*Title:* MicroRNAs in epilepsy: pathophysiology and clinical utility

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**Background:** Temporal lobe epilepsy (TLE) is a common and frequently intractable seizure disorder. The pathogenesis of acquired TLE is thought to involve large-scale alterations to the expression of genes controlling neurotransmitter signalling, ion channels, synaptic structure, cell death, gliosis and inflammation, among others. Identifying mechanisms coordinating gene networks in TLE would improve our understanding of the disease and help identify novel druggable targets or biomarkers. MicroRNAs (miRNAs) are a family of small non-coding RNAs that control levels of multiple proteins by post-transcriptionally decreasing messenger RNA stability and translation. Accordingly, they could represent important regulatory mechanisms and therapeutic targets in epilepsy.

   **Recent developments:** Studies over the past five years found select changes to miRNA levels in the hippocampus of TLE patients and animal models of epilepsy. Among early functional studies was the demonstration that silencing brain-specific miR-134 using antisense oligonucleotides (antagomirs) had potent anti-seizure effects whereas genetic deletion of miR-128 produced fatal epilepsy in mice. Levels of certain miRNAs were also found to be altered in the blood after seizures in rodents. In the past 18 months, functional studies identified nine further miRNAs that appear to influence seizures or hippocampal pathology. Their targets include transcription factors, neurotransmitter signalling components and modulators of neuroinflammation. New approaches to manipulate miRNAs have been tested including injection of mimics (agomirs) to enhance brain levels of miRNAs. Altered miRNA expression has been reported in other types of refractory epilepsy and there has been progress on how miRNA levels are controlled, with studies on DNA methylation indicating epigenetic regulation. Last, differences in circulating miRNAs have been found in epilepsy patient biofluids implying diagnostic potential.

   **Where next?** The recent functional and biomarker studies need to be replicated by other groups to build a more robust evidence base. Researchers need to identify the cell type(s) in which the miRNAs execute their functions and their primary targets to explain the phenotypic effects of modulating miRNAs. There are challenges around the delivery of large molecules such as antisense inhibitors or mimics to the brain of patients and the multi-targeting effects of miRNAs create additional risk of

unanticipated side effects. Potential genetic variation in miRNAs should be explored as a candidate for modifying disease susceptibility. In summary, the latest findings provide exciting advances and a rich source of new miRNA targets but significant challenges remain before their role in the pathogenesis, treatment and diagnosis of epilepsy can be translated to the clinic.

*Keywords:* Antisense; noncoding RNA; hippocampal sclerosis; drug-resistant epilepsy; microRNA; precision medicine

## Introduction

The 68th general assembly of the World Health Organization recently ascertained that epilepsy is one of the most common severe neurological disorders, affecting more than 50 million people worldwide.[1] This and other reports,[2] urge member states to improve investment in epilepsy research and increase research capacity. Six million people in Europe are treated for epilepsy with an estimated annual cost of 14 billion Euros in 2010.[3] Epilepsy is characterized by recurrent seizures, a higher mortality rate, and decreased social participation and quality of life. Current anti-epileptic drugs (AEDs) are ineffective in a third of patients and biomarkers predicting the response to specific AED do not exist in clinical practice.[4] In addition, there is no convincing evidence that the available AEDs impact the underlying pathophysiology and are not merely seizure-suppressive. However, the need for disease-modifying drugs is increasingly recognised.[5] This includes treatment addressing epileptogenesis as well as ictogenesis. Ideally, persons at risk that suffered an initial precipitating injury should be identified by the use of valid biomarkers of epileptogenesis and the occurrence of epilepsy could then be prevented by disease-modifying treatment.[4]

How should anti-epileptogenesis and disease-modification in epilepsy be achieved? Analysis of brain tissue from patients with TLE indicates there is large-scale dysregulation of gene expression. This includes entire networks of genes that regulate pathways including inflammation, gliosis, synaptic structure and function.[6] A disease-modifying treatment in the future may need to target critical "nodes" in these pathways. Alternatively, drugs could target regulators of transcription and RNA processing as well as epigenetic factors.

This *Rapid Review* focuses on recent developments which have seen exciting progress, with several new miRNAs targeted in animal models of epilepsy, a better understanding of their mechanisms, and the first clinical studies to explore biomarker potential. Also, the first curated database on miRNA in epilepsy was established.[7] Termed EpimiRBase, the fully searchable database features up to date information on miRNA expression changes in brain and blood from published studies on experimental and human epilepsy, as well as details of functional studies. Criteria for what

constitutes a miRNA have also been revised during this time with researchers arguing for more strict criteria resulting in only 523 human miRNA "genes" meeting the standards.[8] According to these criteria some miRNAs recently linked to epilepsy may not be bona fide miRNAs.[9]

## Regulation of gene networks by microRNAs

A new layer of gene expression control was discovered in mammals in the early 2000s involving small non-coding RNAs called microRNAs (miRNA). These work by binding to complementary sites in messenger RNA (mRNA) and reducing mRNA stability and translation.[10] It has been estimated that levels of ~60% of all proteins are directly regulated by miRNAs.[11] The biogenesis and mechanism of miRNA action have been reviewed elsewhere,[12, 13] and are summarized in **Figure 1**. After biogenesis, miRNAs are selected by an RNA-induced silencing complex (RISC), a multi-enzyme complex containing Argonaute proteins. The miRNA-containing RISC is then guided to target mRNAs. The target selection is specified by a 7 – 8 nucleotide complementarity match, the "seed" interaction, between the miRNA and mRNA.[14] This leads to degradation of the mRNA or translational inhibition and lower protein levels of the target. An individual miRNA can have dozens of targets, regulating several genes in a single pathway or single genes in several pathways.[15] As an example, genetic deletion of brain-expressed miR-128 resulted in upregulation of 1035 mRNA transcripts of which 154 were predicted direct miR-128 targets; remarkably, 27 fell within a single pathway.[16] This multi-targeting property has obvious advantages for disease-modification in epilepsy since it offers the possibility to disrupt several processes at once. It increases, however, the potential for unwanted or unanticipated side effects of any miRNA-based therapy.

## MicroRNAs in epilepsy

The first study on miRNA in human epilepsy appeared in 2010 and identified an increase in hippocampal levels of miR-146a, a miRNA linked to the control of inflammatory responses.[17] Researchers later reported genome-wide analysis of miRNA expression,[18] and evidence for dysregulation of the miRNA biogenesis pathway,[19] in human epilepsy.

*In vivo* functional data were first reported on five miRNAs (in chronological order); miR-132,[20] miR-34a,[21, 22] miR-134,[23] miR-184,[24] and miR-128.[16] Most notably, studies in mice showed that inhibiting miR-134 after status epilepticus suppressed the development of spontaneous seizures,[23] and genetic deletion of miR-128 resulted in fatal epilepsy.[16] A further nine miRNAs have recently been functionally interrogated using miRNA inhibitors (antisense oligonucleotides targeting miRNAs termed antagomirs) and mimics (agomirs) in *in vivo* models of epilepsy (see Table 1 for a summary, Figure 1 and Supplementary Table S1 for more complete details). The findings suggest miRNAs could be a broad and flexible class of targets for the treatment of seizures and epilepsy-related neuropathology.

A profiling analysis of the mouse brain RISC after status epilepticus induced by intraamygdala microinjection of the excitotoxin kainic acid identified miR-22 as the most abundant miRNA within the contralateral hippocampus.[25] This brain region is largely spared damage in the model and the authors found that blocking miR-22 by intracerebroventricular injection of antagomirs exacerbated neuroinflammation and increased the frequency of spontaneous seizures.[25] Pharmacologic and genetic studies suggested this was largely due to de-repression of the purinergic P2X7 receptor which drives microglia activation and release of the proconvulsive inflammatory cytokine interleukin 1β. An injection of miR-22 mimics reduced spontaneous seizures in mice although the effects lasted only a few days.[25] However, miR-22 may have other targets of relevance to epilepsy. Recent work in *Aplysia* showed that miR-22 targets cytoplasmic polyadenylating binding protein, an RNA-binding protein involved in translation, and impairs maintenance of long-term facilitation, a model of synaptic plasticity and memory[26].

Zhan and colleagues reported that miR-23b was downregulated in mouse cortex after seizures induced by a low dose of kainic acid prompting tests of a miR-23b mimic.[27] The authors found that injection of miR-23b mimic into the mouse ventricle after kainic acid alleviated some of the high amplitude spiking seen on EEG the next day.[27]

Levels of neuronal miR-124 were found to decline following kainic acid-induced status epilepticus in rats.[28] A key target of this miRNA was the neuron restrictive silencing factor, a transcriptional suppressor that coordinately represses various genes during epileptogenesis.[28] However, increasing miR-124 levels in the model with mimics did not affect the later occurrence of spontaneous seizures, a finding put down to the parallel enhancement of inflammation produced by miR-124 acting on microglia.[28] This neatly captures a key challenge with miRNA manipulations; their multi-targeting can impact repair functions or can lead to seizures. A separate study also found levels of miR-124 were lower in experimental and human epilepsy.[29] In that report the pre-treatment of rats with miR-124 mimics reduced seizure severity during pilocarpine-induced status epilepticus and pentylenetetrazole-induced seizures. These findings suggest potential seizure-suppressive effects of miR-124 may be lost where there is pre-existing epileptic pathology although conflicting findings may be due to differences in models, dose and route of mimic administration.

Recent studies showed that miR-155 opposes beneficial functions of microglia in models of motor neuron disease by suppressing multiple genes required for microglia activation, phagocytosis and inflammatory signalling.[30] Targeting miR-155 restored microglia function and protected against neurodegenerative changes, findings that may be relevant for epilepsy. Now, a team has shown that injection of antagomirs against miR-155 before pilocarpine-induced status epilepticus improves post-ictal behaviour in mice.[31]

A screen of brain-expressed miRNAs in both children with TLE and rats subject to pilocarpine-induced status epilepticus identified upregulation of miR-181a.[32] An intracerebroventricular injection of miR-181a mimic produced neuronal death in rats,[33] whereas antagomirs against miR-181a

reduced neuronal death after status epilepticus.[32] These studies did not investigate whether manipulation of miR-181a affected seizures but previous work showed this miRNA can target the GluA2 subunit of the AMPA receptor leading to reduced dendritic spines and miniature excitatory post-synaptic currents[34].

Pilocarpine-induced status epilepticus was reported to upregulate miR-199a in the rat hippocampus and antagomirs against miR-199a reduced seizure severity in the model.[35] This was partly mediated by protection of Sirtuin1 levels, a deacetylase and transcriptional silencer.[35] Interestingly, miR-199a also targets inhibitors of the mechanistic target of rapamycin (mTOR) pathway.[36] Since increased mTOR activity is implicated in common causes of focal pharmacoresistant epilepsy including tuberous sclerosis and cortical dysplasia,[37] miR-199a antagomirs may offer alternative approaches to mTOR inhibition.

miR-203 was upregulated in both experimental and human epilepsy and the inhibitory glycine receptor –β was identified as a potential target.[38] Intranasal injection of antagomirs targeting miR-203 corrected glycine receptor levels after experimental status epilepticus and reduced the frequency of spontaneous seizures in mice.[38]

Status epilepticus induced by pilocarpine in rats upregulated miR-210 in the hippocampus and injection of antagomirs against miR-210 shortly after status epilepticus reduced injury to the hippocampal CA1 subfield.[39] Antagomir treatment also normalized expression of GABAergic signalling components although it was not established whether these were direct targets of miR-210.[39]

Lower levels of miR-219 were found in models of status epilepticus and cerebrospinal fluid samples from TLE patients.[40] Silencing miR-219 in normal mice using antagomirs resulted in spiking and high amplitude discharges on EEG. Co-injection of the NMDA receptor antagonist dizocilpine obviated the EEG changes suggesting the observed phenotype was mediated by de-repression of this

receptor. The authors also showed that pre-treating mice with miR-219 mimic protected against seizures induced by kainic acid.[40] Reduced miR-219 expression may influence brain excitability through other mechanisms, such as altering levels of Tau.[41]

## Pathways under miRNA control in epilepsy

Early functional studies linked miRNA effects on seizures to neuroinflammation and neuronal microstructure. For example, targeting miR-134 altered the number and volume of dendritic spines on excitatory neurons, presumably through its target, Limk1.[23, 42] miR-146a and miR-221/222 can control immune responses through targets such as interleukin-1β and cell adhesion molecules, respectively.[17, 18, 43] miRNA dysregulation probably impacts a wide variety of molecular and cellular pathways in epilepsy including differentiation and migration or cell proliferation. Identification of unsuspected pathways, such as axon guidance,[44, 45] emphasizes that knowledge of epilepsy-associated miRNAs may also help to further unravel the pathogenic mechanisms underlying epilepsy. While it is unknown whether deregulation of axon guidance cue expression influences epileptogenesis, these molecular signals are known to contribute to various neurological disorders through their ability to control neurite growth and guidance, neuron migration and synapse development and function.[46] Axon guidance molecules may be important for the integration of newly differentiated neurons, for example in the dentate gyrus, a process disturbed in epilepsy.[47] It should be noted that for most miRNAs implicated in epilepsy to date, *in vivo* work, functionally linking the miRNA and their targets within the context of epilepsy, e.g. in epilepsy animal models, is lacking. It is also emerging that miRNAs enhance the breadth of their impact by suppressing transcriptional activators and silencers that control expression of large cohorts of genes.[28] Future 'unbiased' systems studies, including assessing impact of miRNAs on the proteome,[48] are required to understand the complex alterations of neuronal function and network defects in epilepsy. **Figure 2**

depicts a theoretical synapse annotated with some of the new epilepsy-associated miRNAs, several previously epilepsy-associated miRNAs, putative target mRNAs and their likely cellular and subcellular sites of action.

## *Cellular resolution of miRNAs in epilepsy*

Understanding the mechanisms by which miRNAs influence epileptic networks requires knowledge of the cell type(s) in which the miRNA and target are expressed. miRNAs display unique cell-specific expression in the brain,[49] but most efforts to discover epilepsy-associated miRNAs use blocks of tissue that contain mixed cell types. This is an obvious confounder that has only rarely been addressed. In one study, a neuronal-specifically tagged Argonaute protein was expressed in mice in order that sequencing could be performed only on the active miRNA in neurons.[16] A recent study used laser capture microdissection to profile the dentate granule cell layer, a relatively homogenous population of excitatory neurons.[45] This identified a set of miRNAs that underwent expression changes during different phases of epileptogenesis in rats and several of these miRNAs were found to be regulated in the same subfield in human epilepsy.[45] The dentate granule cell layer would include cell populations involved in neurogenesis which may express very different sets of miRNAs from mature cells involved in epileptic networks. Nevertheless, this and other approaches to profile miRNA expression in unique cell types,[50] should improve our knowledge of the cellular basis of miRNA-mediated effects in epilepsy.

## *Control of miRNA expression in epilepsy*

It is largely unknown how miRNA expression becomes deregulated in animal models of epilepsy or TLE patients. Presumably some is indirect: changes in the number or function of neurons or glia are accompanied by changes to levels of miRNAs normally expressed in those cells. Other mechanisms may be more specific. The miR-124 gene locus underwent a reduction in histone acetylation after kainic acid-induced status epilepticus in rats, an epigenetic silencing mechanism which may account

for the reduced hippocampal levels of miR-124.[28] A recent study suggested a link between miRNA

levels in epilepsy and methylation of DNA.[51] Increased DNA methylation typically promotes

compaction of chromatin and a reduction in the transcription of genes at those sites. A genome-

wide analysis of DNA methylation using hippocampal tissue from TLE patients found differences in

the methylation state of multiple miRNA genes.[51] For some there was a highly significant association

between methylation status and expression of the miRNA. Interestingly, interleukin-1β upregulates

miR-146a expression in human astrocyte cultures suggesting that the immune system is not only

controlled by miRNAs but can regulate miRNAs as well.[43]


*Genetic variation in miRNAs*

It is intriguing to think that mutations in miRNAs might contribute risk for epilepsy. A number of

candidate gene association studies have now been reported but there is no compelling evidence yet

that genetic epilepsies are caused by mutations or variation in miRNA genes.[52-55] There has been no

systematic effort to examine the contribution of miRNA sequence variation to human epilepsy. This

is about to change. The EpimiRNA consortium is exploring the relationship between sequence

variation in miRNAs and miRNA targets in epilepsy.[56] The project examines whether there is an

enrichment of variants falling in a particular miRNA-related region in a large cohort of epilepsy

patients compared to controls. To the best of our knowledge there are no other similar efforts

underway presently that primarily focus their high throughput DNA sequencing on sites in the

genome that are specifically miRNA-related. The sequencing strategy is focusing on regions that are

most likely to cause epilepsy when mutated. All miRNAs in this set,[57] are included along with a

subset of predicted miRNA targets. To predict the miRNA targets, the miRNAs expressed in human

hippocampus,[58] are used. This ensures focus on targets that are most likely to contribute to epilepsy.

Binding targets are computationally predicted based on sequence complementarity within each

gene's 3' untranslated region. The subset of these targets for which the corresponding miRNA has

been shown in functional studies to regulate the predicted target mRNAs are extracted.[59] Following

this, criteria such as sensitivity to regulatory variation and conservation of regulatory sequence,[60] are included and targets falling in genes predicted to be sensitive to regulatory variation prioritized. Sequencing will generate a set of candidate causal variants in miRNA-related regions, and highlight miRNA-related regions in which variation contributes to epilepsy.

## miRNAs as biomarkers of epilepsy

Evidence has emerged that circulating miRNAs in biofluids may be useful biomarkers of brain injury. This pool of miRNAs is thought to originate from damage or disruption of the blood-brain barrier allowing passage of small quantities of brain-expressed miRNAs. These circulate for some time afterwards due to stable complexing with proteins or encapsulation in extracellular vesicles. Early animal studies suggested specific miRNA biofluid profiles existed for different types of brain injury, including a pattern unique to prolonged seizures,[61] and this has been confirmed more recently.[45] A molecular biomarker of epilepsy would be of enormous benefit for diagnosis, decisions on risk of epilepsy development or monitoring treatment responses.[4] Now, a study identified a set of circulating miRNAs in epilepsy patients.[62] This included increased serum levels of miR-146a, a miRNA already linked to epilepsy. Another set of miRNAs showed differences in blood levels between patients with controlled versus refractory seizures,[63] indicating potential biomarkers of drug-resistant epilepsy. Because the studies included both focal and generalized epilepsies their value for identifying different epilepsies remains uncertain. Additional reports of biofluid miRNAs in epilepsy patients are emerging.[9, 40] A first step toward using miRNAs for diagnostic tests was recently undertaken. Using miR-134 as a known epilepsy-associated miRNA, a team developed an electrochemical detection method for measuring ultra-low levels of miRNAs and showed that plasma miR-134 levels were higher in epilepsy patients compared to controls.[64]

## Opportunities and barriers for miRNA therapeutics

*Limitations of recent functional studies*

Standards for demonstrating effectiveness of potential treatments for epilepsy are rapidly changing.[65] A recent study looking at gene therapy in epilepsy highlighted the challenges of designing a translational study in a disease with symptoms (seizures) which are variable and difficult to quantify.[66] This and other reports emphasise the importance of designing randomised, blinded, intent-to-treat trials to increase the potential for clinical translation. The strength of evidence in most of the recent miRNA functional studies falls short of these objectives. Several are brief reports that lack sufficient detail on experimental design, methodology or data reporting, particularly around performance and analysis of EEG. Dosing of antagomirs and mimics is frequently performed before status epilepticus which limits clinical relevance. There have been reports on anti-seizure effects of miRNA manipulations in more than one *in vivo* model.[16, 23, 42] However, there will need to be *in vivo* validation of a miRNA targeting treatment by an independent group, cross-species corroboration and, ideally, validation in human samples.

Overexpression or knockdown of miRNAs in cultured neuronal networks can be investigated for effects on activity prior to performing in-depth studies in other model systems. This will help to focus *in vivo* research to those with confirmed effect and satisfies commitments to replace, reduce and refine animal use in epilepsy research.[65] Microelectrode array technology was recently used to interrogate the effect of miR-128 knockdown using a miRNA-sponge on *in vitro* neuronal activity.[67] Recordings identified increases in spike and burst rates in the miR-128-depleted neurons characteristic of epileptogenic network activity, highlighting a novel approach to screen candidate epilepsy miRNAs.[67] Microelectrode arrays are not sensitive, however, for miRNAs that impact aspects of epileptogenesis such as immune components.

Most of the new studies have focused on targeting miRNAs before or at the time of status epilepticus. Future studies should also differentiate between treatments that prevent the development of epilepsy (anti-epileptogenic), and treatments that suppress spontaneous seizures in

chronic models (seizure-suppressive). An additional possible distinction is between such treatments that suppress seizures without changing the underlying tendency to seize (seizure-suppressive), and treatments which alter the underlying pathology, and essentially 'cure' epilepsy (disease-modifying). The ideal treatment would fall in the final category, where a one-off administration could reverse the underlying dysregulation of a network of genes, and return neurons/networks to a non-epileptic state.

*Prospects for a miRNA-based treatment for epilepsy*

The multi-targeting actions of miRNAs could make it difficult to predict or avoid off-target effects. Nevertheless, the global RNA interference delivery market is estimated at $25 billion and a number of biotechnology and pharmaceutical companies have therapeutic pipelines that include miRNA-based treatments. Some have reached clinical trials.[68, 69] While encouraging, it is unclear whether these or other companies will focus on epilepsy-based miRNA therapies. Moreover, while RNA-based therapies were once a major focus of biotechnology and pharmaceutical companies this has undergone some decline due to failure to meet expectations and reach the market.[70] Innovations around delivery and formulation are expected to improve this outlook.[70] As long as no small molecule approach is available to specifically influence miRNA levels, miRNA-based treatments will have to rely on large antisense-like molecules such as antagomirs. Getting such large molecules across the blood brain barrier creates additional challenges. The direct injection of antagomirs or mimics into the ventricle or hippocampus in animal studies is unlikely to be translatable to patients except when injections could be performed this way as an alternative to surgical resection of a seizure focus. There may be more creative approaches for delivery of miRNA-based treatments to the brain. Intra-nasal delivery has been reported in animal studies for antagomirs targeting miR-134,[23] and recently miR-203 mimic.[38] Intra-thecal injection has been used in clinical trials of antisense-based therapies.[71] It is unknown, however, whether these routes will be effective at delivering a miRNA-based treatment to a seizure focus. Nanoparticle or other formulations that

encapsulate miRNA-based treatments could facilitative central uptake after systemic injection. For example, cell penetrating peptides or exosome-based cargo systems.[70] Their prolonged duration of action - antagomirs have been shown to reduce levels of their targets in the brain for many weeks[23, 35] – may offset other limitations. A single injection might only be required at the time of an epilepsy-precipitating insult or at infrequent intervals in chronic epilepsy.  Restoring or upregulating miRNAs may be more challenging. Mimic effects do not last as long as antagomirs.[25] Over-expressing small RNAs has also been reported to trigger neurotoxicity due to exceeding the miRNA processing capacity in cells.[72] Techniques for preventing single miRNA:mRNA interactions could limit off-target effects by preventing a miRNA from regulating one particular mRNA while leaving the miRNA to regulate other targets. Gene editing techniques may eventually become useful, to either manipulate expression or edit miRNAs.[73] A final consideration is that intracerebral delivery may not be a prerequisite for all therapeutic effects of miRNAs. Recent work in a stroke model showed systemic but not intracerebral delivery of miR-122 improved functional recovery in rats, indicating targets besides neurons and glia such as cerebral microvessels or immune responses may be important for miRNA-based therapeutic effects on neurologic disorders.[74]

## Conclusions and future directions

Recent studies have considerably expanded the number of miRNAs with potential roles in epilepsy, improved our understanding of their targets, and suggested biomarker potential. In addition to the limitations of the recent work and questions raised above, what are other future directions? A critical test of translation will be whether a miRNA-based treatment in a region of epileptogenic tissue can affect or reverse established epilepsy. More emphasis on cellular resolution by use of cell sorting or expressing tagged RISC components in different cell types will help explain mechanisms. Understanding what controls levels of miRNAs in epilepsy would also be valuable. While miRNAs are considered multi-targeting, most research has focused on single targets; convincing evidence that

miRNAs are multi-targeting in epilepsy is yet to appear. Most work to date has focused on the hippocampus and TLE but this should expand to include other brain regions and causes of epilepsy that feature miRNA dysregulation and thus might benefit from miRNA-based therapeutics.[75] It is possible that current AEDs produce effects on miRNA levels within the brain and it would be important to explore whether this has any contribution toward the efficacy or otherwise of these drugs. Last, researchers might combine miRNA manipulations to increase effect size or mix an antagomir with a mimic. In summary, research on miRNAs promises to make an important impact on our understanding of the causes, treatment and diagnosis of epilepsy.

## Search strategy and selection criteria

A PubMed search was performed on September 14th 2016 using the terms "epilepsy AND microRNA" and "seizure AND microRNA". From this list we focused on published work that featured manipulations of novel miRNAs in *in vivo* epilepsy models since Jan 1, 2015. In addition, we searched for papers during the same period that focused on brain-related functions of the individual microRNAs for which functional interrogations in epilepsy models had been performed. Thus we searched for "brain AND miR-" and included -22, -23b, -124, -128, -134, -155, -181a, -199a, -203, -210, -219. We then selected the most important and relevant articles based on a subjective appraisal of their depth, quality and mechanistic insight that could be relevant to epilepsy. The authors also reviewed their own records and the bibliographies of the papers included. Only articles in English were considered. A search was also made to identify genetics consortia primarily focusing their high throughput DNA sequencing on sites in the genome that are specifically miRNA-related. We searched for "epilepsy miRNA" on NCBI's dbGaP server : http://www.ncbi.nlm.nih.gov/gap, "epilepsy miRNA" on NCBI's GEO database http://www.ncbi.nlm.nih.gov/gds, and "epilepsy miRNA"

on EMBL-EBI's ArrayExpress database: http://www.ebi.ac.uk/arrayexpress/. No other relevant projects were identified.

## Declaration of interest

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## Contributors

DCH performed the literature search, wrote the draft and edited the manuscript and produced the tables and figures. HH, RJP, DBG, JK, JHMP, SS, KL and FR performed literature searches, edited the manuscript and wrote drafts of specific sections.

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**Figure 1** *miRNA biogenesis and overview of miRNA manipulation approaches used in recent functional studies*
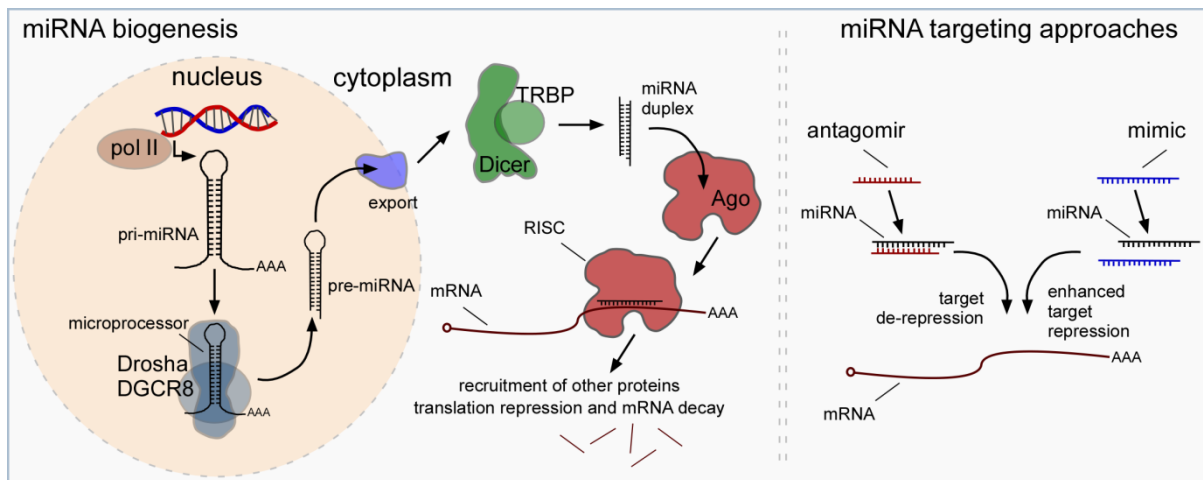


Cartoon presenting the miRNA biogenesis pathway and schematic of recent methods used to functionally interrogate the role of individual miRNAs in animal models of epilepsy. *Left,* miRNA biogenesis begins with transcription of a primary miRNA (pri-miRNA), mainly by RNA polymerase II (pol II), from introns of protein coding genes as well as specific miRNA "gene" loci. The microprocessor complex in the nucleus contains the RNase Drosha and DiGeorge Syndrome Critical Region 8 (DGCR8) which cleave the pri-miRNA to produce a ~60 – 70 nucleotide hairpin structure, the pre-miRNA. The pre-miRNA is then exported to the cytoplasm by exportin 5 and processed further by Dicer, another RNase, to produce the mature ~22 nucleotide duplex miRNA, a process enhanced by transactivation-responsive RNA binding protein (TRBP). One strand of the miRNA ("guide") is bound by an argonaute (Ago) protein which forms the miRNA-induced silencing complex (RISC). Once loaded, the RISC traffics along target mRNAs until sufficient complementary binding is present. This produces stable miRNA:mRNA binding that facilitates mRNA decay or translational repression following recruitment of other factors (not shown) such as GW182 proteins. *Right,* introducing an antisense oligonucleotide sequence complementary to the miRNA can block miRNA function and thereby de-repress a target mRNA. Introducing a miRNA mimic (agomir) will facilitate miRNA-dependent silencing of targets.

**Figure 2** *Sites of action and potential target mechanisms underlying effects of miRNA manipulations on seizures and histopathology.*
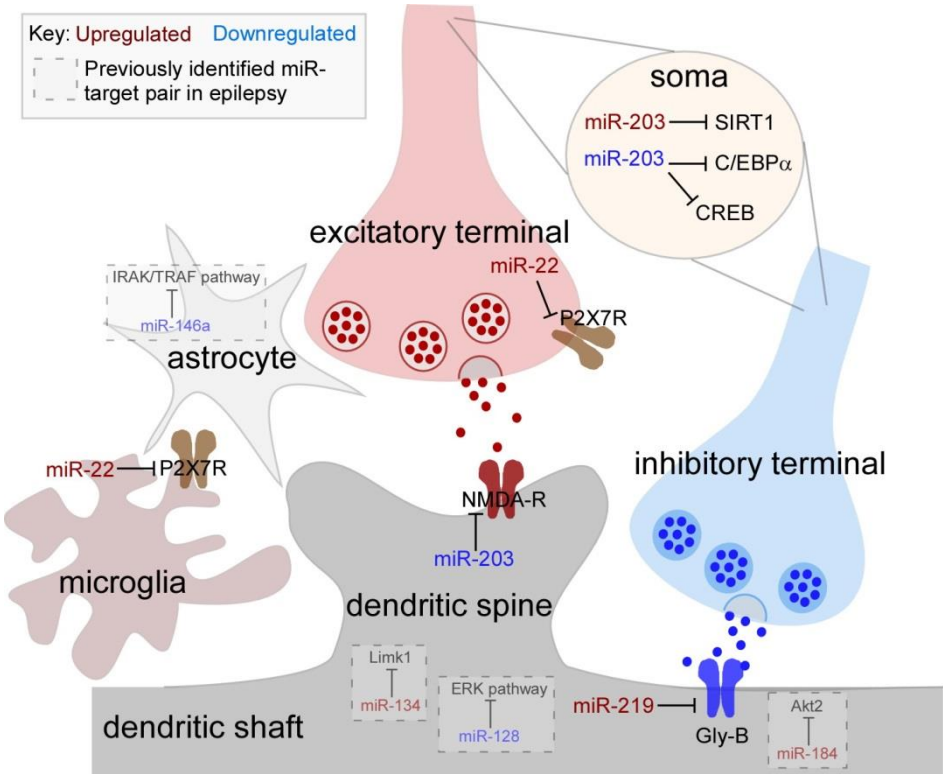


Cartoon presenting a schematic featuring several of the recently identified miRNAs with effects in seizure/epilepsy models depicted alongside putative targets. Figure also includes miRNAs previously linked to epilepsy and their proposed targets (in shadow).

*Key:* C/EBPα, CCAAT enhancer binding protein-α; CREB, cyclic AMP response element binding protein; ERK, extracellular signal-regulated kinase; GAD, glutamate decarboxylase; GAT-1, GABA transporter 1. Gly-B, glycine receptor B; IRAK, Interleukin-1 receptor-associated kinase; Limk1, LIM domain kinase 1; SIRT1, sirtuin 1; TRAF, TNF receptor associated factor; Crossed line on miR-210 intended to signify that regulation of the GABA signalling components has not been proven directly.