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Targeting RNA Modifications for Drug Development: The Role of Epitranscriptomics in Pharmacology

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Abstract

Epitranscriptomics has emerged as an additional regulatory layer of gene expression mediated by chemical modifications on RNA molecules, with important implications for cellular function and disease. This review synthesizes recent advances in epitranscriptomic research, focusing on major RNA modifications, including N^6-methyladenosine, pseudouridine, and 5-methylcytosine, and their mechanistic roles in regulating RNA stability, splicing, localization, and translational efficiency through the coordinated actions of writers, erasers, and readers. It further examines the dynamic regulation of RNA modifications under pathological stress conditions and highlights their contribution to disease pathogenesis, particularly in cancer, metabolic disorders, and neurological diseases. In addition, the review discusses emerging pharmacological strategies targeting epitranscriptomic regulators, including small-molecule inhibitors and RNA-based therapeutic approaches. The evidence collectively indicates that RNA modifications represent promising therapeutic targets, while also underscoring persistent challenges related to target specificity, delivery efficiency, and off-target effects. The review concludes that epitranscriptomics constitutes a rapidly evolving frontier in drug discovery and precision pharmacology by expanding

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current understanding of disease mechanisms and offering new directions for therapeutic innovation.

Keywords: Epitranscriptomics; RNA Modifications; N^6-Methyladenosine; Drug Discovery; Precision Pharmacology

Introduction

The discovery of pseudouridine in 1957 was a key development in molecular biology, exposing RNA's propensity for chemical modifications (Arzumanian *et al.*, 2022). These modifications occur at the post-transcriptional stage of gene expression on numerous RNA molecules, including transfer RNA (tRNA), messenger RNA (mRNA), long non-coding RNA (lncRNA), and ribosomal RNA (rRNA) (Shi *et al.*, 2020). These modifications influence the stability, location, splicing, degradation, and translational efficiency of RNA, although they do not modify the nucleotide sequence itself.

Currently, over 170 post-transcriptional modifications have been discovered in RNA, occurring in organisms ranging from archaea and bacteria to eukaryotes (Qui *et al.*, 2023; Nossent *et al.*, 2023). Some of these modifications include N6-methyladenosine (m^6A), 5-methylcytosine (m^5C), pseudouridine (Ψ), and N1-methyladenosine (m^1A). The most abundant and well-characterized internal modification in eukaryotic mRNAs is N6-methyladenosine (m^6A) (Qui *et al.*, 2023).

These alterations are deposited onto and erased from RNA molecules by the operations of writer (e.g., METTL3, METTL14, NSUN (NSUN1-7 and WTAP) and eraser enzymes (e.g., FTO, ALKBH5, ALKBH1, and ALKBH3). These modifications are recognized and interpreted by reader proteins, y such as THDF1-3, YTHDC1-2, IGF2BP1-3, HNRNPA2B1, and eIF3 (Wilkinson *et al.*, 2022). These enzymes ultimately determine the RNA fate and gene expression. The regulatory action of the readers ultimately controls the RNA destiny and gene expression.

The chemical diversity and regulatory integrity of RNA molecules are, to a significant extent, mediated by a range of covalent chemical modifications of the RNA nucleotides, collectively known as the epitranscriptome. When examining the significance of RNA modifications within the broader landscape of epigenetic control, it is necessary to contrast them with DNA and histone changes, two well-known established mechanisms in

the field of epigenetics. A narrow repertoire of epigenetic alterations in DNA is observed compared to epitranscriptomic modifications. DNA methylation is the best-characterized epigenetic process. It frequently occurs on cytosines (5mC, 5hmC, 5fC, and 5caC) and is the most abundant and lasting epigenetic mark in eukaryotes that is strongly related to gene regulation (Esteve-Puig *et al.*, 2020). Histones, on the other hand, are subject to many post-translational alterations, including acetylation, methylation, and ubiquitination, influencing chromatin structure, and function, consequently regulating access to genetic information (Xu *et al.*, 2022). Histone PTMs are introduced and erased by diverse kinds of enzymes (writers and erasers, respectively), which typically reside in huge protein complexes having various enzymatic activities (Jurkowska, 2024). While both DNA and histone modifications influence gene expression at the transcriptional level, RNA modifications function post-transcriptionally, affecting how and when mRNAs are translated into proteins.

Furthermore, RNA modifications are crucial in cellular functions, regulating mRNA stability and translation, while others may target mRNA for degradation and inhibit its translation (Qui *et al.*, 2023). They play a role in various human diseases, including cancer, neurological disorders, cardiovascular diseases, metabolic disorders, and developmental and genetic abnormalities. The epitranscriptome, with its fast and reversible effects, is excellent for precision-targeted treatments. The growing understanding of RNA modification and its involvement in biological processes has offered new promise in the diagnosis, treatment, and prevention of a range of disorders, making it an interesting target for therapeutic intervention.

Common RNA Modifications

Out of the >170 post-transcriptional RNA modifications documented to date, a (sub)set, including N6-methyladenosine (m^6A), pseudouridine (Ψ), 5-methylcytosine (m^5C), and N1-methyladenosine (m^1A), has been discovered as particularly abundant and functionally relevant. Collectively, these alterations operate as molecular switches and checkpoints that program gene expression events with temporal and spatial accuracy.

N6-Methyladenosine (m6A)

N6-Methyladenosine (m^6A) is one of the most abundant posttranscriptional modifications on RNA's in eukaryotes, effecting various RNA metabolic processes, including RNA alternative splicing, translation efficiency, degradation (Huang *et al.*, 2020). According to Qui *et al.*, (2023) m6A altered site notably localizes at the beginning of the 3'

untranslated region (UTR) at the stop codon, often conserved within the consensus pattern 5'-RRACH-3' (R represents G, A, or U; H stands for U, A, or C). Its deposition is mediated by a multicomponent methyltransferase complex, the adenosines are methylated by m6A-methyltransferases, referred to as m6A 'writers', which are part of the so-called 'm6A writer complex', consisting of methyltransferases METTL3 and METTL14 together with their cofactor Wilms' tumor 1-associating protein (WTAP) (Nossent *et al.*, 2023). Since it is a reversible process, it can be removed by demethylases, which have been found to include FTO and alkylated DNA repair protein AlkB homolog 5 (ALKBH5). Regulation also involves m^{6}A-binding proteins, such as YTH domain family 1–3 (YTHDF1–3), YTHDC1, and YTHDC2, which exert regulatory effects by selective recognition. For instance, m6A residues can alter the stability of target transcripts by engaging with YTHDF2 or increase protein synthesis by connecting with YTHDF1 (Liang *et al.*, 2024).

The emergence of m^{6}A sequencing (m^{6}A-seq) technologies and the identification of m6A modification writers, erasers, and readers have prepared the ground for inquiry into the function of m^{6}A mRNA modification in varied disorders (Arzumanian *et al.*, 2022) Recent findings also tie m6A to oncogenic signaling, indicating its dual activity as both a tumor suppressor and promoter, depending on cellular context (Sun *et al.*, 2023; Cui *et al.*, 2022).

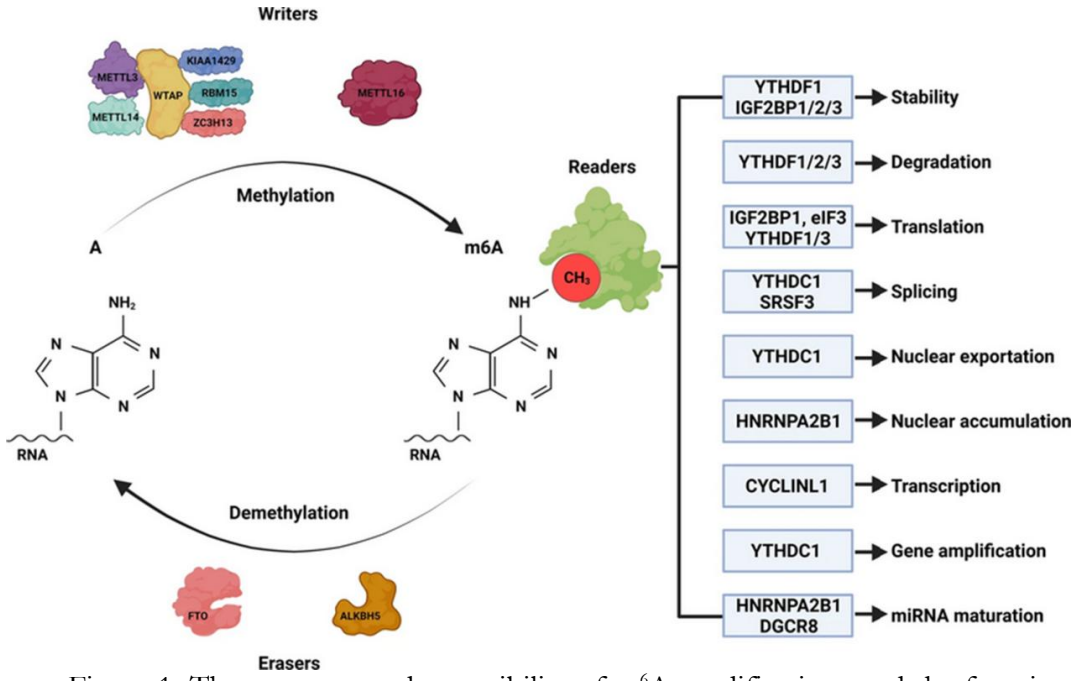

Figure 1: The structure and reversibility of m^{6}A modifications and the function of m6A through recognition by the reader protein (Liu *et al.*, 2023).

Pseudouridine (Ψ)

Pseudouridine (Ψ) was the first modified ribonucleoside identified in 1951 and is the most abundant post-transcriptionally modified nucleotide in RNA from all three domains of life. It is discovered in a wide spectrum of RNA, from tRNA and rRNA to distinct snRNAs (Arzumanian *et al.*, 2022). Pseudouridine (Ψ), a C–C glycosyl isomer arises via Pseudouridylation (isomerization of uridine), mediated by pseudouridine synthases (PUS) which include TruA family (PUS1, PUSL1, PUS3), TruB family (TRUB1, TRUB2), TruD family (PUS7, PUS7L), RluA family (RPUSD1, RPUSD2, RPUSD3, RPUSD4), and PUS10 in human cells (Zhao *et a*l., 2025). Pseudouridylation refers to the process in which the pyrimidine ring of uridine (U) rotates 180° with C 3 and C6 as an axis to form pseudouridine (Ψ), which is the most abundant modified nucleoside in human cells (Barbieri *et al.*, 2020). Isomerization of uridine to pseudouridine requires cleavage of the glycosidic bond between N1 and C1', rotation of the base, and subsequent reattachment of the base to the ribose via C5, resulting in enhanced stabilization of RNA conformations, destabilization, base stalking, altered hydrogen bonding properties and interactions with varied RNA binding proteins (Borchardt *et al.*, 2020). Recent research has revealed Ψ modifications in various RNA types, including messenger RNA (mRNA), transfer RNA (tRNA, tsRNA), ribosomal RNA (rRNA), small nuclear RNA (snRNA), and long noncoding RNA (lncRNA). Inducible pseudouridylation of mRNA occurs under stress, viral infection, or cellular transformation. This modification, defined by a geometrically equivalent Watson–Crick base, enhances the thermodynamic stability of RNA compared with the normal uridine (U)-adenine (A) base pair, and also plays roles in ribosome assembly (Liu *et al.* 2024). Ψ provides crucial tasks in ribosome construction, tRNA function, pre-mRNA splicing, and translation fidelity. Moreover, pseudouridylated nucleotides are increasingly significant in mRNA-based therapeutics, notably in vaccines, as they minimize immunogenicity and enhance translational efficiency (Zhao *et al.*, 2025)

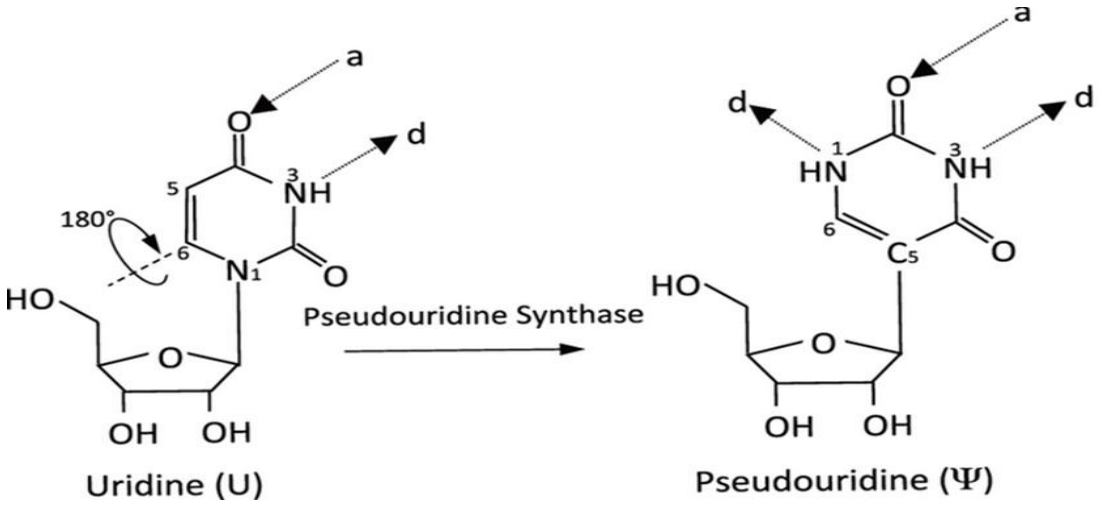

Figure 2: Schematic presentation of the Pseudouridation reaction: It results in an extra hydrogen bond donor (d) and the same number of hydrogen bond acceptors (a) (Zhao *et al.*, 2018).

5-Methylcytosine (m5C)

5-Methylcytosine (m^5C) is a well-characterized mark in DNA that is crucial in gene expression and epigenetic regulation (Shi *et al.*, 2020). However, it also exists abundantly in RNA, tRNA, rRNA, mRNA, and ncRNA., where it affects RNA structure, stability, transport, and translation into proteins (Xue *et al.*, 2022). m^5C is dynamically regulated by "writers,", "erasers", and "readers". m^5C is implanted via a sequence of m5C methyltransferases ("writers"), such as NOP2, NSUN2, NSUN3, NSUN4, NSUN5, NSUN6, NSUN7, DNMT1, TRDMT1, DNMT3A, and DNMT3B (Gu *et al.*, 2021). The elimination process is catalyzed by TETs. The Aly/REF nuclear export factor (ALYREF) might detect and bind to m^5C sites for biological function (Zhang *et al.*, 2021). m^5C is localized in 5'UTRs and coding regions of mRNAs, where it may increase ribosome loading or shield mRNA from endonuclease cleavage (Cheng *et al.*, 2021). Dysregulation of m^5C has been linked in stem cell differentiation, neurological disorders, and tumor metastasis, principally via its impact on non-coding RNAs and cancer-related genes (Han *et al.*, 2023; Xue *et al.*, 2022). Recent sequencing developments (e.g., bisulfite sequencing for RNA) have mapped m^5C patterns with single-nucleotide resolution, showing its universal functional relevance (Han *et al.*, 2023).

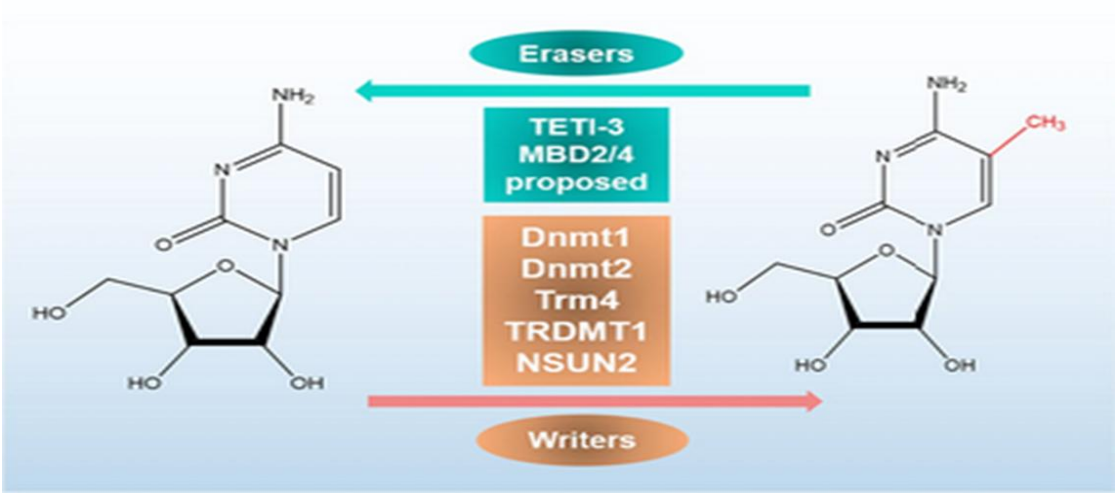

Figure 3: The structure and reversibility of m^5C modifications through the recognition by reader proteins (Song *et al*., 2022).

N 1-Methyladenosine (m1A)

N1-methyladenosine (m^1A) is an adenosine molecule whose N1-position is methylated. m^1A methylation is a pervasive, prolific, and conserved internal post-transcriptional alteration throughout bacterial and eukaryotic RNAs, notably in higher eukaryotic cells (Xiong *et al*., 2022). It is common in several kinds of RNA, including rRNA, tRNA, mRNA, mtRNA, ncRNA, and enhancer RNA (eRNA) (Wang *et al*., 2024). These modifications participate in translation, interpretation of codons, preservation of stability, export and translation in the different types of RNAs (Huang *et al*., 2019; Xiong *et al*., 2022). It is catalyzed by enzymes such as TRMT6/61A. m^1A impacts Watson-Crick base pairing, influencing RNA secondary structure and translation. In contrast to m^6A, m^1A often localizes to the 5' untranslated regions and start codons, where it promotes translation initiation under specific physiological conditions. It is dynamically regulated by ALKBH1, a known m^1A demethylase. The biological relevance of m^1A includes functions in metabolic management, hypoxic stress response, and stem cell pluripotency, and its dysregulation has been identified in several malignancies (Sweef *et al*., 2024; Wang *et al*., 2024).

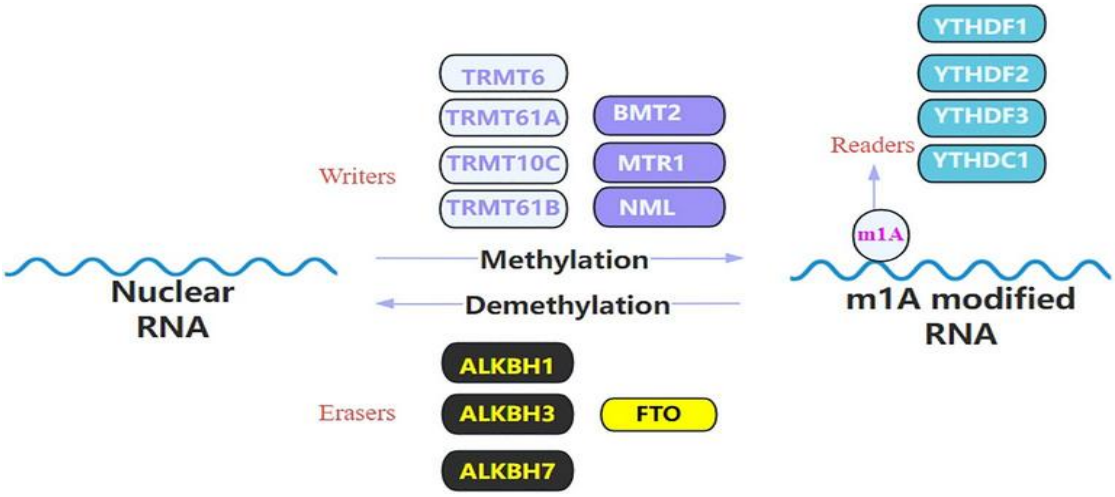

Figure 4: List the identified regulators of m^{1}A modification (Wang *et al*., 2024).

Mechanistic Links between RNA Modifications and Gene Expression

RNA modifications serve as critical posttranscriptional regulators of gene expression programs and their correct deposition is essential for normal development (Artika *et al*., 2025). Accumulating evidence reveals that the dynamics of internal RNA modifications play critical roles in multiple RNA-processing events including splicing, transport, translation, and degradation all of which in turn regulate gene expression. The expression patterns of RNA modifications and also of their regulators have the potential to be used as biomarkers for diseases or absorption of disease-causing hazard, acting as molecular switches that determine the fate and function of specific transcripts (Chen *et al*., 2023).

The role of m^{6}A in the mRNA's life cycle is complex, involving the reader, writer, and eraser. The m^{6}A modification is installed co-transcriptionally by a methyltransferase complex (MTC), which includes the METTL3 catalytic component and other accessory subunits and other accessory subunits such as METTL14 and WTAP (Cheng *et al*., 2024). Demethylases remove this complex, and RNA-binding proteins interpret it. The writer complex binds to mRNAs and transfers the methyl group to specific adenosines, usually containing RRACH motifs. METTL3 has been implicated in various biological processes, including modulation of alternative splicing, stabilization of target mRNAs, regulation of hematopoietic stem cell self-renewal, and involvement in tumorigenesis. m^{6}A erasers, also known as demethylases, shape the m^{6}A epitranscriptome by converting m^{6}A into adenosine

(Chen *et al.,* 2024*)*. The contribution of m^6A modifications to mRNA metabolism and cell fate transition is mediated by m^6A readers, which bind to m6A-modified RNAs and recruit interacting proteins to regulate RNA metabolism (Zhang *et al*., 2023).

Moreover, RNA modifications often occur at key regulatory regions, such as 5' UTRs, stop codons, and splice junctions, further enhancing their influence over transcript isoform diversity and translation efficiency (Höfler and Duss, 2023). As such, RNA modifications have emerged as central modulators of cellular identity and function, playing critical roles in processes ranging from stem cell pluripotency to immune cell activation and tissue repair.

RNA Modification as a Dynamic Regulator under Pathological Stress

One of the most intriguing aspects of RNA modifications is their dynamic behavior under stress conditions. Environmental and pathological stresses including oxidative damage, inflammation, hypoxia, and viral infection trigger widespread changes in the RNA epitranscriptome. These changes help the cell quickly recalibrate its gene expression profile to survive, adapt, or initiate programmed cell death when necessary.

Both m^6A and m^5C pathways play important roles in regulating the cellular response to oxidative stress, a condition caused by disrupted redox homeostasis, including the generation of reactive oxygen species (ROS) promoting their efficient translation to support cellular repair mechanisms and antioxidant responses. Thalhammer and colleagues identified that the *ALKBH5* promoter was up-regulated in response to hypoxia across several different cancer cell lines (Wilkinson *et al*., 2021).

In pathological contexts like neurodegeneration, cancer, and chronic inflammation, stress-induced changes in RNA modifications can become maladaptive. A recent study on Alzheimer's disease (AD) revealed that, postmortem human brains exhibit cell type-specific m^6A dysregulation, with pyramidal neurons containing lower m^6A and METTL3 levels associated with memory deficits, neuronal death, and neurite degeneration (Zhao *et al*., 2021). Similarly, in degenerative conditions like intervertebral disc disease, dysregulated m^6A profiles correlate with extracellular matrix remodeling, inflammatory gene activation, and progression of tissue degeneration (Liu *et al*., 2025).

Importantly, stress-induced RNA modifications are reversible, providing therapeutic opportunities. Modulating these marks using small molecules could restore balanced gene expression under pathological stress and prevent irreversible cellular damage.

Dysregulation of RNA Modifications in Human Disease

The discovery of dynamic and reversible RNA modifications has introduced a new dimension to gene control and cellular homeostasis. Among these, N6-methyladenosine (m^6A), together with other marks like as m^5C, pseudouridine (Ψ), and m1A, operates as a post-transcriptional epigenetic layer that influences RNA splicing, stability, translation, and degradation. Dysregulation of RNA-modifying enzymes known as writers, erasers, and readers has been progressively implicated in the pathophysiology of a wide range of human disorders. These include malignancies, neurological diseases, metabolic abnormalities, and immunological dysregulation. The context-dependent activity of RNA modifications has not only revealed novel disease pathways but has also suggested therapeutic possibilities for targeting RNA methylation systems. The context-dependent activity of RNA modifications has not only revealed novel disease mechanisms but has also opened therapeutic avenues for targeting RNA methylation systems.

Cancer: Aberrant m^6A Regulation in Tumor Progression

Deviant RNA modifications disrupt gene expression by modifying the stability and translational efficiency of mRNAs that encode proteins critical for DNA repair, cell cycle regulation, and apoptosis, potentially resulting in heightened mutation rates and genomic instability (Yang *et al.*, 2022; Bai *et al.*, 2023). Wang *et al.* (2015) established that m^6A influences mRNA translation efficiency, whereas Mendel *et al.* (2021) revealed that m^6A at splice sites obstructs U2AF35 binding, hence inhibiting RNA splicing.

The most critical RNA modifications implicated in tumorgenesis include, 5-methylcytosine (m^5C), N1-methyladenosine (m^1A), N6-methyladenosine (m^6A), pseudouridine (Ψ), and others like 7-methylguanosine (m^7G) and adenosine-to-inosine (A-to-I) editing (Chen *et al.,* 2024). These modifications alter the chemical structure of RNAs, directly affecting gene expression and various biological processes. Of all this modifications m^6A has gained more attention due to it's the following: (1) it is the most abundant internal

mRNA modification in eukaryotes, affecting thousands of transcripts, (2) it is extensively mapped using techniques like MeRIP-seq, making it easier to study, (3) It is dynamics and reversible thus enabling multiple intervention points, (4) plays pivotal roles in cancer hallmarks; cell proliferation, apoptosis, angiogenesis, metastasis, immune evasion, and drug resistance and a lot more there is.

Dysregulated m^6A writers exhibit oncogenic functions: METTL3 augments the translation of critical oncogenic mRNAs such as c-MYC, BCL2, PTEN, and MDM2, while METTL14 upregulated in AML increases m^6A modification on MYB and MYC transcripts, hence facilitating cell survival and proliferation (Guan, 2024; Du *et al.*, 2024). Moreover, METTL3 facilitates epithelial-to-mesenchymal transition (EMT) and metastasis by augmenting the mRNA stability of ZEB1 and SNAIL through m^6A alteration (Ouyang *et al.*, 2024; Li *et al.*, 2025).

Additionally, m^6A erasers including FTO and ALKBH5 are also of pivotal importance. (A) FTO is frequently overexpressed in glioblastoma and AML where it removes m6A modifications from a subset of tumor suppressor transcripts to stabilize them aberrantly. ALKBH5 maintains breast cancer CSC properties by removing methylation from and stabilizing pluripotency-related transcripts including NANOG and SOX2 (Zhang *et al.*, 2021). Collectively, our findings highlight the bifunctional role of m^6A regulators in cancer biology, functioning as either oncogenes or tumor suppressors based on the cellular context.

Neurological Disorders: Role of RNA Methylation in Brain Development

The central nervous system demonstrates elevated RNA methylation activity, encompassing the initial fate determination of neural stem/progenitor cells, the production of neurons and glial cells, the migration of post-mitotic immature neurons, synapse formation, programmed cell death, synaptic reorganization, and the maintenance and reconstruction of neurons during the postnatal period (Park et al., 2020). Research on axonal regeneration in adult mouse dorsal root ganglion demonstrated the significant involvement of m6A methylation in both normal physiology and responses to pathogenic stimuli within the adult mammalian nervous system (Wardowska, 2021). The hippocampus and cortex are the principal brain areas implicated in the pathophysiology of Alzheimer's disease (AD). Preclinical investigations have demonstrated specific functions of RNA methyltransferases and demethylases in cortical development and hippocampus

neurogenesis (Du et al., 2021). For instance, METTL3 significantly impacts the regulation of the neural progenitor pool and neuronal production, in contrast to FTO (Du et al., 2021). Its silencing leads to pronounced abnormalities in dendritic spines and synapses, accompanied by extensive neuronal death in the hippocampus—hallmarks of Alzheimer's disease (Zhao et al., 2021). The examination of human samples from individuals with Alzheimer's disease indicated a decrease in the mRNA expression levels of METTL3 and FTO relative to the control group (Li et al., 2022). In contrast, the downregulation of FTO facilitates memory reconsolidation (Chang et al., 2023), demonstrating that an accurate global m6A methylation level and a delicate equilibrium between methyltransferases and demethylases are essential for optimal brain function.

Pseudouridine and ac4C are two additional RNA markers recognized for their roles in neurodegenerative symptoms by influencing ribosomal efficiency and mitochondrial function. This information indicates an increasing awareness that epitranscriptomic dysregulation is fundamental to brain dysfunction, rather than merely a consequence of disease.

Metabolic and Immune Diseases

RNA changes have been increasingly intimately connected in recent years with immune homeostasis and metabolic signaling circuit regulation. m6A regulates genes linked to insulin sensitivity, adipocyte formation, and the metabolism of fats and carbs. According to Wang et al. (2021), m6A methylation is regarded to be critical for the immune response's operation in both healthy persons and patients with a range of disorders. T cell development and function in the thymus can be severely impacted by abnormalities in the mRNA methylome, which can result in aberrant immunological responses. In the absence of METTL3, T cell differentiation and proliferation have been reported to reduce (Chao et al., 2021). METTL3 decrease in Tregs enables the SOCS gene to become more stable, which prevents the IL-STAT5 pathway's cytokine signaling from being transduced (Lv et al., 2021). METTL3 blockade is probably a significant component in immune homeostasis regulation and the decrease of the onset of numerous autoimmune illnesses (Zhang et al., 2024). METTL3 reduction in dendritic cells inhibits the phenotypic and functional development of Dendritic cells, as seen by lower expression of CD40 and CD80, two costimulatory markers, and restricted IL2 production. In vitro and in vivo activation of T-cell responses is influenced by these METLL3-associated changes in DC activity (Yao et

al., 2021). Alike, YTHDF1 has been found to alter some immune genes translation in DCs, thereby modifying DCs interaction with T cells (Wen et al., 2024). With the rising information regarding RNA methylome and its impact on living organisms' physiological processes, the interest in exploring its function in human diseases is developing. Autoimmune illnesses, next to cancer, have risen to the forefront in the field of epitranscriptomic study. The available evidence about m6A modification may show some association between RNA methylation levels and autoimmune development and progression (Paramasivam et al., 2020). Pathogens also utilize the host's epitranscriptome. For instance, SARS-CoV-2 RNA is m6A-modified, which helps the virus elude immune detection and boosts translation efficiency (Zou et al., 2024). This dynamic interaction between host and pathogen reveals RNA methylation as a critical contact in antiviral defense.

Therapeutic Targeting of RNA Modifications

Small-Molecule Modulators of RNA-Modifying Enzymes

The therapeutic strategy of RNA modifications, particularly m^6A methylation, is based on their reversible nature, making them an attractive target for epitranscriptomic regulation. The enzymatic machinery involved in these modifications, writers, erasers, and readers governs RNA fate and function. This reversible nature allows for the development of small-molecule inhibitors targeting RNA-modifying enzymes, promising for cancer, neurological diseases, and immunotherapy.

Inhibitors of Methyltransferases

METTL3, the catalytic subunit of the m6A methyltransferase complex, is frequently upregulated in tumors and is responsible for installing m6A marks that enhance the translation of oncogenic transcripts. Researchers are developing METTL3 inhibitors to address the increasing roles of the enzyme in various pathologies. The main target of inhibitor design is the MTD of METTL3, which is primarily aimed at competing with the cosubstrate SAM (Fiorentino et al., 2023).

The METTL (methyltransferase-like) proteins are a family of methyltransferases responsible for catalyzing the transfer of a methyl group from a donor molecule (such as S-adenosyl methionine) to specific target sites on RNA molecules (Wong and Eirin-Lopez,

2021). The vertebrate METTL family consists of 33 members, including METTL1, METTL3, METTL5, METTL6, METTL14, and METTL16, each of which possesses a conserved SAM-binding domain residing in part of the overall 7BS structure (Tooley *et al.*, 2023). Although enzymes modifying similar substrates did not share a common ancestor, they do largely group phylogenetically. The primary function of METTL proteins is to add methyl groups to specific nucleotides within substrate molecules, including proteins, nucleic acids, and other small-molecule metabolites. Fourteen of the 33 METTL family members methylate DNA or RNA (Tooley *et al.,* 2023). The most extensively studied RNA methylation target of METTL proteins is m^6A, catalyzed by the METTL3-METTL14 m^6A methyltransferase complex (Jiang *et al.*, 2021). Accumulating evidence has shown that METTL3 may act as a potential therapeutic target, dependent or independent of m^6A modification.

Researchers are developing METTL3 inhibitors to address the increasing roles of the enzyme in various pathologies. The main target of inhibitor design is the MTD of METTL3, which is primarily aimed at competing with the co-substrate SAM (Fiorentino et al., 2023). Most small-molecule inhibitors targeting the METTL family have been developed against METTL3.

Demethylase Inhibitors

FTO and ALKBH5 are m^6A demethylases that remove methyl groups from RNA, thus reversing methylation-induced regulatory effects. Overexpression of FTO in various cancers correlates with chemoresistance, stemness, and poor prognosis. Therefore, pharmacological inhibition of FTO offers a compelling approach to restoring normal RNA methylation profiles.

FTO inhibitors have shown promising clinical potential since their discovery in 2012. Rhein, a natural product, is a significant FTO inhibitor that inhibits FTO's m6A demethylation activity by competing with its active site (Gao et al., 2024). Meclofenamic acid (MA) has been identified as a highly selective FTO inhibitor, leading to an increase in RNA's m6A modifications. Two potent small-molecule FTO inhibitors (CS1, CS2) were reported in 2019, showcasing robust anti-tumor effects across various cancer types (Feng et al., 2024). However, the clinical potential of small-molecule FTO inhibitors is limited by moderate activity, toxicity, and low specificity for leukemia stem cells (LSCs) (Su et al., 2021). Researchers developed a glutathione (GSH) bioimprinted nanocomposite material,

GNPIPP12MA, loaded with FTO inhibitors, which targets the FTO/m6A pathway, synergistically enhancing anti-leukemia effects by depleting GSH (Cao et al., 2022).

The development of ALKBH5 inhibitors has gone through several important stages, including high-throughput screening and computer-aided drug design (ACS Omega, 2021). Some promising ALKBH5 inhibitors include citrate, IOX1, MV1035, Ena15, Ena21, 2-(2-hydroxyethylsulfanyl) acetic acid (RD3), 4-[(methyl)amino]-3,6-dioxo (RD6), DO-2728a, and RD3 and RD6, which demonstrate notable inhibitory effects on cancer cell lines (Fang *et al.*, 2025). These compounds can effectively suppress ALKBH5 activity in both in vitro and in vivo models, thereby affecting m6A-modified RNA levels. With further optimization and preclinical studies, these compounds are expected to become new cancer therapies.

RNA-Targeting Strategies

RNA-modifying enzymes and post-transcriptional changes are critical for gene control, but treatment requires specific instruments targeting changed RNA transcripts. Two methodologies have emerged: antisense and RNAi-based technologies for silenced RNAs and CRISPR-Cas13-based platforms for programmable RNA editing. These approaches allow reversible, adjustable, and sequence-specific RNA function modification without genome mutation, expanding therapeutic options for genetic, inflammatory, and degenerative illnesses.

Antisense Oligonucleotides (ASOs) and siRNA-Based Targeting of Modified RNA

The bulk of RNA-based medications used in the clinic or currently under development are antisense oligonucleotides (ASOs) or double-stranded RNA molecules called short interfering RNAs (siRNAs). ASOs and siRNAs both bind target mRNAs, or pre-mRNAs, via complementary Watson-Crick base pairing, but differ in composition and mechanisms of action.

Antisense Oligonucleotide (ASOs)

Antisense Oligonucleotide ASOs are synthetic RNA sequences that selectively attach to a gene's RNA, causing numerous diseases. They can change mRNA processing or degrade the target transcript. Synthesized ASOs can bind to pre-mRNA, influencing splicing events, inhibiting protein translation, or recruiting RNase H to the target transcript (Crooke *et al.*, 2018). Recent studies have revealed that ASOs can be designed to bind

m6A-modified RNA regions, limiting the recruitment of reader proteins such as YTHDF1 or impeding downstream translation. For example, ASOs targeting m6A-modified circRNAs have shown efficacy in inhibiting tumor proliferation in lung and liver malignancies (Qin *et al.*, 2022)

Short interfering RNA (siRNA)

Short interfering RNA (siRNA), contrary. is a double-stranded construct that stimulates the RNAi pathway, a natural cellular defensive mechanism against RNA viruses. Approximately twenty siRNA-based treatments have reached clinical trial stages, targeting disease-causing genes and variations in diseases such as malignancies, inflammatory disorders, and neuropathies. siRNAs targeted to knock down genes with aberrant methylation patterns have been validated in models of glioblastoma, Huntington's disease, and cardiomyopathy (Li et al., 2024). Small interfering ribonucleic acid (siRNA)-based therapeutics have been developed for the past 20 years. The first siRNA agent, patisiran, got US Food and Drug Administration (FDA) approval in 2018. To date, the FDA has approved 6 siRNA agents: patisiran, givosiran, lumasiran, inclisiran, nedosiran, and vutisiran (U.S. FDA, 2018; Padda et al., 2024) Clinically, FDA-approved siRNA medications such as Patisiran (Onpattro) have established the therapeutic promise of this class, and novel ASO-based therapeutics are under development for RNA methylation-linked ailments, including neurodegeneration and splicing-related diseases (Padda et al., 2024). However, there are still considerable challenges to efficient RNA-based therapy options, including distribution to specific regions or tissues, off-target effects, and treatment longevity. Despite these challenges, siRNA remains a promising therapeutic strategy for different disorders.

CRISPR–Cas13-Based RNA Editing Tools

The advancement and swift adoption of CRISPR/Cas technology have facilitated diverse genetic engineering by the targeted recruitment of a Cas endonuclease via a sequence-specific single guide RNA (sgRNA) (Barrangou and Doudna, 2016). Catalytically inactive Cas (dCas) proteins facilitate the recruitment of other protein modes to specific genomic loci without causing DNA strand breaks (Pickar-Oliver and Gersbach, 2019). Likewise, RNA-targeting dCas13 can be conjugated with several protein effectors that alter RNA, hence facilitating RNA modification engineering (Figure 5A) (Abudayyeh *et al.*, 2017; Cox *et al.*, 2017). Thus, RNA modifications can be added or eliminated at specific loci

without necessitating alterations to the main DNA sequence. Successful targeted installation and removal of m^6A utilizing dCas13-METTL3 and dCas13-ALKBH5, respectively, have been documented (Wilson *et al.*, 2020; Xia *et al.*, 2021; Chen *et al.*, 2022). Likewise, dCas13 conjugated with the deaminase domain of adenosine deaminase acting on RNA type 2 (ADAR2) can facilitate targeted A-to-I RNA editing (Cox *et al.*, 2017; Eisenberg *et al.*, 2018). RNA can be targeted with dCas9 when a single-stranded DNA molecule with a protospacer adjacent motif (PAM) is provided, a method utilized to write and erase m^6A (Rau *et al.*, 2019; Liu et al., 2019; Ying *et al.*, 2020). Alongside CRISPR/Cas-based molecular tools, endogenous RNA modification machinery can be activated by supplying a sequence-specific guide RNA. Two recent studies have shown that the natural pseudouridylation machinery can be utilized to introduce Ψ onto certain RNA transcripts (Figure 5B) (Adachi *et al.*, 2023; Song *et al.*, 2023). Additionally, native ADAR proteins can be utilized for A-to-I editing through the introduction of exogenous circular guide RNA (Katreka *et al.*, 2022). Molecular tools can also be customized to achieve temporal control of RNA modification engineering. For example, Shi *et al.* created a method for inducible m^6A modification, where the release of photo-caged abscisic acid upon UV light exposure is essential for tethering dCas13 with METTL3 or ALKBH5 via adaptor proteins (Figure 5C) (Shi *et al.*, 2022). We believe that the toolset for RNA modification engineering will continue to expand as our understanding of different RNA modifications and their modification machinery advances.

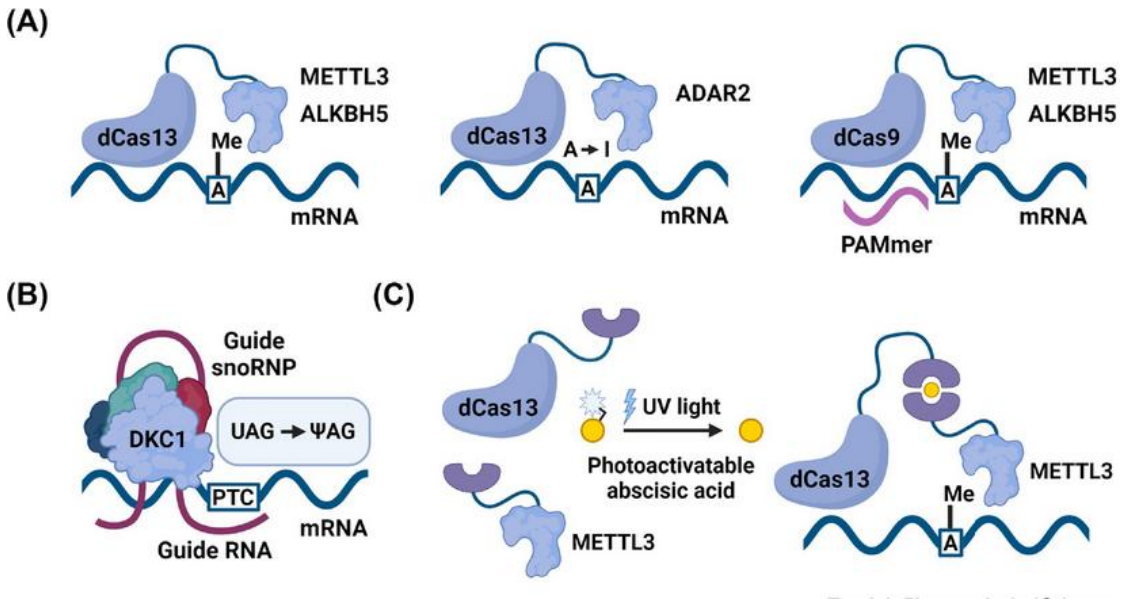

Figure 5: Molecular tools for engineering RNA modifications

High-Throughput Screening for Epitranscriptomic Modulators

High-throughput screening (HTS) has been pivotal in identifying first-in-class small-molecule inhibitors targeting RNA-modifying enzymes. HTS platforms involve the rapid screening of hundreds of thousands of chemical compounds against defined enzymatic or cellular assays to identify hits that modulate activity. For instance, the FTO inhibitor Dac85 was identified via HTS and further optimized into QP73, the first PROTAC degrader targeting FTO. This compound induced potent antileukemic activity by degrading FTO protein selectively and restoring tumor-suppressive m^6A methylation levels (Liu *et al.,* 2024). Similarly, ALKBH5-specific inhibitors have been discovered using fluorescence-based HTS platforms, capable of discriminating between FTO and ALKBH5 activity through their distinct catalytic pockets (Lai *et al*., 2024).

Other platforms utilize microfluidics, chemoproteomics, and m^6A-sensitive biosensors to quantify dynamic methylation levels and screen libraries in cell-based contexts. Integration of HTS with RNA-seq and methylation-specific sequencing has enabled functional validation of inhibitors by monitoring transcriptomic and methylomic shifts in treated cells (Yang *et al*., 2024).

Structure-Based Drug Design Targeting RNA-Modifying Proteins

Structure-based drug design (SBDD) is rapidly evolving to be a fundamental tool for faster and more cost-effective methods of lead drug discovery. SBDD aims to offer a computational replacement to traditional high-throughput screening (HTS) methods of drug discovery (Martin *et al*., 2024). Structure-based drug design (SBDD) optimizes high-throughput screening by allowing for the logical, structure-guided improvement of lead compounds, leveraging high-resolution three-dimensional target data.

The atomic structures (X-ray/cryo-EM) of RNA-modifying enzymes, specifically the METTL3-METTL14 writer complex, demethylases FTO and ALKBH5, and various YTH reader domains, have had a direct impact on inhibitor design by revealing cofactor/substrate pockets and allosteric sites that facilitate small-molecule binding.

The m^6A writer METTL3 has been linked to the initiation and maintenance of acute myeloid leukaemia (AML). Structure-guided campaigns resulted in selective METTL3 binders that reduce AML features in vitro and in vivo. Inhibitors, such as STM2475 and UZH1a, have been observed to occupy the SAM-binding site of METTL3 based on X-ray crystal structures (Moroz-Omori *et al*., 2021; Yankova *et a*l., 2021). STM2475 and UZH1a

exhibit half-maximal inhibitory concentration (IC50) values of 2.2 and 4.6 μM, respectively, in MOLM-13 cells and prevent proliferation of AML cell lines (Moroz-Omori *et al.*, 2021; Yankova *et al.*, 2021).

In 2019, two potential FTO inhibitors, FB23 and FB23-2, were developed using structure-based rational design. These inhibitors directly bind to FTO, selectively inhibiting its m6A demethylase activity. In vitro, simulating FTO deficiency, FB23-2 significantly inhibited human AML cell proliferation and promoted differentiation and apoptosis. In xenograft mouse models, FB23-2 significantly inhibited the progression of human AML cells (Huang *et al.*, 2029). Rhein, a natural product, is a significant FTO inhibitor. Unlike 2-oxoglutarate analogs or metal ion chelators, Rhein inhibits FTO's m^6A demethylation activity by competing with its active site, significantly inhibiting m6A demethylation within cells (Chen *et al.*, 2012).

Through structure-based virtual screening, Chen et al. (2023) recently found two robust FTO inhibitors, CS1 and CS2, with IC_{50} at nanomolar range in AML cells. CS1 and CS2 selectively occupied the FTO catalytic pocket, thus inhibiting FTO demethylase activity by blocking the interaction between m6A-modified substrate and FTO catalytic pocket (Su et al., 2020). Through virtual screening, structural optimization and bioassay, Xie et al. developed a novel small-molecule FTO inhibitor, 18,097, which selectively bound to FTO active site and significantly inhibited breast cancer cell proliferation and metastasis both in vitro and in vivo.

The crystal structure of human ALKBH5, comprising 395 amino acids, was elucidated in 2013 (Shen et al., 2022). ALKBH5 is primarily expressed in the testes and lungs, followed by the spleen, kidneys, and liver, with minimal expression in the heart and brain (Chen et al., 2023). The first generation of m^6A reader inhibitors, including ebselen and fragment-derived YTH binders, were developed by fragment-based screening and structure-guided medicinal chemistry. Li et al., (2020) identified 25 small-molecule ligands of the YTH domain of YTHDC1 by in silico methods, i.e., molecular docking and molecular dynamics simulations. Their crystal structures in the complex with YTHDC1 were solved at high resolution (1.2–1.9 Å). Allosteric modulators impede m^6A recognition by modifying the reader domain conformation.

The current SBDD toolkit for epitranscriptomic targets includes molecular dynamics (MD) to analyze target flexibility and kinetics, AI-assisted docking/virtual

screening, X-ray/cryo-EM validation, and fragment-to-lead approaches that focus on binding efficiency and ADME features. Together, these resources speed up the identification of candidates with attractive preclinical pharmacology, binding kinetics, and selectivity.

Challenges in Drugging the Epitranscriptome

Specificity and Off-Target Effects

This is a great difficulty in RNA modification targeting, because the solution requires great specificity without inflicting undesired molecular damage. These RNA-modifying enzymes, such as METTL3, FTO, and ALKBH5, often have larger substrate profiles in a range of biological situations. Hence, inhibitors that target these enzymes should not affect non-specific changes or inhibition of other proteins with comparable activities (Höfler and Duss, 2023). The same multiplicity of alterations in epitranscriptomic networks means that inadvertent editing or suppression may alter immunological signaling, the cell cycle, or differentiation. To address this, recent studies are employing rational drug design with fragment-based screening and chemical proteomics to identify inhibitors with enhanced target selectivity. Another recent study has developed allosteric inhibitors that block a regulatory domain exclusively found in each enzyme, and hence tailored to be selectively active against related demethylases (Xie *et al.*, 2023).

Delivery and stability of RNA-targeted medicines in cells

Even if very specific inhibitors are produced, getting them into cells is still an issue. Small chemicals, antisense oligonucleotides (ASOs), and CRISPR-Cas13 constructs that target RNA must get past cellular hurdles such as endosomal entrapment, degradation by nucleases, and immunological activation that isn't the target. For instance, many METTL3 and FTO inhibitors function well in the lab but not so effectively in the body because they do not get across the membrane or get removed by the liver very efficiently. Likewise, siRNA or ASO-based treatments that target RNA methylation usually need chemical modifications (like phosphorothioate backbones) or nanoparticle carriers to keep them from breaking down and to transmit them into the body (Liu *et al.*, 2025).

Lipid nanoparticles (LNPs), PEGylated carriers, and customized conjugates like GalNAc for liver-specific delivery are some of the ways that people are working to increase

stability and uptake. But attaining cell–type–specific accuracy is still hard, notably in the brain, pancreas, and blood-forming organs.

Absence of Structural Data for RNA Modification Complexes

Another challenge that makes it hard to find novel medications in this area is that many RNA modification complexes have not been thoroughly described structurally. There are crystal structures for METTL3-METTL14 and some sections of FTO and ALKBH5, but many reader proteins (such as YTHDF3) and cofactors still don't have their structures figured out. Dynamic RNA-protein interactions, exemplified by the development of liquid-liquid phase-separated granules (LLPS), evade capture by standard X-ray crystallography or cryo-electron microscopy. This makes it harder to create medications that target functional RNA-protein interactions in the body (Guo *et al.*, 2021).

Conclusion

The discipline of epitranscriptomics has developed from a specialist domain into a major pillar of modern biomedical science. RNA modifications such as N^6-methyladenosine (m^6A), 5-methylcytosine (m^5C), and pseudouridine (Ψ) have emerged as key regulators of mRNA fate, altering gene expression, cellular plasticity, and disease consequences. Aberrant regulation of RNA-modifying enzymes ncluding writers (e.g., METTL3/METTL14), erasers (e.g., FTO, ALKBH5), and readers (e.g., YTHDFs) has been implicated in the advancement of malignancies, neurological diseases, and immunological dysfunctions. Recent advancements in high-throughput sequencing, direct RNA modification mapping, and single-cell transcriptomics have expanded our capacity to decipher the functional impact of RNA alterations in health and illness. Concurrently, therapeutic breakthroughs ranging from small-molecule inhibitors to RNA-targeting CRISPR-Cas systems are converting epitranscriptomic knowledge into real clinical tactics. The integration of RNA modification signatures into precision medicine, biomarker identification, and drug development pipelines emphasizes the translational momentum of this research. However, to realize the full potential of RNA-targeted therapeutics, obstacles remain: assuring delivery specificity, avoiding off-target effects, standardizing detection technologies, and building adequate regulatory and ethical frameworks. These challenges are surmountable with interdisciplinary collaboration, technological development, and evidence-driven clinical validation.

Recommendations

To promote RNA modification research and its therapeutic application, significant strategic ideas include the establishment of standardized platforms for mapping RNA modifications. This requires upgrading techniques like nanopore direct RNA sequencing and spatial transcriptomics to enhance accuracy and comparability across investigations. Promising medications targeting METTL3, FTO, and ALKBH5 are advancing into clinical trials, backed by mechanisms such as biomarkers and patient stratification using RNA signatures. Incorporating epitranscriptomic profiling into clinical models is critical for predicting responses in disciplines like oncology and neurodegeneration. The use of AI for patient categorization based on transcriptome data is also advocated. Additionally, regulatory frameworks relevant to RNA editing techniques must be devised, addressing ethical problems and ensuring public trust. Cross-sector collaboration and investments in educational programs are necessary for the successful deployment of RNA-based therapeutics

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