaminergic pathway-related transcripts like *Drd3*, *Girk2*, and *Grin1*.²⁷ Increased expression of m⁶A demethylases ALKBH5 and FTO and decreased levels of m⁶A were observed in the striatum of rats subjected to 6-hydroxydopamine (6-OHDA)-induced PD.¹¹⁹ FTO inhibition also protected PC12 cells against 6-OHDA-induced cell death.¹¹⁹

Brain cancer

Several studies showed that the expression of m⁶A writers, erasers, and readers is significantly perturbed in the glioblastoma multiforme (GBM) patients.^{120–122} Notably, modulation of m⁶A machinery in patient-derived glioma stem-like cells (GSCs) altered their self-renewal and tumorigenic properties.^{123,124} Tumor tissue resected from GBM patients showed high levels of the m⁶A writer METTL3 that correlated with poor patient survival.¹²⁰ However, role of METTL3 in regulating the tumorigenic potential of GSCs remains controversial. Initial studies proposed METTL3 to be a tumor suppressor as METTL3 knockdown *in vivo* promoted tumor progression and worsened survival.^{124,125} Furthermore, METTL3 overexpression in a GBM cell line U251 inhibited the migration and proliferation of cells and concomitantly induced apoptosis.¹²⁵ In contrast, silencing METTL3 prevented m⁶A methylation of the glioma reprogramming factor *Sox2*, leading to apoptosis and radiosensitization of GSCs, and reduced tumor size in mice.¹²³ Furthermore, METTL3 was

Figure 5. Role of m^6A methylation in brain development, physiology, and disease. The m^6A , with its multifaceted role in mRNA processing, is considered as the fifth nucleobase in the mammalian brain. In the developing brain, m^6A gradually increases to control axonogenesis, neurogenesis, and gliogenesis. In the adult brain, m^6A maintains essential physiological processes such as synaptic plasticity, cognitive function, and circadian rhythm. The m^6A dysregulation is implicated in many pathological processes, including chronic neurodegeneration, acute brain injury, and tumorigenesis.

METTL3: methyltransferase like-3; METTL14: methyltransferase like-14; WTAP: Wilms' tumor-associated protein; FTO: fat mass and obesity-associated; ALKBH5: alkB homolog 5; YTH: YT521-B homology.

shown to regulate many transcripts related to oncogenic signaling and RNA editing, such as adenosine deaminase RNA specific 1 and apolipoprotein B mRNA editing enzyme catalytic subunit 3A that are critical for glioma progression.¹²⁶ METTL3 was also shown to drive glioma growth by regulating the alternative splicing machinery and SRSFs.¹²⁰ These contradictory suggestions for the role of METTL3 to act both as a tumor suppressor or promoter by different studies might be due to the high heterogeneity of gliomas. Increased levels of the methyltransferase subunit WTAP, as well as m^6A erasers, ALKBH5 and FTO, were shown to predict poor prognosis in GBM patients.^{127–129} Particularly, ALKBH5 demethylates and stabilizes a master transcription factor forkhead box M1 that activates proliferation-inducing genes in GSCs, and its depletion or inhibition prevented migration and invasiveness of GBM cells in mice.^{129,130} Treatment of GSCs with FTO inhibitors, meclofenamic acid or R-2-hydroxyglutarate, suppressed their growth,

stemness, and tumorigenicity.^{124,131} Expression of m^6A readers (YTHDF1, YTHDF2, YTHDF3, and YTHDC2) was also shown to be upregulated in GBMs.¹²¹ YTHDC1 deficiency reduced the sphere formation capacity of GBM cells.¹²⁰ Collectively, the above studies suggest that m^6A signaling is integral to GBM pathogenesis.

Other CNS disorders

The m^6A RNA methylation is also shown to be involved in various other neurodevelopmental and neuropsychiatric conditions. Certain genetic variants of FTO strongly confer risk for major depressive disorder (MDD) and attention-deficit/hyperactivity disorder.^{132–135} An SNP in ALKBH5 is associated with various clinical features of MDD, including anxiety, retardation, and cognitive dysfunction.¹³⁶ ALKBH5 was further shown to demethylate and stabilize a mood-associated transcript *FAAH* that contributes to depression-like behavior in mice.¹³⁷

ALKBH5 was also found to be a causative gene for a rare neurodevelopmental disorder called Smith–Magenis syndrome.¹³⁸ The m⁶A reader YTHDC2 was reported to be a potential risk locus for the Autism spectrum disorder in the Japanese population.¹³⁹ The m⁶A SNP (G to A mutation) in the METTL21B gene was found to correlate with multiple sclerosis.¹⁴⁰ Overall, key regulators of m⁶A methylation are implicated in various CNS disorders.

Conclusion and future directions

The brain has evolved multiple RNA regulatory mechanisms to optimize energy expenditure, plasticity, and spatial regulation in polarized structures. Conceptually, these mechanisms involve binding RBPs and noncoding RNAs to the sequence elements present in the RNA, thus recruiting diverse cellular machinery to affect the fate of target RNA.¹⁴¹ Additionally, RNA acquires chemical modifications to ascertain the fidelity and dynamics of this process. To that end, m⁶A methylation is an epitranscriptomic mechanism primarily employed by neurons to tightly control gene expression during development and disease (Figure 5). More importantly, the reversible nature of m⁶A makes it a powerful tool to achieve context- and stimulus-dependent regulation. Among the two well-characterized m⁶A erasers, FTO is dysregulated in various CNS pathologies ranging from brain tumors to acute and chronic brain damage. The lack of compensatory demethylation by ALKBH5 indicates that each eraser controls distinct targets in the brain.

Although the m⁶A dysregulation was observed in various neurological diseases, its functional significance in disease outcomes is not yet completely understood. A challenge is to understand the functional significance of hundreds of transcripts of distinct functional classes that show m⁶A methylation. However, new studies are emerging to show that dosing (number of m⁶A consensus motifs and their methylation at a given time) is important to promote a functional change.¹⁴² Techniques like CRISPR-based site-specific m⁶A editing might allow studying its role in disease outcomes.^{50–52} Currently, several small-molecule inhibitors of m⁶A editing enzymes are also available to decipher the roles of writers, erasers, and readers.^{130,143–148} Finally, several studies showed the cross-talk between m⁶A and other regulators such as epigenetic modifications and microbiome.^{80,81,93,149}

Acknowledgements

Figures are created using Biorender.com.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was partly supported by the NIH grant RO1 NS109459 and AHA grant 20PRE35120233.

ORCID iD

Anil K Chokkalla <https://orcid.org/0000-0002-0101-0417>

References

1. Boccaletto P, Machnicka MA, Purta E, et al. MODOMICS: a database of RNA modification pathways. 2017 update. *Nucleic Acids Res* 2018; 46: D303–D307.
2. Jung Y and Goldman D. Role of RNA modifications in brain and behavior. *Genes Brain Behav* 2018; 17: e12444.
3. Desrosiers R, Friderici K and Rottman F. Identification of methylated nucleosides in messenger RNA from novikoff hepatoma cells. *Proc Natl Acad Sci USA* 1974; 71: 3971–3975.
4. Wei CM, Gershowitz A and Moss B. Methylated nucleotides block 5' terminus of HeLa cell messenger RNA. *Cell* 1975; 4: 379–386.
5. Adams JM and Cory S. Modified nucleosides and bizarre 5'-termini in mouse myeloma mRNA. *Nature* 1975; 255: 28–33.
6. Meyer KD, Saletore Y, Zumbo P, et al. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell* 2012; 149: 1635–1646.
7. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, et al. Topology of the human and mouse m⁶A RNA methylomes revealed by m⁶A-seq. *Nature* 2012; 485: 201–206.
8. Liu N, Parisien M, Dai Q, et al. Probing N⁶-methyladenosine RNA modification status at single nucleotide resolution in mRNA and long noncoding RNA. *RNA* 2013; 19: 1848–1856.
9. Widagdo J and Anggono V. The m⁶A-epitranscriptomic signature in neurobiology: from neurodevelopment to brain plasticity. *J Neurochem* 2018; 147: 137–152.
10. Ries RJ, Zaccara S, Klein P, et al. m(6)a enhances the phase separation potential of mRNA. *Nature* 2019; 571: 424–428.
11. Widagdo J, Zhao QY, Kempen MJ, et al. Experience-dependent accumulation of N⁶-methyladenosine in the prefrontal cortex is associated with memory processes in mice. *J Neurosci* 2016; 36: 6771–6777.
12. Merkurjev D, Hong WT, Iida K, et al. Synaptic N(6)-methyladenosine (m(6)A) epitranscriptome reveals functional partitioning of localized transcripts. *Nat Neurosci* 2018; 21: 1004–1014.
13. Chang M, Lv H, Zhang W, et al. Region-specific RNA m(6)a methylation represents a new layer of control in

- the gene regulatory network in the mouse brain. *Open Biol* 2017; 7: 170166.
14. Liu J, Yue Y, Han D, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. *Nat Chem Biol* 2014; 10: 93–95.
 15. Scholler E, Weichmann F, Treiber T, et al. Interactions, localization, and phosphorylation of the m(6)a generating METTL3-METTL14-WTAP complex. *RNA* 2018; 24: 499–512.
 16. Yoon KJ, Ringeling FR, Vissers C, et al. Temporal control of mammalian cortical neurogenesis by m(6)a methylation. *Cell* 2017; 171: 877.e17–889.e17.
 17. Geula S, Moshitch-Moshkovitz S, Dominissini D, et al. Stem cells. m6A mRNA methylation facilitates resolution of naive pluripotency toward differentiation. *Science* 2015; 347: 1002–1006.
 18. Horiuchi K, Umetani M, Minami T, et al. Wilms' tumor 1-associating protein regulates G2/M transition through stabilization of cyclin A2 mRNA. *Proc Natl Acad Sci USA* 2006; 103: 17278–17283.
 19. Yue Y, Liu J, Cui X, et al. VIRMA mediates preferential m(6)a mRNA methylation in 3'UTR and near stop codon and associates with alternative polyadenylation. *Cell Discov* 2018; 4: 10.
 20. Shi H, Wei J and He C. Where, when, and how: context-dependent functions of RNA methylation writers, readers, and erasers. *Mol Cell* 2019; 74: 640–650.
 21. Gershoni-Emek N, Mazza A, Chein M, et al. Proteomic analysis of dynein-Interacting proteins in amyotrophic lateral sclerosis synaptosomes reveals alterations in the RNA-binding protein Staufen1. *Mol Cell Proteomics* 2016; 15: 506–522.
 22. Jia G, Fu Y, Zhao X, et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. *Nat Chem Biol* 2011; 7: 885–887.
 23. Zheng G, Dahl JA, Niu Y, et al. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. *Mol Cell* 2013; 49: 18–29.
 24. Gulati P, Avezov E, Ma M, et al. Fat mass and obesity-related (FTO) shuttles between the nucleus and cytoplasm. *Biosci Rep* 2014; 34.
 25. Yu J, Chen M, Huang H, et al. Dynamic m6A modification regulates local translation of mRNA in axons. *Nucleic Acids Res* 2018; 46: 1412–1423.
 26. Wei J, Liu F, Lu Z, et al. Differential m(6)A, m(6)Am, and m(1)A demethylation mediated by FTO in the cell nucleus and cytoplasm. *Mol Cell* 2018; 71: 973.e5–985.e5.
 27. Hess ME, Hess S, Meyer KD, et al. The fat mass and obesity associated gene (fto) regulates activity of the dopaminergic midbrain circuitry. *Nat Neurosci* 2013; 16: 1042–1048.
 28. Li L, Zang L, Zhang F, et al. Fat mass and obesity-associated (FTO) protein regulates adult neurogenesis. *Hum Mol Genet* 2017; 26: 2398–2411.
 29. Gao X, Shin YH, Li M, et al. The fat mass and obesity associated gene FTO functions in the brain to regulate postnatal growth in mice. *PLoS One* 2010; 5: e14005.
 30. Boissel S, Reish O, Proulx K, et al. Loss-of-function mutation in the dioxygenase-encoding *FTO* gene causes severe growth retardation and multiple malformations. *Am J Hum Genet* 2009; 85: 106–111.
 31. Zou S, Toh JD, Wong KH, et al. N(6)-methyladenosine: a conformational marker that regulates the substrate specificity of human demethylases FTO and ALKBH5. *Sci Rep* 2016; 6: 25677.
 32. Tsujikawa K, Koike K, Kitae K, et al. Expression and sub-cellular localization of human ABH family molecules. *J Cell Mol Med* 2007; 11: 1105–1116.
 33. Song H, Wang Y, Wang R, et al. SFPQ is an FTO-binding protein that facilitates the demethylation substrate preference. *Cell Chem Biol* 2020; 27: 283.e6–291.e6.
 34. Zhu T, Roundtree IA, Wang P, et al. Crystal structure of the YTH domain of YTHDF2 reveals mechanism for recognition of N6-methyladenosine. *Cell Res* 2014; 24: 1493–1496.
 35. Luo S and Tong L. Molecular basis for the recognition of methylated adenines in RNA by the eukaryotic YTH domain. *Proc Natl Acad Sci USA* 2014; 111: 13834–13839.
 36. Patil DP, Chen CK, Pickering BF, et al. m(6)a RNA methylation promotes XIST-mediated transcriptional repression. *Nature* 2016; 537: 369–373.
 37. Roost C, Lynch SR, Batista PJ, et al. Structure and thermodynamics of N6-methyladenosine in RNA: a spring-loaded base modification. *J Am Chem Soc* 2015; 137: 2107–2115.
 38. Liu N, Dai Q, Zheng G, et al. N(6)-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions. *Nature* 2015; 518: 560–564.
 39. Alarcon CR, Lee H, Goodarzi H, et al. N6-methyladenosine marks primary microRNAs for processing. *Nature* 2015; 519: 482–485.
 40. Edupuganti RR, Geiger S, Lindeboom RG, et al. N(6)-methyladenosine (m(6)A) recruits and repels proteins to regulate mRNA homeostasis. *Nat Struct Mol Biol* 2017; 24: 870–878.
 41. Huang H, Weng H, Sun W, et al. Recognition of RNA N(6)-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. *Nat Cell Biol* 2018; 20: 285–295.
 42. Wu R, Li A, Sun B, et al. A novel m(6)a reader Prrc2a controls oligodendroglial specification and myelination. *Cell Res* 2019; 29: 23–41.
 43. Edens BM, Vissers C, Su J, et al. FMRP modulates neural differentiation through m(6)A-dependent mRNA nuclear export. *Cell Rep* 2019; 28: 845.e5–854.e5.
 44. Bringmann P and Luhrmann R. Antibodies specific for N6-methyladenosine react with intact snRNPs U2 and U4/U6. *FEBS Lett* 1987; 213: 309–315.
 45. Thuring K, Schmid K, Keller P, et al. Analysis of methylated RNA. *Methods Mol Biol* 2017; 1562: 3–18.
 46. Linder B, Grozhik AV, Olarerin-George AO, et al. Single-nucleotide-resolution mapping of m6A and

- m6Am throughout the transcriptome. *Nat Methods* 2015; 12: 767–772.
47. Maurer J and Jaffrey SR. FTO, m(6) am, and the hypothesis of reversible epitranscriptomic mRNA modifications. *FEBS Lett* 2018; 592: 2012–2022.
 48. Garcia-Campos MA, Edelheit S, Toth U, et al. Deciphering the “m(6)a code” via antibody-independent quantitative profiling. *Cell* 2019; 178: 731e16–747.e16.
 49. Meyer KD. DART-seq: an antibody-free method for global m(6)a detection. *Nat Methods* 2019; 16: 1275–1280.
 50. Rauch S, He C and Dickinson BC. Targeted m(6)a reader proteins to study epitranscriptomic regulation of single RNAs. *J Am Chem Soc* 2018; 140: 11974–11981.
 51. Liu XM, Zhou J, Mao Y, et al. Programmable RNA N(6)-methyladenosine editing by CRISPR-Cas9 conjugates. *Nat Chem Biol* 2019; 15: 865–871.
 52. Li J, Chen Z, Chen F, et al. Targeted mRNA demethylation using an engineered dCas13b-ALKBH5 fusion protein. *Nucleic Acids Res* 2020; 48: 5684–5694.
 53. Chokkalla AK, Mehta SL, Kim T, et al. Transient focal ischemia significantly alters the m(6)a epitranscriptomic tagging of RNAs in the brain. *Stroke* 2019; 50: 2912–2921.
 54. Wu Q, Yuan X, Han R, et al. Epitranscriptomic mechanisms of N6-methyladenosine methylation regulating mammalian hypertension development by determined spontaneously hypertensive rats pericytes. *Epigenomics* 2019; 11: 1359–1370.
 55. Zhou J, Wan J, Gao X, et al. Dynamic m(6)a mRNA methylation directs translational control of heat shock response. *Nature* 2015; 526: 591–594.
 56. Fry NJ, Law BA, Ilkayeva OR, et al. N(6)-methyladenosine is required for the hypoxic stabilization of specific mRNAs. *RNA* 2017; 23: 1444–1455.
 57. Ping XL, Sun BF, Wang L, et al. Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. *Cell Res* 2014; 24: 177–189.
 58. Chen M and Manley JL. Mechanisms of alternative splicing regulation: insights from molecular and genomics approaches. *Nat Rev Mol Cell Biol* 2009; 10: 741–754.
 59. Zhao X, Yang Y, Sun BF, et al. FTO-dependent demethylation of N6-methyladenosine regulates mRNA splicing and is required for adipogenesis. *Cell Res* 2014; 24: 1403–1419.
 60. Xiao W, Adhikari S, Dahal U, et al. Nuclear m(6)a reader YTHDC1 regulates mRNA splicing. *Mol Cell* 2016; 61: 507–519.
 61. Wickramasinghe VO and Laskey RA. Control of mammalian gene expression by selective mRNA export. *Nat Rev Mol Cell Biol* 2015; 16: 431–442.
 62. Lesbirel S, Viphakone N, Parker M, et al. The m(6)A-methylase complex recruits TREX and regulates mRNA export. *Sci Rep* 2018; 8: 13827.
 63. Roundtree IA, Luo GZ, Zhang Z, et al. YTHDC1 mediates nuclear export of N(6)-methyladenosine methylated mRNAs. *Elife* 2017; 6: e31311.
 64. Hsu PJ, Shi H, Zhu AC, et al. The RNA-binding protein FMRP facilitates the nuclear export of N(6)-methyladenosine-containing mRNAs. *J Biol Chem* 2019; 294: 19889–19895.
 65. Bolognani F and Perrone-Bizzozero NI. RNA-protein interactions and control of mRNA stability in neurons. *J Neurosci Res* 2008; 86: 481–489.
 66. Zhang F, Kang Y, Wang M, et al. Fragile X mental retardation protein modulates the stability of its m6A-marked messenger RNA targets. *Hum Mol Genet* 2018; 27: 3936–3950.
 67. Sommer S, Lavi U and Darnell JE Jr. The absolute frequency of labeled N-6-methyladenosine in HeLa cell messenger RNA decreases with label time. *J Mol Biol* 1978; 124: 487–499.
 68. Wang X, Zhao BS, Roundtree IA, et al. N(6)-methyladenosine modulates messenger RNA translation efficiency. *Cell* 2015; 161: 1388–1399.
 69. Li A, Chen YS, Ping XL, et al. Cytoplasmic m(6)a reader YTHDF3 promotes mRNA translation. *Cell Res* 2017; 27: 444–447.
 70. Meyer KD, Patil DP, Zhou J, et al. 5' UTR m(6)a promotes Cap-Independent translation. *Cell* 2015; 163: 999–1010.
 71. Yang Y, Fan X, Mao M, et al. Extensive translation of circular RNAs driven by N(6)-methyladenosine. *Cell Res* 2017; 27: 626–641.
 72. Wang X, Lu Z, Gomez A, et al. N6-methyladenosine-dependent regulation of messenger RNA stability. *Nature* 2014; 505: 117–120.
 73. Du H, Zhao Y, He J, et al. YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex. *Nat Commun* 2016; 7: 12626.
 74. Park OH, Ha H, Lee Y, et al. Endoribonucleolytic cleavage of m(6)A-Containing RNAs by RNase P/ MRP complex. *Mol Cell* 2019; 74: 494.e8–507.e8.
 75. Yang X, Liu QL, Xu W, et al. m(6)a promotes R-loop formation to facilitate transcription termination. *Cell Res* 2019; 29: 1035–1038.
 76. Abakir A, Giles TC, Cristini A, et al. N(6)-methyladenosine regulates the stability of RNA:DNA hybrids in human cells. *Nat Genet* 2020; 52: 48–55.
 77. Liu J, Dou X, Chen C, et al. N(6)-methyladenosine of chromosome-associated regulatory RNA regulates chromatin state and transcription. *Science* 2020; 367: 580–586.
 78. Yao B, Christian KM, He C, et al. Epigenetic mechanisms in neurogenesis. *Nat Rev Neurosci* 2016; 17: 537–549.
 79. Li M, Zhao X, Wang W, et al. Ythdf2-mediated m(6)a mRNA clearance modulates neural development in mice. *Genome Biol* 2018; 19: 69.
 80. Wang Y, Li Y, Yue M, et al. N(6)-methyladenosine RNA modification regulates embryonic neural stem cell self-renewal through histone modifications. *Nat Neurosci* 2018; 21: 195–206.
 81. Chen J, Zhang YC, Huang C, et al. m⁶A regulates neurogenesis and neuronal development by modulating histone methyltransferase Ezh2. *Genomics Proteomics Bioinformatics* 2019; 17: 154–168.

82. Spychala A and Ruther U. FTO affects hippocampal function by regulation of BDNF processing. *PLoS One* 2019; 14: e0211937.
83. Xu H, Dzhashiashvili Y, Shah A, et al. m⁶A mRNA methylation is essential for oligodendrocyte maturation and CNS myelination. *Neuron* 2020; 105: 293.e5–309.e5.
84. Wang CX, Cui GS, Liu X, et al. METTL3-mediated m⁶A modification is required for cerebellar development. *PLoS Biol* 2018; 16: e2004880.
85. Ma C, Chang M, Lv H, et al. RNA m⁶A methylation participates in regulation of postnatal development of the mouse cerebellum. *Genome Biol* 2018; 19: 68.
86. Zhuang M, Li X, Zhu J, et al. The m⁶A reader YTHDF1 regulates axon guidance through translational control of Robo3.1 expression. *Nucleic Acids Res* 2019; 47: 4765–4777.
87. Shi H, Zhang X, Weng YL, et al. m⁶A facilitates hippocampus-dependent learning and memory through YTHDF1. *Nature* 2018; 563: 249–253.
88. Koranda JL, Dore L, Shi H, et al. Mettl14 is essential for epitranscriptomic regulation of striatal function and learning. *Neuron* 2018; 99: 283.e5–292.e5.
89. Fustin J-M, Doi M, Yamaguchi Y, et al. RNA-methylation-dependent RNA processing controls the speed of the circadian clock. *Cell* 2013; 155: 793–806.
90. Fustin JM, Kojima R, Itoh K, et al. Two Ckldelta transcripts regulated by m⁶A methylation code for two antagonistic kinases in the control of the circadian clock. *Proc Natl Acad Sci USA* 2018; 115: 5980–5985.
91. Walters BJ, Mercaldo V, Gillon CJ, et al. The role of the RNA demethylase FTO (fat mass and obesity-associated) and mRNA methylation in hippocampal memory formation. *Neuropsychopharmacology* 2017; 42: 1502–1510.
92. Wang L, Liu X, Luo X, et al. Genetic variants in the fat mass- and obesity-associated (FTO) gene are associated with alcohol dependence. *J Mol Neurosci* 2013; 51: 416–424.
93. Sun L, Ma L, Zhang H, et al. Fto deficiency reduces anxiety- and depression-like behaviors in mice via alterations in gut microbiota. *Theranostics* 2019; 9: 721–733.
94. Anders M, Chelysheva I, Goebel I, et al. Dynamic m(6)a methylation facilitates mRNA triaging to stress granules. *Life Sci Alliance* 2018; 1: e201800113.
95. Xiang Y, Laurent B, Hsu CH, et al. RNA m(6)a methylation regulates the ultraviolet-induced DNA damage response. *Nature* 2017; 543: 573–576.
96. Engel M, Eggert C, Kaplick PM, et al. The role of m(6) a/m-RNA methylation in stress response regulation. *Neuron* 2018; 99: 389.e9–403.e9.
97. Vemuganti R. Stroke: molecular mechanisms and therapies. *Neuromolecular Med* 2019; 21: 323–324.
98. Bertoglia MJ, Morris-Blanco KC and Vemuganti R. Epigenetic mechanisms of neurodegenerative diseases and acute brain injury. *Neurochem Int* 2020; 133: 104642.
99. Morris-Blanco KC, Chokkalla AK, Bertoglia MJ, et al. TET3 regulates DNA hydroxymethylation of neuroprotective genes following focal ischemia. *J Cereb Blood Flow Metab.* Epub ahead of print 2020. <https://doi.org/10.1177/0271678X20912965>
100. Winter Y, Back T, Scherag A, et al. Evaluation of the obesity genes FTO and MC4R and the type 2 diabetes mellitus gene TCF7L2 for contribution to stroke risk: the Mannheim–Heidelberg stroke study. *Obes Facts* 2011; 4: 290–296.
101. Mo XB, Lei SF and Zhang Yh Zhang H. Integrative analysis identified IRF6 and NDST1 as potential causal genes for ischemic stroke. *Front Neurol* 2019; 10: 517.
102. Xu K, Mo Y, Li D, et al. N(6)-methyladenosine demethylases Alkbh5/fto regulate cerebral ischemia-reperfusion injury. *Ther Adv Chronic Dis* 2020; 11: 2040622320916024.
103. Kim W, Kang MS, Kim TH, et al. Ischemia-related changes of fat-mass and obesity-associated protein expression in the gerbil hippocampus. *Metab Brain Dis* 2020; 35: 335–342.
104. Diao MY, Zhu Y, Yang J, et al. Hypothermia protects neurons against ischemia/reperfusion-induced pyroptosis via m⁶A-mediated activation of PTEN and the PI3K/akt/GSK-3beta signaling pathway. *Brain Res Bull* 2020; 159: 25–31.
105. Wang LJ, Xue Y, Li H, 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* 2020; 24: 4981–4991.
106. Wang LJ, Xue Y, Huo R, et al. N6-methyladenosine methyltransferase METTL3 affects the phenotype of cerebral arteriovenous malformation via modulating notch signaling pathway. *J Biomed Sci* 2020; 27: 62.
107. Menorca RM, Fussell TS and Elfär JC. Nerve physiology: mechanisms of injury and recovery. *Hand Clin* 2013; 29: 317–330.
108. Weng YL, An R, Cassin J, et al. An intrinsic epigenetic barrier for functional axon regeneration. *Neuron* 2017; 94: 337.e6–346.e6.
109. Weng YL, Wang X, An R, et al. Epitranscriptomic m(6) a regulation of axon regeneration in the adult mammalian nervous system. *Neuron* 2018; 97: 313.e6–325.e6.
110. Wang Y, Mao J, Wang X, et al. Genome-wide screening of altered m⁶A-tagged transcript profiles in the hippocampus after traumatic brain injury in mice. *Epigenomics* 2019; 11: 805–819.
111. Yu J, Zhang Y, Ma H, et al. Epitranscriptomic profiling of N6-methyladenosine-related RNA methylation in rat cerebral cortex following traumatic brain injury. *Mol Brain* 2020; 13: 11.
112. Esposito M and Sherr GL. Epigenetic modifications in Alzheimer's neuropathology and therapeutics. *Front Neurosci* 2019; 13: 476.
113. Han M, Liu Z, Xu Y, et al. Abnormality of m⁶A mRNA methylation is involved in Alzheimer's disease. *Front Neurosci* 2020; 14: 98.
114. Li H, Ren Y, Mao K, et al. FTO is involved in Alzheimer's disease by targeting TSC1-mTOR-Tau

- signaling. *Biochem Biophys Res Commun* 2018; 498: 234–239.
115. Keller L, Xu W, Wang HX, et al. The obesity related gene, FTO, interacts with APOE, and is associated with Alzheimer's disease risk: a prospective cohort study. *J Alzheimers Dis* 2011; 23: 461–469.
 116. Reitz C, Tosto G, Mayeux R, et al.; The NIA-LOAD/ NCRAD Family Study Group and the Alzheimer's Disease Neuroimaging Initiative. Genetic variants in the fat and obesity associated (FTO) gene and risk of Alzheimer's disease. *PLoS One* 2012; 7: e50354.
 117. Ai SX, Xu Q, Hu YC, et al. Hypomethylation of SNCA in blood of patients with sporadic Parkinson's disease. *J Neurol Sci* 2014; 337: 123–128.
 118. Leng Y and Chuang DM. Endogenous alpha-synuclein is induced by valproic acid through histone deacetylase inhibition and participates in neuroprotection against glutamate-induced excitotoxicity. *J Neurosci* 2006; 26: 7502–7512.
 119. Chen X, Yu C, Guo M, et al. Down-regulation of m6A mRNA methylation is involved in dopaminergic neuronal death. *ACS Chem Neurosci* 2019; 10: 2355–2363.
 120. Li F, Yi Y, Miao Y, et al. N(6)-methyladenosine modulates nonsense-mediated mRNA decay in human glioblastoma. *Cancer Res* 2019; 79: 5785–5798.
 121. Wang W, Li J, Lin F, et al. Identification of N(6)-methyladenosine-related lncRNAs for patients with primary glioblastoma. *Neurosurg Rev*. Epub ahead of print 2020. <https://link.springer.com/article/10.1007/s10143-020-01238-x>
 122. Chai RC, Wu F, Wang QX, et al. m⁶A RNA methylation regulators contribute to malignant progression and have clinical prognostic impact in gliomas. *Aging (Albany NY)* 2019; 11: 1204–1225.
 123. Visvanathan A, Patil V, Arora A, et al. Essential role of METTL3-mediated m(6)a modification in glioma stem-like cells maintenance and radioresistance. *Oncogene* 2018; 37: 522–533.
 124. Cui Q, Shi H, Ye P, et al. m(6)a RNA methylation regulates the self-renewal and tumorigenesis of glioblastoma stem cells. *Cell Rep* 2017; 18: 2622–2634.
 125. Li F, Zhang C and Zhang G. m6A RNA methylation controls proliferation of human glioma cells by influencing cell apoptosis. *Cytogenet Genome Res* 2019; 159: 119–125.
 126. Visvanathan A, Patil V, Abdulla S, et al. N(6)-methyladenosine landscape of glioma stem-like cells: METTL3 is essential for the expression of actively transcribed genes and sustenance of the oncogenic signaling. *Genes (Basel)* 2019; 10: 141.
 127. Jin DI, Lee SW, Han ME, et al. Expression and roles of Wilms' tumor 1-associating protein in glioblastoma. *Cancer Sci* 2012; 103: 2102–2109.
 128. Xi Z, Xue Y, Zheng J, et al. WTAP expression predicts poor prognosis in malignant glioma patients. *J Mol Neurosci* 2016; 60: 131–136.
 129. Zhang S, Zhao BS, Zhou A, et al. m⁶A demethylase ALKBH5 maintains tumorigenicity of glioblastoma stem-like cells by sustaining FOXM1 expression and cell proliferation program. *Cancer Cell* 2017; 31: 591.e6–606.e6.
 130. Malacrida A, Rivara M, Di Domizio A, et al. 3D proteome-wide scale screening and activity evaluation of a new ALKBH5 inhibitor in U87 glioblastoma cell line. *Bioorg Med Chem* 2020; 28: 115300.
 131. Su R, Dong L, Li C, et al. R-2HG exhibits anti-tumor activity by targeting FTO/m(6)a/MYC/CEBPA signaling. *Cell* 2018; 172: 90.e23–105.e23.
 132. Milaneschi Y, Lamers F, Mbarek H, et al. The effect of FTO rs9939609 on major depression differs across MDD subtypes. *Mol Psychiatry* 2014; 19: 960–962.
 133. Samaan Z, Anand SS, Zhang X, et al. The protective effect of the obesity-associated rs9939609 a variant in fat mass- and obesity-associated gene on depression. *Mol Psychiatry* 2013; 18: 1281–1286.
 134. Velders FP, De Wit JE, Jansen PW, et al. FTO at rs9939609, food responsiveness, emotional control and symptoms of ADHD in preschool children. *PLoS One* 2012; 7: e49131.
 135. Choudhry Z, Sengupta SM, Grizenko N, et al. Association between obesity-related gene FTO and ADHD. *Obesity (Silver Spring)* 2013; 21: E738–E744.
 136. Du T, Rao S, Wu L, et al. An association study of the m6A genes with major depressive disorder in Chinese Han population. *J Affect Disord* 2015; 183: 279–286.
 137. Huang R, Zhang Y and Bai Y. et al. N⁶-Methyladenosine modification of fatty acid amide hydrolase messenger RNA in circular RNA STAG1-regulated astrocyte dysfunction and depressive-like behaviors. *Biol Psychiatry* 2020; 88: 392–404.
 138. Ricard G, Molina J, Chrast J, et al. Phenotypic consequences of copy number variation: insights from Smith–Magenis and Potocki–Lupski syndrome mouse models. *PLoS Biol* 2010; 8: e1000543.
 139. Liu X, Shimada T, Otowa T, et al. Genome-wide association study of autism spectrum disorder in the East Asian populations. *Autism Res* 2016; 9: 340–349.
 140. Mo XB, Lei SF, Qian QY, et al. Integrative analysis revealed potential causal genetic and epigenetic factors for multiple sclerosis. *J Neurol* 2019; 266: 2699–2709.
 141. Darnell RB. RNA protein interaction in neurons. *Annu Rev Neurosci* 2013; 36: 243–270.
 142. Lewis CJ, Pan T and Kalsotra A. RNA modifications and structures cooperate to guide RNA-protein interactions. *Nat Rev Mol Cell Biol* 2017; 18: 202–210.
 143. Huang Y, Su R, Sheng Y, et al. Small-molecule targeting of oncogenic FTO demethylase in acute myeloid leukemia. *Cancer Cell* 2019; 35: 677.e10–691.e10.
 144. Su R, Dong L, Li Y, et al. Effective novel FTO inhibitors show potent anti-cancer efficacy and suppress drug resistance. *Blood* 2019; 134: 233.
 145. Zheng G, Cox T, Tribbey L, et al. Synthesis of a FTO inhibitor with anticonvulsant activity. *ACS Chem Neurosci* 2014; 5: 658–665.
 146. Huang Y, Yan J, Li Q, et al. Meclofenamic acid selectively inhibits FTO demethylation of m6A over ALKBH5. *Nucleic Acids Res* 2015; 43: 373–384.

147. Peng S, Xiao W, Ju D, et al. Identification of entacapone as a chemical inhibitor of FTO mediating metabolic regulation through FOXO1. *Sci Transl Med* 2019; 11: eaau7116.
148. Selberg S, Blokhina D, Aatonen M, et al. Discovery of small molecules that activate RNA methylation through cooperative binding to the METTL3-14-WTAP complex active site. *Cell Rep* 2019; 26: 3762.e5–3771.e5.
149. Jabs S, Biton A, Becavin C, et al. Impact of the gut microbiota on the m(6)a epitranscriptome of mouse cecum and liver. *Nat Commun* 2020; 11: 1344.