

Review paper

## Decoding the m6A-miRNA crosstalk in epileptogenesis: From writer-eraser-reader dynamics to RNA-based precision therapeutics

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### Abstract

**Background:** Epileptogenesis is a dynamic process characterized by continuous molecular, cellular, and network-level changes that result in persistent hyperexcitable neuronal activity and recurring seizures. While well-known, classical mechanisms (such as neurotransmitter imbalance, synaptic remodelling, neuroinflammation, and ion channel dysfunction) are not as well studied as RNA-based epigenetic regulation as an integrated mechanism. **Mechanisms:** The roles of N6-methyladenosine (m6A) epitranscriptomic modifications and microRNA (miRNA)-mediated posttranscriptional regulation have been thoroughly explored as independent processes, but no systematic overview of the crosstalk between different stages of epileptogenesis (acute insult, latency, and chronic epilepsy) has been conducted. This is a novel review of an integrative m6A-miRNA regulatory mechanism in which the m6A writer, eraser, and reader proteins (METTL3, METTL14, FTO and ALKBH5) directly regulate the biogenesis and maturation of miRNAs, and miRNAs and, in turn, fine-tune the expression of m6A-containing enzymes, creating a self-reinforcing regulatory loop that contributes to neuroinflammation, synaptic remodelling, oxidative stress, and neuronal dysfunction. **Implications:** Based on this, we review and integrate new dual-pathway therapeutic strategies, such as antiepileptic drugs, mTOR pathway modulators, anti-inflammatory compounds, and RNA-based therapies (e.g. miRNA mimics and antagomirs), that target pathways not previously synthesized in the epilepsy literature. The review also highlights emerging exosome-based nanocarriers to enhance blood-brain barrier penetration of such RNA-based cargo. **Conclusion:** This review, which combines mechanistic and translational approaches, provides a new conceptual framework for discovering disease-modifying biomarkers and targets, and for rethinking epileptogenesis as an interconnected epitranscriptomic network rather than a series of isolated molecular events.

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### Keywords

Epitranscriptomics; m6A-miRNA integration; neuroinflammation; dysregulation; RNA-based therapies

### Introduction

Epileptogenesis is the sequence of events in which the normal brain becomes prone to seizures. According to the International League Against Epilepsy (ILAE), epilepsy is a group of chronic CNS disorders characterized by abnormal spikes in the brain's electrical activity that cause recurrent and repetitive seizures [1]. Cerebral insults such as traumatic brain injury (TBI), stroke, infection, and genetic variations initiate a cascade of molecular, cellular, and network-like changes that lead to a dynamic process of epileptogenesis [2]. The

incidence of epilepsy is approximately 50 new cases *per* 100,000 people annually [3]. The clinical manifestations and severity of epilepsy vary individually [4]. In the context of epileptogenesis, the term latency period is often used interchangeably, as it refers to the time between the insult and the first spontaneous seizure. This phase is crucial for ceasing the progression of epilepsy [5].

miRNAs are well-established contributors to disease progression, and epileptogenesis involves complex dysregulation of gene expression. Two layers are implicated in this process: transformed miRNA expression and m6A-mediated RNA modification [6]. These dysregulated systems affect CNS activity. While miRNAs regulate gene expression at the posttranscriptional level, m6A modification acts as an additional epitranscriptomic regulatory mechanism that controls RNA stability and translation and splicing. However, miRNA expression dynamically changes during epileptogenesis, with several miRNAs associated with immune and inflammatory responses. Studies have shown that the m6A-related enzymes methyltransferase-like 3 (METTL3), fat mass and obesity-associated protein (FTO), and alkane monooxygenase alkylation B (AlkB) homolog 5 (ALKBH5) influence epilepsy [7]. Dysregulated miRNAs and m6A modifications do not operate in isolation but represent interconnected regulatory layers that drive the pathological process of epileptogenesis [8].

Dysregulated synaptic plasticity drives neuronal hyperexcitability and is influenced by miRNA- and m6A-mediated gene regulation [9]. Altered neurotransmitter homeostasis disrupts the inhibitory and excitatory balance, thereby promoting epileptogenesis. Disturbances in the glutamate transport system contribute to excitotoxicity and impair gabaminergic function [9]. Additionally, m6A-associated proteins such as YTH domain-containing 2 (YTHDC2) have been shown to influence glutamate dynamics and enhance excitotoxic responses [10]. Moreover, ionic changes increase neuronal excitability. During a seizure, there is a massive influx of Na<sup>+</sup> and Ca<sup>2+</sup> into neurons and persistent high extracellular K<sup>+</sup> concentrations. Glial networks, particularly connexins and gap junctions, are essential for maintaining ionic homeostasis, and their disruption impairs K<sup>+</sup> buffering and clearance, resulting in excitotoxicity. Ionic channels mediated by miRNAs modify the regulatory processes [11].

This review summarizes evidence that the m6A-miRNA regulatory axis regulates several pathogenic mechanisms in epileptogenesis, including neuroinflammation, synaptic remodelling, neuronal hyperexcitability, oxidative stress, mitochondrial dysfunction, and ionic disturbances. Additionally, we investigated these pathways' mechanisms during the latent period, chronic epilepsy, and different stages of disease emergence.

Recent studies have shed important light on m6A-mediated epitranscriptomic control in epilepsy, specifically via METTL3/METTL14 [12], or the contribution of miRNA dysregulation to epileptogenesis [13]. However, these two regulatory mechanisms have largely been considered independently. Consequently, a comprehensive understanding of how m6A modification and miRNA biogenesis interact across the stages of epileptogenesis remains limited. Moreover, the therapeutic implications of this reciprocal regulatory network have not been systematically explored. The primary objective of this review is to examine the complex and dynamic interplay between N6-methyladenosine (m6A) RNA modification and microRNA (miRNA) regulation during epileptogenesis. These two types of epitranscriptomic mechanisms are tightly coupled. miRNAs can regulate m6A writers, erasers, and readers, whereas m6A modifications regulate miRNA biogenesis, maturation, and stability, and ultimately, miRNA regulation. This reciprocal mechanism is key to understanding how epitranscriptomic changes shape the molecular processes underlying epilepsy. Finally, the translational impact of the m6A-miRNA axis as a therapeutic target and a clinically significant biomarker is the main emphasis of this review. Antagomirs, m6A machinery modulators, and miRNA mimics are examples of novel RNA-based treatments that show promise for precision therapy. Furthermore, circulating

miRNAs and m6A-associated molecular signatures may serve as prognostic and clinical biomarkers to improve early epilepsy detection, patient stratification, and personalized therapy.

### **Trajectory of epileptogenesis**

Epilepsy is one of the most common neurological disorders and is characterized by an abnormal burst of electrical activity, rapid neuronal firing, and recurrent, unprovoked seizures. Unlike electrical abnormalities, epileptogenesis involves alterations at the molecular and cellular circuit levels and is initiated by responses such as strokes, TBI, infections, or status epilepticus [14].

#### *Reorganization of synapses and circuit hyperexcitability*

The earliest manifestation of epileptogenesis is synaptic plasticity and circuit reorganization [15]. Reorganization is the primary mechanism of neuroplasticity, allowing the brain to adapt its internal circuitry; however, aberrant damage shifts the brain toward a hyperexcitable state. Synaptic remodelling is region-specific; in temporal lobe epilepsy (TLE), the temporoammonic pathway to the hippocampal CA1 region undergoes activity-dependent strengthening [16]. Simultaneously, the silencing of hilar mossy cells deprives inhibitory interneurons within the dentate gyrus, weakening the inhibitory “gate” and allowing the glutamatergic networks to dominate, resulting in seizure amplification [17]. Quantitative analysis showed that synaptic rewiring caused a 2.5-fold increase in dendritic spine density in the CA1 region [5]. Experimental studies further demonstrated that long-term potentiation (LTP), which supports learning and memory, becomes aberrantly activated after brain injury and contributes to pathological responses [18].

Recent research shows that the interplay between miRNAs and m6A-mediated pathways contributes to synaptic architecture and circuit stability. Specific miRNAs, such as miR-132 and miR-134, are strongly implicated in dendritic spine remodelling [5]. Moreover, m6A-dependent RNA metabolism influences synaptic structure, and dysregulated epitranscriptomic factors contribute to chronic seizure susceptibility [16].

#### *Ionic imbalance*

The brain has multiple mechanisms of ionic concentration and neurotransmitter homeostasis to maintain neuronal excitability. The main ions involved in epileptogenesis are potassium, sodium, calcium, and chloride, as they contribute to signalling neuronal excitability and firing [19].

Astrocytic dysfunction impairs extracellular potassium buffering by altering Kir4.1 channel activity, and whereas mutations in the ion channel genes sodium voltage-gated channel alpha subunit 1 (SCN1A) and potassium voltage-gated channel subfamily Q member 2 (KCNQ2) lower the seizure threshold [11]. Increased intracellular calcium through voltage-gated calcium channels activates excitotoxic cascades and causes neuronal injury [20]. Neurotransmitter imbalance also plays a central role in epileptogenesis progression. Hyperactivation of glutamatergic N-methyl-D-aspartate NMDA and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid AMPA receptors, together with impaired GABAergic inhibition, disrupts the excitatory-inhibitory (E/I) balance within hippocampal circuits [7]. miRNAs directly regulate neurotransmitter-associated signalling pathways; for example, miR-124 increases cAMP response element-binding protein 1 (CREB1) and NMDA receptor signalling, whereas miR-34 impairs GABAergic transmission [7,21].

#### *Glial activation, neuroinflammation and integrated epitranscriptomic regulation*

Inflammation causes structural and functional changes and disrupts the highly packed endothelial network of the blood-brain barrier (BBB) [22]. Brain trauma initiates multiple organized events that include oedema and infiltration of different immune cells. Activation of reactive glial cells *via* the mTOR pathway mainly involves microglia, and astrocytes release proinflammatory mediators, including cytokines (IL-1 $\beta$ , IL-6 and TNF),

chemokines, protectins and lipid mediators, to promote neuronal excitability, exacerbate seizure activity, and lower the seizure threshold [23]. Recent studies have shown evidence of innate immune involvement in epileptic tissues [24]. Pathogenic processes involve high levels of cytokines, especially IL-1 $\beta$ , which affect glutamate release by impairing astrocytes' glutamate uptake and reducing their ability to clear glutamate [25].

This injury also promotes a robust release of proinflammatory chemicals, infiltration of lymphocytes and leukocytes, increased glial activity, and alterations in the inhibitory circuits [26,27]. miRNAs and m6A are linked to inflammatory reactions. miR-155 regulates cytokine production through suppression of cytokine signalling 1 (SOCS1) and Src homology 2 domain-containing inositol 5-phosphatase 1 (SHIP1) [23].

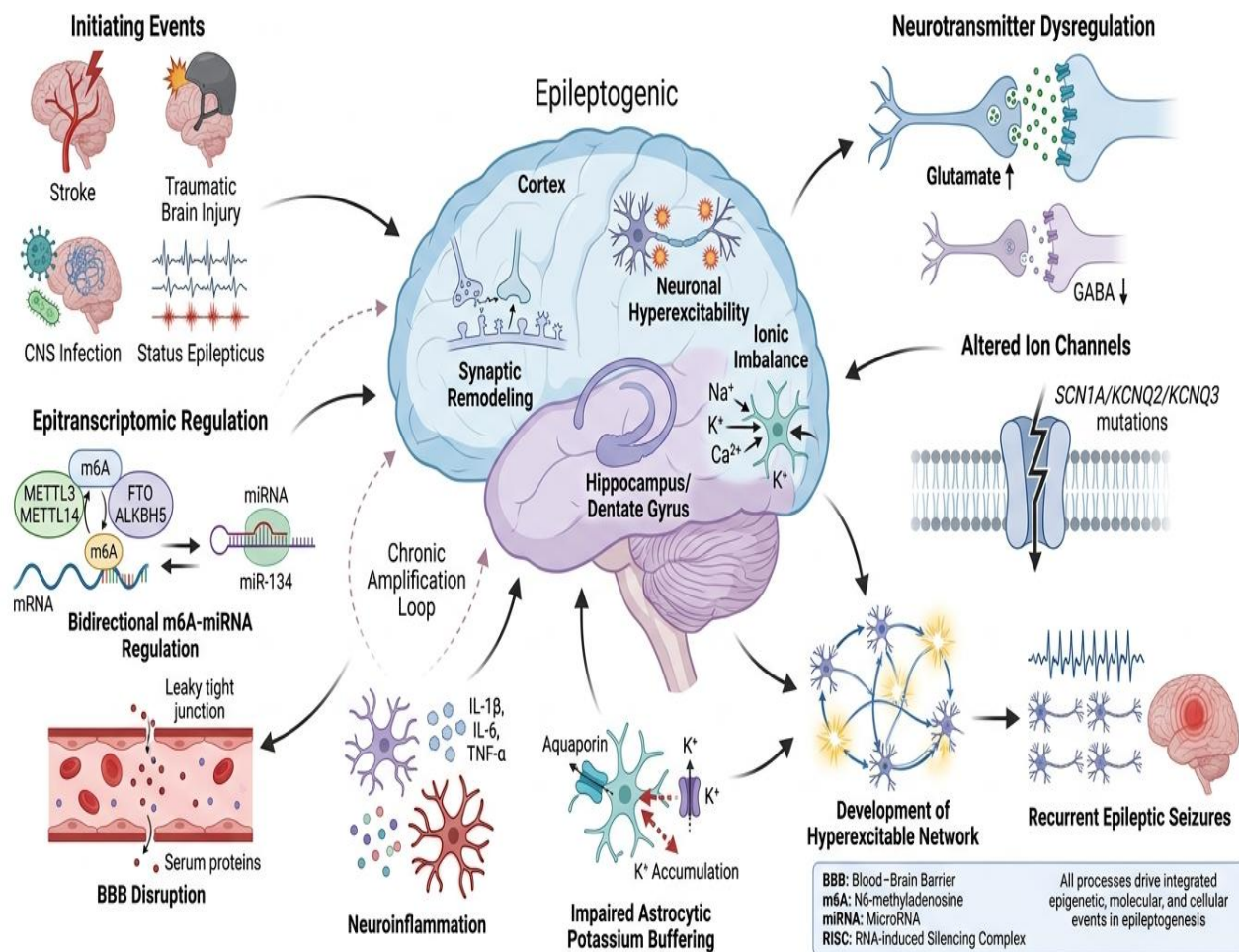
In inflammation, another important epileptogenic driver is calpain activation, which amplifies inflammation [24]. Because of increased calcium influx, calpain overactivity degrades GABAergic proteins and GABA transporters, including the vesicular GABA transporter (VGAT), increasing glutamate effects and contributing to seizure progression [9]. These findings suggest that glial polarization and inflammatory responses are epitranscriptomic [28-31].

A key feature of this regulatory network is the two-way communication between the m6A machinery and miRNAs: m6A writers (METTL3 and METTL14) add methyl groups to pri-miRNAs, which in turn increases the efficiency with which DGCR8 recognizes pri-miRNAs and Drosha processes them into mature miRNAs. Many mature miRNAs directly target the transcripts of m6A writers, erasers and reader proteins, creating a feedback loop that continuously influences the epitranscriptomic landscape.

Epileptogenesis should therefore be regarded as a progressive biological process rather than a single pathological event. This process involves a coordinated series of molecular changes in which both miRNAs and m6A RNA methylation act together to regulate neuronal function. m6A modification influences RNA stability, splicing, translation, degradation and maturation, thereby affecting the expression of numerous genes involved in neuronal homeostasis. In addition, the methylation of pri-miRNAs enhances their recognition by the DiGeorge syndrome critical region 8 (DGCR8) component of the microprocessor complex, promoting efficient miRNA biogenesis and strengthening the functional crosstalk between these two epitranscriptomic regulatory systems [32]. Collectively, ionic imbalance, neuroinflammation, synaptic remodelling and dysregulated circuitry networks are epitranscriptomic coordinates. miRNA- and m6A-dependent dysregulated networks include circuit instability and glial activation networks and ultimately drive chronic seizure conditions (Figure 1).

### Writers, erasers and readers: Decoding the role of m6A in epileptogenesis

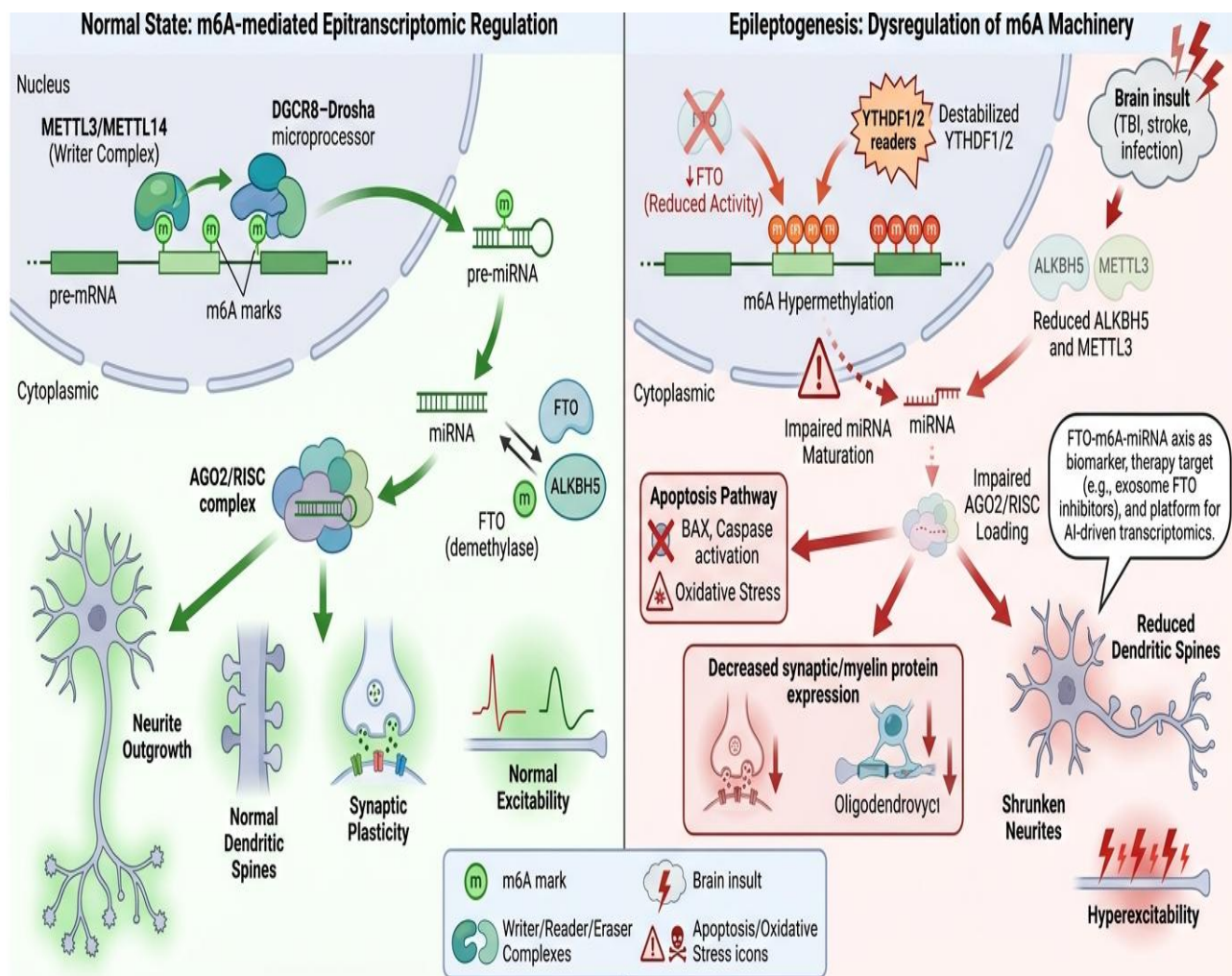
The most prevalent internal modification of mRNAs in eukaryotes is m6A (N6-methyladenosine) [33]. The enzyme responsible for the addition of methyl groups is METTL3, which is commonly referred to as a writer enzyme [34]. Approximately 60 % of mRNAs undergo epitranscriptomic modifications, the most significant of which is m6A, highly abundant in brain tissue. m6A acts as a major regulator and determines the fate of mRNA metabolism, including splicing and the formation of mature mRNA. Export from the nucleus to the cytoplasm. Translation, which regulates protein synthesis. Altered m6A modifications following brain injury represent maladaptive gene expression. m6A modification alters RNA metabolism and structure without changing the nucleotide sequence [35]. In addition to miRNA-mediated regulation, epitranscriptomic modifications such as m6A provide an additional regulatory layer in epileptogenesis [36]. m6A modification acts as writers (METTL3 /METTL14), catalysing the addition of m6A to mRNAs. Erasers (FTO/ALKBH5): Demethylate m6A by removing methyl groups. Readers (YTH domain family proteins YTHDF1/2/3) are RNA-binding proteins (RBPs) that regulate RNA fate and protein translation [37]. Thus, this modification is influenced by methylases, dimethylases, and RNA-binding proteins (Figure 2).



**Figure 1.** The figure summarizes the key molecular and cellular mechanisms involved in epileptogenesis, the process by which brain injury leads to chronic recurrent seizures. Initiating events such as stroke, traumatic brain injury, infection, and status epilepticus trigger synaptic reorganization, neuronal hyperexcitability, ionic imbalance, and neuroinflammatory responses. Alterations in ion channels, impaired astrocytic potassium regulation, excessive glutamate signalling, and reduced GABAergic inhibition contribute to excitotoxicity and abnormal neuronal activity. At the same time, microglial and astrocytic activation, cytokine release, blood-brain barrier disruption, and epitranscriptomic regulation through miRNAs and m6A modifications further increase seizure susceptibility. Together, these interconnected processes promote the development of a hyperexcitable neural network that ultimately results in recurrent epileptic seizures

Brain insults such as traumatic brain injury, stroke, and central nervous system infections can disrupt the expression of the m6A regulatory machinery, thereby contributing to epileptogenesis. Altered m6A signalling influences the expression of genes involved in synaptic plasticity, neuronal excitability, apoptosis, inflammation, and oxidative stress. These molecular changes promote neuronal injury by increasing proapoptotic mediators such as BAX and caspases, increasing inflammatory cytokine production, and causing excessive oxidative damage [12]. Evidence from hippocampal tissue obtained from patients with temporal lobe epilepsy (TLE) further revealed an inverse relationship between FTO expression and global m6A levels, suggesting that impaired RNA demethylation is a characteristic feature of this disease. Reduced FTO activity leads to m6A hypermethylation, disrupting epitranscriptomic homeostasis [38,39]. In addition, experimental studies indicate that FTO deficiency enhances lysosomal pathway activity while impairing autophagic processes, both of which have been implicated in neuronal dysfunction and susceptibility to seizures.

The instability of m6A reader proteins, such as YTHDF1 and YTHDF2, has also been linked to the molecular basis of epilepsy, in addition to FTO. Abnormal activity of these reader proteins interferes with the production of synaptic proteins, such as synapsin-1, and myelin-associated proteins, which are essential for efficient neuronal communication [40-42].



**Figure 2.** This figure shows the role of m6A modification as an epitranscriptomic regulator of mRNAs and miRNAs in the brain and how it is dysregulated during epileptogenesis. In parallel, m6A modification of pre-mRNAs by the writer complex METTL3/METTL14 enhances recognition by the DGCR8-Drosha microprocessor for the maturation of pre-miRNAs, which then facilitates the loading of AGO2/RISC and thus modulates neurite outgrowth, dendritic spine density, synaptic plasticity, and neuronal excitability. After a brain insult, for example, traumatic brain injury, stroke or infection, this machinery becomes disrupted: FTO activity is decreased and the activity of the reader YTHDF1/2 is destabilized, which leads to m6A hypermethylation, a further increase in proapoptotic and inflammatory signalling (BAX, activation of caspases, and oxidative stress) and the impairment of reader-dependent expression of synaptic and myelin proteins (due to a reduction in ALKBH5/METTL3). These results suggest that the FTO-m6A-miRNA axis could explain how epilepsy develops and could serve as a candidate biomarker, a basis for a new therapeutic approach involving exosome-delivered FTO-inhibitory molecules, and a tool for identifying potential targets *via* AI-driven transcriptomic analysis

Excessive m6A accumulation has been linked to impaired brain growth and neurodevelopment, whereas reduced ALKBH5 expression interferes with normal cerebellar development [43]. Similarly, reduced METTL3 activity alters dendritic spine morphology and postsynaptic density organization, impairing synaptic connections. Taken as a whole, these changes worsen oxidative stress, mitochondrial malfunction, and neuroinflammation, all of which are known factors involved in the development of epilepsy and seizures [40].

m6A modification has recently been identified as a significant regulator of miRNA biosynthesis and maturation. Methyl groups are deposited on primary miRNA transcripts (pri-miRNAs) by m6A writer proteins, especially METTL3 and METTL14. This facilitates detection by the DGCR8-Drosha microprocessor complex and encourages effective processing into mature miRNAs [13,41]. Through this process, m6A indirectly affects the excitability of neurons, neuronal plasticity, and dendritic spine morphology processes that are crucial to the development of epileptogenesis. In addition, m6A-associated molecular signatures have shown promise as biomarkers for distinguishing different forms of epilepsy, highlighting their potential diagnostic value.

### Stage-specific miRNA dysregulation in epileptogenesis

Mature microRNAs are small, single-stranded molecules that regulate gene expression [42]. They originate from double-stranded stem-loop precursor structures, which Dicer processes into a functional strand that acts as a posttranscriptional gene regulator (PTGR) with the RNA-induced silencing complex (RISC) [44]. The brain is highly enriched in miRNAs [45], which primarily bind to the 3' untranslated regions (UTRs) of target mRNAs to prevent translation or mRNA degradation [46]. miRNAs regulate protein expression, neuronal communication, and cortical rhythms [2]. Any dysregulation of miRNAs contributes to seizure initiation and progression [46]. Meta-analyses of experimental rat models and human tissue samples show that miRNAs contribute to seizure progression [2]. Oxidative stress, neuroinflammation, apoptosis, mitochondrial dysfunction, synaptic re-circuitry, glial dysfunction, and neurotransmitter imbalance are the key drivers of seizure induction and are also influenced by proteins regulated by miRNAs [13,45]. Additionally, miRNA levels vary across different levels of epileptogenesis, emphasizing the need for temporal and system-level analysis.

#### *miRNA influence on neurotransmitters*

The primary mechanism through which miRNAs influence epileptogenesis is the regulation of neurotransmitter systems. miRNAs contribute to secondary neuronal damage [47]. Specific miRNAs contribute to the hormonal imbalance [47]. Glutamate acts through multiple receptor subtypes, including AMPA and NMDA receptors [48]. Specific miRNAs maintain the E/I balance [49]. Brain-specific miR-124 is downregulated in TLE and increases expression of cAMP-response element-binding protein (CREB1), promoting hyperexcitability by targeting the NMDAR1 subunit of the NMDA receptor [6,50]. Silencing of miR-219 increases NR1 and calcium/calmodulin-dependent protein kinase II (CaMKII) expression, promoting electrical bursts and seizure activity [51]. For GABA receptors, miR-34 family upregulation contributes to status epilepticus and chronic temporal lobe epilepsy by targeting GABRA2/3, thereby impairing GABAergic transmission [52,53].

#### *miRNAs and synapses*

Beyond inflammatory pathways, miRNAs also support synaptic integrity and plasticity. Neuronal network stability depends on synaptic plasticity, and disruption of this process leads to epileptogenesis [54]. miR-132 downregulation in TLE increases p250GAP expression, promoting hippocampal excitatory circuitry [55]. The expression level of miR-132 varies during different seizure stages. miR-134 increases network excitability by targeting LIM domain kinase 1 (LIMK1).

#### *Role of miRNAs in cell death*

Different miRNAs regulate genes in the metabolic oxidative system [56]. In TLE mice, kainic acid induced hippocampal damage increases miR-24a, which compromises mitochondrial antioxidant enzymes such as manganese superoxide dismutase (MnSOD) and superoxide dismutase 2 (SOD2) [57].

Seizure activity contributes to neuronal apoptosis, leading to progressive neuronal loss and the destruction of normal neural networks. This process is largely mediated through the regulation of BCL2 family proteins and caspase-dependent apoptotic pathways. Following status epilepticus, miR-34a, a downstream target of the p53 signalling pathway, is significantly upregulated and suppresses the antiapoptotic protein BCL2, thereby promoting neuronal cell death [44]. Similarly, increased miR-141 expression favours apoptosis by increasing the activity of the Bcl-2-associated X protein (BAX) and activating caspases-3 and -9, shifting the balance from cell survival toward programmed cell death. Collectively, these findings indicate that several seizure-induced miRNAs function as proapoptotic regulators, contributing to neuronal degeneration and the progression of epileptogenesis [44] (Table 1).

**Table 1.** Evidence based classification of miRNA-m6A relationships relevant to epileptogenesis

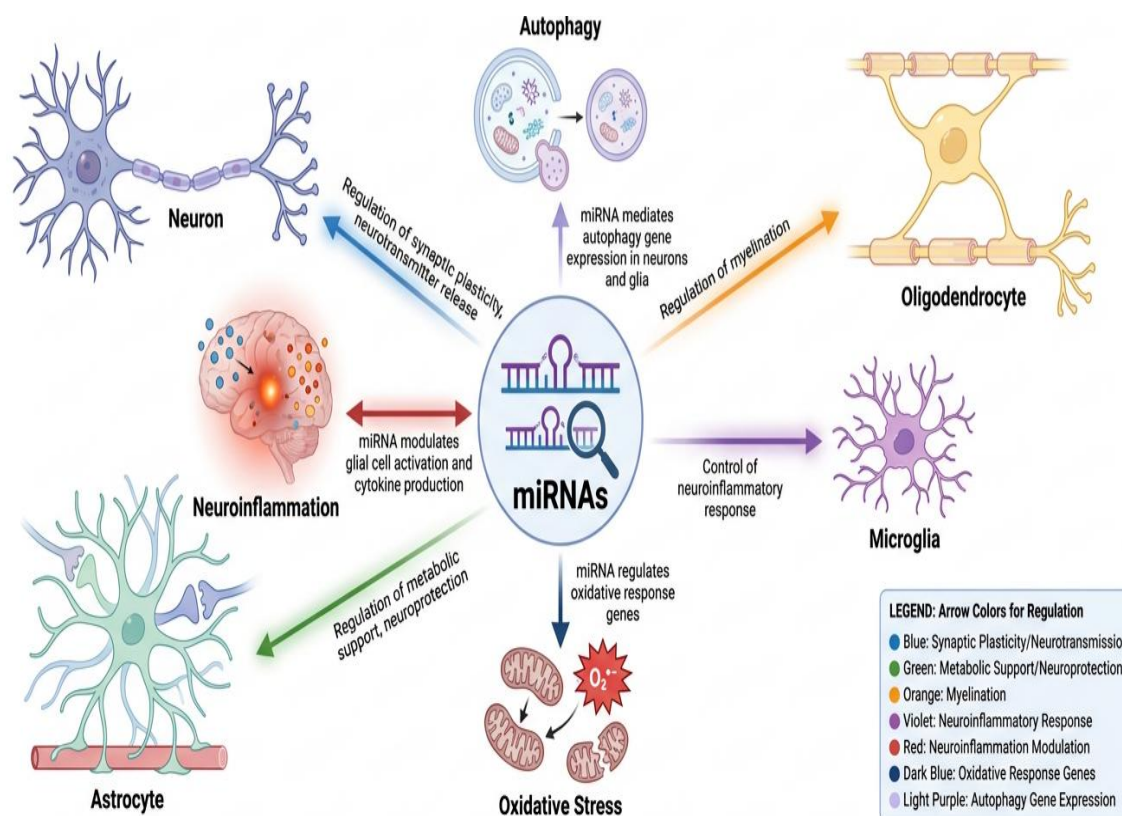
| miRNA      | m6A-related evidence                                                        | Epilepsy-associated target/ pathway | Role in epileptogenesis                                                                   | Evidence status                                      | Expression trend              | Ref.    |
|------------|-----------------------------------------------------------------------------|-------------------------------------|-------------------------------------------------------------------------------------------|------------------------------------------------------|-------------------------------|---------|
| miR-134    | m6A-controlled maturation and synaptic regulation <i>via</i> METTL3/METTL14 | LIMK1                               | Propagates seizures <i>via</i> synaptic hyperexcitability and dendritic spine remodelling | Proposed/ indirect                                   | Upregulated                   | [45,58] |
| miR-146a   | m6A-linked inflammatory miRNA progression and glial activation              | TRAF6, IRAK1, CFH                   | Releases cytokines and activates astrocytes                                               | Proposed/ indirect                                   | Upregulated                   | [41,44] |
| miR-132    | m6A-driven neuronal plasticity pathway                                      | P250GAP, BDNF                       | Promotes aberrant synaptic activity and excitatory circuit formation                      | Epilepsy evidence only; m6A relationship unconfirmed | Upregulated                   | [45,55] |
| miR-124    | m6A dysregulation changes miRNA stability                                   | CREB1, Bax/Bcl2                     | Controls neuronal survival and glutamate transmission                                     | Epilepsy evidence only; m6A relationship unconfirmed | Down-regulated                | [46,50] |
| miR-155    | Increased inflammatory signalling and reactive gliosis linked to m6A        | SHIP1, SOCS1                        | Elevates cytokine activity and microglial activation                                      | Indirect/ extrapolated                               | Upregulated                   | [59,60] |
| miR-34a    | Association with m6A regulators drives apoptotic signalling                 | SIRT1, GABRA2/3                     | Impairs GABA activity by promoting apoptosis                                              | Epilepsy evidence only; m6A relationship unconfirmed | Upregulated                   | [44,53] |
| miR-27a-3p | Potential m6A-dependent posttranscriptional regulation                      | MAP2K4                              | Amplifies inflammatory response and neuronal excitation                                   | Epilepsy evidence only; m6A relationship unconfirmed | Upregulated                   | [44]    |
| miR-23a    | Oxidative pathways associated with m6A dysregulation                        | MnSOD/SOD2                          | Impairs mitochondrial function and antioxidant defence                                    | Epilepsy evidence only; m6A relationship unconfirmed | Context-dependent (up in TLE) | [57]    |
| miR-141    | m6A-mediated apoptotic response                                             | STAT4, CXCL12                       | Promotes neuronal death by activating caspase pathways                                    | Epilepsy evidence only; m6A relationship unconfirmed | Upregulated                   | [61]    |
| miR-222    | m6A-related inflammatory epitranscriptomic regulation                       | STAT3, PARP1                        | Amplifies apoptotic pathways and inflammatory response                                    | Proposed/ insufficient evidence                      | Upregulated                   | [44]    |

### *Integrative role of miRNAs in epileptogenesis*

Every stage of epileptogenesis involves miRNAs, whose expression varies constantly as the illness worsens. Many miRNAs, such as miR-155 and miR-134, become dysregulated after initial brain injury and contribute to key pathological processes, including neuroinflammation, synaptic remodelling, and neuronal dysfunction [58,59,62,63]. In addition to their regulatory roles, miRNAs have emerged as promising minimally invasive biomarkers for epilepsy. miR-146a and miR-134 have shown potential as biomarkers for diagnosis and prognosis, whereas exosomal miRNAs are particularly intriguing since they can cross the BBB and facilitate intercellular communication, making them attractive candidates for liquid biopsy-based approaches [64-67]. Moreover, multiomics studies have repeatedly linked abnormal miRNA expression to inflammatory pathways, metabolic dysregulation, and poor synaptic transmission in both experimental models and epileptic patients. Altogether, these results indicate that miRNAs are involved in epileptogenesis and are potential biomarkers and therapeutic targets.

Further evidence has shown that N6-methyladenosine (m6A) RNA methylation and the regulatory roles of miRNAs are closely related. Changes in m6A affect the processing, maturation, stability, and abundance of specific miRNAs, which in turn alters their downstream regulatory consequences. However, a bidirectional regulatory connection exists because many mature miRNAs directly target transcripts encoding m6A writers, erasers, and readers. During epileptogenesis, m6A modification and miRNAs work as an integrated regulatory network that modifies gene expression rather than as distinct mechanisms. The cellular miRNA landscape can be altered by changes in m6A signalling, and RNA methylation patterns can be further altered by miRNA-

mediated control of m6A-related enzymes. This reciprocal feedback loop is likely to influence whether the molecular responses initiated after seizures or brain injury promote tissue recovery or instead drive the persistent neuronal hyperexcitability that characterizes chronic epilepsy [45] (Figure 3).



**Figure 3.** This diagram shows the pivotal role of microRNAs (miRNAs) in the regulation of essential cellular and molecular functions in the central nervous system (CNS). These findings illustrate how miRNAs modulate pathways involved in CNS cell function in major CNS cell types, such as neurons, astrocytes, oligodendrocytes, and microglia, including synaptic plasticity, neurotransmitter release, metabolic support, neuroprotection, myelination, and the neuroinflammatory response. Moreover, the diagram highlights miRNAs' role in several pathological mechanisms, including oxidative stress and autophagy, and their ability to modulate the expression of genes involved in the oxidative response and autophagy in neurons and glial cells, respectively

## Sequential integration of emerging empirical datasets and novel molecular axes

### Human temporal lobe epilepsy m6A profiling

One problem with early research into the epitranscriptome was the idea that epileptogenesis results from widespread, global changes in total m6A levels in the brain [68]. Quantitative profiling of resected hippocampal tissue from human temporal lobe epilepsy (TLE) patients (sclerotic TLE, non-sclerotic TLE, and non-epileptic control specimens) reveals a much more complex pathophysiological picture. Total m6A in the hippocampus of human TLE does not differ significantly from non-epileptic controls ( $P = 0.5321$ ), based on colorimetric and MS quantification of polyA-tailed RNA [69]. Western blot and densitometric analyses, however, reveal that the enzyme machinery is very severely disrupted at the protein level. In human TLE patients, catalytic writers METTL3 ( $P = 0.0001$ , Welch's t-test) and METTL14 ( $P = 0.0188$ , Mann-Whitney U test) are significantly downregulated [69]. At the same time, the major m6A erasers FTO ( $P = 0.0156$ ) and ALKBH5 ( $P < 0.0001$ ) are extremely depleted. These results challenge the theory of TLE pathogenesis based on chronic hyper- or hypomethylation [69]. Concomitant loss of FTO and ALKBH5 (two enzymes that control m6A) traps selected neuronal and glial networks in a hypermethylated, pathologically dysregulated state, preventing clearance of m6A marks from these mRNAs and non-coding transcripts [12].

*Fat mass and obesity-associated protein, nuclear receptor subfamily 4 group A member 2 and YTH N6-methyladenosine RNA binding protein 2 axis in post-traumatic epilepsy*

Beyond TLE, there is recent empirical evidence supporting a novel, mechanistically unique m6A regulatory circuit involving FTO, nuclear receptor subfamily 4 group A member 2 (NR4A2) and the YTH N6-methyladenosine RNA binding protein 2 (YTHDF2) in TBI-induced post-traumatic epilepsy (PTE) [70]. In the case of severe craniocerebral trauma, expression of FTO is substantially reduced in the neurons of the hippocampus. Under normal conditions, FTO demethylates m6A in NR4A2 mRNA, stabilizing these transcripts and supporting transcription networks that protect the nervous system and promote anti-inflammatory responses. FTO downregulation in PTE induces pathological hypermethylation of NR4A2 mRNA. The cytoplasmic m6A reader YTHDF2 specifically targets these hypermethylated adenosine residues on NR4A2 mRNA and brings the carbon catabolite repression 4-negative on TATA-less (CCR4-NOT) deadenylase complex to NR4A2 mRNA to promote NR4A2 mRNA decay and protein depletion [70]. Loss of NR4A2 directly increases seizure susceptibility, aggravates apoptosis in hippocampal neurons, and disrupts BBB structure. Experimental rescue by viral-mediated FTO overexpression removes m6A marks from NR4A2, blocks YTHDF2-mediated degradation, restores NR4A2 protein and drastically lowers post-traumatic seizure frequency and BBB leakage *in vivo* [71].

*METTL3-related neuronal injury*

METTL3 acts as an immediate-early epitranscriptomic switch in the control of neuronal survival and structural plasticity during acute epileptogenesis. Serum response factor (SRF) moves and attaches to the promoter area in response to severe seizure activity. METTL3 was found to have a significant increase in transcription and nuclear protein content within 24 hours after status epilepticus ( $P = 0.029$ ) [12]. Increased METTL3 selectively modifies transcripts associated with neuronal excitability and cell survival, such as the GTP-binding protein Gem and Nrf2. Moreover, nuclear METTL3 hypermethylates the pri-miR-134 and pri-miR-132 stem-loop structures, indicating their nuclear degradation by HNRNPA2B1 and the Drosha-DGCR8 Microprocessor complex [12]. This quick processing results in excessive production of mature pro-epileptogenic miRNAs, which target structural synaptic proteins (such as Limk1), causing dendritic spine dysgenesis, mossy fibre sprouting, and permanent excitotoxic neuronal damage. METTL3 knockdown or pharmacological inhibition blunts pri-miRNA processing, maintains neuronal viability, and reduces seizure-induced neurodegeneration [12].

*Clinical and pre-clinical evidence for mi-RNA 134*

MicroRNA-134 (miR-134) is one of the best clinically validated non-coding RNA targets, connecting molecular discovery with therapeutic translation in epilepsy research. Pathologically increased in TLE foci in humans and in experimental TLE, miR-134 is localized at the neuronal dendritic level, where it inhibits translation of LIM domain kinase 1 (Limk1) mRNA, resulting in enlarged neuronal dendritic spines, higher levels of AMPA receptors, and hyper-excitability of synapses. Recent preclinical and early clinical-phase studies have shown that antisense oligonucleotide (ASO) "antagomirs" against miR-134 (antagomiR-134/antimiR-134) can achieve potent, long-lasting disease-modifying effects [69]. AntagomiR-134 following status epilepticus results in a long-lasting reduction in spontaneous recurrent seizures, mitigates hippocampal gliosis, and does not cause behavioural toxicity or impair memory retention. The discovery of m6A methylation of pri-miR-134 as its control mechanism opens a new line of treatment, as m6A methylation inhibitors can either activate FTO or inhibit its catalytic activity, both of which would reduce the levels of miR-134, and thus have a more powerful effect in disease modification than conventional antiseizure drugs [69].

*Functional validation of mi-RNA-155*

Recent meta-analyses (2025/2026) of microRNA expression in human epilepsy cohorts of resected brain tissue and peripheral blood plasma (PBP) samples of epilepsy patients validate miR-155 as a significantly

upregulated microRNA in both sample types. Meta-analysis of pooled clinical datasets shows that higher plasma miR-155 levels are associated with seizure frequency, disease duration and conventional antiseizure medication (c-ESM) resistance. Meta-analytic pooling of clinical datasets shows that higher circulating miR-155 levels are associated with increased seizure frequency, disease duration, and conventional antiseizure medication (c-ESM) resistance [72]. Activated astrocytes and microglial cells enrich miR-155 in the cytoplasm around human epileptic lesions. Pathological expression of miR-155 downregulates Matrix Metalloproteinase 3 (MMP3) and upregulates the nuclear factor kappa B (NF- $\kappa$ B) signalling pathway, triggering the recruitment of inflammatory-activated A1 reactive astrocytes, which are responsible for the release of pro-inflammatory mediators IL- $\beta$ , TNF- $\alpha$ , and for breaking down the components of the extracellular matrix of the blood-brain barrier [72]. Silencing miR-155 restores MMP3, maintains the BBB, inhibits M1 microglial polarization, and suppresses network hyperexcitability in chronic epilepsy models [73].

### Critical re-evaluation of m6A-miRNA interactive machinery

#### *Epitranscriptomic control of pri-miRNA processing kinetics*

The biogenesis of miRNAs starts in the nucleus, where the RNase III enzyme Drosha cleaves long pri-miRNAs (pri-miRNAs) along with the essential cofactor DiGeorge syndrome critical region gene 8 (DGCR8) [74]. Epitranscriptomic modifications, particularly m6A RNA methylation, regulate the rate and specificity of this processing cascade. The catalytic components of the m6A writer complex, methyltransferase-like 3 (METTL3) and methyltransferase-like 14 (METTL14), selectively place marks within the hairpin stem-loops of pri-miRNAs with the consensus RGAC motif. This m6A mark serves as a structural recognition motif for several nuclear reader proteins, mainly Heterogeneous Nuclear Ribonucleoprotein A2/B1 (HNRNPA2B1) and DGCR8. HNRNPA2B1 directly binds m6A-modified pri-miRNAs, inducing local RNA conformational alterations termed the epitranscriptomic switch that facilitate the recruitment and positioning of the Microprocessor complex. This interaction accelerates nuclear cleavage of pri-miRNAs into precursor microRNAs (pre-miRNAs) and does not involve the rate-limiting steps of non-methylated miRNA biogenesis [75].

#### *Cell-type-specific epigenetic crosstalk in synaptic remodelling and neuroinflammation*

Epileptogenesis is characterized by complex changes in cellular circuits, including structural synaptic remodelling, dendritic spine enlargement, astrocytic dysfunction, and microglial neuroinflammation. The mechanism of action between m6A modification and miRNAs is reciprocal and occurs in different CNS compartments, resulting in cell-type-specific regulatory loops. The m6A reader protein YTH domain-containing 2 (YTHDC2) directly regulates extracellular glutamate homeostasis in reactive astrocytes [12]. YTHDC2 selectively binds m6A-modified glutamate-cystine antiporter (xCT) transcripts, which encode the SLC7A11 subunit. In TLE with hippocampal sclerosis, high YTHDC2 activity anchors m6A-modified xCT transcripts in reactive astrocytes, promotes antiporter exchange, raises external glutamate to neurotoxic levels, and triggers recurrent network seizures. At the same time, certain miRNAs from the astroglia regulate inflammatory signalling and BBB integrity [72]. miR-132-3p is a microRNA that is pathologically upregulated in astrocytes after prolonged status epilepticus and is a direct target of methyl-CpG-binding protein 2 (MeCP2) [72]. A1 reactive astrocyte polarization is associated with increased release of pro-inflammatory cytokines such as Interleukin-1 beta and tumour necrosis factor-alpha, leading to further neuronal death and increased network excitability. In human lesions, miR-155 is localized in the cytoplasm of activated astrocytes and acts as a central regulator of neuroinflammation, controlling expression of matrix metalloproteinase 3 (MMP3) and affecting matrix stability of the BBB in chronic seizures [72]. This non-coding network is further complicated by microglial activation and intercellular communication. Microglial miR-146a exhibits a dual protective/pathological role: its level is associated with neuroinflammation severity, but its experimental

downregulation decreases glial fibrillary acidic protein (GFAP) expression, reduces hippocampal gliosis, and suppresses caspase-9-mediated apoptotic cascades. Moreover, intercellular crosstalk involves direct transfer of exosome-derived miR-200c-3p to microglial cells, which are generated by the neurons. When taken up, miR-200c-3p regulates the expression of the key regulatory component of the m6A writer complex, zinc finger CCH-type containing 13 (ZC3H13), which in turn down-regulates the expression of the key regulatory component of the m6A reader complex, dual-specificity phosphatase 9 (DUSP9), and activates downstream extracellular signal-regulated kinase (ERK) signalling to modulate microglial activation state [72].

### Dual-pathway therapeutic strategies: Targeting the m6A-miRNA axis

Epileptogenesis is a dynamic condition characterized by alterations in the brain's activity and structure, leading to abrupt, continuous seizure conditions. The optimal time for treatment with novel, disease-modifying approaches is during the latent phase. Pharmacotherapy is suitable only for symptomatic relief and has failed to manage the underlying causes [76]. Many patients are resistant to traditional treatment, prompting the development of other targeted disease options [76]. These disturbances include neurotransmitter imbalances, overactivity of the mTOR pathway, and ionic imbalances, all triggered by brain damage such as stroke, infection, and TBI. Identifying the cause and taking appropriate measures to halt the process is crucial. For this purpose, biomarkers are the gold standard for identifying the cause and type of epilepsy [77].

Conventionally, first-line antiepileptic drugs (AEDs) primarily stop the seizures but have a limited ability to prevent or modify the underlying process of epileptogenesis [78]. Because of this restriction, there is growing interest in treatments that focus on the biological mechanisms underlying the disease's progression rather than only relieving symptoms of seizures. Combinations of second- and third-generation AEDs, such as lamotrigine, levetiracetam, topiramate, and eslicarbazepine, have shown synergistic antiepileptogenic benefits in preclinical tests by lowering glutamate release, oxidative stress, and excitotoxicity [79-82]. Moreover, several mechanism-based therapeutic approaches have shown encouraging results. Inhibition of the mTOR signalling pathway with rapamycin has produced both anticonvulsant and neuroprotective properties, particularly when rapamycin is administered during the early stages following status epilepticus [83,84]. Similarly, the COX-2 inhibitor celecoxib has been reported to increase the seizure threshold while attenuating NLRP3 inflammasome-driven neuro-inflammation [85,86]. Other promising strategies include the inhibition of adenosine kinase to restore endogenous adenosine-mediated antiseizure signalling in drug-resistant epilepsy models [87] and mesenchymal stem cell (MSC) therapy, which has demonstrated anti-inflammatory and neuroprotective benefits in preclinical studies and early clinical investigations [88]. Ketogenic dietary therapy, which shifts brain metabolism toward ketone body utilization, similarly reduces neuronal excitability and provides a complementary, non-pharmacological disease-modifying option, particularly in drug-resistant epilepsy [89]. The precision genomic and RNA-targeted therapeutic techniques covered in the next sections build on these developments, collectively reflecting a broader shift from symptom-oriented treatment to mechanism-based therapies.

The involvement of miRNAs and m6A modifications in chronic seizures disrupts molecular and cellular events, leading to a wide range of genetic alterations and the translation of pro-epileptogenic proteins [45] (Table 2). These are highly specific approaches rather than conventional targets, as they offer intervention at both the transcriptional and posttranscriptional levels [46]. Dysregulated m6A machinery modulates glutamate transport and astrocytic function, thereby decreasing seizure frequency. A promising compound, 7d, negatively regulates the 'eraser' FTO, thereby increasing m6A activity, and is anticonvulsant in experimental models. Other miRNA-based therapeutic avenues include antagomirs, which silence proepileptic proteins, and mimics, which restore miRNA loss due to injury [46]. In temporal lobe epilepsy (TLE), elevated miR-134, which causes aberrant dendritic remodelling and altered spine density, can be reversed by antagomir-based inhibition [13,90-93]. Likewise,

increased miR-146a expression results in apoptosis and neuroinflammation [44, 94-97]. Identifying the m6A site on miRNAs is necessary to silence these aberrant miRNAs [13].

**Table 2.** Modern genomic and RNA-based therapeutic approaches for epileptogenesis

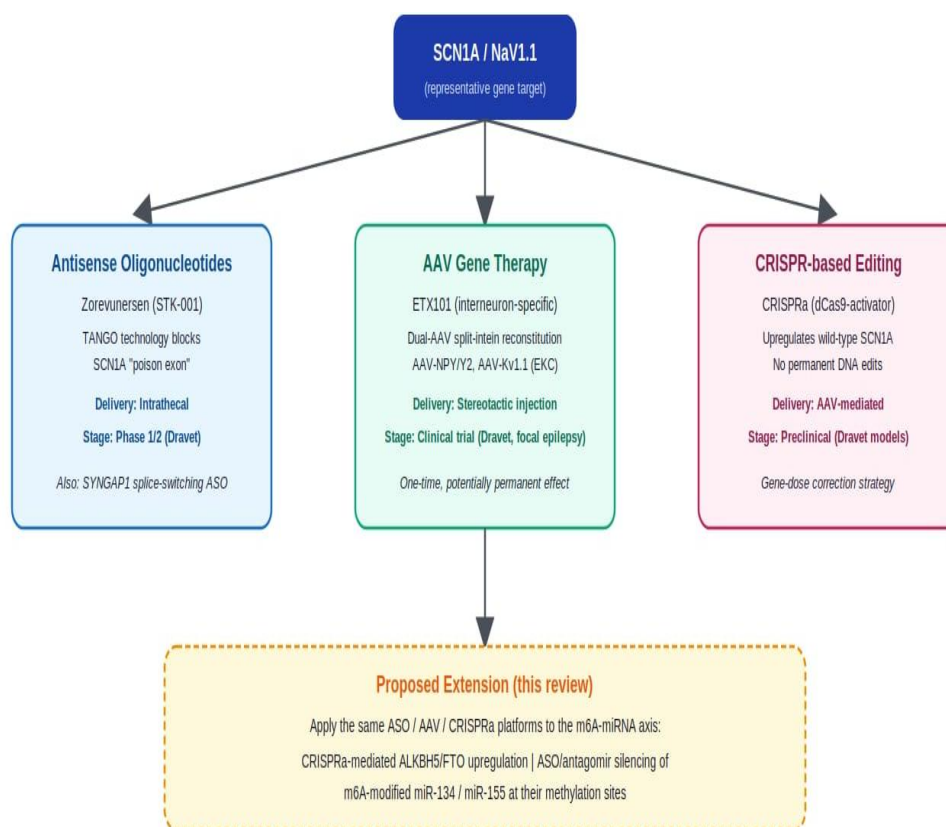
| Approaches                                               | Molecular target & mechanism                                     | Disease model / stage              | Outcome                                         | Delivery route                 | Ref.      |
|----------------------------------------------------------|------------------------------------------------------------------|------------------------------------|-------------------------------------------------|--------------------------------|-----------|
| Antagomir miR-134                                        | Activates LIMK-1 signaling; stabilizes dendritic spine structure | Preclinical                        | Reduced aberrant dendritic remodelling          | ICV/ stereotactic injection    | [58]      |
| Antagomir miR-155                                        | Suppresses inflammatory signalling                               | Preclinical                        | Reduced seizure severity                        | ICV/ stereotactic injection    | [98]      |
| Antagomir miR-34a                                        | Promotes neuronal survival pathways                              | Preclinical                        | Improved neuronal survival                      | ICV/ stereotactic injection    | [59]      |
| Antagomir miR-132                                        | Reduces neuronal death and dendritic spine abnormality           | Preclinical                        | Reduced neuronal death                          | ICV/ stereotactic injection    | [55]      |
| Mimic miR-146a                                           | Restores miR-146a levels                                         | Preclinical                        | Neuroprotective, anti-inflammatory              | ICV/ stereotactic injection    | [59]      |
| ASO - Zorevunersen (STK-001, TANGO)                      | Blocks SCN1A poison exon; restores Nav1.1                        | Dravet syndrome; Phase 1/2 + OLE   | Sustained seizure reduction; improved cognition | Intrathecal injection          | [99]      |
| Splice-switching ASO                                     | Corrects SYNGAP1 alt. 3' splice site                             | SYNGAP1 epilepsy; preclinical      | Restored SYNGAP1 protein                        | Intrathecal/ ICV (preclinical) | [99]      |
| AAV gene therapy (ETX101)                                | Interneuron-specific Nav1.1 restoration                          | Dravet syndrome; clinical trial    | Improved survival; reduced seizures             | Stereotactic intraparenchymal  | [99]      |
| Dual-AAV split-intein reconstitution                     | Full-length SCN1A <i>via</i> two AAV vectors                     | Dravet mouse models; preclinical   | Reduced mortality/seizures                      | Stereotactic intraparenchymal  | [99]      |
| AAV-NPY / Y2 receptor                                    | Neuropeptide Y overexpression                                    | Focal epilepsy; clinical trial     | Reduced seizure frequency                       | Stereotactic intrahippocampal  | [100]     |
| AAV/lentivirus engineered K <sup>+</sup> channel (Kv1.1) | Enhanced neuronal repolarization                                 | Focal epilepsy models; preclinical | Suppressed focal epileptic activity             | Stereotactic focal injection   | [100]     |
| RNAi knockdown - GluK5                                   | Reduced kainate receptor signalling                              | Focal epilepsy; preclinical        | Seizure suppression                             | Intrathecal/ ICV               | [100]     |
| CRISPRa (dCas9-activator)                                | Upregulates wild-type SCN1A dosage                               | Dravet mouse models; preclinical   | Increased Nav1.1; improved network activity     | AAV-mediated stereotactic      | [100,101] |

By focusing on fixing the underlying genetic flaws rather than just managing seizures, breakthroughs in precision genomic medicine are revolutionizing the treatment of epilepsy beyond RNA-targeted medicinal methods. Antisense oligonucleotides (ASOs) have advanced the most toward clinical use among these tactics. Zorevunersen (STK-001), developed with targeted augmentation of nuclear gene output (TANGO) technology, is one such example. Zorevunersen promotes the synthesis of functional Nav1.1 sodium channels by blocking the insertion of a naturally occurring "poison exon" in SCN1A pre-mRNA. Clinical research, including the MONARCH and ADMIRAL trials and their open-label extension, has shown that patients with Dravet syndrome have improved cognitive outcomes and sustained reductions in seizure frequency, consistent with the underlying Nav1.1 interneuronopathy mechanism [99,100,102]. A similar splice-switching ASO strategy has also been investigated for SYNGAP1-related epilepsy, where accentuation of an alternative 3' splice site prevents nonsense-mediated decay and restores SynGAP1 protein expression [99].

Gene replacement therapy with adeno-associated virus (AAV) vectors can provide long-lasting therapeutic benefits with a single injection. It has become a promising approach for the treatment of epilepsy. ETX101, a tailored AAV vector designed to restore Nav1.1 expression specifically in GABAergic interneurons and enhance inhibitory neurotransmission, is a prominent example. However, the low packaging capacity of conventional vectors is a significant obstacle to AAV-based therapy. To overcome this limitation, researchers have developed dual-AAV platforms using intein-mediated protein reconstitution or Cre/lox recombination. These platforms enable the delivery of big genes, such as SCN1A, which are larger than what a single AAV vector can carry [99].

Beyond gene replacement, several viral vector-based treatment strategies are being assessed in early clinical stages and advanced preclinical research. These include enhancing the NaV $\beta$ 1 subunit, delivering neuropeptide Y (NPY) and its Y2 receptor *via* AAV, and creating potassium channels derived from Kv1.1 to reduce focal neural hyperexcitability. To reduce seizure activity, RNA interference-based techniques that target the kainate receptor subunit GluK5 are also being researched concurrently. Taken together, these novel gene therapy techniques show the increasing potential of precision molecular therapies to alter the fundamental mechanisms of epilepsy instead of only managing its symptoms [100].

Genome-editing technology further expands precision therapeutic options. CRISPR activation (CRISPRa) increases endogenous gene expression by fusing catalytically inactive Cas9 (dCas9) to transcriptional activators rather than creating persistent DNA fragments. CRISPRa effectively increased the expression of the intact wild-type SCN1A allele in Dravet syndrome experimental mice, resulting in elevated NaV1.1 levels and improved neuronal performance [100,101] (Figure 4).



**Figure 4.** Modern genomic and RNA-based therapeutic platforms for epilepsy (antisense oligonucleotides, AAV gene therapy, and CRISPR-based gene-dose correction) and their proposed convergence with the m6A-miRNA regulatory axis central to this review

Crucially, these new genomic technologies may target the m6A-miRNA regulatory network this research focuses on. For example, ASOs or miRNA antagomirs can block harmful m6A-regulated miRNAs such as miR-134 and miR-155, whereas CRISPRa could elevate the expression of m6A demethylases like ALKBH5 or FTO. Although these approaches have not yet been systematically investigated in epilepsy, integrating epitranscriptomic regulation with precision genomic medicine represents a promising avenue for future research. Such strategies may provide opportunities to modify the molecular pathways that drive epileptogenesis rather than treating seizures alone (Table 2).

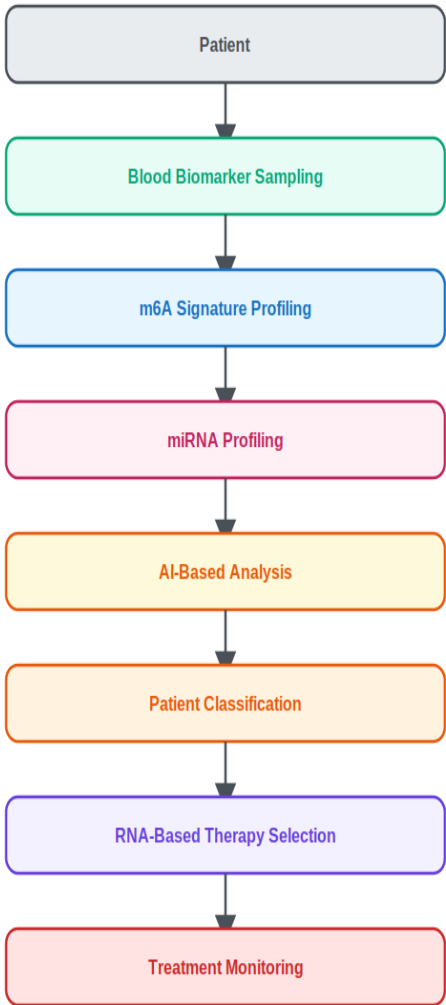
Precise genomic editing and regulation control are essential to overcome the limitations of genomic interventions and improve therapeutic effectiveness. Unlike current antiseizure medications that primarily

suppress seizures, therapies targeting the m6A-miRNA regulatory network may modify the underlying disease process by preventing neuronal remodelling, neuroinflammation, and maladaptive synaptic plasticity (Table 3).

**Table 3.** Molecular abnormalities and corresponding therapeutic strategies

| Molecular abnormality                                                                                        | Therapeutic strategy                                                                                              |
|--------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| Aberrant overexpression of METTL3 leading to excessive m6A RNA methylation                                   | Development and application of selective METTL3 inhibitors to restore normal epitranscriptomic regulation         |
| Dysregulated FTO activity resulting in altered RNA demethylation                                             | Use of FTO activators or inhibitors, depending on the underlying molecular alterations and therapeutic objectives |
| Increased expression of miR-134, which is associated with neuronal hyperexcitability and seizure progression | Therapeutic inhibition using antagomir-134 to reduce pathological miR-134 activity                                |
| Impaired function or altered expression of YTHDF reader proteins affecting m6A-mediated RNA metabolism       | Small-molecule inhibitors                                                                                         |
| Sustained neuroinflammatory responses that contribute to epileptogenesis and disease progression             | Combination therapy                                                                                               |

Given the multilayered nature of epileptogenesis, future treatment is unlikely to rely on a single agent. A combination epitranscriptomic therapy approach-pairing a conventional antiseizure drug with a miRNA inhibitor, a METTL3 inhibitor, and exosome-based delivery-may target the m6A-miRNA network at multiple nodes simultaneously, reducing the likelihood of compensatory resistance *via* single-agent strategies (Figure 5).



**Figure 5.** Proposed personalized treatment roadmap: from patient blood sampling through m6A and miRNA signature profiling and AI-based classification to RNA-based therapy selection and treatment monitoring

**Future perspective and challenges**

The key hurdle in the newly established m6A-miRNA field of epilepsy is to shift the focus from association to causal and spatially resolved mechanisms. Strong evidence supports independent roles for m6A and

miRNA in epileptogenesis, although direct experimental testing of specific m6A-miRNA interactions has been limited. Future studies should therefore focus on mechanistic resolution rather than further descriptive profiling.

The initial step is to validate whether candidate miRNAs like miR-134, miR-146a, miR-155, miR-124 and miR-132 are indeed modified by m6A in the epileptic brain. Sequences need to be identified, but that is not enough. To confirm the presence and abundance of m6A modification, as well as its disease-stage dependence, use site-specific approaches such as miCLIP, MeRIP-qPCR, and orthogonal biochemical validation.

It remains unclear whether m6A modifications affect miRNA maturation or only miRNA expression. Future studies should measure pri-miRNA, pre-miRNA, and mature miRNA levels after altering METTL3, METTL14, FTO or ALKBH5 levels. The protein interactions with RNA should also be examined for association with DGCR8 and for microprocessor recruitment following m6A modification in epileptic neurons and glial cells.

The process of epileptogenesis occurs in three phases: acute, latent and chronic, and is a process involving neurons, astrocytes, microglia, oligodendrocytes, and endothelial cells. Thus, bulk-tissue measures can mask opposing effects across cell populations. Single-cell and spatially resolved transcriptomic methods, alongside spatial RNA-modification mapping, should identify disease-stage- or cell-type-specific interactions between m6A and miRNA.

A key gap to overcome is the lack of validation of m6A-miRNA interactions in human epilepsy. Moving forward, integrating resected human TLE tissue, matched blood, cerebrospinal fluid samples, and clinical phenotype with m6A and miRNA profiling should be implemented in future studies. Special consideration should be given to the duration of the illness, drug resistance, hippocampal sclerosis, and drug exposure.

The potential regulatory role of m6A in miRNA maturation has not yet been explored, and m6A may also affect miRNA-target interactions. m6A modification can regulate miRNA loading, target selection, or efficiency. AGO2-associated RNA profiling, together with m6A mapping, can determine whether m6A modification affects miRNA loading, target selection, or regulatory efficiency. These experiments may uncover mechanisms that are not identified by typical miRNA expression profiling.

Therapeutic development should not indiscriminately target global m6A levels. The evidence indicates that m6A function is context-dependent, with effects varying by transcript, cell type, disease stage, and regulatory enzyme. The long-term goal should therefore be to restore the regulatory circuits altered in a pathological manner, rather than to inhibit or activate the m6A pathway in general.

MiRNAs and m6A signatures may be present in the bloodstream, but this needs rigorous testing. Future biomarker studies will need large, longitudinal cohorts, and whether peripheral markers accurately reflect brain pathological processes needs to be determined. Finally, the availability of miRNA abundance, m6A-associated transcripts, clinical variables, EEG characteristics, and imaging could enable more comprehensive prediction.

The key next step is to identify experimentally which factors in the proposed m6A-miRNA network is causal in epileptogenesis and which are merely downstream reactions to seizures. Resolving this distinction will be a key step in advancing the proposed network from a conceptual framework to a validated therapeutic and biomarker platform.

Addressing the current knowledge gaps summarized in (Table 4) will not only improve our understanding of the molecular basis of epileptogenesis but also facilitate the development of more precise diagnostic tools and disease-modifying therapies for epilepsy.

**Table 4.** Knowledge gaps and future research priorities

| Knowledge gap                                                                                                     | Why it matters                                                                                                 | Required Experiment                                                                                                             |
|-------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|
| The role of METTL3 in epilepsy has been extensively investigated, particularly in regulating m6A RNA methylation. | Its cell type-specific functions within different neuronal and glial populations remain poorly understood.     | Apply single-cell and spatial transcriptomic approaches to define cell-specific m6A regulatory networks during epileptogenesis. |
| <b>miR-134</b> has been consistently implicated in neuronal excitability and seizure progression.                 | Whether its expression and function are directly regulated by m6A modification has not been fully established. | Combine epitranscriptomic, transcriptomic and miRNA profiling to clarify the interaction between m6A and miR-134                |
| Does m6A modify the epigenetic expression of epilepsy associated miRNAs directly?                                 | Distinguishes association from causality.                                                                      | miCLIP/MeRIP-qPCR                                                                                                               |
| Are their maturation processes affected by METTL3/METTL14?                                                        | Initiates mechanism.                                                                                           | METTL3/METTL14 perturbation + pri-/mature-miRNA analysis                                                                        |
| Is there any specific miRNA that FTO/ALKBH5 demethylates?                                                         | Defines eraser-dependent regulation.                                                                           | RIP/MeRIP + catalytic mutants                                                                                                   |
| Do effects occur in neurons or in glia?                                                                           | Epilepsy is a heterogeneous disease at the cellular level.                                                     | Single-cell/ Spatial RNA-seq.                                                                                                   |
| Does m6A affect the targeting of miRNAs (not their levels)?                                                       | Could show a new mechanism?                                                                                    | AGO2-RIP/CLIP + targetome                                                                                                       |

## Conclusion

Epileptogenesis is now known to be a molecular disease, driven by a dynamic molecular regulatory pathway at the epitranscriptomic level, not a purely electrical disturbance. In this review, the authors do not consider m6A RNA methylation and microRNA-mediated gene silencing as independent pathways, but propose a mutual circuit: m6A writers, erasers, and readers regulate miRNA biogenesis, while mature miRNAs regulate m6A machinery expression. This loop is replicated in each of the key mechanisms underlying epileptogenesis (synaptic restructuring, ionic and neurotransmitter imbalance, glial activation and neuro-inflammation, oxidative stress, programmed cell death) and seems to function differently during the acute, latent and chronic phases of the disease.

The field remains far from conclusive, with only a few circuits mechanistically resolved that support this axis (namely, FTO/NR4A2/YTHDF2 in post-traumatic epilepsy, METTL3-dependent processing of pri-miR-134/ /pri-miR-132 after status epilepticus, and clinically validated miR-134 and miR-155 targets). Most suggested miRNA-m6A interactions in epilepsy are correlative; the next challenge is to understand the causal relationship through site-specific mapping (miCLIP; MeRIP-qPCR), perturbation of individual writers/erasers, and single-cell or spatially resolved profiling approaches that distinguish disease drivers from downstream bystander changes.

Rethought this way, the m6A-miRNA axis offers an opportunity to develop novel disease-modifying therapies, rather than only symptom-modulating drugs like traditional antiepileptic agents. A possible pathway for dual-pathway strategies, combining RNA-based interventions (antagomirs, miRNA mimics, m6A-modulating small molecules) with precision genomic tools (ASOs, AAV gene therapy, CRISPRa) and exosome-mediated BBB delivery, is the way to achieve a therapy acting on the regulatory network itself, rather than on single receptors or channels. Achieving this will rely on the causal and cell-type-resolution gaps revealed here, translating the m6A-miRNA axis into a clinically usable platform for early diagnosis, biomarker development, and precision epilepsy therapeutics.

## Abbreviations

|        |                                                             |
|--------|-------------------------------------------------------------|
| AAV    | - Adeno-associated virus                                    |
| ALKBH5 | - AlkB homolog 5                                            |
| ASO    | - Antisense oligonucleotide                                 |
| CRISPR | - Clustered regularly interspaced short palindromic repeats |

|         |                                                              |
|---------|--------------------------------------------------------------|
| CRISPRa | - CRISPR activation (dCas9-based transcriptional activation) |
| BAX     | - Bcl-2-associated X protein                                 |
| BBB     | - Blood-brain barrier                                        |
| BDNF    | - Brain-derived neurotrophic factor                          |
| CaMKII  | - Calcium/calmodulin-dependent protein kinase II             |
| CFH     | - Complement factor H                                        |
| CREB1   | - cAMP response element-binding protein 1                    |
| DGCR8   | - DiGeorge syndrome critical region 8                        |
| FTO     | - Fat mass and obesity-associated protein                    |
| ILAE    | - International League Against Epilepsy                      |
| IRAK1   | - Interleukin-1 receptor-associated kinase 1                 |
| LIMK1   | - LIM Domain Kinase 1                                        |
| lncRNA  | - Long non-coding RNA                                        |
| TANGO   | - Targeted augmentation of nuclear gene output               |
| m6A     | - N6-methyladenosine                                         |
| METTL3  | - Methyltransferase-like 3                                   |
| METTL14 | - Methyltransferase-like 14                                  |
| MnSOD   | - Manganese superoxide dismutase                             |
| mTOR    | - Mechanistic Target of Rapamycin                            |
| PARP1   | - Poly(ADP-ribose) polymerase 1                              |
| PTGR    | - Post-transcriptional gene regulation                       |
| RISC    | - RNA-Induced Silencing Complex                              |
| SHIP1   | - Src homology 2 domain-containing inositol 5-phosphatase 1  |
| SOCS1   | - Suppressor of cytokine signalling 1                        |
| SOD2    | - Superoxide dismutase 2                                     |
| STAT3   | - Signal transducer and activator of transcription 3         |
| STAT4   | - Signal transducer and activator of transcription 4         |
| TRAF6   | - TNF receptor-associated factor 6                           |
| UTR     | - Untranslated region                                        |
| VGAT    | - Vesicular GABA transporter                                 |
| YTHDC2  | - YTH domain containing 2                                    |
| YTHDF   | - YTH domain family protein                                  |

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