HHS Public Access

Author manuscript

Transl Stroke Res. Author manuscript; available in PMC 2022 February 01.

Published in final edited form as:

Transl Stroke Res. 2022 February ; 13(1): 1–11. doi:10.1007/s12975-021-00927-z.

Epitranscriptomic Modifications Modulate Normal and Pathological Functions in CNS

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Abstract

RNA is more than just a combination of four genetically encoded nucleobases as it carries extra information in the form of epitranscriptomic modifications. Diverse chemical groups attach covalently to RNA to enhance the plasticity of cellular transcriptome. The reversible and dynamic nature of epitranscriptomic modifications allows RNAs to achieve rapid and context-specific gene regulation. Dedicated cellular machinery comprising of writers, erasers, and readers drives the epitranscriptomic signaling. Epitranscriptomic modifications control crucial steps of mRNA metabolism such as splicing, export, localization, stability, degradation, and translation. The majority of the epitranscriptomic modifications are highly abundant in the brain and contribute to activity-dependent gene expression. Thus, they regulate the vital physiological processes of the brain, such as synaptic plasticity, neurogenesis, and stress response. Furthermore, epitranscriptomic alterations influence the progression of several neurologic disorders. This review discussed the molecular mechanisms of epitranscriptomic regulation in neurodevelopmental and neuropathological conditions with the goal to identify novel therapeutic targets.

Keywords

RNA modifications; N^6-Methyladenosine; N^1-Methyladenosine; Inosine; 5-Methylcytosine; Pseudouridine; Brain; Stroke

Introduction

Analogous to epigenetics representing DNA and histone modifications, the epitranscriptome refers to RNA modifications. To date, 172 types of epitranscriptomic modifications are identified, which are far more diverse than the currently known 51 epigenetic modifications [1, 2]. In an RNA, adenosine, cytidine, guanosine, uridine, and ribose can be modified by the addition of functional groups such as methyl, acyl, thioalkyl, and glycosyl groups

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Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest The authors declare no competing interests.

[1]. Methyl group modifications are highly pervasive with 72 variants [1]. Apart from the covalent addition of these functional groups, epitranscriptomic modifications also include post-transcriptional nucleobase exchange and isomerization [3]. The well-characterized epitranscriptomic modifications include N^6-methyladenosine (m^6A), N^1-methyladenosine (m^1A), inosine (I), 5-methylcytosine (m^5C), and pseudouridine (ψ) [4] (Fig. 1). Among various classes of RNAs, the tRNAs show the highest prevalence of epitranscriptomic modifications (~ 25% of tRNAs show modifications), which are thought to stabilize their tertiary structure [5]. However, transcriptome-wide profiling of modified RNAs after selective enrichment showed the widespread distribution of epitranscriptomic modifications in both mRNAs and other classes of noncoding RNAs [3]. Identification of machinery responsible for depositing (writers) and removing (erasers) of m^6A in RNAs and deciphering of m^6A-modified RNAs (readers) garnered attention to epitranscriptomics. For example, the discovery of m^6A demethylase fat mass and obesity-associated protein (FTO) spurred the interest to investigate the reversibility and dynamic existence of other epitranscriptomic modifications [6].

Epitranscriptomic modifications fine-tune gene expression by regulating multiple steps of mRNA processing such as splicing, export, stability, degradation, and translation [7]. Importantly, several epitranscriptomic modifications, including m^6A, m^1A, and I, are enriched in the brain and essential for CNS physiological functions such as synaptic transmission and neurogenesis [8–10]. Epitranscriptomic alterations are also associated with neurologic dysfunction, and a better understanding of these molecular mechanisms will provide novel therapeutic targets [4]. This review delineates the role of some of the most abundant and well-studied epitranscriptomic modifications, including m^6A, m^1A, I, m^5C, and ψ, and the key epitranscriptomic enzymes involved in the post-transcriptional gene regulation in brain development and diseases, with an emphasis on acute brain injuries, chronic neurodegeneration, tumorigenesis, and neuropsychiatric disorders.

N^6-Methyladenosine (m^6A)

The m^6A over-represents the mammalian epitranscriptome with an abundance ranging 0.1–0.4% of total adenines [11]. It is prevalent at the RR**A**CH sequence motif (R can be adenosine or cytosine or uracil, A is adenosine that can be modified to m^6A, C is cytosine, and H can be guanosine or adenosine) present in the 3′-UTR of mRNAs and deposited by the multi-subunit writer complex consisting of methyltransferase like (METTL) 3 and 4 and Wilms tumor-associated protein (WTAP) [8, 12]. Two demethylases, FTO and alkB homology (ALKBH) 5, erase m^6A methylation [6, 13]. The m^6A readers bind and recruit diverse cellular machinery to the methylated mRNAs to mediate the downstream signaling. The well-characterized m^6A readers include YT521-B homology (YTH) domain family/containing proteins (YTHDF1, 2, and 3, and YTHDC1 and 2), each with distinct functions [14]. The m^6A methylation is known to regulate multiple steps of mRNA processing such as splicing, export, localization, stability, translation, and degradation, thereby fine-tuning the gene expression [4]. Notably, CNS shows the highest m^6A abundance in the mammalian body [8]. As m^6A and its machinery are highly enriched in axons and dendrites, it is thought to mediate the activity-dependent gene regulation [15]. The m^6A levels gradually increase in the brain during development and are indispensable for neurogenesis, axonogenesis, and

gliogenesis [4]. The FTO-deficient mice show severe developmental brain deficits, including microcephaly, postnatal growth retardation, and aberrant dopaminergic neurotransmission [16]. Interestingly, m^6A crosstalk with epigenetic modifications such as H3K27me3 at the proliferation-related gene loci during neurogenesis [17].

The m^6A also controls axon guidance and elongation by promoting the local translation of key axonal transcripts such as Gap43 and Robo3.1 [18, 19]. In the adult brain, m^6A acts as a circadian pacesetter by decreasing the levels of crucial clock transcripts such as CK1δ, which otherwise prolongs the oscillation [20]. More importantly, m^6A shapes the local proteome at the synapse by regulating the translation of over 1,000 synaptic plasticity-related transcripts in a YTHDF1-dependent manner [15]. Therefore, attenuating m^6A-reliant neural plasticity impairs adaptive behaviors such as anxiety, depression, addiction, and cognition [21]. Moreover, neuronal m^6A is sensitive to stressful stimuli such as heat shock and hypoxia, suggesting its dynamic regulatory potential [22, 23]. Because of these striking abilities, m^6A signaling is implicated in the progression of acute CNS injuries, chronic neurodegeneration, tumorigenesis, and neuropsychiatric disorders [4]. For example, we recently reported that cerebral ischemia downregulates FTO, thereby inducing m^6A hypermethylation in inflammatory transcripts such as Tnf-α and IL-6, and apoptotic transcripts such as Fas and Bcl2a1c [24]. Curtailing m^6A hypermethylation by FTO overexpression was shown to decrease oxygen–glucose deprivation-induced apoptosis in the cortical neurons [25]. In contrast, traumatic brain injury decreases METTL3 expression in the hippocampus, leading to hypomethylation of neuronal metabolism-related transcripts such as Pde12 and Qsox2 [26]. Furthermore, spinal cord injury (SCI) was shown to induce hypomethylation of neural regeneration-related transcripts such as Hsp90ab1 and Igf2bp1, preventing their degradation [27]. Therefore, m^6A drives neurotoxic or neuroprotective signaling after acute injury to the CNS in a context-specific manner. Interestingly, FTO protein levels were shown to be significantly elevated in both Alzheimer's disease (AD) and Parkinson's disease (PD) rodent brains [28, 29]. Additionally, FTO knockout ameliorated the AD-associated cognitive decline, and its inhibition reduced PD-associated neuronal loss in rodents [28, 29]. Thus, targeting FTO appears to be a viable therapeutic approach to prevent chronic neurodegeneration.

The m^6A dysregulation due to increased expression of writers (METTL3 and WTAP) and erasers (FTO and ALKBH5) was shown to predict poor prognosis and survival in glioblastoma multiforme (GBM) patients [30]. Of note, METT3 is proposed to act as both an oncogene and a tumor suppressor [31, 32], whereas FTO and ALKBH5 display oncogenic potential as their inhibition reduced glioma growth and tumorigenicity in mice [30]. Mechanistically, altered m^6A methylation of several master regulators, including RNA editing enzymes such as ADAR1 and transcription factors such as Sox2 and FOXM1, drives GBM pathogenesis [33, 34]. The SNPs in m^6A effectors such as FTO, ALKBH5, and YTHDC2 are also associated with various neuropsychiatric disorders such as major depressive disorder, attention-deficit/hyperactivity disorder, and autism spectrum disorder [35–37]. Overall, m^6A warrants further research to evaluate its therapeutic potential in various neurological diseases due to its high abundance and pervasive functional repertoire.

N^1-Methyladenosine (m^1A)

Although the m^1A in the mammalian RNA was first identified in 1961, it remains a poorly characterized epitranscriptomic mark [38]. The m^1A was initially discovered in tRNAs and rRNAs and recently shown in nuclear and mitochondrial mRNAs [9, 39–41]. Approximately 0.02% of adenines in the human transcriptome are m^1A modified, and therefore, m^1A is ten times lower in abundance than m^6A [9, 41]. Under alkaline conditions, m^1A undergoes Dimroth rearrangement to form m^6A, making it challenging to measure accurately [42]. In contrast to m^6A, the m^1A is predominantly localized in the 5′-UTR region near the translation initiation sites [40]. The positive charge conferred by m^1A is thought to influence RNA–protein interactions, thereby correlating with the translation efficiency [9]. Interestingly, m^1A methylases are organelle-specific with cytosolic m^1A deposited by tRNA methyltransferase (TRMT) 6/61A complex, and mitochondrial m^1A deposited by TRMT10C/61B complex [40]. Like m^6A, m^1A is also a reversible epitranscriptomic mark and erased by ALKBH3 in the mRNA and ALKBH1 in the tRNA [39, 43]. Based on the proteomic profiling of the m^1A interactome, recent studies proposed YTH domain-containing proteins as the putative m^1A readers [44, 45]. Despite its low abundance, m^1A is dynamically regulated by cellular stress. Specifically, ischemia reduces and heat shock increases the global m^1A levels [9, 23]. Moreover, m^1A safeguards mRNAs in the stress granules during heat shock stress and promotes their translation during recovery, indicating its protective function [46]. Notably, the brain has the highest abundance of m^1A, followed by kidney, muscle, heart, and liver [9]. However, as the transcriptome-wide profile of m^1A in the brain is not yet evaluated, its function in brain physiology and diseases is not yet understood. Piecemeal evidence indicates the association of m^1A machinery with GBM progression. The expression levels of TRMT6 and TRMT61A were found to be higher in the aggressive form of GBM compared to the low-grade tumors [47]. Furthermore, the mRNA expression of TRMT61A was observed to be decreased in GBM cells treated with an anticancer agent [48]. The m^1A is embedded within the tertiary fold configuration in the tRNA and is thought to be crucial for stabilizing its three-dimensional structure [49]. The tRNAs are cleaved to produce tRNA halves (tiRNAs) by ribonuclease angiogenin following cellular stress, exposing the m^1A tag [50, 51]. Interestingly, the m^1A-tagged tiRNA levels were shown to be significantly elevated after stroke [52]. Furthermore, treatment of anti-inflammatory, neuroprotective agent minocycline reduced the m^1A-tagged tiRNA levels in PC12 cells subjected to oxygen–glucose deprivation [53]. More importantly, the plasma concentration of the m^1A, an indirect measure of tiRNA levels, is significantly increased in both ischemic and hemorrhagic stroke patients compared to healthy controls and correlated with the infarct size and hematoma volume [54]. All these studies show the possibility of m^1A as a stroke biomarker. Furthermore, m^1A levels were reported to be significantly increased in the urine of AD patients compared to control subjects [55]. However, the role of m^1A in mRNA metabolism after stroke and other neurological diseases remains to be investigated in detail.

Inosine (I)

In addition to the covalent modifications of RNA like m^6A and m^1A, epitranscriptomic regulation also comprises RNA editing that involves alteration of the mRNA nucleotides

relative to the genomic sequence. The conversion of adenosine to inosine (A-to-I) by adenosine deaminase that acts on RNA (ADAR) family of enzymes is an example of this type of modification that is highly prevalent with over 2 million sites in the human transcriptome [56]. To date, 3 mammalian ADARs have been identified, with ADAR1 and 2 being enzymatically active and ADAR3 being a brain-specific negative modulator of editing [57]. A-to-I editing occurs in the double-stranded RNA structures found within the Alu repetitive elements of pre-mRNAs and noncoding RNAs [58]. Although the sequence motif preferred by ADARs remains elusive, several *trans* regulators such as Pin1, WWP2, and AIMP2 were shown to control the editing activity [10, 59]. Greater than 75% of these sites are present in the intronic regions, whereas only 0.17% occur in the exonic regions of mRNAs, and the remaining are intergenic [60]. Interestingly, inosine is recognized by the translation machinery as guanosine, and therefore, A-to-I editing directly modulates the function of ion channels and neurotransmitter receptors, including GluA2, 5-HT_{2C}R, and K_v1.1 [61]. A-to-I editing was also shown in the noncoding RNAs, where it influenced splicing, translation, and miRNA targeting [62]. Brain shows the highest expression of ADAR1 and 2 and A-to-I editing activity in the mammalian body [10]. Furthermore, neurons exhibit a higher editing activity relative to other cell types within the brain [63]. A-to-I editing is known to be elevated spatiotemporally during brain development and its dysregulation was extensively reported in several neurological disorders [64–66]. A-to-I editing was shown to be reduced in the transcripts such as 5-HT_{2C}R and K_v1.1 following SCI due to the downregulation of ADAR2 [67]. Similarly, the editing levels in GluA2 mRNA were reported to be reduced after cerebral ischemia due to the loss of ADAR2 and its overexpression protected the hippocampal neurons from ischemic injury [68]. Recent studies also reported a significant decrease in the total RNA editing during GBM progression [69] along with downregulation of ADAR2 and upregulation of ADAR3 in GBM patients [70, 71]. Furthermore, several transcripts related to neuronal signaling such as GluA2, GluK1, GluK2, and GABRA3 and glioma growth such as CDC14B were shown to undergo hypo-editing in GBM [69, 72, 73]. In medulloblastomas, A-to-I editing was observed to be diminished in a key Hedgehog signaling-related transcript—GLI1, subsequently inhibiting its transcriptional activity [74]. The A-to-I editing of several glutamate receptors such as GRIA2, 3, and 4 and ion channels such as UNC80 was significantly downregulated in the hippocampus and frontal and temporal lobes of AD patients compared to healthy controls [75]. Another recent study reported differential RNA editing of the transcripts related to endocytic and inflammatory pathways such as TREM2 and BIN1 in the blood of the multi-ethnic AD disease cohort [76]. ADeditome database cataloged 108,010 RNA editing events from 1,524 AD patient samples that were associated with disease progression [77]. The hypo-editing of GluA2 due to the loss of ADAR2 was shown to be related to the death of motor neurons in amyotrophic lateral sclerosis (ALS) patients [78]. Furthermore, AAV9-mediated overexpression of ADAR2 in the ADAR2-deficient mice prevented motor neuron death and improved motor function [79]. Taken together, ADAR2-mediated epitranscriptomic editing of neural receptors is impaired in brain tumors and in acute and chronic neurodegenerative conditions.

5-Methylcytosine (m^5C)

5-Methylcytosine (5mC) is a well-characterized epigenetic modification in DNA. The rRNAs, tRNAs, and mRNAs are also shown to undergo methylation of cytosine at the 5^{th} position (m^5C) [80]. The abundance of m^5C in RNAs is variable across species, ranging from 0.03 to 0.1% of total cytosines [81]. Similar to m^1A, the m^5C is also enriched near the translation initiation sites in the 5′ UTR region of mRNA [82]. Two isoforms of NOP2/Sun domain family (NSUN) methyltransferases were shown to deposit m^5C in the mRNA based on the sequence context. While NSUN2 methylates the **C**NGGG motif in the 5′ UTR, NSUN6 methylates the **C**TCCA motif in the 3′ UTR [83, 84]. Furthermore, the ten-eleven translocation (Tet) family of enzymes Tet1, Tet2, and Tet3 that oxidize 5mC to 5-hydroxymethylcytosine (5hmC) in DNA also catalyze m^5C to hm^5C in the RNA, but the prevalence of hm^5C in comparison to m^5C is very low (1:5,000) [85]. The mRNA export adaptor Aly/REF export factor (ALYREF) directly binds to m^5C and promotes the nuclear export of the methylated mRNAs [82]. In addition, Y-box binding protein 1 (YBX1) binds and stabilizes the m^5C-modified mRNAs during maternal to zygotic transition in zebrafish [86]. Furthermore, m^5C in the coding regions of mRNAs positively correlates with their translation efficiency, particularly m^5C coordinates with m^6A to promote the translation of p21 mRNA [83, 87]. NSUN6-dependant m^5C methylation in the 3′ UTR was shown to promote translation termination [84]. Intriguingly, m^5C is induced at the sites of DNA double-stranded breaks and serves as a signal to recruit the DNA repair proteins such as RAD51 and RAD52 to promote homologous recombination [88]. This indicates that the regulatory potential of m^5C and m^6A might be similar despite a 3–10 times lower abundance of m^5C [89]. Despite their overlapping functions, the transcriptome-wide crosstalk between m^6A and m^5C is not yet evaluated. The m^5C seems to be important for normal brain function. In humans, a missense mutation in the NSUN2 gene was linked to intellectual disability [90]. Furthermore, NSUN2 is enriched in the Purkinje cells of the cerebellum, hinting that mutant NSUN2 interferes with the GABAergic cerebellar circuitry in humans [90]. A causal link was identified between homozygous splice mutation in NSUN2 and Dubowitz syndrome, clinically characterized by neurological abnormalities such as microcephaly and behavioral deficits like speech delay [91]. More importantly, NSUN2 knockout mice show severe neurodevelopmental defects, such as decreased neuronal cell size and impaired synaptogenesis [92]. Mechanistically, loss of NSUN2 in the neuroepithelial stem cells of the developing human brain causes hypomethylation and subsequent cleavage of tRNAs, ultimately inhibiting the migration and differentiation of neural progenitors [93]. Moreover, the loss of m^5C in noncoding vault RNAs due to NSUN2 deficiency impairs the generation of microRNA-like molecules that regulate the intellectual disability-associated ion channel proteins [94]. A recent study found that the mRNA expression of NSUN6 was significantly decreased in human GBM samples compared to healthy controls [84]. These studies indicate that altered m^5C might promote neurological dysfunction in various conditions. It was also reported that hm^5C is widespread in RNAs in the brainstem, hippocampus, amygdala, cortex, and cerebellum of mice [95]. Furthermore, the abundance of RNA hm^5C was shown to be decreased significantly in the hippocampus, substantia nigra, and striatum of the mice subjected to MPTP-induced

PD [95]. This indicates the potential interplay between m^5C and hm^5C in RNAs during neuropathological conditions.

Pseudouridine (ψ)

The ψ formed by uridine isomerization is the first epitranscriptomic modification discovered [96]. The ψ is the second most highly abundant epitranscriptomic modification after m^6A in mammalian mRNA, observed in ~ 0.3% of the total uridines [97]. The ψ is known to stabilize the secondary structure of tRNAs and rRNAs by providing an extra hydrogen bond, but its prominence in mRNAs is not yet studied in detail [98]. Transcriptome-wide mapping of ψ conservatively identified 260 sites in 238 mRNAs of yeast and HeLa cells [99]. More importantly, ψ is regulated in mRNAs in response to environmental cues such as nutrient deprivation, oxidative stress, and heat shock [97, 99, 100]. Among the 13 pseudouridine synthases (PUSs) that catalyze the pseudouridylation in various types of RNA, PUS 1, 4, 6, and 7 can generate ψ in mRNAs [101–103]. There are no known erasers for this epitranscriptomic mark, suggesting that ψ might be irreversible. A recent study demonstrated that methionine aminoacyl $tRNA^{Met}$ synthetase (MRS) functions as ψ reader and reduces the translation initiation of YEF3 mRNA in the yeast [103]. Another study showed that mRNA pseudouridylation impedes translation elongation [104]. Although these studies show that ψ affects translation fidelity, the mechanism of how it influences the ribosome function remains elusive. Multiple studies linked the aberrant pseudouridylation with neurological disorders. Humans with PUS3 mutation display intellectual disability and PUS1 mutation develop mild cognitive impairment, probably due to perturbed tRNA pseudouridylation [105, 106]. A recent study demonstrated the beneficial role of pseudouridylation in myotonic dystrophy type 2 (DM2) patients. Mechanistically, pseudouridylation within the toxic CCUG repeats moderately prevents the sequestration of splicing regulator Muscleblind-like 1 protein, which otherwise drives the DM2 pathology [107]. Overall, ψ is a widespread epitranscriptomic mark, but its function during brain development and diseases is still unexplored.

Clinical Significance

Epitranscriptomic imbalance is increasingly recognized as a molecular hallmark of various CNS diseases (Fig. 2). Reversing the epitranscriptomic alterations by targeting its machinery has proven to be a novel therapeutic strategy for acute and chronic neurological disorders [4]. However, the evidence for designing drugs to modulate epitranscriptome is still emerging. High-throughput screens identified several small molecules that target m^6A effectors. Of note, seven small molecule inhibitors of m^6A demethylase FTO are identified [108]. Among them, R-2-hydroxyglutarate displayed anti-glioma activity by inhibiting FTO and thereby modulating the transcription factors *c-MYC* and *CEBPA* [109]. Another FTO inhibitor, entacapone, delayed PD-associated motor dysfunction in humans [110, 111]. Furthermore, the redox cofactor nicotinamide adenine dinucleotide phosphate (NADP) was found to be a highly potent small molecule activator of FTO [112]. In addition, STM2457 was found to selectively inhibit m^6A methylase subunit—METTL3, whereas the piperazine derivative compound 4 was found to activate the m^6A methylase complex [113, 114]. Future studies are needed to explore the therapeutic benefits of these epitranscriptomic drugs in

various CNS diseases. For example, NADP and STM2457 may be tested for ischemic stroke as they nullify the m^6A hypermethylation (Chokkalla AK, Stroke, 2019). In addition to m^6A, certain small molecules target RNA hm^5C effectors Tet 1, 2, and 3. Particularly, ascorbic acid activates, whereas dimethyloxallyl glycine inhibits, Tet proteins [115, 116]. However, if these compounds modulate RNA hm^5C in CNS diseases remains unknown. Instead of global attenuation of epitranscriptomic modifications by small molecules that target epitranscriptomic enzymes, the development of mRNA substrate-selective inhibitors/activators is highly desired. For example, a short helix-threading peptide binds near the RNA A-to-I editing site in the $5\text{-}HT_{2C}R$ mRNA and selectively inhibits its editing by ADAR2 [117]. Future studies should exploit this concept for selectively modulating the epitranscriptomic modification within a single transcript.

The epitranscriptomic modifications display enormous potential to serve as diagnostic and prognostic biomarkers in addition to their therapeutic utility. During RNA turnover in the cells, unmodified nucleosides such as adenosine are typically recycled via the salvage pathway [118]. In contrast, the modified nucleosides such as inosine are released into the extracellular space and subsequently detected in blood and urine [119, 120]. Mass spectrometry-based profiling identified 20 types of modified nucleosides, including m^6A, m^1A, I, and m^5C, in human plasma [121]. More importantly, these modified nucleosides are highly abundant and accounted for 49% of total nucleosides [121]. Furthermore, pulse-chase labeling revealed that extracellular m^6A nucleosides are major byproducts of mRNA and rRNA catabolism [121]. Inosine levels were reported to be markedly elevated in the blood of stroke, multiple sclerosis, and epileptic patients, whereas decreased in the blood of major depressive disorder and saliva of AD subjects [122–126]. Moreover, the urinary concentration of ψ was observed to be significantly increased in patients with AD and post-stroke depression [55, 127]. Additionally, stratification of glioblastoma patients based on RNA A-to-I editing profiles identified a novel sex-dependent high-risk patient subgroup [128]. These studies suggest the possibility of developing point-of-care diagnostic and therapeutic tools for CNS diseases based on epitranscriptomic modifications.

Future Perspectives

Collectively, epitranscriptomic modifications form an additional layer to control gene expression during the physiological and pathological processes of the brain. Despite being sparse, their dynamic regulation by writers and erasers drives activity-dependent gene expression in the brain. In addition to the above-discussed modifications, the functions of several less abundant modifications such as N^4-acetylcytosine (ac^4C), N^6,2′-O-dimethyladenosine (m^6Am), 2′-O-methylation (Nm), and N^7-methylguanosine (m^7G) are currently being unfolded [89]. A major challenge in studying these modifications is the lack of specific antibodies and chemical reagents. Several controversial findings reported antibody cross-reactivity among epitranscriptomic modifications. For example, the m^1A antibody cross-reacts with m^7G, skewing the previously reported transcriptome-wide m^1A prevalence [129]. Likewise, m^6A antibody cross-reacts with m^6Am [130]. Hence, the development of antibody-independent techniques is necessary to map these modifications more accurately. Interestingly, certain epitranscriptomic modifications such as m^5C and m^6A exist in proximity with each other and co-regulate the fate of the transcript [87].

Hence, future studies should implement holistic approaches to understand their interplay resulting in synergy or competition. For example, techniques such as modified RNA bisulfite sequencing can simultaneously map the co-occurrence of multiple modifications, such as m^5C, Ψ, and m^1A, at single-nucleotide resolution [131]. Although the writers and erasers for various epitranscriptomic modifications are well-characterized, our understanding of the readers and their mechanisms of action, especially for modifications such as ψ, is limited. Unbiased methods, such as mass spectrometry-based proteome profiling after pulldown with modified bait RNA probes, may be applied to comprehensively elucidate the readers and their interactome [132]. Surprisingly, certain readers like YTHDF proteins bind both m^6A and m^1A, further convoluting the epitranscriptomic crosstalk [14, 44, 45]. Although several studies exposed the prevalence of epitranscriptomic alterations in CNS diseases, their mechanistic link to disease pathology remains obscure. Importantly, whether these changes serve as disease drivers or merely disease manifestations (cause or effect) has to be examined. This requires evaluating the temporal landscape of epitranscriptomic modifications during disease pathogenesis. For example, the dynamic changes in epitranscriptomic signaling can be investigated by employing auxin-inducible degron systems to achieve transient and sharp degradation of epitranscriptomic machinery at a particular stage of the disease [133]. Moreover, most of the current studies are restricted to just profiling these modifications in CNS disease models. Overall, the application of various knockout/overexpression mouse models and small molecule modulators of epitranscriptomic enzymes will lead to a better understanding of the RNA modifications in neurological disorders.

Funding

This study was partly supported by the NIH grant RO1 NS109459 and AHA grant 20PRE35120233.

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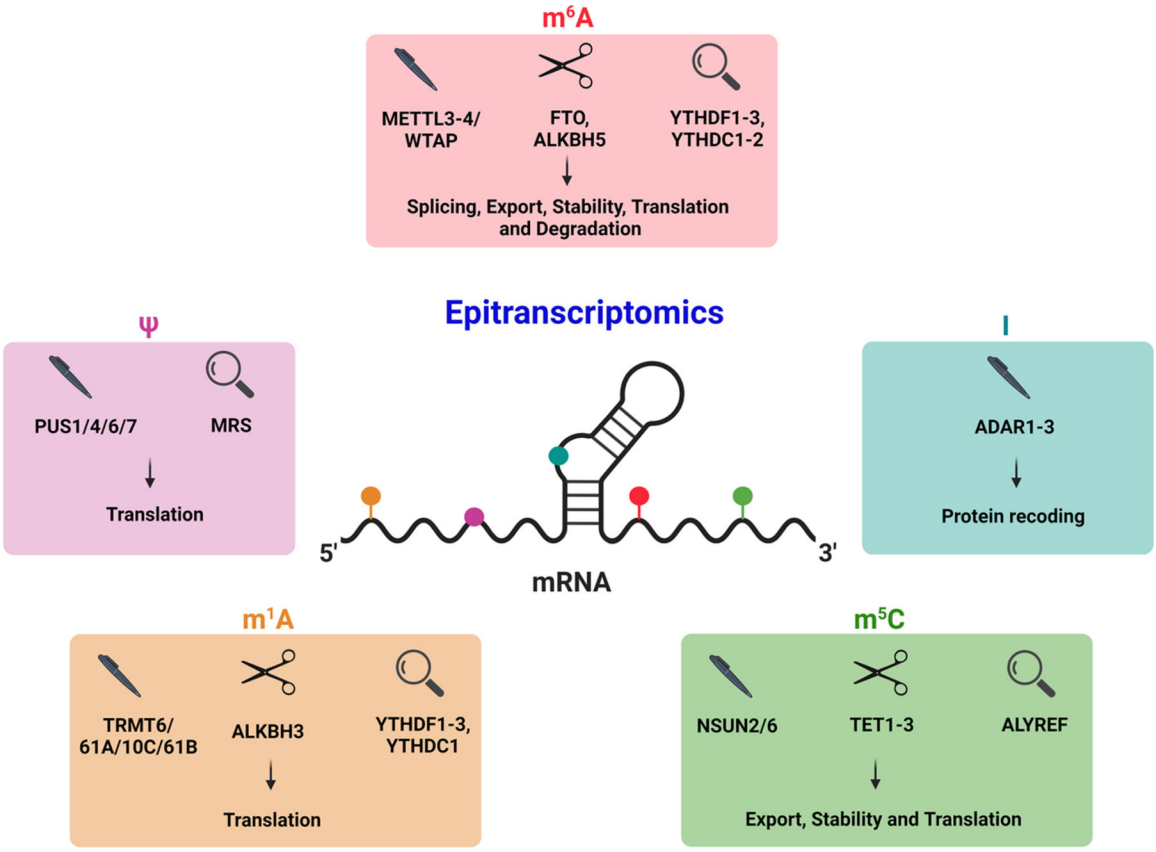

Fig. 1.

Overview of major epitranscriptomic modifications in the brain. The most abundant and well-studied epitranscriptomic modifications in the brain include N1-methyladenosine (m^1A), pseudouridine (ψ), inosine (I), N6-methyladenosine (m^6A), and 5-methylcytosine (m^5C). The m^1A, m^6A, and m^5C involve the addition of methyl group, whereas I and ψ are base exchange modifications. These modifications are controlled by a set of writers (marker), erasers (scissors), and readers (magnifier) to dictate the fate of modified mRNAs. They regulate various steps of mRNA processing such as splicing, export, localization, stability, translation, degradation, and protein recoding

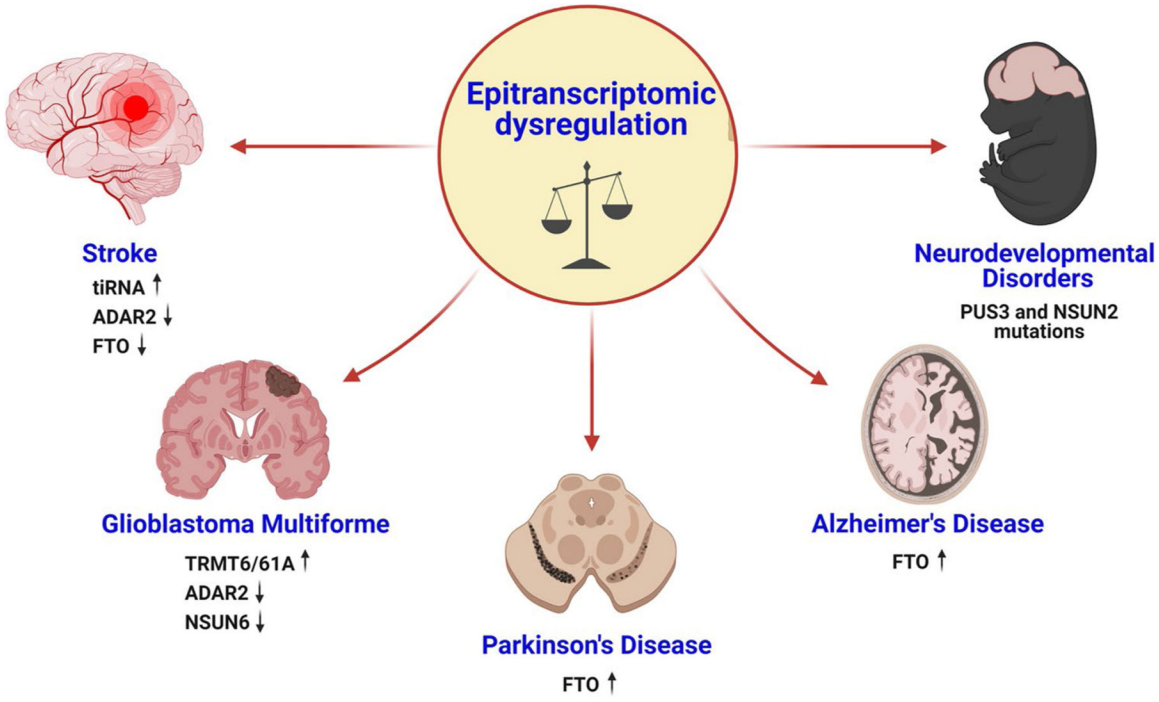

Fig. 2.

Epitranscriptomic imbalance in major CNS diseases. The altered expression of writers and erasers, including ADAR2, FTO, TRMT6/61A, and NSUN6, mediates several brain disorders such as stroke, glioblastoma multiforme, Parkinson's disease, and Alzheimer's disease. Additionally, the mutations in certain epitranscriptomic enzymes such as PUS3 and NSUN2 drive neurodevelopmental disorders