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Advances in brain epitranscriptomics research and translational opportunities

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Abstract

Various chemical modifications of all RNA transcripts, or epitranscriptomics, have emerged as crucial regulators of RNA metabolism, attracting significant interest from both basic and clinical researchers due to their diverse functions in biological processes and immense clinical potential as highlighted by the recent profound success of RNA modifications in improving COVID-19 mRNA vaccines. Rapid accumulation of evidence underscores the critical involvement of various RNA modifications in governing normal neural development and brain functions as well as pathogenesis of brain disorders. Here we provide an overview of RNA modifications and recent advancements in epitranscriptomic studies utilizing animal models to elucidate important roles of RNA modifications in regulating mammalian neurogenesis, gliogenesis, synaptic formation, and brain function. Moreover, we emphasize the pivotal involvement of RNA modifications and their regulators in the pathogenesis of various human brain disorders, encompassing neurodevelopmental disorders, brain tumors, psychiatric and neurodegenerative disorders. Furthermore, we discuss potential translational opportunities afforded by RNA

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modifications in combatting brain disorders, including their use as biomarkers, in the development of drugs or gene therapies targeting epitranscriptomic pathways, and in applications for mRNA-based vaccines and therapies. We also address current limitations and challenges hindering the widespread clinical application of epitranscriptomic research, along with the improvements necessary for future progress.

INTRODUCTION

In a classic view of molecular biology, coded genetic information flows from double-stranded DNA to single-stranded RNA during transcription, and then to protein during translation [1]. More than 170 chemically distinct modifications of all kinds of RNA transcripts, named epitranscriptomics, orchestrate the transcription and translation of genetic information and the dynamic and reversible RNA modifications are analogous to the epigenetic code formed by DNA and histone modifications [2–7]. Accumulative evidence has demonstrated diverse and important roles of epitranscriptomics in modulating nearly all aspects of RNA metabolism to regulate various biological processes, including development, aging, and diseases [8–11]. The translational potential of epitranscriptomics has been highlighted by the recent success of COVID-19 mRNA vaccines utilizing a N1-methylpseudouridine modification to reduce immunogenicity and enhance efficacy [12]. Notably, epitranscriptomics also play important roles in the nervous system, ranging from neural development, circuit activities, to pathology, including psychiatric disorders [13–18]. Here we review recent advances in our understanding of epitranscriptomic mechanisms in healthy and diseased mammalian brains. We also discuss translational opportunities and new directions of epitranscriptomic research of the nervous system. Interested readers can consult general reviews regarding epitranscriptomics [7, 8, 10, 19–22].

GENERAL INTRODUCTION OF EPITRANSCRIPTOMICS

Although RNA modifications were discovered about a century ago, only recently has our knowledge of modification sites and functions expanded significantly, mainly due to the development of mass-spectrometry and high-throughput sequencing methods for mapping modification sites, some at single-nucleotide resolution, and identification of writers, readers, and erasers for different modifications [2, 4, 6, 7, 23–33] (Table S1). RNA modifications have been found in different types of RNA, including messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), microRNA (miRNA), long non-coding RNA, small nuclear RNA, small nucleolar RNA (snoRNA), Piwi-interacting RNA (piRNA), chromosome-related regulatory RNAs (CarRNAs), enhancer RNA (eRNA), circular RNA, and mitochondrial RNA [5, 7, 8, 19, 20, 22, 34–74] (Fig. 1 and Table S2). The types of RNA modifications are also very diverse, including N^6-methyladenosine (m^6A), pseudo-uridine (Ψ), C^5- methylcytosine (m^5C), N^3-methylcytosine (m^3C), N^1-methyladenosine (m^1A), 2′-O-methylation, 2'-O-methyladenosine (m^6Am), N^7-methylguanosine (m^7G), A-to-I editing, glycosylated RNA and various tRNA modifications (Fig. 1 and Table S2). Beyond being the byproduct of passive incorporation of modified ribonucleotides, RNA modifications are often actively deposited on specific sites by "writer" proteins, potentially suppressed by "suppressor" factors, recognized and decoded

by “reader” proteins, and eliminated by “eraser” proteins, rendering the epitranscriptome reversible and dynamic. RNA modifications are critically involved in regulating almost every aspect of RNA metabolism, including transcription, splicing, decay, stability, translation, translocation, catalytic function, interaction with proteins, charging, and RNA structure (Fig. 1 and Table S2). Thus, it is not surprising that RNA modifications have been shown to play vital roles in regulating the nervous system, ranging from neurodevelopment and physiological brain functions to mental disorders.

ROLES AND MECHANISMS OF RNA MODIFICATIONS REVEALED IN ANIMAL STUDIES

Neurogenesis and neuronal development

Neurogenesis involves proliferation and differentiation of neural progenitor cells (NPCs) and migration and maturation of immature neurons, and these processes are precisely coordinated to generate layered or nuclear structures and functional neuronal connectivity of the brain [75, 76]. An increasing number of studies via genetic manipulation of writers, readers, and erasers of various RNA modifications using animal and human cellular models has demonstrated extensive involvement of epitranscriptomics in regulating diverse aspects of neurogenesis (Fig. 2).

The most well-studied example is m^6A mRNA modification, the most abundant internal modification of mRNA. The level of m^6A RNA modification in the brain is dynamic, which is low at embryonic stages and elevated in adulthood [23]. Deletion of m^6A methyltransferases *Mettl14* and *Mettl3*, or m^6A reader *Fmr1*, or double knockout of m^6A readers *Ythdf1/2*, lead to similar defects in mouse cortical development, with some exceptions, that are generally characterized by prolonged cell cycle of NPCs, decreased generation of neurons in the cortex, and a smaller brain size [18, 37, 77–80] (Fig. 2a). In contrast, depletion of m^6A erasers, *Fto* and *Alkbh5*, does not appear to affect embryonic brain development [18, 77, 78] except under exposure to hypobaric hypoxia, which leads to aberrant NPC proliferation and differentiation in the postnatal cerebellum of mice with *Alkbh5* deletion [81]. It has been proposed that mRNA m^6A modification ensures the degradation of the old and pre-patterned mRNA and the translation of new mRNA in a fast and precise fashion to enhance the temporal resolution and accuracy of transcriptome/proteome changes during NPC differentiation [18]. With more m^6A modification regulators being identified, such as methyltransferase *Mettl16* and m^6A readers *Ythdc1/2*, *Prrc2a*, and *Igf2bp1/2/3*, characterization of neurogenesis phenotypes caused by genetic manipulation of these genes in a spatiotemporal conditional fashion will greatly expand our knowledge of functions and mechanisms of m^6A mRNA modification in regulating development of the nervous system.

In addition to m^6A modifications on mRNA, modifications of other types of RNA also regulate neurogenesis. For example, knockdown of *Mettl5*, which encodes an 18S rRNA m^6A methyltransferase, causes a microcephalic phenotype in *Zebra*fi*sh* [34]. Knockout of *Mettl8*, which encodes a m^3C methyltransferase only for mitochondria $tRNA^{Thr/Ser(UCN)}$, both in mice and in human induced pluripotent stem cell (iPSC)-derived forebrain organoids

leads to impaired NPC maintenance and increased neuronal differentiation by attenuating mitochondrial protein translation and function [46]. Knockout of *Nsun2*, which encodes an m^5C methyltransferase, leads to deficits in neural differentiation and a reduced number of upper-layer neurons in the mouse cerebral cortex [82]. Knockout of *Nsun5*, which encodes a 28S rRNA m^5C methyltransferase, impairs radial neuronal migration and disrupts the lamination of deep/superficial layer neurons in the mouse cortex [83]. Knockout of *Wdr4*, which encodes a member of the METTL1/WDR4 complex responsible for RNA m^7G modification, leads to embryonic lethality of mice at E9.5–10.5 with severe abnormality of brain development [84].

Beyond embryonic development, neurogenesis occurs in the dentate gyrus of adult mammalian brains, which is important for learning, memory, and mood regulation, and its dysregulation has been associated with psychiatric disorders [85, 86]. *Mettl3* knockdown and *Fto* knockout in adult NPCs lead to similar phenotypes with decreased NPC proliferation and neuronal differentiation [87–89] (Fig. 2b). However, another study showed that conditional *Fto* knockout in adult NPCs in vivo transiently increases their proliferation and neuronal differentiation, leading to decreased adult neurogenesis over the long-term [90]. The disparities in the roles of FTO in regulating adult neurogenesis may be attributed to the use of conventional or conditional knockout mice in different studies. Besides, FTO appears to affect adult neurogenesis but largely not embryonic brain development, which may be due to the cell-type-specific effects and distinct m^6A-modified mRNA targets of FTO in these two quite different systems in which embryonic brain development involves the dividing stem cells undergoing massive proliferation and differentiation, while adult neurogenesis involves the activation of long-term quiescent neural stem cells, warranting future clarification. Despite these advances, future functional studies in animal and human brain organoid models are needed to provide a comprehensive understanding of the roles of diverse RNA modifications beyond m^6A in regulating embryonic and adult neurogenesis.

Gliogenesis

Glial cells are as numerous as neurons in the human brain and are important for providing metabolic support to neurons and regulating neural specification, synaptic plasticity, myelination, and cellular debris clearance [91, 92]. Compared to neurogenesis, the role of epitranscriptomics in regulating gliogenesis is much less known. Knockout of *Mettl14* in oligodendrocyte progenitor cells (OPCs) and post-mitotic oligodendrocytes leads to a reduced number of mature oligodendrocytes in the mouse brain [93] (Fig. 2c). Knockout of m^6A reader *Prrc2a* in oligodendroglia cells inhibits OPC generation and proliferation, while it promotes oligodendrocyte differentiation [36]. Knockout of *Nsun5* impairs OPC proliferation and reduces numbers of OPCs and oligodendrocytes [94, 95]. All these mice exhibit deficits in myelination, highlighting the importance of epitranscriptomics in regulating myelination. Regarding astrocyte generation, conditional knockout of *Mettl14* in embryonic neural stem cells causes deficits in astrocyte generation in the postnatal mouse cortex [18]. Knockout of *Mettl3* in retinal progenitor cells of the retina leads to extended and elevated generation of Müller glial cells during late-stage retinogenesis [96]. Besides, deletion of *Ythdf2* in neurospheres leads to a dramatic reduction of $GFAP^+$ astrocytes upon differentiation [80]. Despite these advances, it will also be interesting to investigate potential

roles of epitranscriptomics in other cell types in the nervous system, such as microglia, choroid plexus, blood vessels, tanycytes, and ependymal cells.

Synaptic transmission

m^{6}A methylated RNA and components of RNA modification machinery, such as FTO, METTL14, and YTHDF1/2/3, are localized near synapses [97–100]. Knockout of *Mettl3* or *Ythdf1* in the mouse hippocampus impairs synaptic transmission and long-term potentiation [101, 102], and knockout mice for *Ftsj1* encoding a 2'-O-methyltransferase exhibit immature filopodia-like spines with fewer mushroom-type spines [103] (Fig. 2d). In addition, A-to-I editing of mRNAs occurs at high levels in the human brain and regulates synaptic transmission [104]. Pre-mRNAs of serotonin (5-HT) 2 C receptors (5-HT2CRs), AMPA-type glutamate receptors, and γ-aminobutyric acid type A receptors are heavily edited in a cell-type and brain-region-specific fashion and produce various protein isoforms that form receptors with different permeabilities and neurotransmission efficiencies [104]. As the most studied example, AMPA receptors lacking GluA2 subunits or containing GluA2 subunits encoded by unedited *Grin2a* are Ca^{2+}-permeable, whereas GluA2 subunits encoded by an edited version of *Grin2a* form Ca^{2+}-impermeable AMPA receptors [104] (Fig. 2d). Together, these studies point towards an important role of RNA modifications in modulating synaptic properties in the brain.

Learning and memory

Neuronal activity induces dynamic changes of m^{6}A and m^{5}C landscapes [13, 102, 105–107] and decreases A-to-I editing of *Grik1* and *CaV1.3* mRNA [108, 109] in various brain regions, affecting multiple steps of learning and memory formation [13, 97, 100–102, 109] (Figs. 2d, 3a). For example, postnatal knockout of *Mettl3* or *Ythdf1* in forebrain excitatory neurons in the mouse hippocampus leads to deficits in long-term memory formation [13, 101, 102], whereas *Mettl14* knockout in the striatum alters dopamine signaling and impairs striatal learning [110]. In contrast, knockdown of *Fto* enhances fear memory [97, 111]. As one potential mechanism, the interaction between CYFIP2 and DPYSL2 RNA binding proteins and m^{6}A-modified lncRNA metastasis-associated lung adenocarcinoma transcript 1 *(Malat1*) in synapses of the medial prefrontal cortex (PFC) is important for the consolidation of fear-extinction memory [100]. Moreover, a recent study of *Qtrt1* (queuine tRNA-ribosyltransferase 1) knockout mice indicates that queuosine (Q) on tRNA has emerged as an essential component of learning and memory formation, exerting a greater influence in female mice [74] (Fig. 3a). In the future, systematic time-resolved characterization of all changes in RNA modifications and their signaling upon neuronal activation will provide a more complete picture on roles of RNA modifications and their combinations in learning and memory. Furthermore, achieving subcellular resolution is crucial, as one study discovered an upregulation of nuclear levels of METTL14 and FTO but a downregulation of postsynaptic FTO after a memory-inducing behavioral experience in mice [112].

Stress

Some stress responses in mice are also accompanied by dynamic m^{6}A and m^{6}Am level changes (antibodies used for the detection recognize both modifications (see Table S1))

in mRNA with hypomethylation in the PFC and hippocampus and hypermethylation in the amygdala [113, 114] (Fig. 3b). Chronic unpredictable stress-induced depressive-like behaviors in mice are attenuated by overexpression of circular RNA circSTAG1 that restores m^6A levels on *Faah* mRNA via interaction with m^6A RNA demethylase ALKBH5 [113]. RNA editing of 5-HT2CRs can produce 24 various isoforms that exhibit different activities and mice expressing the unedited isoform of 5-*HT2CR* mRNA showed anxiety-like and depression-like behavior and an increase in the behavior of despair [115, 116] (Fig. 3b). This phenotype is opposite in heterozygous *Adar2* knockout mice, which are resistant to behavioral despair [117]. This is likely because multiple pre-RNAs of receptors and ion channels are edited in wild-type animals; thus, a decrease in ADAR2 levels affects not only mRNA of Serotonin (5-HT) 2 C receptors but all other neuronal receptors resulting in the different phenotypes observed in these two studies. In addition, *Nsun2* neuronal deficient mice with alterations in m^5C methylation show decreased translational efficiencies of glycine-rich proteins in neurons and altered glutamatergic synaptic signaling and antidepressant-like behavioral phenotypes [107] (Fig. 3b). Together, these studies revealed critical roles of various RNA modifications in stress-related behaviors and further studies can address the underlying molecular mechanisms.

Substance abuse

A common feature of many substance abuse disorders is dysregulation of dopaminergic midbrain circuitry. Changes in RNA modifications due to substance use may contribute to the formation of an addiction cycle (Fig. 3c). FTO is downregulated, and m^6A levels of thousands of transcripts, many of which are involved in synaptic functions, are altered in the hippocampus of cocaine-conditioned mice [118], whereas *Fto*-deficient mice exhibit an increased sensitivity toward cocaine-induced locomotor activity and reward-stimulatory actions of cocaine [119]. Cocaine-seeking behavior in rats is accompanied by increased synaptic expression of calcium-permeable AMPA receptor channels in the nucleus accumbens (NAc), a key brain structure related to the rewarding effect of substance abuse. After 7 days of cocaine abstinence, ADAR2 expression and editing of the GluA2 subunit of the AMPA receptor are reduced in the NAc. Editing of the GluA2 subunit leads to the formation of calcium-impermeable AMPA receptors, while a decrease in editing leads to a higher amount of calcium-permeable AMPA receptors, promoting the reinstatement of cocaine-seeking behavior [120]. In addition, chronic consumption of ethanol is accompanied by ADAR1 and ADAR2 regulation and increased levels of RNA editing of Htr2C pre-mRNA in the NAc [121]. NAc-specific *Adar2* knockout mice showed increased preference for ethanol induced by chronic exposure [122]. The discovery of the role of RNA modifications in substance abuse disorders opens avenues for developing novel treatments for addiction by modulating RNA modification levels.

Neuronal degeneration

The m^6A pathway is altered in mouse models of multiple neurodegenerative disorders (Fig. 3d). The global m^6A level is increased in the hippocampus of mice modeling Huntington's disease (HD) [112], spinal cord samples from ALS patients, and rat models of ALS [123], but decreased in the brains of Alzheimer disease (AD) patients [124, 125], mouse models of cognitive decline [125], and in the striatum in rat models of Parkinson's

disease [126] (Fig. 3d, g). *Mettl3* knockdown in the adult mouse hippocampus leads to neurodegeneration, gliosis, and impairment in recognition memory that recapitulate AD symptoms in patients [124]. Mechanistically, *Mettl3* knockdown in cultured mouse [125] and rat [124] primary neurons leads to impaired synaptic translation and decreased neuronal activity [125] and dysregulation of cell cycle genes and subsequent postsynaptic deficits and neurite degeneration, which can be rescued by FTO inhibition [124]. In the brain of 5xFAD mice modeling AD, global m^6A and METTL3 levels are decreased, whereas FTO levels are increased (Fig. 3d). Neuronal knockout or knockdown of *Fto* in the dorsal hippocampal CA1 region rescues cognitive deficits in the 3xTg mice modeling AD [127] and mice modeling HD [112] correspondingly. In mice modeling Nasu-Hakola disease, a heritable neurodegenerative disease resembling AD, METTL3, METTL14, and WTAP levels are decreased [128]. In contrast, the APP/PS1 mouse model of AD exhibits an increased level in global m^6A methylation in the cortex and hippocampus [129]. This difference in various mouse models for AD requires further investigation. Overall, dysregulation of the m^6A pathway appears to be a common feature of neurodegenerative diseases and raises the possibility of modulating the FTO level to manage the development and symptoms of neurodegenerative diseases.

Neuronal injuries and axon regeneration

Axonal injury in the adult peripheral and central nervous systems in mice results in a transient increase of m^6A levels on regeneration-associated genes and protein translation machinery components, which leads to increased translation of these mRNA in a Mettl14- and Ythdf1-dependent fashion [130] (Fig. 3e). On the other hand, the FTO level is increased after peripheral nerve injury in rats, leading to a decrease in m^6A levels on euchromatic histone lysine methyltransferase 2 mRNA [131], a key regulator of transcription and neuropathic pain symptoms. Studies of traumatic brain injury in rodent models uncovered dynamic injury-induced changes in m^6A pathway components, including METTL3 [132], METTL14 and FTO [133], as well as many hypermethylated and hypomethylated transcripts [132, 133]. The post-ischemic stroke recovery success is also linked to dynamic epitranscriptomic changes, including a decrease of FTO [134, 135] and increased *Alkbh5* and global m^6A/m^6Am levels [134, 135]. Transient focal cerebral ischemia in rats leads to decreased protein levels of YTHDF1, YTHDF2, and YTHDF3 and an increase of YTHDC1 and YTHDC2 in the ipsilateral cortex after reoxygenation [136] (Fig. 3f). Ischemic injury is accompanied by a massive Ca^{2+} overload and death of the ischemic-vulnerable neurons, which is characterized by reduced expression of ADAR2 post-ischemia [137–139] (Fig. 3f). RNA editing by ADAR2 of *Grin2a* pre-mRNA leads to the formation of Ca^{2+}-impermeable AMPA receptor channels, thus protecting neurons from the massive Ca^{2+} influx [140]. As a result, overexpression of *Adar2* protects vulnerable CA1 neurons during an ischemic injury in rats [139]. Time-resolved characterization of epitranscriptomic changes occurring post-injury may provide insight into developing therapeutic interventions to improve neuronal regeneration and stroke recovery by temporarily altering patterns of RNA modifications in affected brain regions.

EPITRANSCRIPTOMIC DYSREGULATION IN HUMAN BRAIN DISORDERS

Neurodevelopmental disorders

Mutations or aberrant expression of some genes encoding writers, readers, and erasers of different RNA modifications are correlated with microcephaly, intellectual disability, developmental delay, autism spectrum disorders, and other neurodevelopmental disorders in human patients [19, 34, 51, 60, 83, 141–159] (Fig. 4 and Table S3). Notably, most of these mutant genes encode regulators of cytosolic/mitochondrial tRNA modifications, which are usually critically involved in modulating the folding and codon-anticodon recognition of tRNA to tune cytosolic/mitochondrial protein translation, reflecting the importance of tRNA modifications and protein translation in brain development. Besides, the necessity of depositing methyl groups to diverse RNAs in brain development reemphasizes the importance of methyl donor-folic acid supplement during pregnancy. Indeed, compared with control samples, significantly reduced mRNA m^6A modification levels and *Mettl3* expression levels were observed in the brain tissues of mouse models with neural tube defects (NTD) induced by administration of retinoic acid and ethionine [160, 161]. Interestingly, treatment of S-adenosylmethionine (SAM), a methyl donor, can restore reduced mRNA m^6A modification levels in the NTD mouse model, while there is no evidence indicating that SAM administration can also rescue neural tube defects [161], which needs further examination. Despite these observed connections between human neurodevelopmental disorders and mutations in epitranscriptomic regulators, causal links remain to be established in animal or human brain organoid models for most cases.

Glioblastoma and neuroblastoma

The epitranscriptome is also critically involved in the pathogenesis of primary brain tumors, including glioblastoma multiforme (GBM) and neuroblastoma [22, 51, 162–186] (Fig. 4 and Table S4). GBM is often associated with high levels of expression of both m^6A methyltransferase-related proteins, METTL3 and WTAP, and ALKBH5 (Fig. 3g), while higher glioma grades and poorer clinical outcomes are found to be correlated with decreased FTO expression and increased expression of YTHDF2 and YTHDF3 [174–179]. Functionally, knockdown of *METTL3*, *METTL14*, *YTHDC1* and *FTO*, or overexpression of *METTL14*, *WTAP*, *YTHDF1* and *IGF2BP3* promotes glioma stem cell (GSC) proliferation, self-renewal, and tumorigenesis; on the contrary, overexpression of *METTL3* and *FTO*, silencing *ALKBH5*, *WTAP*, *METTL3*, *YTHDF1*, *YTHDF2* and *IFG2BP3*, or treatment with FTO inhibitors (including MA2, R-2HG, and FTO-04) inhibits proliferation and self-renewal of GSCs and GSC-induced tumorigenesis [174, 175, 178–187]. The variable effects of manipulating different m^6A regulators on GSC proliferation and tumorigenesis in different studies probably reflects different cancer cell lines examined. Overall, WTAP and YTHDF proteins seem to play a consistently oncogenic role in promoting GBM tumorigenesis, raising the possibility of developing inhibitors to treat GBM.

In addition, higher expression levels of regulators including *DKC1* encoding a snoRNA-dependent Ψ synthase, *PUS7* encoding a RNA-independent Ψ synthase, several m^5C RNA methyltransferases, *FTSJ2* encoding a rRNA 2′-O-methyltransferase, *TRMT6* encoding a m^1A RNA methyltransferase, and *METTL1* encoding a m^7G tRNA methyltransferase,

have been reported to be associated with patients with glioma and neuroblastoma and often predict a poorer prognosis [22, 51, 165–172] (Table S4). At the molecular level, overexpression of *PUS7* and *NSUN5* promotes GSC proliferation, self-renewal and growth, whereas knockdown of *PUS7*, *DKC1*, *NSUN5*, *TRMT6*, and *METTL1* and treatment with PUS7 inhibitors attenuates glioma cell proliferation, migration, and tumorigenesis [22, 165, 166, 168, 170–172]. Collectively, aberrant expression levels of multiple regulators of RNA modifications, including m^6A, Ψ, m^5C, 2'-O-methylation, m^7G, and m^1A, have the potential to be biomarkers for predicting the occurrence and prognosis of brain tumors as well as potential therapeutic targets of brain tumors. As oncogenes or tumor suppresser genes are often master genes regulated by RNA modifications, inhibitors and activators of these RNA modification regulators may be effective in attenuating brain tumors, such as METTL3 inhibitors (STM2457, UZH1a), FTO inhibitors (MA2, R-2HG, and FTO-04), YTHDF1 inhibitors (Ebselen), PUS7 inhibitors (C17), and DKC1 inhibitors (pyrazofurin) [165, 183, 185, 188–191]. In contrast to disparate roles of m^6A mRNA modifications in regulating glioma tumorigenesis, which may be attributed to the fact that significantly more studies using various glioma samples have been conducted on m^6A compared to other types of RNA modifications, the oncogenic roles of some RNA modifications, such as m^7G and their modifiers, have been relatively consistent to date in glioma, thus deserving more effort to develop small molecule inhibitors to test their therapeutic efficacy.

Psychiatric disorders

Multiple studies have revealed alterations in RNA modifications in patients with psychiatric disorders (Figs. 3 and 4). Global m^6A/m methylation levels in whole blood were transiently reduced after an acute stressful challenge and glucocorticoid stimulation in healthy subjects, but not in patients with major depressive disorders (Fig. 5a) [114]. In addition, the level of circular RNA circSTAG1, which regulates the m^6A methylation level of *Faah* mRNA in the mouse hippocampus, was significantly decreased in the plasma and whole blood of patients with depression [113]. A number of genetic variants have been found to be associated with an increased risk for major depressive disorders, such as *FTO* (rs9939609) [192] and *ALKBH5* (rs12936694) [193]. A pilot study also found changes in m^6A methylation of mRNAs involved in inflammation and immune responses in postmortem NAc from three males with alcohol use disorder compared to three matched control subjects [194]. In another study, increased expression and decreased m^6A methylation of BDNF-AS lncRNA was found in the postmortem amygdala in early-onset alcohol use disorders [195]. An association study of patients with major depressive disorder, bipolar disorder, and schizophrenia found rs9983925 and rs4819035 polymorphisms in *ADARB1* to be associated with suicide risk [196, 197], indicating that alterations in RNA editing may also be a risk factor for suicidal behavior. There are region-specific alterations of RNA editing and expression levels of cyclic nucleotide phosphodiesterase 8 A mRNA in the cortex [197] and 5-HT2CR pre-mRNA in the neocortex [198] of patients of major depressive disorders with suicide risk. *ADAR2* expression is often decreased in the postmortem brains of patients with schizophrenia and bipolar disorder and an RNA editing deficit was found in many cases of schizophrenia [117, 199]. To note, there is a trend for RNA hypoediting in schizophrenia patients of European descent [200]. These studies showed a promising

direction for developing biomarkers for early diagnostics of psychiatric disorders and prediction of suicide behavior (Fig. 5a).

Neurodegenerative disorders

The expression of m^6A machinery components is altered in patients with multiple sclerosis (MS) compared to matched controls (Fig. 4), leading to increased m^6A levels in cerebrospinal fluid from patients with relapsing–remitting MS and progressive MS [201]. Similarly, an increase in m^6A levels and hypermethylation of mRNAs and lncRNAs were found in postmortem spinal samples from patients with sporadic ALS [123]. In contrast, reduced levels of m^6A in mRNA and m^6A regulators were found in the postmortem brains of patients with AD, even at early stages of the disease (Fig. 3g) [124]. Genetic studies have also revealed an association of *FTO* (rs9939609) with a higher risk for AD and dementia [202]. Alterations in A-to-I RNA editing levels were also found in postmortem brain samples of patients with AD, mainly in the hippocampus and, to a lesser degree, in the temporal and frontal lobes [203, 204]. In addition, decreased *ADAR2* expression was found in motor neurons of patients with sporadic ALS [205] (Fig. 3g). Together, these preliminary studies have identified genetic associations among RNA modification regulators with different neurodegenerative disorders and dysregulated RNA modifications in patient samples (Fig. 4). Future large cohort studies are needed to validate these initial findings to establish potential biomarkers for disease detection and to identify therapeutic targets for intervention.

TRANSLATIONAL OPPORTUNITIES

Aberrant expression or mutations of RNA modification regulators and dysregulated RNA modification levels in various brain disorders, such as neurodevelopmental diseases, psychiatric disorders, and brain tumors, highlights not only the biological significance of RNA modifications in regulating the nervous system, but also the potential translational opportunities of applications of epitranscriptomics in the diagnosis and treatment of brain disorders.

Epitranscriptomics as biomarkers for human brain disorders

Aberrant levels of RNA modifications and RNA modification regulators that accompany the progression of brain disorders can potentially serve as diagnostic biomarkers and prognosis predictors (Fig. 5a). For example, patients with relapsing–remitting MS and progressive MS have increased m^6A levels in cerebrospinal fluid compared to healthy controls [201]. The m^6A/m levels in blood after glucocorticoid stimulation is also a promising biomarker of major depressive disorder [114]. An RNA editing signature in whole blood was successfully used to identify patients with bipolar disorder among depressed patients to facilitate appropriate treatment in a timely manner [206]. Also, aberrant expression levels of multiple regulators of RNA modifications, including pseudouridine, m^7G, and m^1A, have the potential to be biomarkers for predicting the occurrence and prognosis of brain tumors. The main challenge for developing epitranscriptomics-based biomarker panels is establishing and expanding the strong association between disease conditions and epitranscriptomic

signatures in biosamples, such as blood or cerebrospinal fluid, from a large cohort of patients with diverse genetic backgrounds.

Drugs or gene therapies that target epitranscriptomic pathways

Realization of clinical applications of RNA modifications in treating diseases requires more comprehensive understanding of key dysregulated RNA modifications and molecular pathways involved in pathogenesis and the development of efficient and specific strategies for manipulating RNA modifications, such as agonists or inhibitors of RNA modification regulators and CRISPR/Cas13-based or engineered snoRNA-mediated RNA modification editing [207, 208] (Fig. 5b). Inhibitors of RNA modification regulators, which can be screened from various natural products or synthetic chemicals, are gaining more interest for treating brain disorders, including glioma and neurodegenerative diseases [127, 183, 185, 188–191]. For example, inhibitors of m^6A eraser-FTO and pseudouridine synthase PUS7 have been reported to attenuate proliferation and tumorigenesis of GSCs [165, 183, 185, 187], suggesting their therapeutic potential in treating brain tumors. *Fto* knockdown in the dorsal hippocampal CA1 region improved behavior and cognitive defects in a mouse model of HD [112], and the neuronal *Fto* knockout rescued the cognitive deficits in 3xTg mouse model of AD [127], suggesting the potential of FTO inhibitors to mitigate symptoms in human neurodegenerative diseases. Overexpression of circular RNA *circSTAG1* prevents ALKBH5 translocation into the nucleus, restoring m^6A levels on *Faah* mRNA and attenuating depressive-like behaviors in chronic unpredictable stress-treated mice [113], suggesting the potential of ALKBH5 inhibitors for depression treatment. In addition, two recent studies showed that directed site-specific pseudouridylation of premature termination codons (PTCs) mediated by engineered small nucleolar RNAs (snoRNAs) enhances nonsense suppression and PTC readthrough to produce full-length functional proteins in mammalian cells, revealing the therapeutical potential of targeted pseudouridylation in treating PTC-associated brain disorders, such as spinal muscular atrophy caused by nonsense mutations in the *SMN1* gene [208, 209].

Mechanism-based therapies

A better understanding of cellular mechanisms underlying deficits due to mutations of epitranscriptomic pathways can suggest mechanism-based downstream interventions (Fig. 5c). For example, in MELAS disease affecting mitochondria, mutations in U34 of mitochondrial $tRNA^{Leu(UAA)}$ leads to deficiency of its 5-taurinomethyluridine (τm^5U) modification and impaired mitochondrial function [68, 210]. Deficiency in mitochondrial function in MELAS could be restored by expression of the other isoacceptor-mitochondrial-$tRNA^{Leu(UAG)}$ with a mutated anticodon to (UAA), which can be τm^5U34 modified [68, 211], indicating that introducing tRNA or other RNAs with proper modification can restore defects in diseases caused by hypomodified tRNA or other RNAs. In another example, impaired NPC maintenance in mice or human forebrain organoids with knockout of *Mettl8* encoding mitochondrial $tRNA^{Thr/Ser(UCN)}$ m^3C methytransferase is due to attenuated mitochondrial activity, whereas pharmacologically enhancing mitochondrial activity with piracetam treatment can largely restore NPC maintenance [46].

mRNA vaccines and therapy

One of the best examples of a clinical application of epitranscriptomics is the recent success of COVID-19 mRNA vaccines utilizing RNA modifications to enhance efficacy and reduce immunogenicity [12, 212]. In addition, several RNA-based drugs have been approved for various neurological diseases by the FDA, mainly focused on siRNA and antisense oligonucleotides (AONs) to reduce expression of genes with pathogenic mutations or correct splicing defects of mutated pathogenic genes [213]. Utilization of RNA modifications when designing antisense oligonucleotides improves their binding affinity to target RNAs and their resistance to nuclease degradation [213]. Furthermore, the profound success of COVID-19 mRNA vaccines led the field to re-evaluate potential applications of RNA modifications in mRNA vaccines and mRNA therapy for attenuating brain disorders, such as neurodegenerative disorders and brain tumors [12, 212] (Fig. 5d). For example, given the inefficiency of Aβ vaccine in ameliorating symptoms of AD [214], mRNA vaccines against toxic Aβ protein could be an alternative. Similarly, it will also be promising to develop mRNA vaccines against brain tumor-specific antigens or viruses infecting brain tissue, such as ZIKA virus and viral encephalitis [215–217]. Although transdifferentiation from astrocytes to neurons in vivo through infecting astrocytes with AAV virus expressing factors such as NeuroD1, remains controversial [218, 219], this strategy may be improved by *NeuroD1* mRNA therapy with an astrocyte-specific delivery strategy. In the case of expressing exogenous proteins, such as neurotrophic factors to improve neuronal function and activity in neurodegenerative disorders, reversible and transient mRNA therapy could be a competitive and safe choice compared to AAV virus-based gene therapies [220, 221].

FUTURE DIRECTIONS

To date, clinical applications of epitranscriptomics for treating brain disorders is still in a nascent stage. Despite many correlations between mutations or aberrant expression of RNA modification regulators and brain disorders in patients, causal links need to be validated in animal or human brain organoid models with corresponding genetic manipulations. It will also be useful to systematically evaluate levels of various RNA modifications and different RNA modification regulators in biosamples from patients with neurodevelopmental, psychiatric disorders or brain tumors versus healthy people to identify potential biomarkers. These advances will lead to a more comprehensive understanding of pathogenesis mechanisms of brain disorders and lay the foundation for unraveling the therapeutic potential of manipulating levels of some specific RNA modifications or their regulators.

Detection and mapping methods

In recent years, a plethora of innovative methods for detecting RNA modifications [6, 23–33] (Table S1) has played a pivotal role in enhancing our understanding of their roles in the pathogenesis of brain disorders. However, to pave the way for the creation of safe and efficacious epitranscriptome-based therapeutics, there remains a pressing need for continued advancements in detection methodologies. Considering the high complexity and tissue specificity of RNA modifications' repertoire, development of RNA modification detection methods that retain spatial and cell-type information in the brain

will provide an exciting opportunity to understand the complexity of brain region- and cell type-specific roles of RNA modifications in brain tissues, including human postmortem brains. A combination of spatial transcriptomics with sequencing-based methods for RNA modification detection and single-cell RNA modification analysis is the next logical step in method development. In addition, understanding the crosstalk between different RNA modifications and epitranscriptomic codes on one RNA molecule is an exciting new direction that might help to decipher the complexity of RNA modification-based regulation in the nervous system. Methods are needed to simultaneously detect different RNA modifications on one RNA molecule. Direct RNA sequencing, such as Nanopore sequencing, has great potential to become a gold standard in the RNA modification detection field should technical and computational challenges be addressed. Moreover, for diagnostic purposes, working with human samples requires the development of RNA modification detection methods with clinical feasibility that do not rely on transgenic protein expression or culturing cells.

Understanding RNA modification mechanisms of action in disease progression

Despite recent significant advances, only a handful of the over 170 RNA modifications have been investigated for their roles in regulating the nervous system, while the function of many others, such as RNA glycosylation on the cell surface, remains ambiguous. In addition, the roles of RNA modifications in other types of cells in the brain beyond neurons, such as oligodendrocytes, astrocytes, microglia, choroid plexus, brain blood vessels, tanycytes, and ependymal cells, are much less clear. Comprehensive knowledge of the epitranscriptomic landscapes in diverse types of cells in the brain would help us better understand their roles in the pathogenesis of brain disorders involving different cell types and develop cell-type-specific therapeutic interventions to treat brain disorders.

While mouse models for human brain disorders are very useful for developing new strategies for managing disease symptoms and progression, caution should be taken while transferring knowledge gained from mice models to patients, especially in the case of multifactorial diseases, such as AD. For instance, *Fto* downregulation mitigates symptoms in mouse models of HD [112] and AD [127], yet the APP/PS1 mouse model of AD is characterized by an increase in global m^6A methylation in the cortex and hippocampus [129]. This warrants more research to better understand the molecular mechanisms of how specific RNA modifications and their combinations affect disease progression, in which human cell-based models, such as brain organoids, will be particularly useful.

Developing novel therapeutics

Regarding the clinical use of the small molecule inhibitors of RNA modification regulators in attenuating brain disorders and taking glioma as an example [183, 185, 188–191], manipulating the same RNA modifications can have opposite effects in different glioma cells, either promoting or attenuating tumorigenesis. Therefore, personalized medicine should be a priority, depending on the directionality of the change of RNA modifications and affected key downstream genes in diseases. Other issues also need to be considered, such as blood-brain-barrier (BBB) penetration, and the specificity and side effects of small molecules. For example, some drugs designed for inhibiting m^5C RNA methyltransferases

can often have cross-activity on other targets, like m^5C DNA methyltransferases, which may have unexpected consequences [22]. Interestingly, photoactivated compounds, such as a caged molecule activator of METTL3/14, can potentially increase the spatiotemporal specificity and reduce side effects of small molecule drugs of RNA modifiers in vivo [222, 223]. Furthermore, the specificity of manipulating RNA modifications can be improved by taking advantage of CRISPR/Cas13-based RNA modification editing. Like CRISPR/Cas9-mediated genome-editing, use of the CRISPR/Cas13 system makes it possible to target specific key RNA molecules and manipulate their specific RNA modifications [207, 224]. CRISPR/Cas13 fused with RNA modification regulators, such as FTO, METTL3/14, METTL1, and YTHDFs, to specifically remove, deposit, or target the modifications of specific RNAs, especially for key master regulator genes of signaling pathways, genes with mutations or aberrant expression, and tumor suppresser genes or oncogenes, would potentially reduce side effects of manipulating all RNA modifications.

Currently, applications of RNA modifications in mRNA vaccines and therapy mainly focus on the 5′ cap analog and uridine moieties. Whether combining other types of RNA chemical modifications with varying compositions and depositing RNA modifications to different regions of mRNA (5’ UTR, 3’ UTR, and open reading frame) can further improve the expression efficacy and reduce the immunogenicity of mRNA-based vaccines and therapy warrants more investigation in the future [212]. Moreover, the lack of safe and efficient delivery methods of mRNA therapy into the nervous system still greatly hinders its clinical applications and requires significant improvement [212, 213].

CONCLUSION

Epitranscriptomics holds great promise in revolutionizing diagnosis and treatment of brain disorders. RNA modifications, which influence various biological processes in the nervous system, have been linked to a wide range of neurodevelopmental, neurological, and psychiatric disorders. Epitranscriptomics-based therapies may potentially offer advantages such as flexibility, safety, and robust therapeutic effects, making them an attractive approach for treating brain disorders. Moreover, RNA modifications can be directly utilized to enhance the effectiveness of mRNA vaccines and may serve as valuable biomarkers for brain tumors and psychiatric disorders. However, widespread clinical application of epitranscriptomics-based therapies necessitates a deeper understanding of the causal relationship between altered RNA modifications and levels of their regulators and development of brain disorders. It is crucial to systematically evaluate RNA modifications and their regulators in both healthy and diseased brains, while also developing more sensitive detection methods capable of preserving spatial information and being readily implemented in clinical settings. Development of novel animal models and human brain organoids specific to brain disorders will be essential in identifying new strategies for managing symptoms and disease progression through epitranscriptomic modulation. Finally, gaining a comprehensive understanding of the epitranscriptome functions across diverse types of brain cells will expand our knowledge on the role of neuroepitranscriptomics in the pathogenesis of brain disorders involving multiple cell types, contributing to the development of cell-type-specific therapeutic interventions. Together, these research efforts

hold the potential to transform our understanding of molecular mechanisms underlying brain disorders and pave the way for the development of new and more effective therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Crick F Central dogma of molecular biology. Nature. 1970;227:561–3. [PubMed: 4913914]
2. Helm M, Motorin Y. Detecting RNA modifications in the epitranscriptome: predict and validate. Nat Rev Genet 2017;18:275–91. [PubMed: 28216634]
3. Zhao BS, Roundtree IA, He C. Post-transcriptional gene regulation by mRNA modifications. Nat Rev Mol Cell Biol 2017;18:31–42. [PubMed: 27808276]
4. Wiener D, Schwartz S. The epitranscriptome beyond m(6)A. Nat Rev Genet 2021;22:119–31. [PubMed: 33188361]
5. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 2011;7:885–7. [PubMed: 22002720]
6. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, et al. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature. 2012;485:201–6. [PubMed: 22575960]
7. Zaccara S, Ries RJ, Jaffrey SR. Reading, writing and erasing mRNA methylation. Nat Rev Mol Cell Biol 2019;20:608–24. [PubMed: 31520073]
8. Jiang X, Liu B, Nie Z, Duan L, Xiong Q, Jin Z, et al. The role of m6A modification in the biological functions and diseases. Signal Transduct Target Ther 2021;6:74. [PubMed: 33611339]
9. Delaunay S, Frye M. RNA modifications regulating cell fate in cancer. Nat Cell Biol 2019;21:552–9. [PubMed: 31048770]
10. Roundtree IA, Evans ME, Pan T, He C. Dynamic RNA Modifications in Gene Expression Regulation. Cell. 2017;169:1187–1200. [PubMed: 28622506]
11. Frye M, Harada BT, Behm M, He C. RNA modifications modulate gene expression during development. Science. 2018;361:1346–9. [PubMed: 30262497]
12. Morais P, Adachi H, Yu YT. The Critical Contribution of Pseudouridine to mRNA COVID-19 Vaccines. Front Cell Dev Biol 2021;9:789427. [PubMed: 34805188]
13. Shi H, Zhang X, Weng YL, Lu Z, Liu Y, Lu Z, et al. m6A facilitates hippocampus-dependent learning and memory through YTHDF1. Nature. 2018;563:249–53. [PubMed: 30401835]
14. Vissers C, Sinha A, Ming GL, Song H. The epitranscriptome in stem cell biology and neural development. Neurobiol Dis 2020;146:105139. [PubMed: 33065280]
15. Shafik AM, Allen EG, Jin P. Dynamic N6-methyladenosine RNA methylation in brain and diseases. Epigenomics. 2020;12:371–80. [PubMed: 32081027]
16. Livneh I, Moshitch-Moshkovitz S, Amariglio N, Rechavi G, Dominissini D. The m(6)A epitranscriptome: transcriptome plasticity in brain development and function. Nat Rev Neurosci 2020;21:36–51. [PubMed: 31804615]

17. Shafik AM, Allen EG, Jin P. Epitranscriptomic dynamics in brain development and disease. Mol Psychiatry 2022;27:3633–46. [PubMed: 35474104]
18. Yoon KJ, Ringeling FR, Vissers C, Jacob F, Pokrass M, Jimenez-Cyrus D, et al. Temporal Control of Mammalian Cortical Neurogenesis by m(6)A Methylation. Cell. 2017;171:877–89 e817. [PubMed: 28965759]
19. Bohnsack KE, Hobartner C, Bohnsack MT. Eukaryotic 5-methylcytosine (m(5)C) RNA Methyltransferases: Mechanisms, Cellular Functions, and Links to Disease. Genes. 2019;10:102. [PubMed: 30704115]
20. He PC, He C. m(6) A RNA methylation: from mechanisms to therapeutic potential. EMBO J 2021;40:e105977. [PubMed: 33470439]
21. Kumar S, Mohapatra T. Deciphering Epitranscriptome: Modification of mRNA Bases Provides a New Perspective for Post-transcriptional Regulation of Gene Expression. Front Cell Dev Biol 2021;9:628415. [PubMed: 33816473]
22. Nombela P, Miguel-Lopez B, Blanco S. The role of m(6)A, m(5)C and Psi RNA modifications in cancer: novel therapeutic opportunities. Mol cancer. 2021;20:18. [PubMed: 33461542]
23. Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR. Comprehensive analysis of mRNA methylation reveals enrichment in 3′ UTRs and near stop codons. Cell. 2012;149:1635–46. [PubMed: 22608085]
24. Schaefer M, Pollex T, Hanna K, Lyko F. RNA cytosine methylation analysis by bisulfite sequencing. Nucleic Acids Res 2009;37:e12. [PubMed: 19059995]
25. Linder B, Grozhik AV, Olarerin-George AO, Meydan C, Mason CE, Jaffrey SR. Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. Nat Methods. 2015;12:767–72. [PubMed: 26121403]
26. Molinie B, Wang J, Lim KS, Hillebrand R, Lu ZX, Van Wittenberghe N, et al. M6A-LAIC-seq reveals the census and complexity of the m6A epitranscriptome. Nat Methods. 2016;13:692–8. [PubMed: 27376769]
27. Khoddami V, Cairns BR. Identification of direct targets and modified bases of RNA cytosine methyltransferases. Nat Biotechnol 2013;31:458. [PubMed: 23604283]
28. Li X, Zhu P, Ma S, Song J, Bai J, Sun F, et al. Chemical pulldown reveals dynamic pseudouridylation of the mammalian transcriptome. Nat Chem Biol 2015;11:592–7. [PubMed: 26075521]
29. Carlile TM, Rojas-Duran MF, Gilbert WV. Pseudo-Seq: Genome-Wide Detection of Pseudouridine Modifications in RNA. Methods Enzymol 2015;560:219. [PubMed: 26253973]
30. Meyer KD. DART-seq: an antibody-free method for global m6A detection. Nat Methods. 2019;16:1275–80. [PubMed: 31548708]
31. Liu N, Parisien M, Dai Q, Zheng G, He C, Pan T. Probing N6-methyladenosine RNA modification status at single nucleotide resolution in mRNA and long noncoding RNA. RNA 2013;19:1848–56. [PubMed: 24141618]
32. Mirza AH, Attarwala N, Gross SS, Chen Q, Jaffrey SR. Selective detection of m6A derived from mRNA using the Phospho-tag m6A assay. bioRxiv 2022;493172.
33. Ensinck I, Sideri T, Modic M, et al. m6A-ELISA, a simple method for quantifying N6-methyladenosine from mRNA populations. RNA 2023;29:705–12. [PubMed: 36759126]
34. Richard EM, Polla DL, Assir MZ, Contreras M, Shahzad M, Khan AA, et al. Bi-allelic variants in METTL5 cause autosomal-recessive intellectual disability and microcephaly. Am J Hum Genet 2019;105:869–78. [PubMed: 31564433]
35. Wang X, Feng J, Xue Y, Guan Z, Zhang D, Liu Z, et al. Structural basis of N(6)-adenosine methylation by the METTL3-METTL14 complex. Nature. 2016;534:575–8. [PubMed: 27281194]
36. Wu R, Li A, Sun B, Sun JG, Zhang J, Zhang T, et al. A novel m(6)A reader Prrc2a controls oligodendroglial specification and myelination. Cell Res 2019;29:23–41. [PubMed: 30514900]
37. Edens BM, Vissers C, Su J, Arumugam S, Xu Z, Shi H, et al. FMRP Modulates Neural Differentiation through m(6)A-Dependent mRNA Nuclear Export. Cell Rep 2019;28:845–54.e845. [PubMed: 31340148]

38. Martinez NM, Su A, Burns MC, Nussbacher JK, Schaening C, Sathe S, et al. Pseudouridine synthases modify human pre-mRNA co-transcriptionally and affect pre-mRNA processing. Mol Cell. 2022;82:645–59.e649. [PubMed: 35051350]
39. Karijolich J, Yu YT. Spliceosomal snRNA modifications and their function. RNA Biol. 2010;7:192–204. [PubMed: 20215871]
40. Borchardt EK, Martinez NM, Gilbert WV. Regulation and function of RNA pseudouridylation in human cells. Annu Rev Genet 2020;54:309–36. [PubMed: 32870730]
41. Li X, Ma S, Yi C. Pseudouridine: the fifth RNA nucleotide with renewed interests. Curr Opin Chem Biol 2016;33:108–16. [PubMed: 27348156]
42. Chen YS, Yang WL, Zhao YL, Yang YG. Dynamic transcriptomic m(5) C and its regulatory role in RNA processing. Wiley Interdiscip Rev RNA 2021;12:e1639. [PubMed: 33438329]
43. Yang X, Yang Y, Sun BF, Chen YS, Xu JW, Lai WY, et al. 5-methylcytosine promotes mRNA export - NSUN2 as the methyltransferase and ALYREF as an mC reader. Cell Res 2017;27:606–25. [PubMed: 28418038]
44. Kleiber N, Lemus-Diaz N, Stiller C, Heinrichs M, Mai MM, Hackert P, et al. The RNA methyltransferase METTL8 installs m(3)C(32) in mitochondrial tRNAs(Thr/Ser(-UCN)) to optimise tRNA structure and mitochondrial translation. Nat Commun 2022;13:209. [PubMed: 35017528]
45. Scholler E, Marks J, Marchand V, Bruckmann A, Powell CA, Reichold M, et al. Balancing of mitochondrial translation through METTL8-mediated m(3)C modification of mitochondrial tRNAs. Mol Cell. 2021;81:4810–25.e4812. [PubMed: 34774131]
46. Zhang F, Yoon K, Zhang DY, Kim NS, Ming GL, Song H. Epitranscriptomic regulation of cortical neurogenesis via Mettl8-dependent mitochondrial tRNA m(3) C modification. Cell Stem Cell. 2023;30:300–11.e311. [PubMed: 36764294]
47. Xu L, Liu X, Sheng N, Oo KS, Liang J, Chionh YH, et al. Three distinct 3-methylcytidine (m(3)C) methyltransferases modify tRNA and mRNA in mice and humans. J Biol Chem 2017;292:14695–703. [PubMed: 28655767]
48. Ignatova VV, Kaiser S, Ho JSY, Bing X, Stolz P, Tan YX, et al. METTL6 is a tRNA m(3)C methyltransferase that regulates pluripotency and tumor cell growth. Sci Adv 2020;6:eaaz4551. [PubMed: 32923617]
49. Shima H, Igarashi K. N 1-methyladenosine (m1A) RNA modification: the key to ribosome control. J Biochem 2020;167:535–9. [PubMed: 32129871]
50. Zhang C, Jia G. Reversible RNA Modification N(1)-methyladenosine (m(1)A) in mRNA and tRNA. Genomics Proteom Bioinforma 2018;16:155–61.
51. Dimitrova DG, Teysset L, Carré C. RNA 2'-O-Methylation (Nm) Modification in Human Diseases. Genes 2019;10:117. [PubMed: 30764532]
52. Ayadi L, Galvanin A, Pichot F, Marchand V, Motorin Y. RNA ribose methylation (2'-O-methylation): Occurrence, biosynthesis and biological functions. Biochim Biophys Acta Gene Regul Mech 2019;1862:253–69. [PubMed: 30572123]
53. Oerum S, Meynier V, Catala M, Tisne C. A comprehensive review of m6A/m6Am RNA methyltransferase structures. Nucleic Acids Res 2021;49:7239–55. [PubMed: 34023900]
54. Liu J, Li K, Cai J, Zhang M, Zhang X, Xiong X, et al. Landscape and Regulation of m(6)A and m(6)Am Methylome across Human and Mouse Tissues. Mol Cell. 2020;77:426–40.e426. [PubMed: 31676230]
55. Boulias K, Toczydlowska-Socha D, Hawley BR, Liberman N, Takashima K, Zaccara S, et al. Identification of the m(6)Am Methyltransferase PCIF1 Reveals the Location and Functions of m(6)Am in the Transcriptome. Mol Cell. 2019;75:631–43 e638. [PubMed: 31279658]
56. Cowling VH. Regulation of mRNA cap methylation. Biochem J 2009;425:295–302. [PubMed: 20025612]
57. Ramanathan A, Robb GB, Chan SH. mRNA capping: biological functions and applications. Nucleic Acids Res 2016;44:7511–26. [PubMed: 27317694]
58. Pandolfini L, Barbieri I, Bannister AJ, Hendrick A, Andrews B, Webster N, et al. METTL1 Promotes let-7 MicroRNA Processing via m7G Methylation. Mol Cell. 2019;74:1278–90.e1279. [PubMed: 31031083]

59. Alexandrov A, Martzen MR, Phizicky EM. Two proteins that form a complex are required for 7-methylguanosine modification of yeast tRNA. RNA 2002;8:1253–66. [PubMed: 12403464]
60. Cheng W, Gao A, Lin H, Zhang W. Novel roles of METTL1/WDR4 in tumor via m(7)G methylation. Mol Ther Oncolytics 2022;26:27–34. [PubMed: 35784404]
61. Malbec L, Zhang T, Chen YS, Zhang Y, Sun BF, Shi BY, et al. Dynamic methylome of internal mRNA N(7)-methylguanosine and its regulatory role in translation. Cell Res 2019;29:927–41. [PubMed: 31520064]
62. Chalk AM, Taylor S, Heraud-Farlow JE, Walkley CR. The majority of A-to-I RNA editing is not required for mammalian homeostasis. Genome Biol 2019;20:268. [PubMed: 31815657]
63. Wang H, Chen S, Wei J, Song G, Zhao Y. A-to-I RNA Editing in Cancer: From Evaluating the Editing Level to Exploring the Editing Effects. Front Oncol 2020;10:632187. [PubMed: 33643923]
64. Torres AG, Pineyro D, Filonava L, Stracker TH, Batlle E, Ribas de Pouplana L. A-to-I editing on tRNAs: biochemical, biological and evolutionary implications. FEBS Lett 2014;588:4279–86. [PubMed: 25263703]
65. Flynn RA, Pedram K, Malaker SA, Batista PJ, Smith BAH, Johnson AG, et al. Small RNAs are modified with N-glycans and displayed on the surface of living cells. Cell. 2021;184:3109–24.e3122. [PubMed: 34004145]
66. Suzuki T The expanding world of tRNA modifications and their disease relevance. Nat Rev Mol Cell Biol 2021;22:375–92. [PubMed: 33658722]
67. Zhang LS, Xiong QP, Pena Perez S, Liu C, Wei J, Le C, et al. ALKBH7-mediated demethylation regulates mitochondrial polycistronic RNA processing. Nat Cell Biol 2021;23:684–91. [PubMed: 34253897]
68. Torres AG, Batlle E, Ribas de Pouplana L. Role of tRNA modifications in human diseases. Trends Mol Med 2014;20:306–14. [PubMed: 24581449]
69. Suzuki T, Suzuki T. A complete landscape of post-transcriptional modifications in mammalian mitochondrial tRNAs. Nucleic Acids Res 2014;42:7346–57. [PubMed: 24831542]
70. Schimmel P The emerging complexity of the tRNA world: mammalian tRNAs beyond protein synthesis. Nat Rev Mol Cell Biol 2018;19:45–58. [PubMed: 28875994]
71. Kirchner S, Ignatova Z. Emerging roles of tRNA in adaptive translation, signalling dynamics and disease. Nat Rev Genet 2015;16:98–112. [PubMed: 25534324]
72. Dai X, Wang T, Gonzalez G, Wang Y. Identification of YTH Domain-Containing Proteins as the Readers for N1-Methyladenosine in RNA. Anal Chem 2018;90:6380–4. [PubMed: 29791134]
73. Drazkowska K, Tomecki R, Warminski M, Baran N, Cysewski D, Depaix A, et al. 2'-O-Methylation of the second transcribed nucleotide within the mRNA 5' cap impacts the protein production level in a cell-specific manner and contributes to RNA immune evasion. Nucleic Acids Res 2022;50:9051–71. [PubMed: 36018811]
74. Cirzi C, Dyckow J, Legrand C, Schott J, Guo W, Perez Hernandez D, et al. Queuosine-tRNA promotes sex-dependent learning and memory formation by maintaining codon-biased translation elongation speed. EMBO J 2023;42:e112507. [PubMed: 37609797]
75. Huttner WB, Kosodo Y. Symmetric versus asymmetric cell division during neurogenesis in the developing vertebrate central nervous system. Curr Opin cell Biol 2005;17:648–57. [PubMed: 16243506]
76. Ayala R, Shu T, Tsai LH. Trekking across the brain: the journey of neuronal migration. Cell. 2007;128:29–43. [PubMed: 17218253]
77. Wang Y, Li Y, Yue M, Wang J, Kumar S, Wechsler-Reya RJ, et al. N(6)-methyladenosine RNA modification regulates embryonic neural stem cell self-renewal through histone modifications. Nat Neurosci 2018;21:195–206. [PubMed: 29335608]
78. Du K, Zhang Z, Zeng Z, Tang J, Lee T, Sun T. Distinct roles of Fto and Mettl3 in controlling development of the cerebral cortex through transcriptional and translational regulations. Cell Death Dis 2021;12:700. [PubMed: 34262022]
79. Niu F, Che P, Yang Z, Zhang J, Yang L, Zhuang M, et al. m(6)A regulation of cortical and retinal neurogenesis is mediated by the redundant m(6)A readers YTHDFs. iScience. 2022;25:104908. [PubMed: 36039295]

80. Li M, Zhao X, Wang W, Shi H, Pan Q, Lu Z, et al. Ythdf2-mediated m(6)A mRNA clearance modulates neural development in mice. Genome Biol 2018;19:69. [PubMed: 29855337]
81. Ma C, Chang M, Lv H, Zhang ZW, Zhang W, He X, et al. RNA m(6)A methylation participates in regulation of postnatal development of the mouse cerebellum. Genome Biol 2018;19:68. [PubMed: 29855379]
82. Flores JV, Cordero-Espinoza L, Oeztuerk-Winder F, Andersson-Rolf A, Selmi T, Blanco S, et al. Cytosine-5 RNA Methylation Regulates Neural Stem Cell Differentiation and Motility. Stem Cell Rep 2017;8:112–24.
83. Chen P, Zhang T, Yuan Z, Shen B, Chen L. Expression of the RNA methyltransferase Nsun5 is essential for developing cerebral cortex. Mol Brain. 2019;12:74. [PubMed: 31462248]
84. Cheng IC, Chen BC, Shuai HH, Chien FC, Chen P, Hsieh TS. Wuho Is a New Member in Maintaining Genome Stability through its Interaction with Flap Endonuclease 1. PLoS Biol 2016;14:e1002349. [PubMed: 26751069]
85. Ming GL, Song H. Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 2005;28:223–50. [PubMed: 16022595]
86. Ming GL, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 2011;70:687–702. [PubMed: 21609825]
87. Chen J, Zhang YC, Huang C, Shen H, Sun B, Cheng X, et al. m(6)A Regulates Neurogenesis and Neuronal Development by Modulating Histone Methyltransferase Ezh2. Genomics Proteom Bioinforma 2019;17:154–68.
88. Sun W, Zhang B, Bie Q, Ma N, Liu N, Shao Z. The Role of RNA Methylation in Regulating Stem Cell Fate and Function-Focus on m(6)A. Stem Cells Int 2021;2021:8874360. [PubMed: 34745269]
89. Li L, Zang L, Zhang F, Chen J, Shen H, Shu L, et al. Fat mass and obesity-associated (FTO) protein regulates adult neurogenesis. Hum Mol Genet 2017;26:2398–411. [PubMed: 28398475]
90. Cao Y, Zhuang Y, Chen J, Xu W, Shou Y, Huang X, et al. Dynamic effects of Fto in regulating the proliferation and differentiation of adult neural stem cells of mice. Hum Mol Genet 2020;29:727–35. [PubMed: 31751468]
91. Salas IH, Burgado J, Allen NJ. Glia: victims or villains of the aging brain? Neurobiol Dis 2020;143:105008. [PubMed: 32622920]
92. Lago-Baldaia I, Fernandes VM, Ackerman SD. More Than Mortar: Glia as Architects of Nervous System Development and Disease. Front Cell Developmental Biol 2020;8:611269.
93. Xu H, Dzhashiashvili Y, Shah A, Kunjamma RB, Weng YL, Elbaz B, et al. m(6)A mRNA Methylation Is Essential for Oligodendrocyte Maturation and CNS Myelination. Neuron 2020;105:293–309.e295. [PubMed: 31901304]
94. Zhang T, Chen P, Li W, Sha S, Wang Y, Yuan Z, et al. Cognitive deficits in mice lacking Nsun5, a cytosine-5 RNA methyltransferase, with impairment of oligodendrocyte precursor cells. Glia 2019;67:688–702. [PubMed: 30485550]
95. Yuan Z, Chen P, Zhang T, Shen B, Chen L. Agenesis and Hypomyelination of Corpus Callosum in Mice Lacking Nsun5, an RNA Methyltransferase. Cells. 2019;8:552. [PubMed: 31174389]
96. Xin Y, He Q, Liang H, et al. m6A epitranscriptomic modification regulates neural progenitor-to-glial cell transition in the retina. Elife. 2022;11:e79994. [PubMed: 36459087]
97. Walters BJ, Mercaldo V, Gillon CJ, Yip M, Neve RL, Boyce FM, et al. The Role of The RNA Demethylase FTO (Fat Mass and Obesity-Associated) and mRNA Methylation in Hippocampal Memory Formation. Neuropsychopharmacology. 2017;42:1502–10. [PubMed: 28205605]
98. Merkurjev D, Hong W-T, Iida K. Synaptic N6-methyladenosine (m6A) epitranscriptome reveals functional partitioning of localized transcripts. Nat Neurosci 2018;21:1004–14. [PubMed: 29950670]
99. Flamand MN, Meyer KD. m6A and YTHDF proteins contribute to the localization of select neuronal mRNAs. Nucleic Acids Res 2022;50:4464–83. [PubMed: 35438793]
100. Madugalle SU, Liau WS, Zhao Q, et al. Synapse-Enriched m6A-Modified Malat1 Interacts with the Novel m6A Reader, DPYSL2, and Is Required for Fear-Extinction Memory. J Neurosci 2023;43:7084–100. [PubMed: 37669863]

101. Zhang Z, Wang M, Xie D, Huang Z, Zhang L, Yang Y, et al. METTL3-mediated N6-methyladenosine mRNA modification enhances long-term memory consolidation. Cell Res 2018;28:1050–61. [PubMed: 30297870]
102. Shi H, Zhang X, Weng YL, Lu Z, Liu Y, Lu Z, et al. m(6)A facilitates hippocampus-dependent learning and memory through YTHDF1. Nature. 2018;563:249–53. [PubMed: 30401835]
103. Nagayoshi Y, Chujo T, Hirata S, et al. Loss of Ftsj1 perturbs codon-specific translation efficiency in the brain and is associated with X-linked intellectual disability. Sci Adv 2021;7:eabf3072. [PubMed: 33771871]
104. Goldeck M, Gopal A, Jantsch MF, Mansouri Khosravi HR, Rajendra V, Vesely C. How RNA editing keeps an I on physiology. Am J Physiol-Cell Physiol 2022;323:C1496–C1511. [PubMed: 36036447]
105. Gowda NKC, Nawalpuri B, Ramakrishna S, Jhaveri V, Muddashetty RS. NMDAR mediated dynamic changes in m6A inversely correlates with neuronal translation. Sci Rep 2022; 12.
106. Xu X, Johnson Z, Xie H. Neuronal Depolarization Induced RNA m5C Methylation Changes in Mouse Cortical Neurons. Biology. 2022;11:988. [PubMed: 36101370]
107. Blaze J, Navickas A, Phillips HL, Heissel S, Plaza-Jennings A, Miglani S, et al. Neuronal Nsun2 deficiency produces tRNA epitranscriptomic alterations and proteomic shifts impacting synaptic signaling and behavior. Nat Commun 2021;12.
108. Brande-Eilat N, Golumbic YN, Zaidan H, Gaisler-Salomon I. Acquisition of conditioned fear is followed by region-specific changes in RNA editing of glutamate receptors. Stress. 2015;18:309–18. [PubMed: 26383032]
109. Zhai J, Navakkode S, Yeow SQZ, Krishna-K K, Liang MC, Koh JH, et al. Loss of CaV1.3 RNA editing enhances mouse hippocampal plasticity, learning, and memory. Proc Natl Acad Sci USA 2022;119:e2203883119. [PubMed: 35914168]
110. Koranda JL, Dore L, Shi H, Patel MJ, Vaasjo LO, Rao MN, et al. Mettl14 is essential for epitranscriptomic regulation of striatal function and learning. Neuron 2018;99:283. [PubMed: 30056831]
111. Widagdo J, Zhao QY, Kempen MJ, Tan MC, Ratnu VS, Wei W, et al. Experience-Dependent Accumulation of N6-Methyladenosine in the Prefrontal Cortex Is Associated with Memory Processes in Mice. J Neurosci 2016;36:6771. [PubMed: 27335407]
112. Pupak A, Singh A, Sancho-Balsells A, Alcalá-Vida R, Espina M, Giralt A, et al. Altered m6A RNA methylation contributes to hippocampal memory deficits in Huntington's disease mice. Cell Mol Life Sci 2022;79:416. [PubMed: 35819730]
113. Huang R, Zhang Y, Bai Y, Han B, Ju M, Chen B, 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. [PubMed: 32387133]
114. Engel M, Eggert C, Kaplick PM, Eder M, Röh S, Tietze L, et al. The Role of m6A/m-RNA Methylation in Stress Response Regulation. Neuron 2018;99:389–403.e389. [PubMed: 30048615]
115. Aoki M, Watanabe Y, Yoshimoto K, Tsujimura A, Yamamoto T, Kanamura N, et al. Involvement of serotonin 2C receptor RNA editing in accumbal neuropeptide Y expression and behavioural despair. Eur J Neurosci 2016;43:1219–28. [PubMed: 26950265]
116. Mombereau C, Kawahara Y, Gundersen BB, Nishikura K, Blendy JA. Functional relevance of serotonin 2C receptor mRNA editing in antidepressant- and anxiety-like behaviors. Neuropharmacology. 2010;59:468. [PubMed: 20624407]
117. Kubota-Sakashita M, Iwamoto K, Bundo M, Kato T. A role of ADAR2 and RNA editing of glutamate receptors in mood disorders and schizophrenia. Mol Brain. 2014;7:5. [PubMed: 24443933]
118. Xue A, Huang Y, Li M, Wei Q, Bu Q. Comprehensive analysis of differential m6A RNA methylomes in the Hippocampus of Cocaine-conditioned mice. Mol Neurobiol 2021;58:3759–68. [PubMed: 33826069]

119. Hess ME, Hess S, Meyer KD, Verhagen LAW, Koch L, Brönneke HS, et al. The fat mass and obesity associated gene (Fto) regulates activity of the dopaminergic midbrain circuitry. Nat Neurosci 2013;16:1042–8. [PubMed: 23817550]
120. Schmidt HD, McFarland KN, Darnell SB, Huizenga MN, Sangrey GR, Cha JHJ, et al. ADAR2-dependent GluA2 editing regulates cocaine seeking. Mol psychiatry 2015;20:1460. [PubMed: 25349168]
121. Watanabe Y, Yoshimoto K, Tatebe H, Kita M, Nishikura K, Kimura M, et al. Enhancement of alcohol drinking in mice depends on alterations in RNA editing of serotonin 2C receptors. Int J Neuropsychopharmacol 2014;17:739–51. [PubMed: 24345557]
122. Shirahase T, Watanabe Y, Tsujimura A, Kwak S, Yamamoto T, Kanamura N, et al. Ethanol preference and drinking behavior are controlled by RNA editing in the nucleus accumbens. Front Behav Neurosci 2019;12:331. [PubMed: 30697154]
123. McMillan M, Gomez N, Hsieh C, Bekier M, Li X, Miguez R, et al. RNA methylation influences TDP43 binding and disease pathogenesis in models of amyotrophic lateral sclerosis and frontotemporal dementia. Molecular Cell. 2023;83:219–36.e7. [PubMed: 36634675]
124. Zhao F, Xu Y, Gao S, Qin L, Austria Q, Siedlak SL, et al. METTL3-dependent RNA m6A dysregulation contributes to neurodegeneration in Alzheimer's disease through aberrant cell cycle events. Mol Neurodegener. 2021;16:70. [PubMed: 34593014]
125. Castro-Hernandez R, Berulava T, Metelova M, Epple R, Pena Centeno T, Richter J, et al. Conserved reduction of m(6)A RNA modifications during aging and neurodegeneration is linked to changes in synaptic transcripts. Proc Natl Acad Sci USA 2023;120:e2204933120. [PubMed: 36812208]
126. Chen X, Yu C, Guo M, Zheng X, Ali S, Huang H, et al. Down-Regulation of m6A mRNA Methylation Is Involved in Dopaminergic Neuronal Death. ACS Chem Neurosci 2019;10:2355–63. [PubMed: 30835997]
127. Li H, Ren Y, Mao K, Hua F, Yang Y, Wei N, et al. FTO is involved in Alzheimer's disease by targeting TSC1-mTOR-Tau signaling. Biochem Biophys Res Commun 2018;498:234–9. [PubMed: 29501742]
128. Lv Z, Xu T, Li R, et al. Downregulation of m6A Methyltransferase in the Hippocampus of Tyrobp−/− Mice and Implications for Learning and Memory Deficits. Front Neurosci 2022;16:739201. [PubMed: 35386591]
129. Han M, Liu Z, Xu Y, et al. Abnormality of m6A mRNA Methylation Is Involved in Alzheimer's Disease. Front Neurosci 2020;14:98. [PubMed: 32184705]
130. Yi-Lan Weng A, Wang X, An R, Liu K, Song H, Ming. Correspondence G-l. Epitranscriptomic m 6 A Regulation of Axon Regeneration in the Adult Mammalian Nervous System. Neuron 2018;97:313–25.e6. [PubMed: 29346752]
131. Li Y, Guo X, Sun L, et al. N6-Methyladenosine Demethylase FTO Contributes to Neuropathic Pain by Stabilizing G9a Expression in Primary Sensory Neurons. Adv Sci 2020;7:1902402.
132. Wang Y, Mao J, Wang X, Lin Y, Hou G, Zhu J, et al. Genome-wide screening of altered m6A-tagged transcript profiles in the hippocampus after traumatic brain injury in mice. Epigenomics. 2019;11:805–19. [PubMed: 30882247]
133. 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. [PubMed: 31992337]
134. Chokkalla AK, Mehta SL, Kim TH, Chelluboina B, Kim J, Vemuganti R. Transient Focal Ischemia Significantly Alters the m6A Epitranscriptomic Tagging of RNAs in the Brain. Stroke. 2019;50:2912–21. [PubMed: 31436138]
135. Xu K, Mo Y, Li D, et al. N6-methyladenosine demethylases Alkbh5/Fto regulate cerebral ischemia-reperfusion injury. Ther Adv Chronic Dis 2020;11:2040622320916024.
136. Zhang Z, Wang Q, Zhao X, Shao L, Liu G, Zheng X, et al. YTHDC1 mitigates ischemic stroke by promoting Akt phosphorylation through destabilizing PTEN mRNA. Cell Death Dis 2020;11:977. [PubMed: 33188203]
137. Hume RI, Dingledine R, Heinemann SF. Identification of a site in glutamate receptor subunits that controls calcium permeability. Science. 1991;253:1028–31. [PubMed: 1653450]

138. Burnashev N, Monyer H, Seeburg PH, Sakmann B. Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. Neuron 1992;8:189–98. [PubMed: 1370372]
139. Peng PL, Zhong X, Tu W, Soundarapandian MM, Molner P, Zhu D, et al. ADAR2-dependent RNA editing of AMPA receptor subunit GluR2 determines vulnerability of neurons in forebrain ischemia. Neuron 2006;49:719–33. [PubMed: 16504947]
140. Wright A, Vissel B. The essential role of AMPA receptor GluR2 subunit RNA editing in the normal and diseased brain. Front Mol Neurosci 2012;5:34. [PubMed: 22514516]
141. Caglayan AO, Tuysuz B, Coskun S, Quon J, Harmanci AS, Baranoski JF, et al. A patient with a novel homozygous missense mutation in FTO and concomitant nonsense mutation in CETP. J Hum Genet 2016;61:395–403. [PubMed: 26740239]
142. Van Haute L, Dietmann S, Kremer L, Hussain S, Pearce SF, Powell CA, et al. Deficient methylation and formylation of mt-tRNA(Met) wobble cytosine in a patient carrying mutations in NSUN3. Nat Commun 2016;7:12039. [PubMed: 27356879]
143. Pober BR. Williams-Beuren syndrome. N Engl J Med 2010;362:239–52. [PubMed: 20089974]
144. Freude K, Hoffmann K, Jensen LR, Delatycki MB, des Portes V, Moser B, et al. Mutations in the FTSJ1 gene coding for a novel S-adenosylmethionine-binding protein cause nonsyndromic X-linked mental retardation. Am J Hum Genet 2004;75:305–9. [PubMed: 15162322]
145. de Brouwer APM, Abou Jamra R, Kortel N, Soyris C, Polla DL, Safra M, et al. Variants in PUS7 Cause Intellectual Disability with Speech Delay, Microcephaly, Short Stature, and Aggressive Behavior. Am J Hum Genet 2018;103:1045–52. [PubMed: 30526862]
146. Shaheen R, Tasak M, Maddirevula S, Abdel-Salam GMH, Sayed ISM, Alazami AM, et al. PUS7 mutations impair pseudouridylation in humans and cause intellectual disability and microcephaly. Hum Genet 2019;138:231–9. [PubMed: 30778726]
147. Igoillo-Esteve M, Genin A, Lambert N, Desir J, Pirson I, Abdulkarim B, et al. tRNA methyltransferase homolog gene TRMT10A mutation in young onset diabetes and primary microcephaly in humans. PLoS Genet 2013;9:e1003888. [PubMed: 24204302]
148. Cohen JS, Srivastava S, Farwell KD, Lu HM, Zeng W, Lu H, et al. ELP2 is a novel gene implicated in neurodevelopmental disabilities. Am J Med Genet A 2015;167:1391–5. [PubMed: 25847581]
149. Hayhurst H, de Coo IFM, Piekutowska-Abramczuk D, Alston CL, Sharma S, Thompson K, et al. Leigh syndrome caused by mutations in MTFMT is associated with a better prognosis. Ann Clin Transl Neurol 2019;6:515–24. [PubMed: 30911575]
150. Neeve VC, Pyle A, Boczonadi V, Gomez-Duran A, Griffin H, Santibanez-Koref M, et al. Clinical and functional characterisation of the combined respiratory chain defect in two sisters due to autosomal recessive mutations in MTFMT. Mitochondrion 2013;13:743–8. [PubMed: 23499752]
151. Michaud J, Kudoh J, Berry A, Bonne-Tamir B, Lalioti MD, Rossier C, et al. Isolation and characterization of a human chromosome 21q22.3 gene (WDR4) and its mouse homologue that code for a WD-repeat protein. Genomics 2000;68:71–79. [PubMed: 10950928]
152. Braun DA, Shril S, Sinha A, Schneider R, Tan W, Ashraf S, et al. Mutations in WDR4 as a new cause of Galloway-Mowat syndrome. Am J Med Genet A 2018;176:2460–5. [PubMed: 30079490]
153. Shaheen R, Abdel-Salam GM, Guy MP, Alomar R, Abdel-Hamid MS, Afifi HH, et al. Mutation in WDR4 impairs tRNA m(7)G46 methylation and causes a distinct form of microcephalic primordial dwarfism. Genome Biol 2015;16:210. [PubMed: 26416026]
154. Blaesius K, Abbasi AA, Tahir TH, Tietze A, Picker-Minh S, Ali G, et al. Mutations in the tRNA methyltransferase 1 gene TRMT1 cause congenital microcephaly, isolated inferior vermian hypoplasia and cystic leukomalacia in addition to intellectual disability. Am J Med Genet A 2018;176:2517–21. [PubMed: 30289604]
155. Salehi Chaleshtori AR, Miyake N, Ahmadvand M, Bashti O, Matsumoto N, Noruzinia M. A novel 8-bp duplication in ADAT3 causes mild intellectual disability. Hum Genome Var 2018;5:7. [PubMed: 29796286]

156. Thomas E, Lewis AM, Yang Y, Chanprasert S, Potocki L, Scott DA. Novel Missense Variants in ADAT3 as a Cause of Syndromic Intellectual Disability. J Pediatr Genet 2019;8:244–51. [PubMed: 31687266]

157. Martinez FJ, Lee JH, Lee JE, Blanco S, Nickerson E, Gabriel S, et al. Whole exome sequencing identifies a splicing mutation in NSUN2 as a cause of a Dubowitz-like syndrome. J Med Genet 2012;49:380–5. [PubMed: 22577224]

158. Abbasi-Moheb L, Mertel S, Gonsior M, Nouri-Vahid L, Kahrizi K, Cirak S, et al. Mutations in NSUN2 cause autosomal-recessive intellectual disability. Am J Hum Genet 2012;90:847–55. [PubMed: 22541559]

159. Khan MA, Rafiq MA, Noor A, Hussain S, Flores JV, Rupp V, et al. Mutation in NSUN2, which encodes an RNA methyltransferase, causes autosomal-recessive intellectual disability. Am J Hum Genet 2012;90:856–63. [PubMed: 22541562]

160. Yang J, Xu J, Zhang L, Li Y, Chen M. Identifying key m(6)A-methylated lncRNAs and genes associated with neural tube defects via integrative MeRIP and RNA sequencing analyses. Front Genet 2022;13:974357. [PubMed: 36482889]

161. Zhang L, Cao R, Li D, Sun Y, Zhang J, Wang X, et al. Ethionine-mediated reduction of S-adenosylmethionine is responsible for the neural tube defects in the developing mouse embryo-mediated m6A modification and is involved in neural tube defects via modulating Wnt/beta-catenin signaling pathway. Epigenetics Chromatin 2021;14:52. [PubMed: 34863249]

162. Gupta T, Malkin MG, Huang S. tRNA function and dysregulation in cancer. Front Cell Dev Biol 2022;10:886642. [PubMed: 35721477]

163. Song P, Tayier S, Cai Z, Jia G. RNA methylation in mammalian development and cancer. Cell Biol Toxicol 2021;37:811–31. [PubMed: 34272618]

164. Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee US. Glioblastoma Multiforme: A Review of its Epidemiology and Pathogenesis through Clinical Presentation and Treatment. Asian Pac J Cancer Prev 2017;18:3–9. [PubMed: 28239999]

165. Cui Q, Yin K, Zhang X, Ye P, Chen X, Chao J, et al. Targeting PUS7 suppresses tRNA pseudouridylation and glioblastoma tumorigenesis. Nat Cancer. 2021;2:932–49. [PubMed: 35121864]

166. Miao FA, Chu K, Chen HR, Zhang M, Shi PC, Bai J, et al. Increased DKC1 expression in glioma and its significance in tumor cell proliferation, migration and invasion. Invest N. Drugs. 2019;37:1177–86.

167. Wang P, Wu M, Tu Z, Tao C, Hu Q, Li K, et al. Identification of RNA: 5-Methylcytosine Methyltransferases-Related Signature for Predicting Prognosis in Glioma. Front Oncol 2020;10:1119. [PubMed: 32974125]

168. Janin M, Ortiz-Barahona V, de Moura MC, Martinez-Cardus A, Llinas-Arias P, Soler M, et al. Epigenetic loss of RNA-methyltransferase NSUN5 in glioma targets ribosomes to drive a stress adaptive translational program. Acta Neuropathol 2019;138:1053–74. [PubMed: 31428936]

169. Huang QR, Li JW, Pan XB. A novel risk signature with 6 RNA binding proteins for prognosis prediction in patients with glioblastoma. Med (Baltim) 2021;100:e28065.

170. Wang B, Niu L, Wang Z, Zhao Z. RNA m1A Methyltransferase TRMT6 Predicts Poorer Prognosis and Promotes Malignant Behavior in Glioma. Front Mol Biosci 2021;8:692130. [PubMed: 34631793]

171. Huang Y, Ma J, Yang C, Wei P, Yang M, Han H, et al. METTL1 promotes neuroblastoma development through m(7)G tRNA modification and selective oncogenic gene translation. Biomark Res 2022;10:68. [PubMed: 36071474]

172. Li L, Yang Y, Wang Z, Xu C, Huang J, Li G. Prognostic role of METTL1 in glioma. Cancer Cell Int 2021;21:633. [PubMed: 34838021]

173. Dome A, Dymova M, Richter V, Stepanov G. Post-Transcriptional Modifications of RNA as Regulators of Apoptosis in Glioblastoma. Int J Mol Sci 2022;23:9272. [PubMed: 36012529]

174. Zhang S, Zhao S, Qi Y, Li B, Wang H, Pan Z, et al. SPI1-induced downregulation of FTO promotes GBM progression by regulating pri-miR-10a processing in an m6A-dependent manner. Mol Ther Nucleic Acids. 2022;27:699–717. [PubMed: 35317283]

175. Zhang S, Zhao BS, Zhou A, Lin K, Zheng S, Lu Z, et al. m(6)A Demethylase ALKBH5 Maintains Tumorigenicity of Glioblastoma Stem-like Cells by Sustaining FOXM1 Expression and Cell Proliferation Program. Cancer Cell. 2017;31:591–606 e596. [PubMed: 28344040]

176. Xi Z, Xue Y, Zheng J, Liu X, Ma J, Liu Y. WTAP expression predicts poor prognosis in malignant glioma patients. J Mol Neurosci 2016;60:131–6. [PubMed: 27370540]

177. Pan T, Wu F, Li L, Wu S, Zhou F, Zhang P, et al. The role m(6)A RNA methylation is CNS development and glioma pathogenesis. Mol Brain. 2021;14:119. [PubMed: 34281602]

178. Li F, Yi Y, Miao Y, Long W, Long T, Chen S, et al. N(6)-Methyladenosine Modulates Nonsense-Mediated mRNA Decay in Human Glioblastoma. Cancer Res 2019;79:5785–98. [PubMed: 31530567]

179. Dixit D, Prager BC, Gimple RC, Poh HX, Wang Y, Wu Q, et al. The RNA m6A Reader YTHDF2 Maintains Oncogene Expression and Is a Targetable Dependency in Glioblastoma Stem Cells. Cancer Discov 2021;11:480–99. [PubMed: 33023892]

180. Zhu X, Yang H, Zhang M, Wu X, Jiang L, Liu X, et al. YTHDC1-mediated VPS25 regulates cell cycle by targeting JAK-STAT signaling in human glioma cells. Cancer Cell Int 2021;21:645. [PubMed: 34863175]

181. Xu C, Yuan B, He T, Ding B, Li S. Prognostic values of YTHDF1 regulated negatively by mir-3436 in Glioma. J Cell Mol Med 2020;24:7538–49. [PubMed: 32449290]

182. Suvasini R, Shruti B, Thota B, Shinde SV, Friedmann-Morvinski D, Nawaz Z, et al. Insulin growth factor-2 binding protein 3 (IGF2BP3) is a glioblastoma-specific marker that activates phosphatidylinositol 3-kinase/mitogen-activated protein kinase (PI3K/MAPK) pathways by modulating IGF-2. J Biol Chem 2011;286:25882–90. [PubMed: 21613208]

183. Su R, Dong L, Li C, Nachtergaele S, Wunderlich M, Qing Y, et al. R-2HG Exhibits Anti-tumor Activity by Targeting FTO/m(6)A/MYC/CEBPA Signaling. Cell. 2018;172:90–105 e123. [PubMed: 29249359]

184. Miao YQ, Chen W, Zhou J, Shen Q, Sun Y, Li T, et al. N(6)-adenosine-methyltransferase-14 promotes glioma tumorigenesis by repressing argininosuccinate synthase 1 expression in an m6A-dependent manner. Bioengineered. 2022;13:1858–71. [PubMed: 35012429]

185. Huff S, Tiwari SK, Gonzalez GM, Wang Y, Rana TM. m(6)A-RNA Demethylase FTO Inhibitors Impair Self-Renewal in Glioblastoma Stem Cells. ACS Chem Biol 2021;16:324–33. [PubMed: 33412003]

186. Jin DI, Lee SW, Han ME, Kim HJ, Seo SA, Hur GY, et al. Expression and roles of Wilms' tumor 1-associating protein in glioblastoma. Cancer Sci 2012;103:2102–9. [PubMed: 22957919]

187. Cui Q, Shi H, Ye P, Li L, Qu Q, Sun G, et al. m(6)A RNA Methylation Regulates the Self-Renewal and Tumorigenesis of Glioblastoma Stem Cells. Cell Rep 2017;18:2622–34. [PubMed: 28297667]

188. Rocchi L, Barbosa AJ, Onofrillo C, Del Rio A, Montanaro L. Inhibition of human dyskerin as a new approach to target ribosome biogenesis. PLoS One. 2014;9:e101971. [PubMed: 25010840]

189. Micaelli M, Dalle Vedove A, Cerofolini L, Vigna J, Sighel D, Zaccara S, et al. Small-Molecule Ebselen Binds to YTHDF Proteins Interfering with the Recognition of N (6)-Methyladenosine-Modified RNAs. ACS Pharm Transl Sci 2022;5:872–91.

190. Yankova E, Blackaby W, Albertella M, Rak J, De Braekeleer E, Tsagkogeorga G, et al. Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia. Nature. 2021;593:597–601. [PubMed: 33902106]

191. Moroz-Omori EV, Huang D, Kumar Bedi R, Cheriyamkunnel SJ, Bochenkova E, Dolbois A, et al. METTL3 Inhibitors for Epitranscriptomic Modulation of Cellular Processes. ChemMedChem. 2021;16:3035–43. [PubMed: 34237194]

192. Rivera M, Locke AE, Corre T, Czamara D, Wolf C, Ching-Lopez A, et al. Interaction between the FTO gene, body mass index and depression: meta-analysis of 13701 individuals. Br J Psychiatry 2017;211:70. [PubMed: 28642257]

193. Du T, Rao S, Wu L, Ye N, Liu Z, Hu H, et al. An association study of the m6A genes with major depressive disorder in Chinese Han population. J Affect Disord 2015;183:279–86. [PubMed: 26047305]

194. Liu Y, Zhang H. RNA m6A Modification Changes in Postmortem Nucleus Accumbens of Subjects with Alcohol Use Disorder: A Pilot Study. Genes 2022;13:958. [PubMed: 35741720]

195. Bohnsack JP, Teppen T, Kyzar EJ, Dzitoyeva S, Pandey SC. The lncRNA BDNF-AS is an epigenetic regulator in the human amygdala in early onset alcohol use disorders. Transl Psychiatry 2019;9:34. [PubMed: 30728347]

196. Karanović J, Šviković S, Pantović M, Durica S, Brajušković G, Damjanović A, et al. Joint effect of ADARB1 gene, HTR2C gene and stressful life events on suicide attempt risk in patients with major psychiatric disorders. World J Biol Psychiatry 2015;16:261–71. [PubMed: 25732952]

197. Chimienti F, Cavarec L, Vincent L, et al. Correction: Brain region-specific alterations of RNA editing in PDE8A mRNA in suicide decedents. Transl Psychiatry 2019;9:112. [PubMed: 30872571]

198. Weissmann D, van der Laan S, Underwood MD, Salvetat N, Cavarec L, Vincent L, et al. Region-specific alterations of A-to-I RNA editing of serotonin 2c receptor in the cortex of suicides with major depression. Transl Psychiatry 2016;6:e878. [PubMed: 27576167]

199. Breen MS, Dobbyn A, Li Q, et al. Global landscape and genetic regulation of RNA editing in cortical samples from individuals with schizophrenia. Nat Neurosci 2019;22:1402–12. [PubMed: 31455887]

200. Choudhury M, Fu T, Amoah K, Jun HI, Chan TW, Park S, et al. Widespread RNA hypoediting in schizophrenia and its relevance to mitochondrial function. Sci Adv 2023;9:eade9997. [PubMed: 37027465]

201. Ye F, Wang T, Wu X, Liang J, Li J, Sheng W. N6-Methyladenosine RNA modification in cerebrospinal fluid as a novel potential diagnostic biomarker for progressive multiple sclerosis. J Transl Med 2021;19:316. [PubMed: 34294105]

202. Keller L, Xu W, Wang HX, Winblad B, Fratiglioni L, Graff C. The Obesity Related Gene, FTO, Interacts with APOE, and is Associated with Alzheimer's Disease Risk: A Prospective Cohort Study. J Alzheimer's Dis 2011;23:461–9. [PubMed: 21098976]

203. Khermesh K, D'Erchia AM, Barak M, Annese A, Wachtel C, Levanon EY, et al. Reduced levels of protein recoding by A-to-I RNA editing in Alzheimer's disease. RNA 2016;22:290. [PubMed: 26655226]

204. Ma Y, Dammer EB, Felsky D, Duong DM, Klein HU, White CC, et al. Atlas of RNA editing events affecting protein expression in aged and Alzheimer's disease human brain tissue. Nat Commun 2021;12:1–16. [PubMed: 33397941]

205. Aizawa H, Sawada J, Hideyama T, Yamashita T, Katayama T, Hasebe N, et al. TDP-43 pathology in sporadic ALS occurs in motor neurons lacking the RNA editing enzyme ADAR2. Acta Neuropathol 2010;120:75–84. [PubMed: 20372915]

206. Salvetat N, Checa-Robles FJ, Patel V, Cayzac C, Dubuc B, Chimienti F, et al. A game changer for bipolar disorder diagnosis using RNA editing-based biomarkers. Transl Psychiatry 2022;12:1–10. [PubMed: 35013113]

207. Lo N, Xu X, Soares F, He HH. The Basis and Promise of Programmable RNA Editing and Modification. Front Genet 2022;13:834413. [PubMed: 35154288]

208. Song J, Dong L, Sun H, Luo N, Huang Q, Li K, et al. CRISPR-free, programmable RNA pseudouridylation to suppress premature termination codons. Mol Cell. 2023;83:139–55.e139. [PubMed: 36521489]

209. Adachi H, Pan Y, He X, Chen JL, Klein B, Platenburg G, et al. Targeted pseudouridylation: an approach for suppressing nonsense mutations in disease genes. Mol Cell. 2023;83:637–51.e639. [PubMed: 36764303]

210. Yasukawa T, Suzuki T, Ueda T, Ohta S, Watanabe K. Modification defect at anticodon wobble nucleotide of mitochondrial tRNAs(Leu)(UUR) with pathogenic mutations of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. J Biol Chem 2000;275:4251–7. [PubMed: 10660592]

211. Kirino Y, Yasukawa T, Marjavaara SK, Jacobs HT, Holt IJ, Watanabe K, et al. Acquisition of the wobble modification in mitochondrial tRNALeu(CUN) bearing the G12300A mutation suppresses the MELAS molecular defect. Hum Mol Genet 2006;15:897–904. [PubMed: 16446307]

212. Rohner E, Yang R, Foo KS, Goedel A, Chien KR. Unlocking the promise of mRNA therapeutics. Nat Biotechnol 2022;40:1586–1600. [PubMed: 36329321]

213. Anthony K RNA-based therapeutics for neurological diseases. RNA Biol 2022;19:176–90. [PubMed: 35067193]

214. Mantile F, Prisco A. Vaccination against β-Amyloid as a Strategy for the Prevention of Alzheimer's Disease. Biology. 2020;9:425. [PubMed: 33260956]

215. Tang H, Hammack C, Ogden SC, Wen Z, Qian X, Li Y, et al. Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth. Cell Stem Cell. 2016;18:587–90. [PubMed: 26952870]

216. Bohmwald K, Andrade CA, Galvez NMS, Mora VP, Munoz JT, Kalergis AM. The causes and long-term consequences of viral encephalitis. Front Cell Neurosci. 2021;15:755875. [PubMed: 34916908]

217. Barbier AJ, Jiang AY, Zhang P, Wooster R, Anderson DG. The clinical progress of mRNA vaccines and immunotherapies. Nat Biotechnol 2022;40:840–54. [PubMed: 35534554]

218. Rao Y, Du S, Yang B, Wang Y, Li Y, Li R, et al. NeuroD1 induces microglial apoptosis and cannot induce microglia-to-neuron cross-lineage reprogramming. Neuron 2021;109:4094–108.e4095. [PubMed: 34875233]

219. Wang LL, Serrano C, Zhong X, Ma S, Zou Y, Zhang CL. Revisiting astrocyte to neuron conversion with lineage tracing in vivo. Cell. 2021;184:5465–81 e5416. [PubMed: 34582787]

220. Martier R, Konstantinova P. Gene therapy for neurodegenerative diseases: slowing down the ticking clock. Front Neurosci 2020;14:580179. [PubMed: 33071748]

221. Parambi DGT, Alharbi KS, Kumar R, Harilal S, Batiha GE, Cruz-Martins N, et al. Gene therapy approach with an emphasis on growth factors: theoretical and clinical outcomes in neurodegenerative diseases. Mol Neurobiol 2022;59:191–233. [PubMed: 34655056]

222. Deng LJ, Deng WQ, Fan SR, Chen MF, Qi M, Lyu WY, et al. m6A modification: recent advances, anticancer targeted drug discovery and beyond. Mol Cancer. 2022;21:52. [PubMed: 35164788]

223. Lan L, Sun YJ, Jin XY, Xie LJ, Liu L, Cheng L. A Light-Controllable Chemical Modulation of m(6) A RNA Methylation. Angew Chem Int Ed Engl 2021;60:18116–121. [PubMed: 34107156]

224. Tang T, Han Y, Wang Y, Huang H, Qian P. Programmable System of Cas13-Mediated RNA Modification and Its Biological and Biomedical Applications. Front cell Dev Biol 2021;9:677587. [PubMed: 34386490]

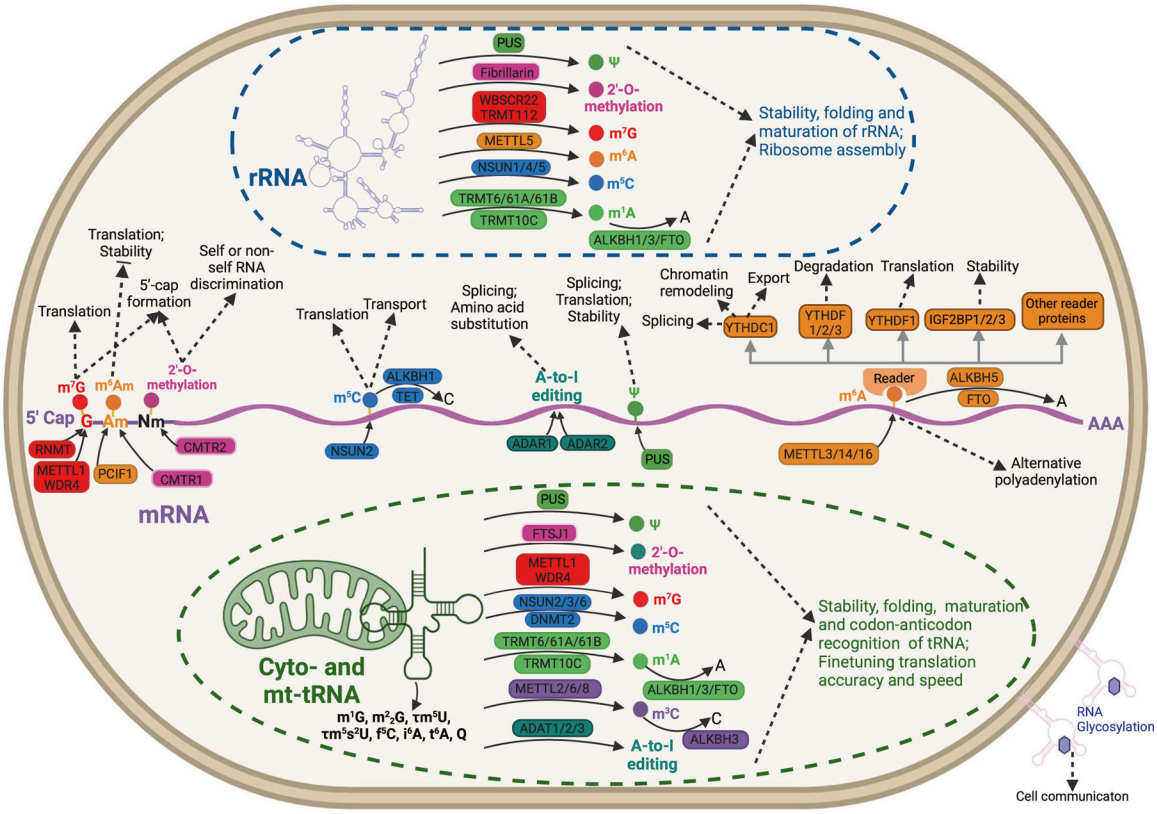

Fig. 1. Epitranscriptome landscape.

Schematic illustration of several important RNA modifications focusing on mRNA, tRNA and rRNAs, as well as their writers, erasers, readers, and general molecular functions. The nucleus or cytoplasm localization of RNA modifications and their regulators are not specified. m^6A: N^6-methyladenosine; Ψ: Pseudo-uridine; m^5C: C^5-methylcytosine; m^3C: N^3-methylcytosine; m^1A: N^1-methyladenosine; m^6Am: 2'-O-methyladenosine; m^7G: N^7-methylguanosine; m^1G: N1-methylguanosine; m^2_2G: N2, N2 dimethylguanosine; τm^5U: 5-taurinomethyluridine; τm^5s^2U: 5-taurinomethyl-2-thiouridine; f^5C: 5-formylcytidine; i^6A: N^6-isopentenyladenosine; t^6A: N^6-threonylcarbamoyladenosine; Q: queuosinylation.

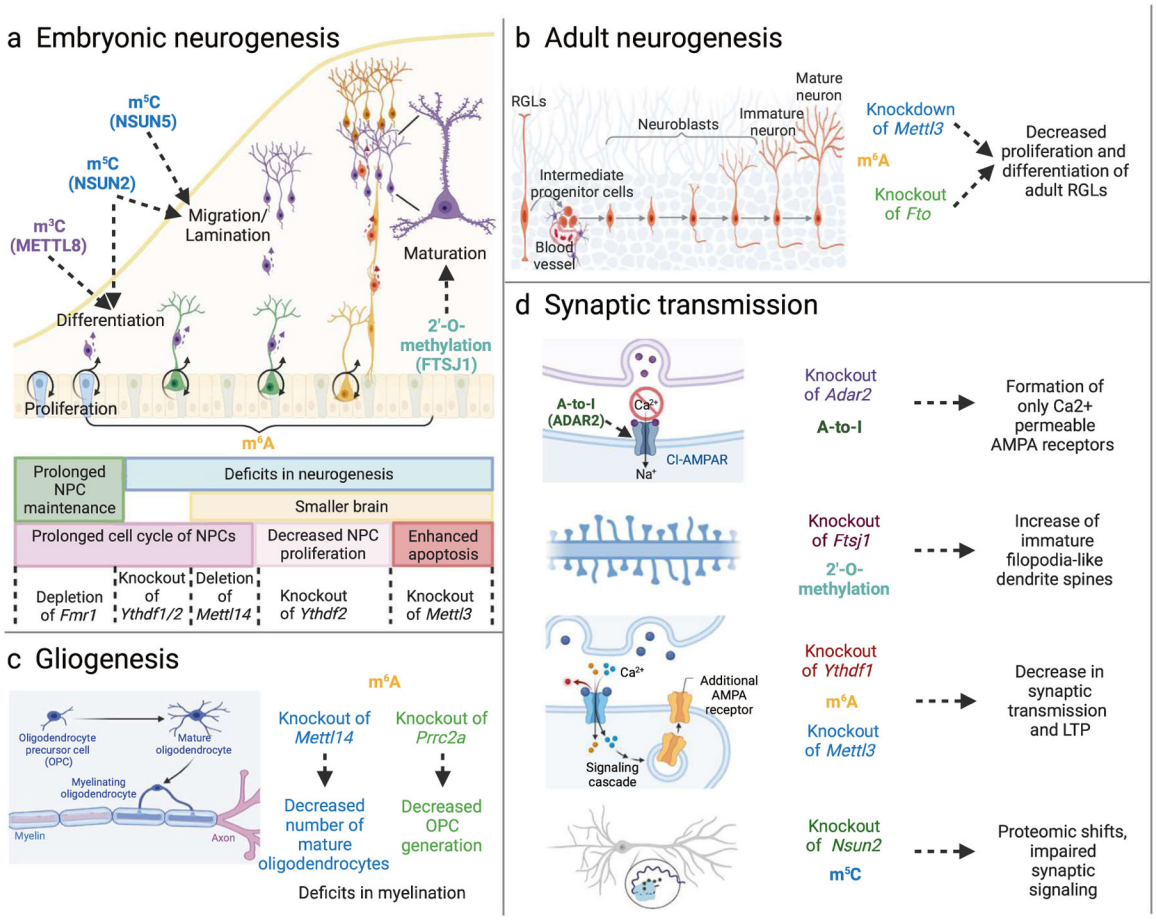

Fig. 2. Epitranscriptomics in the nervous system development.

Involvement of epitranscriptomics in regulating brain development, including embryonic neurogenesis (**a**), adult neurogenesis (**b**), gliogenesis (**c**), and synaptic transmission (**d**). Examples of brain development-related phenotypes in the mouse models with loss of function of several RNA modification regulators, like m^6A, m^5C, m^3C, 2′-O-methylation, and A-to-I editing, are illustrated. A-to-I: A-to-I editing. LTP: Long-term potentiation.

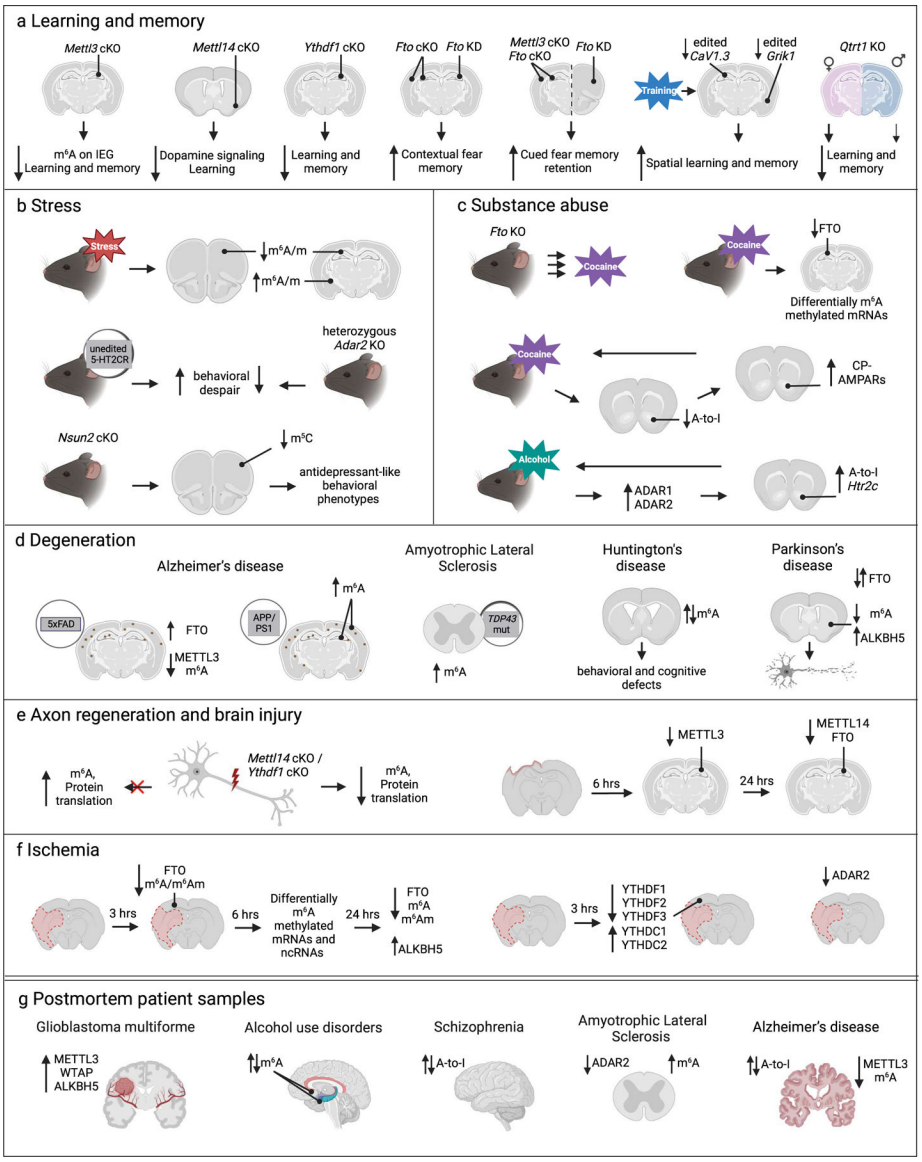

Fig. 3. Functions of RNA modificaions in the adult nervous system.

Functions of RNA modifications in (**a**) learning and memory, (**b**) stress response, (**c**) neurodegeneration, (**d**) substance abuse disorders, (**e**) axon regeneration and brain injury, (**f**) post-ischemia recovery in the adult nervous system revealed by the gain- and loss-of-function experiments in mice and (**g**) in the adult human brain based on data from postmortem patient samples. TBI traumatic brain injury, CI-AMPAR calcium-impermeable AMPA receptor, CP-AMPAR calcium-permeable AMPA receptor; ↑↓: changes were observed in both directions depending on the brain region, environmental factors or specific transcripts.

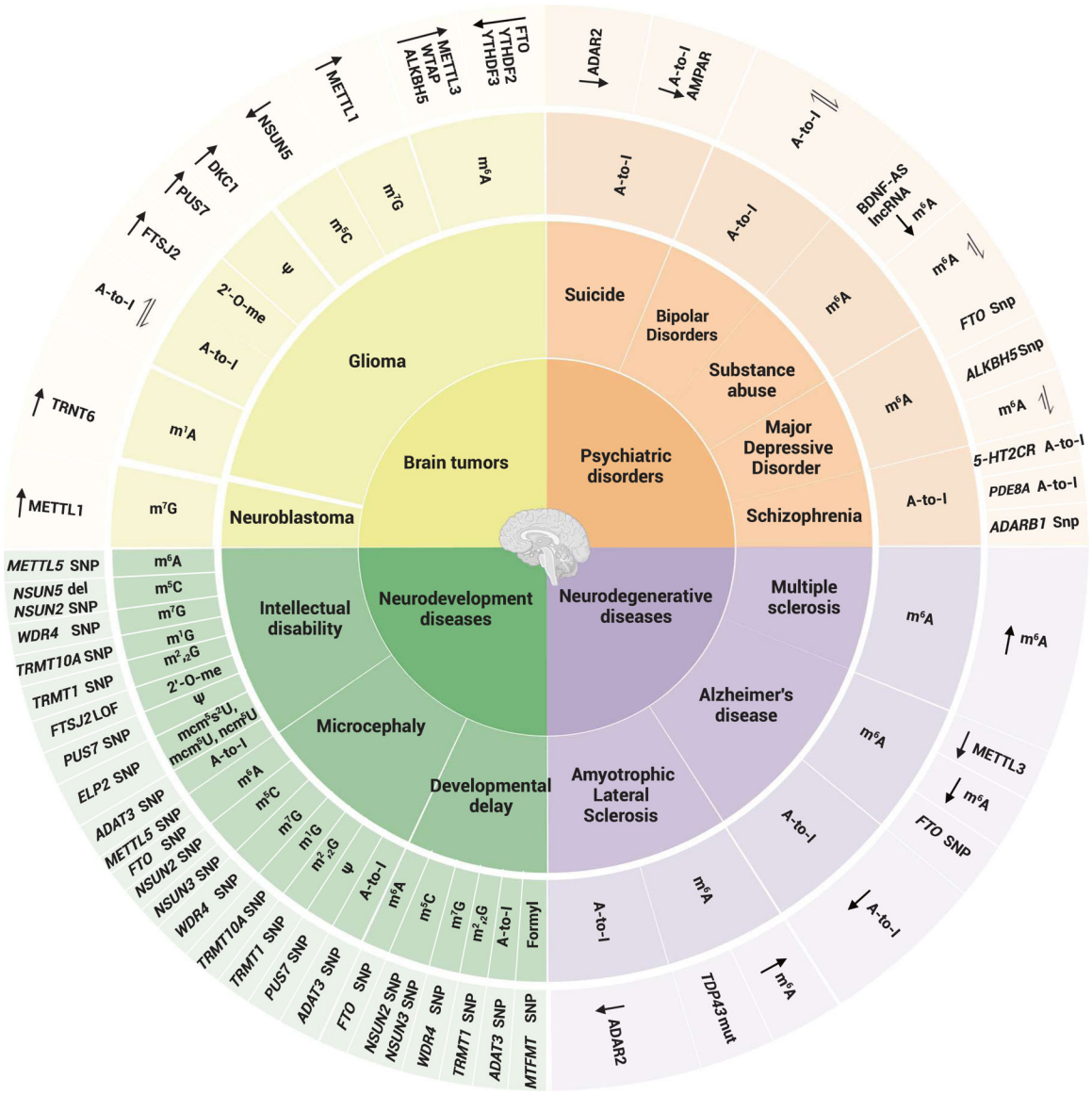

Fig. 4. Alterations in RNA modification pathways associated with brain disorders in humans.

mcm^5U: 5-methoxycarbonylmethyl uridine; mcm^5s^2U: 5-methoxycarbonylmethyl-2-thiouridine; ncm^5U: 5-carbamoylmethyl uridine.

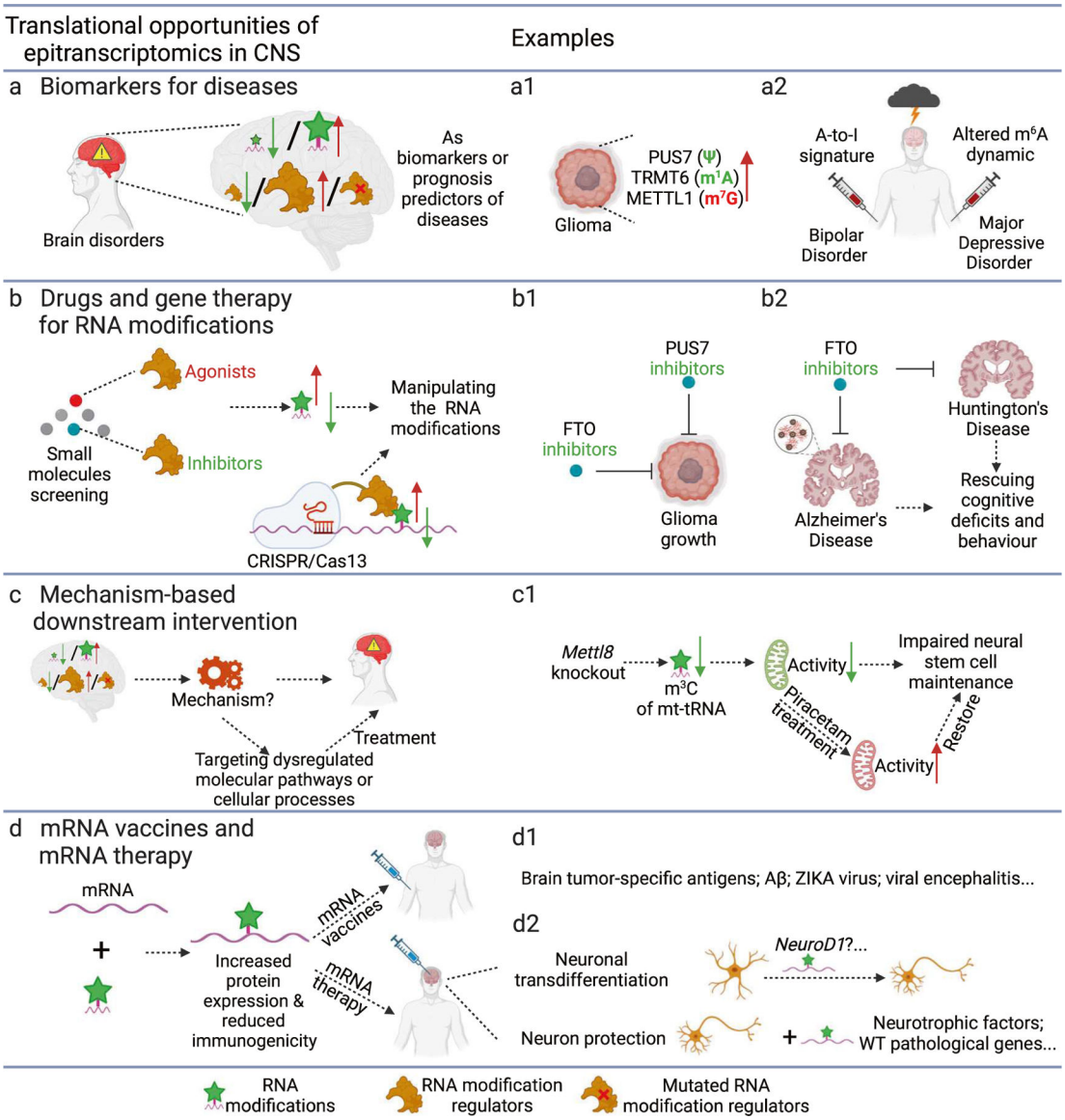

Fig. 5. Potential translational opportunities of epitranscriptomics in the nervous system.

a Application of epitranscriptomics as biomarkers for human brain disorders. The levels of RNA modifications, expression levels of RNA modification regulators, and mutations of RNA modification regulators can potentially be established as biomarkers for diagnostics or prognosis predictors of brain disorders, such as glioma (**a1**) or psychiatric disorders (**a2**). **b** Drugs and gene therapy for RNA modifications. Inhibitors of RNA modification regulators or CRISPR/Cas13-based RNA modification editing can be developed and used to manipulate the levels of RNA modifications and alleviate symptoms of brain disorders. For instance, FTO (the m^6A eraser) and PUS7 (Ψ writer) inhibitors have the potential of attenuating the growth of GSCs (**b1**), and FTO inhibitors have the potential of restoring the cognitive deficits in AD and HD patients based on *Fto* KD and KO studies in mouse models (**b2**). **c** Mechanism-based downstream intervention. Deciphering dysregulated downstream molecular pathways mediating the pathogenesis of epitranscriptomics-related brain disorders

can help to attenuate diseases by targeting and restoring specific dysregulated downstream molecular pathways or cellular processes. For example, impaired NPC maintenance caused by attenuated mitochondria activity in *Mettl8* (mt-tRNA m^3C writer) knockout mice can be rescued by pharmacologically enhancing mitochondria function with piracetam treatment (**c1**). **d** mRNA vaccines and mRNA therapy. Application of RNA modifications can enhance the protein expression and reduce the immunogenicity of mRNA introduced into the cells, potentially increasing the efficacy of mRNA vaccines (**d1**) and mRNA therapy (**d2**) targeting various brain disorders.