Could silencing of brain-enriched miR-9 reduce seizures in drug resistant epileptic developing brains?

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Seizure is the most common paediatric neurological disorder, with up to 10% of children having at least one attack of seizures during childhood. Despite the great expansion of seizure treatments in children in the recent years, many patients are refractory to medical treatment. The discovery of microRNAs (miRNAs) revolutionized the world of molecular biology. Extensive studies have suggested crucial roles for miRNAs in human biology of health and disease. Recently, miRNAs proved to be important players in understanding the pathogenesis of epilepsy and seizure disorders. MiRNAs have furthermore emerged as promising therapeutic targets for multiple neurological and non-neurological disorders. MiR-9 is a brain-enriched miRNA which plays an important role in brain development and is found to be upregulated in an immature rat model with seizures and also in children and adults with epilepsy. Therefore, we postulate that miR-9 might be a potential molecular therapeutic target for anti-convulsant therapy in the developing brains.

 Keywords: MiRNA-9; Epilepsy; Developing brain; Therapeutic target


Introduction

Epilepsy affecting more than 50 million people worldwide, representing the second most common neurological disorder [1]. Medically intractable epilepsy is common in children with tragic long term consequences of recurrent seizures on the developing brain [2, 3].

Neonates and infants are at particularly high risk for seizures with the largest number of newly diagnosed seizure disorders occurring during this time [4, 5]. The peak of seizure susceptibility seems to occur during the intense period of rapid brain growth and synaptogenesis that occurs in the developing brain. The immature brain shows diminished inhibition and increased excitation, and this enhanced excitability increases the propensity for seizures and epileptogenesis in infancy and early childhood period compared with adults. Moreover, inherited forms of epilepsy and epileptogenic malformation of cortical development usually presented with seizures early in life.

MiRNAs are an endogenous class of small (18-24) nucleotide, long, non-coding RNAs that function to regulate post-transcriptional gene expression by directing mRNA destabilization, translational repression, or combination of the two [6]. Aberrant expression or function of miRNAs is convincingly linked to multiple pediatric diseases including, neurological, cardiovascular and chronic diseases [7,8] and suggests to play an important role in epilepsy pathogenesis in the developing brain [10-13]. Targeting the specific miRNAs involved in neurodevelopment have been researched in attempt to explore pathways and can serve the development and discovery of new therapeutic modalities. Seizure-induced neuronal death in the adult brain has been reported to
alter by targeting miR34a, miR-132, and miR-184. Targeting brain-enriched miR-134 showed a neuroprotective effect and furthermore reduced both status epilepticus (SE) seizure severity and recurrence of later spontaneous seizures [14-17]. Additionally, miR-128 reduced expression in the mice postnatal neurons caused increase motor activity and fatal epilepsy [18].

The suboptimal efficacy of currently available antiepileptics in the developing brains, along with their additional spectrum of significant adverse effects, emphasizes the urgency to extend research of the molecular and cellular pathogenic mechanisms of epilepsy in the developing brains. Therapeutic miRNA-targets may develop to become effective anticonvulsant/antiepileptic drugs.

**MiRNAs and epilepsy pathogenesis**

In the last few years miRNAs served greatly in better understanding the complex pathogenic mechanisms involved in the development of epilepsy; especially drug resistant mesial temporal lobe epilepsy (MTLE). Loss of Dicer, which is essential for production of most mature miRNAs in the astrocytes or neurons, leads to miRNA downregulation, seizures, and cognitive deficits [19]. Subsequent experimental evidence showed that Dicer loss leads to reduction of mature miRNA levels in the human temporal lobe epilepsy (TLE) [20]. Dicer loss and failure of mature miRNA expression seems to be important pathogenic features of hippocampal sclerosis (HS) in patients with TLE. MiRNA profiling studies in both the acute and chronic stages of MTLE showed altered expression of miRNAs in the whole hippocampus in animal models [21-24], as well as in the human hippocampus [25].

The role of neuroinflammation in the pathogenesis and maintenance of the epileptic state is increasingly recognized. Many miRNAs have been studied and found to be associated with the neuroinflammatory process such as miR-146a, miR-155, miR-132 and miR-21. Aronica et al. reported altered expression of inflammation-related miR-146a in epileptic rats and TLE patients [26]. The involvement of miR-146a and its interactive relationship with IL-1β in different stages of MTLE development was also indicated by Omran et al. 2012, who showed also upregulation of miR-146a expression in children with MTLE [10]. Later, Ashhab et al. 2013 found a direct relationship between miR-155 and tumor necrosis factor alpha (TNF-α) in astrocytes with similar expression patterns of miR-155 and TNF-α in different stages of MTLE in an immature rat model [12]. MiR-21, an inflammation related miRNA, is also upregulated in children with MTLE [11]. It is suggested to downregulate Neurotrophin-3 factor following SE and thereby be responsible for the increased neuronal cell loss following SE [27].

Neuroprotective mechanisms of miRNAs are extremely important to be evaluated as a possible new therapeutic mechanism. MiR-132 which has been previously associated with synaptic plasticity [28] was found to be upregulated in acute, latent and chronic stages of MTLE development in an immature rat model, with highest expression in the acute stage [11]. Targeting miR-132 protected against seizure-induced neuronal death [18]. Similarly, upregulation of the pro-apoptotic miR-34a were observed in seizure-induced neuronal death or apoptosis, and targeting miR-34a results in inhibition of activated caspase-3 protein and therefore exerts a neuroprotective effect [29].

**Role of miR-9 in brain development and epilepsy**

MiR-9 gained recently great attention as one of the highest miRNAs expressed in the developing brain and specific miRNAs to the central nervous system (CNS) of vertebrates. Its expression levels were shown to be dynamically regulated during brain development, and during in vitro induced neurogenesis [30]. MiR-9 promotes neuronal fate determination in the developing CNS as well as influencing specification of neuronal subtype and regulating axonal growth, branching and targeting [31-33]. It also plays a significant role in early neural tube patterning [34].

In seizure related stages of MTLE in immature rats as well in children and adults with MTLE, miR-9 is upregulated [13, 28]. MiR-9 was also found to be upregulated in response to epileptic preconditioning [16] and in the chronic stage of MTLE [22]. Its expression was dysregulated in conjunction with some epilepsy risk factors including neonatal hypoxic-ischemic brain damage [35], traumatic brain injury [36], meningitis [37] and brain tumours [38]. MiR-9 has also been shown to be a pro-inflammatory miRNA and is involved in modulating the nuclear factor Kappa-B (NF-κB)-dependent inflammatory response [39]. NF-κB is an important player in the epileptogenic process [40, 41], which increases the importance of studying the function of miR-9 in epilepsy development.

**MiRNAs as therapeutic targets**

Because each endogenous miRNA can potentially regulate several hundred targets, a single miRNA pharmacological modulation can affect numerous cellular pathways through changes in the transcriptome [42-44]. It is not surprising that synthetic exogenous miRNAs are widely considered to be a very promising therapeutics.
Recently, targeting miRNAs for therapeutic benefits is gaining great attention in different medical fields. The development of miRNA-based therapeutics depends on two major approaches: inhibitors or antagonists to reduce endogenous levels of the miRNA and could be applied to reduce miRNAs with pathogenic function in stressed cells or diseased tissues and mimics to increase effective levels of a miRNA which can used to restore a beneficial miRNAs lost function. Antagonists reduce miRNA function through a mechanism appears to differ depending on the chemistry of the molecules, either through and includes activation of degradation mechanisms and sequestration as a heteroduplex [45]. Tiny LNAs which have shorter sequences share common seed regions of miRNA families that may enable blockade of multiple miRNA members and potentiate targeting efficacy [46]. MiRNAs as therapeutic targets faced some challenges including safety, delivery and potential off-target effects. Despite these challenges, in vivo miRNA manipulation to regulate disease-related processes is giving initial and promising results in multiple CNS diseases including cerebral ischemia [47], neonatal hypoxic ischemia [48], and epilepsy [17].

**MiRNA and epilepsy treatment**

Research shows that inhibition of a single mature miRNA can provide a new therapeutic target for epilepsy treatment. Antagonists have been used to target four miRNAs in adult epilepsy animal models, including miR-132, miR-34a, miR-184 and miR-134. Targeting miR-132 by intracerebroventricular administration of anantagomir protected against seizure-induced neuronal death after the induction of SE in mice, but no effects were found in SE duration or severity of the seizure [15]. In lithium-pilocarpine epilepsy model, miR-34a downregulation through intracerebroventricular antagonists injection, reduced hippocampal neuronal death [29]. Inhibiting miR-184 expression in vivo resulted in neuronal death after preconditioning seizures and increased seizure-induced neuronal death following SE in previously preconditioned animals, without altering seizure durations [16].

Silencing miR-134 produced the most desirable effects. Targeting miR-134 with a single intracerebroventricular microinjection of LNA-antagomirs reduced 50-70% of kainite induced SE and strongly reduced the hippocampal damage [17]. Very recently, Wang et al. 2014 provided direct evidence that inhibition of miR-134 can block SE-like discharges and is neuroprotective in hippocampal neuronal cultures and suggested that inhibiting miR-134 may be a potential candidate for the clinical treatment of SE [49].

Just earlier, Peng et al. 2013 showed significant upregulation of brain-enriched miR-124 and miR-134 in the seizure related stages of MTLE development in the immature rats and predicts that both can be potential targets for anticonvulsant drugs in the epileptic developing brains [11]. Modulation of the (miR-146a-IL-1β) and (miR-155- TNF-α) pathways suggested as therapeutic targets for MTLE treatment in the developing brains [10, 12].

**The hypothesis**

Epilepsy is a chronic brain disorder that affects 1-2% of the worldwide population and contributes to 0.7% of the global burden of disease. A major proportion of epileptic patients develop the condition during childhood, and approximately 25% of them have medically intractable epilepsy. Recurrent seizures in children are associated with behavioural and psychiatric problems, poor quality of life of the child and family, and increased risk of injury and sudden death. There is a great challenge facing epilepsy researchers for understanding the neurobiology of pharmacoresistance epilepsies and the development of effective anticonvulsant and antiepileptic drugs (AEDs) that work in the immature brain.

Since 2010 miRNAs entered the focus of many epilepsy researchers as promising targets for better understanding of epilepsy pathogenesis in adult and developing brains. In 2012 a brilliant first study in vivo showed that silencing brain-enriched miR-134 reduced seizure severity during SE and reduced recurrent spontaneous seizures in adult brains. The high expression of miR-9 in the developing brain and its important role in CNS development is concomitant with its upregulation in the hypocapmus of immature rats in the seizure related stages and also in children and adults with MTLE. Moreover, it is dysregulated in multiple conditions which are considered epilepsy risk factor and is linked to the NF-κB pathway. Therefore, we postulate that silencing of miR-9 in the developing brains may represent a novel molecular therapeutic target for anticonvulsant drugs in the developing brains.

**Conclusion**

Despite great effort in the management of epilepsy, new antiepileptic drugs and respective surgery, the treatment of epilepsy and seizure control especially in the developing brains remain a major challenge. There is no single traditional treatment strategy for a complete cure of epilepsy. This leads to growing demand for searching about novel molecular therapeutic targets for treatment of seizures and epilepsy. The main aim of this hypothesis is to consider targeting miR-9 as a new therapeutic target
for anticonvulsant drugs in the developing brains. This may add a new strategy for treatment of children with seizures especially with drug resistant seizure disorders.

Conflicts of Interest
The authors report no conflicts of interest.

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References


