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Targeting of microRNA-21-5p protects against seizure damage in a kainic acid-induced status epilepticus model via PTEN-mTOR

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ABSTRACT

Objective: Studies have shown that microRNAs play a role in the development of epilepsy by regulating downstream target messenger (m)RNA. The present study aims to determine the changes associated with microRNA-21-5p (miR-21-5p) during epileptogenesis in a kainic acid rat model, and to assess whether the PTEN-mTOR pathway is a target of miR-21-5p.

Method: Reverse transcription polymerase chain reaction (RT-PCR) was used to examine the quantitative expressions of miR-21-5p and PTEN, and Western blotting was used to test the activity of mTOR in the acute, latent, and chronic stages of epileptogenesis. The antagomir of miR-21-5p was injected into the intracerebroventricular space using a microsyringe. Neuronal death and epilepsy discharge were assessed by Nissl staining and electroencephalography (EEG), respectively. The Morris water maze (MWM) was used to assess the cognitive impairment in rats after status epilepticus (SE).

Results: Both miR-21-5p and mTOR were upregulated and PTEN was downregulated in rats during acute, latent, and chronic stages of epileptogenesis when compared with those of the control. After using antagomir miR-21-5p in vivo, miR-21-5p and mTOR decreased and the expression of PTEN increased compared with that in the SE model. The silencing of miR-21-5p diminished the number of abnormal spikes on EEG and decreased the number of neuron deletions on Nissl staining. The cognitive and memory impairment caused by epilepsy could also be improved after miR-21-5p knockdown in vivo.

Conclusion: The results of the present study demonstrate that PTEN-mTOR is the target of miR-21-5p in a kainic acid model of epilepsy. The knockout of miR-21-5p decreases the neuronal damage in stages of epileptogenesis. The miR-21-5p/PTEN/mTOR axis may be a potential target for preventing and treating seizures and epileptic damage.

1. Introduction

Epilepsy is a common disease of the central nervous system, affecting more than 50 million patients worldwide; one-third of these cases are refractory epilepsy resistant to anti-epileptic medications. The pathogenesis of epilepsy is mainly caused by the excitatory and inhibitory imbalance of the central nervous system and is closely related to a neurotransmitter imbalance, as well as ion channel, glial cell, genetic, and immune abnormalities (Avoli et al., 2002; Vezzani, 2014). As the most common type of refractory partial epilepsy, temporal lobe epilepsy (TLE) is characterized by pathological features, including hippocampal sclerosis and neuronal network alterations. TLE are

Abbreviations: ANOVA, analysis of variance; Ant-Scr, antagomir scrambled oligonucleotide; DG, dentate gyrus; EEG, electroencephalogram; FDR, false discovery rate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IPI, initial precipitating injury; KA, kainic acid; Ant-21-5p, miR-21-5p antagomir; MWM, morris water maze; PDCD4, programmed cell death 4; PFA, paraformaldehyde; PTEN, phosphatase and tensin homolog; p-S6, phosphorylation levels of the S6 ribosomal protein; RECK, reverse-inducing cystine-rich protein with kazal motifs; RT-PCR, reverse transcription polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEM, standard error of the mean; SE, status epilepticus; TIMP3, tissue inhibitor of metalloproteinase-3; TLE, temporal lobe epilepsy; UTR, untranslated region

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caused by multifactor and related with different forms of initial precipitating injury (IPI), such as status epilepticus (SE). Epileptogenesis refers to a period of epilepsy development in which a normal brain turns into a debilitated brain by spontaneous recurrent seizures. The dynamic process of epileptogenesis is divided into three periods: the IPI period, the latent period, and the chronic period (Younus and Reddy, 2017). Therefore some researchers focus on neuroprotection and reversing epileptogenesis by the suppression of seizures for the treatment of epilepsy (Curia et al., 2014; Gualtieri et al., 2012). As such, the perspective of molecular research during epileptogenesis may provide novel ideas for the treatment of epilepsy.

MicroRNA (miRNA) is a single strand of non-coding RNA consisting of 18–25 nucleotides derived from endogenous small hairpin precursors. This molecule can bind to the 3 'or 5' untranslated region (UTR) of the target mRNA and inhibit mRNA translation or directly degrade mRNA. Evidence has revealed that microRNA plays a key role in a variety of neurological diseases, for example, microRNA-101 in the development of neuronal excitability (Lippi et al., 2016), including epilepsy (Henshall, 2013). MiRNA can be used as a target for the study of epilepsy for the following reasons: 1) a single miRNA can control multiple genes that play important roles in signaling pathways. More than 60% of proteins can serve as targets for miRNAs. 2) MiRNA can participate in the regulation of pathological processes related to epileptogenesis, including neuronal apoptosis, glial hyperplasia, and inflammation (Henshall et al., 2016). Scientific studies have shown that during epilepsy, the miRNA profile, including the expression of miR-21-5p, changes (Alsharafi et al., 2015a,b). MiR-21-5p is associated with cell proliferation and apoptotic cell death by regulating the cell cycle through targeting phosphatase and tensin homolog (PTEN), programmed cell death 4 (PDCD4), reverse-inducing cystine-rich protein with kazal motifs (RECK), and tissue inhibitor of metalloproteinase-3 (TIMP3) in the central nervous system (Wang et al., 2015). In addition, miR-21-5p is involved in regulating those genes that cause apoptosis in many diseases (Huang et al., 2016; Wang et al., 2017, 2017). However, the role of miR-21-5p in epilepsy remains unknown.

PTEN is a recently discovered tumor suppressor gene that has both lipid and protein dual phosphatase activity. This protein inhibits AKT/mTOR (the mammalian target of rapamycin) activity by dephosphorylating PIP3 (Garcia-Junco-Clemente and Golshani, 2014). The mTOR pathway regulates a series of biological processes such as cell growth, differentiation, neuronal morphology, protein synthesis, and cortical development. The function of PTEN/mTOR signaling pathway is as an important intrinsic control molecule for neurogenesis and neural regeneration in the nervous system. The mTOR pathway has been considered a new target in the treatment of epilepsy in recent studies (Switon et al., 2017). In adult neuron subset-specific PTEN knockout mice, the inhibition of mTOR with rapamycin suppressed epileptiform activity by reducing the mTORC1 and mTORC2 pathway and improving baseline EEG activity (Nguyen et al., 2015). The study of cortical dysplasia, as a common cause of epilepsy in children has revealed that the mTOR pathway is most significantly associated with neuronal migration and development, and PTEN as an upstream gene in the mTOR pathway may be targeted by miR-21-5p (Lee et al., 2014).

Several studies have shown that the biological effect of miR-21 is related to the PTEN-mTOR pathway, as observed in bladder cancer cell lines (Yang et al., 2015) and in breast cancer cells (Yu et al., 2016). The silencing of miR-21-5p could reduce PTEN-mTOR activity, with therapeutic effects on these disease processes. However, the relationship between miR-21-5p and PTEN-mTOR in epilepsy has not been elucidated. In the present study, we focused on the expression level of miR-21-5p in a kainic acid-induced SE model obtained during the acute, latent, and chronic periods of epileptogenesis and assessed changes in the expression of PTEN and mTOR activity. We investigated whether PTEN-mTOR participated in the pathological process mediated by miR-21-5p during epileptogenesis. We then knocked out miR-21-5p in vivo with an antagomir to decrease its expression, and observed that neuronal damage and cognitive impairment, are correlated with levels of PTEN-mTOR using Nissl staining and the Morris water maze (MWM). Electroencephalogram (EEG) recordings were used to detect alterations of epileptic discharge after the alleviation of miR-21-5p.

2. Materials and methods

2.1. Animals

All adult male Wistar rats used in the present study were purchased from the Animal Center of Harbin Medical University (Harbin, People's Republic of China). The rats weighing 200–250 g were housed under standard laboratory conditions (12-h light–12-h dark cycle). Before the operation, the animals were acclimated for at least 1 week. The rats were provided food and water ad libitum. All experiments were approved by the Ethics Committee of the First Clinical College of Harbin Medical University and according to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Seizure model

The rats were anesthetized with isoflurane (5% induction, 1% to 3% maintenance) and then fixed on the stereotactic frame. The microsyringe was injected into the right CA3 region of the hippocampus after boring a hole in the skull at were 5.6 mm posterior to the bregma (AP), 4.5 mm lateral to the midline from the right side (L), and 5.5 mm ventral to the surface (V) of the skull. Then, 2 μL of kainic acid (KA; 0.4 μg in 1 μL of saline) was injected into the CA3 of each rat at a rate of 1 μL/minute. The criteria for modeling success was established for a stage 4 or 5 seizure, according to modified Racine's scale: stage 1–behavioral arrest with mouth/facial movements, stage 2–head nodding, stage 3–forelimb clonus, stage 4–rearing, and stage 5–rearing and falling (Racine, 1972; Zeng et al., 2009), while observing seizure activity in the rats. To reduce the mortality rate, diazepam (10 mg/kg, i.p.) was administered more than 90 min from the onset of a seizure. The control group was injected with an equal volume of saline instead of KA.

2.3. Intracerebroventricular injections

The miR-21-5p antagomir (Ant-21-5p; Ribobio Guangzhou, People's Republic of China) was used to specifically target antagonized miR-21-5p; this molecule was injected into the right lateral ventricle of the rats, while an antagomir scrambled oligonucleotide (Ant-Scr; Ribobio Guangzhou) was used as the control. The following coordinates from bregma were used: AP = −3.0 mm, L = −2.2 mm, V = −2.8 mm. Each rat was injected with 1 nmol Ant-21-5p or Ant-Scr by microsyringe after anesthetization with isoflurane (5% induction, 1% to 3% maintenance) and the final concentration was 0.5 nmol/μL when dissolved in artificial cerebrospinal fluid. After 24 h, the rats were submitted to KA-induced SE.

2.4. Tissue preparation

The rats were sacrificed following pentobarbital overdose. For real-time polymerase chain reaction (RT-PCR) and Western blotting, the hippocampus was microdissected from the whole brain, which was placed on ice at −80 °C after rapid decapitation. For Nissl staining, the rats were perfused through the heart with 0.1 mol/L of a phosphate-buffered solution followed by 4% paraformaldehyde (PFA); then, the rat brain was quickly removed and placed in PFA (4 °C) overnight. Subsequently, the brain was dehydrated and embedded in paraffin.

2.5. Nissl staining

The paraffin-embedded hippocampus tissue (n = 6 for each group,