Published online: March 30, 2019

IMR Press

Journal of Integrative Neuroscience

ORIGINAL RESEARCH

Molecular expression and functional analysis of genes in children with temporal lobe epilepsy

Xiaojuan Wu[1], Yajie Wang[1], Zhenrong Sun[2], Shouchen Ren[1], Weili Yang[1], Yaxian Deng[1], Chaoxia Tian[1], Yazhen Yu[1] and Baoqin Gao[1,*]

[1]*Department of Pediatrics, Beijing Tiantan Hospital, Capital Medical University, Beijing, 100050, China*

[2]*Department of Neurosurgery, Beijing Tiantan Hospital, Capital Medical University, Beijing, 100050, China*

*Correspondence: gaobaoqin@bjtth.org (Baoqin Gao)

DOI: 10.31083/j.jin.2019.01.13

Temporal lobe epilepsy is the most common form of epilepsy. However, for this type of condition, antiseizure medication is not effective for children. As miRNAs are involved in the development of temporal lobe epilepsy in children, they may provide potential therapeutic approaches for treatment. The primary aim of this study was to explore the expression and function of miR-135a-5p in children with temporal lobe epilepsy. Hippocampal slices from either normal (control) children or children with temporal lobe epilepsy were used to detect the expression of miR-135a-5p and its target gene caspase activity and apoptosis inhibitor 1. To further explore the role of miR-135a-5p in the development of temporal lobe epilepsy in children, primary hippocampal neurons from newborn rats were cultured *in vitro* in a magnesium-free medium to mimic the temporal lobe epilepsy condition in children. The effect of transfection of miR-135a-5p inhibitor into cells was also assessed. Apoptosis and proliferation of hippocampus cells was respectively assessed by flow cytometry or 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The level of miR-135a-5p was significantly increased in both children with temporal lobe epilepsy and the epileptiform discharge model that employed newborn rat hippocampal neurons; whereas, the expression of caspase activity and apoptosis inhibitor 1 was downregulated by overexpression of miR-135a-5p. Moreover, miR-135a-5p mediated the pro-apoptotic effect of temporal lobe epilepsy via repressing caspase activity and apoptosis inhibitor 1 expression. Additionally, miR-135a-5p reduced cell survival in the temporal lobe epilepsy condition. Overexpression of miR-135a-5p induced cell apoptosis through inhibition of caspase activity and apoptosis inhibitor 1 expression and suppressed cell survival in children with temporal lobe epilepsy.

Keywords

miR-135a-5p; CAAP1; temporal lobe epilepsy; functional analysis

Abbreviation

TLE: Temporal lobe epilepsy

UTR: untranslated regions

1. Introduction

Epilepsy is a chronic disorder of the nervous system characterized by recurring seizures due to abnormal neuronal excitability (Henshall et al., 2014). It is estimated that the incidence of epilepsy in children ranges from 33 to 82 per 100,000 children per year (Nickels et al., 2011). Temporal lobe epilepsy (TLE) is the most common form of epilepsy with focal seizures originating in the temporal lobe (Thom, 2004). Unfortunately, children with TLE are often resistant to a single antiseizure medication and they need to take two or more appropriate medications (Nickels et al., 2011). However, many children still have seizures after treatment with a single antiseizure medication (Nickels et al., 2011). Given the unsuccessful medication trials, surgical treatment is an alternative. However, it is dependent on exact identification of the ictal-onset zone (Nickels et al., 2011; Gleissner et al., 2005). Thus, efforts to identify novel therapeutic targets for the treatment of TLE are currently ongoing.

miRNAs are a class of short non-coding RNAs that post-transcriptionally regulate gene expression by either mRNA degradation or translational inhibition of target mRNAs through imperfectly pairing of the 3'-untranslated regions (3'-UTR), with the latter being involved in various biological functions including cell proliferation, cell death, development, and metabolism (Bartel, 2009). Recently, numerous studies have indicated a potential role for miRNAs in the development and treatment of TLE both in animal models and in humans, including children. For instance, the implication of miR-146a, miR-221, and miR-222 in the inflammatory response occurring in TLE has been demonstrated (He et al., 2016; Alsharafi et al., 2015; Aronica et al., 2010; Ashhab et al., 2013a,b). miRNAs can govern the process of TLE by negatively regulating TLE-related gene expression levels (Henshall et al., 2014; He et al., 2016; Kan et al., 2012; McKiernan et al., 2012).

A series of studies have investigated miRNA changes in human TLE (Alsharafi et al., 2015; McKiernan et al., 2012). A recent study revealed upregulation of brain-specific miR-135a-5p in adults with TLE (Alsharafi et al., 2015). However, the expression of miR-135a-5p in children with TLE is unclear as TLE in children is different from the syndrome observed in adults (Nickels et al., 2011). Furthermore, the role of miR-135a-5p in TLE is still unknown. Previous studies have identified that TLE induces apopto-

sis in neurons (Henshall and Simon, 2005; Henshall, 2007). More importantly, the miR-135a-5p-targeted gene CAAP1 predicted by TargetScan and miRanda is the inhibitor of a mitochondrial apoptosis pathway (Zhang et al., 2011). This suggests that miR-135a-5p may be involved in TLE-mediated apoptosis through regulating CAAP1. The primary aim of this study was to investigate the expression and function of miR-135a-5p in children with TLE.

2. Materials and methods

2.1. Subjects

Frozen tissue samples from brain donors diagnosed with TLE (n = 15, age 11.2 ± 2.6 years, onset age 7.4 ± 1.3 years) and control individuals (n = 15, age 10.6 ± 3.7 years) were analyzed. Samples were stored at -80°C. Three slices (400 μm thick) of hippocampus Area CA3 from TLE subjects or normal control subjects were collected by surgical excision of the epileptic focus using a manual tissue chopper. All samples were supplied by the Department of Neurosurgery, Beijing Tiantan Hospital, Capital Medical University. The study was approved by the Ethics Committee of the Capital Medical University (No.KY2015-006-01). Written consents were collected from all subjects before the study.

2.2. Primary culture of hippocampal neurons from newborn rats

Wistar rats were purchased from the animal center of Hubei College of Traditional Chinese Medicine and housed under standard laboratory condition. One male rat was housed with a female rat. Primary hippocampal cells from 100 P0 newborn rats were cultured as described previously (Nunez et al., 2008). Briefly, three slices of hippocampus were obtained from the brain and dissociated to single cells after digestion with 0.5% Trypsin at 37°C for 15 minutes. Cells were then washed twice with Hank's buffered saline solution and treated with DNase I. Neurons were placed on glass coverslips with plating medium in a humidified 37°C incubator (5% CO_2). After confirming cell viability and that they had attached, cells were cultured with Neurobasal medium. One-third of the medium was replaced with fresh Neurobasal medium once a week.

2.3. Establishment of epileptiform discharge model in rat hippocampal neurons

To mimic TLE condition in children, primary hippocampal neurons from newborn rats were cultured in magnesium-free medium (145 g NaCl, 2.5 g KCl, 10 g HEPES, 2 g $CaCl_2$, 10 g glucose, 0.002 g glycine dissolved in 1000 mL distilled water, pH 7.2) at 37°C for three hours, after which hippocampal epileptiform activity was induced (Lewis et al., 1990; Sombati and Delorenzo, 1995).

2.4. Real-Time PCR

miRNA and mRNA were isolated from human brain tissue and cultured rat hippocampal neurons, respectively, by using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and respectively reverse transcribed by either the qScript^{TM} microRNA cDNA Synthesis Kit for miRNA (Quanta BioSciences, Gaithersburg, MD, USA) or the QuantiTect Reverse Transcription Kit for mRNA (Qiagen, Valencia, CA, USA). Real-time PCR was performed using the Maxima SYBR Green/ROX qPCR Master Mix assay (Thermo Fisher Scientific, Rockford, IL, USA) with GAPDH or U6 as internal controls in the StepOnePlus system (Applied Biosystem, Foster City, CA, USA). Following amplification in triplicate for each sample, Ct values were acquired with manual thresholds by 7500 System SDS software (Applied Biosystem). Relative expression levels were normalized to the expression level of GAPDH or U6 according to the ΔΔCT method. The expression levels between different groups were compared using $2^{-\Delta\Delta Ct}$, while p-values $\leq$ 0.05 were considered statistically significant. Primer sequences were: miR-135a-5p: 5'-TCACATAGGAATAAAAAGCCATA-3'; *Caap1* F: 5'-CTGTTCCTCCACCAGAAGATG-3'; *Caap1* R: 5'-ACCAGCTTTCATTAGGGCTTTA-3'.

2.5. Western blotting

Western blotting was performed as previously described (Zhong et al., 2014). Human brain tissues and cultured rat primary hippocampal neurons were sonicated in ice-cold lysis buffer according to the manufacturer's instruction (Cell Signaling Technology, Danvers, MA, USA) with a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) to extract protein. Equal amounts of protein (30 μg) from each group or the Precision Plus Protein Standard used for marker (Bio-Rad Laboratories, Hercules, CA, USA) were resolved by SDS-PAGE, transferred onto PVDF membranes and immunoblotted with primary antibodies for CAAP1 (human)/Caap1 (rat) and GAPDH at 1:1000 dilutions in 5% nonfat milk. *CAAP1* antibody (NBP1-86644) was obtained from Novus Biologicals (Littleton, CO, USA). HRP-conjugated goat anti-rabbit (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) secondary antibody was used to detect primary antibody. The intensities of target protein bands were assessed by densitometry and normalized to those of GAPDH (Abcam). The signals were detected by the SuperSignal West Femto Maximum Sensitivity Substrate kits (Thermo Fisher Scientific) and the densitometric intensities were determined using Image J.

2.6. Transfection

miR-135a-5p inhibitor was synthesized by Life Technologies (Grand Island, NY, USA). Cultured rat primary hippocampal neurons were transfected at a confluency of 85–90% on a 96 well plate. Prior to transfection, regular medium was replaced with serum-free Opti-MEM® I medium. 5 μL of 100 μM miR-135a-5p inhibitor and 5 μL Lipofectamine^{TM} RNAiMAX (Invitrogen, Carlsbad, CA, USA) were added into 50 μL Opti-MEM® I medium and incubated for five minutes at room temperature. Cells were then transfected with miR-135a-5p inhibitor mixed with Lipofectamine^{TM} RNAiMAX and incubated at 37°C for six hours. After transfection, Opti-MEM® I medium was replaced with regular medium. After 24 hours cells were cultured in a magnesium-free medium at 37°C for three hours. The sequence of miR-135a-5p inhibitor was: 5'-UCACAUAGGAAUAAAAAGCCAUA-3'.

2.7. Flow cytometric apoptosis assay

1×10^6 cultured rat primary hippocampal neurons were harvested from each group and washed by incubation buffer (10 mmol/L HEPES/NaOH, pH 7.4, 140 mmol/L NaCl, 5 mmol/L $CaCl_2$). Hippocampal neurons were then suspended in 100 μL PBS with 1 μg Annexin V-FITC (Becton Dickinson, Franklin Lakes, NJ, USA) and Propidium iodide (PI) (Thermo Fisher Scientific), followed by dark incubation at room temperature for 10–15 minutes. After a single wash with incubation buffer, SA-FLOUS

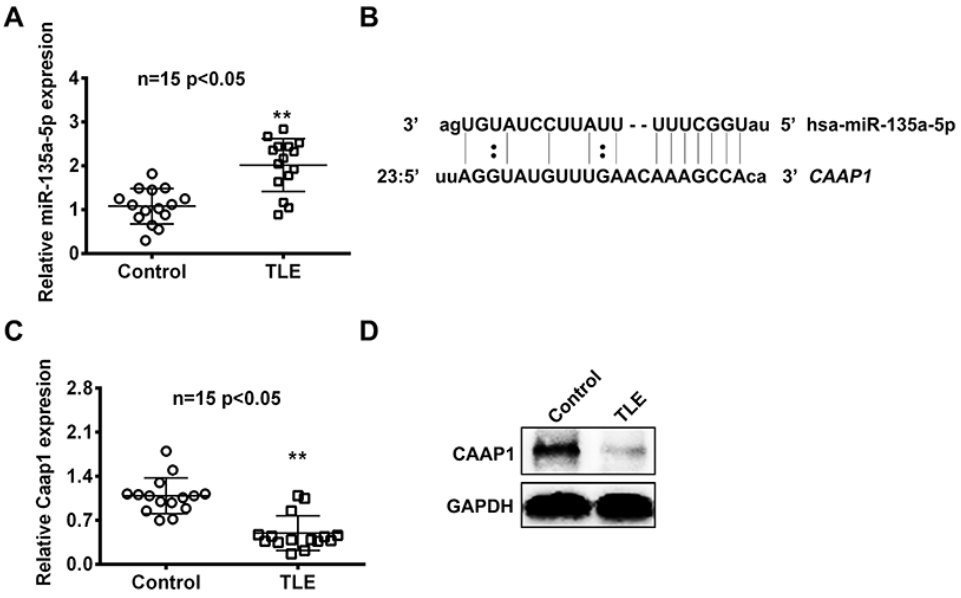

Figure 1. miR-135a-5p expression is increased in temporal lobe epilepsy. A: Quantitative qRT-PCR analyses of miR-135a-5p levels in hippocampus of children. B: Prediction of miR-135a-5p binding site in *Caap1* miRNA 3′-UTR by microRNA website (www.microrna.org/microrna/home.do). C: mRNA level of *Caap1* in hippocampus of children. D: Protein abundance of Caap1 in hippocampus of children. qRT-PCR was performed using 15 samples from control or TLE children respectively. Experiments repeated three times (n = 3). $^{**}p < 0.05$ vs. Control group.

was added to each sample and dark incubated at 4°C for 20 minutes before analysis by flow cytometry.

2.8. MTT assay

A MTT assay kit (Abcam, Cambridge, MA, USA) was used to detect the survival of cultured rat primary hippocampal neurons (Chen et al., 2012). Medium was discarded from the cell cultures in the 96 well plate. Then 50 μL of serum-free media and 50 μL of MTT solution were added into each well and plates were incubated at 37°C for three hours. After incubation, 150 μL of MTT solvent was added into each well. The plate was shaken on an orbital shaker for 15 minutes and absorbance was measured at OD = 590 nm within one hour after shaking.

2.9. Statistical Analysis

Data is reported as mean ± standard error (±SE). Statistical differences were determined by use of the Student's *t*-test between two groups and two-way ANOVA between multiple groups. After the two-way ANOVA analysis, a Tukey's multiple-comparison test was conducted to estimate the significance of the differences. All statistical analyses were conducted by the SigmaStat 3.5 software. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. miR-135a-5p expression is upregulated in the hippocampus of children with TLE

The clinical characteristics of brain donors are summarized in Table. 1. Compared to the control group (n = 15), miR-135a-5p expression was significantly increased in the hippocampus of 80% of the children diagnosed with TLE (n = 15) detected by qPCR (Fig. 1A). In contrast, as the predicted target gene of miR-135a-5p (Fig. 1B), CAAP1 gene expression in hippocampal neurons from 87% of the children with TLE (n = 15) was decreased compared to that in control group (Fig. 1C and Fig. 1D). These data suggested that miR-135a-5p was also upregulated in children with TLE, as previously reported for adult cases (Alsharafi et al., 2015). Moreover, the upregulation of miR-135a-5p suppressed the expression of its target gene CAAP1 in TLE. However, the expression of miR-135a-5p and CAAP1 were not significantly changed in all children with TLE, suggesting that other genes may be involved in TLE formation.

3.2. miR-135a-5p expression is induced in cultured rat hippocampal neurons that displayed epileptiform activity

To mimic the TLE condition in children and to further investigate the expression of miR-135a-5p and *Caap1 in vitro*, primary hippocampal cells from newborn rats were cultured. After three hours of treatment in magnesium-free medium, the expression of miR-135a-5p was increased significantly compared to that of control hippocampal cells (Fig. 2A). Moreover, the overexpression of miR-135a-5p repressed the expression of *Caap1* in hip-

Table 1. Clinical characteristics of brain donors

	IVUS	FFR
Parameters	TLE children	Control children
Number	15	15
Age, (years), mean (SD)	11.2 (2.6)	10.6 (3.7)
Male gender, n (%)	7 (46.7)	8 (53.3)
Onset age, (years), mean (SD)	7.4 (1.3)	-
Seizure type, n (%)	CPS, 9(60) SGTC, 6 (40)	-
Family history, n (%)	3 (20)	-

pocampal neurons cultured in magnesium-free medium (Fig. 2B and Fig. 2C). These data suggested that miR-135a-5p expression was upregulated, whereas, *Caap1* expression was decreased both *in vivo* and *in vitro* under the TLE condition.

3.3. miR-135a-5p induces TLE-like hippocampal cell apoptosis

To determine whether miR-135a-5p mediates the pro-apoptotic effect of hippocampal cells in children with TLE, the miR-135a-5p inhibitor was employed. In cultured hippocampal neurons apoptosis was detected by flow cytometry. Treatment of magnesium-free medium dramatically triggered cell apoptosis compared to that in the control group (Fig. 3Aand Fig. 3B). However, transfection of miR-135a-5p inhibitor into cells cultured in magnesium-free medium resulted in a significantly lower number of apoptotic cells (Fig. 3A and Fig. 3B). Additionally, treatment by miR-135a-5p inhibitor did not affect apoptosis of control cells (Fig. 3A and Fig. 3B). Moreover, the addition of miR-135a-5p inhibitor to cells treated with magnesium-free medium prevented the expression of Caap1 inhibited by the overexpression of miR-135a-5p (Fig. 4A and Fig. 4B). These data collectively suggest that miR-135a-5p mediates TLE-induced apoptosis of hippocampal cells through suppression of Caap1 expression.

3.4. miR-135a-5p inhibits TLE-like hippocampal cell survival

To further explore the role of miR-135a-5p in children with TLE, a MTT assay was employed to detect the proliferation of hippocampal neurons. After culture in magnesium-free medium, cell survival assessed by cell metabolic activity was reduced compared to that of control cells. Conversely, transfection by the miR-135a-5p inhibitor significantly increased cell survival (Fig. 5). Moreover, the addition of miR-135a-5p inhibitor did not promote cell survival under normal condition (Fig. 5). The above data suggests that miR-135a-5p was also involved in the regulation of neuron survival in TLE.

4. Discussion

Epilepsy, including TLE, is characterized by unprovoked seizures (Henshall et al., 2014; Thom, 2004). Mounting evidence from animal models and human patients including children, have shown that seizures trigger apoptosis in neurons and lead to consequent brain injury and epileptogenesis (Henshall and Simon, 2005; Henshall, 2007; Yamamoto et al., 2006). Defining the mechanisms that underly seizure-induced neuronal apoptosis in TLE may be helpful for identifying novel strategies to protect the brain against seizures.

In this study, miR-135a-5p was found to be upregulated in children with TLE. Recent studies have indicated that miR-135a-5p is a novel apoptosis inducer. For example, in malignant glioma, miR-135a-5p is capable of inducing apoptosis (Wu et al., 2012; Zhang et al., 2016). Similarly, miR-135a-5p plays a key role on apoptosis in ovarian cancer cells (Tang et al., 2013). Furthermore, miR-135a-5p enhances hydrogen peroxide-induced apoptosis in rat cardiomyoblast (Liu et al., 2017). Additionally, inhibition of miR-135a-5p protects A549 cells from apoptosis (Zhao et al., 2014). In the aforementioned studies, overexpression of miR-135a-5p is essential to trigger apoptosis and they all indicate that miR-135a-5p mediates neuronal cell apoptosis that could cause TLE in children. Indeed, results from this study provide further evidence in support of this hypothesis by mimicking the TLE condition seen in children in an *in vitro* hippocampal cell model based on newborn rats.

Apoptosis can be activated by intrinsic pathways through disruption of intracellular organelle function, such as by mitochondrial dysfunction, or by an extrinsic pathway via activation of death receptors (Danial and Korsmeyer, 2004). Both of these pathways have been found to be triggered in TLE (Henshall, 2007). However, several studies have demonstrated that miR-135a-5p is solely involved in a mitochondria-dependent apoptosis pathway (Wu et al., 2012; Liu et al., 2017; Zhao et al., 2014), which suggests miR-135a-5p could regulate the intrinsic pathway of apoptosis in children with TLE. Although previous studies have shown that miR-135a-5p initiates apoptosis by downregulating expression of pro-apoptotic Bcl-2 family members, including Bcl-2 and Bcl-xL (Zhao et al., 2014; Navarro et al., 2009), a novel target gene of miR-135a-5p was identified in this study and its expressed protein is also an anti-apoptotic protein. A previous report has suggested that *Caap1* modulates a mitochondrial apoptosis pathway in a caspase-10 dependent manner (Zhang et al., 2011). The present study indicates that *CAAP1* expression was decreased in children with TLE and inhibition of miR-135a-5p restores the mRNA level of *Caap1* in cultured rat hippocampal neurons that displayed epileptiform activity while the miR-135a-5p-induced apoptosis was suppressed.

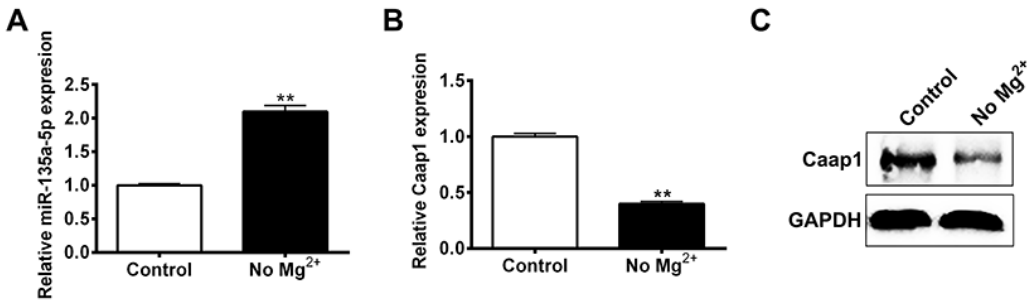

Figure 2. miR-135a-5p is upregulated in TLE model in newborn rat hippocampal neurons. A: Quantitative qRT-PCR analyses of miR-135a-5p levels in primary hippocampal neurons from new born rats. B: mRNA level of *Caap1* in hippocampus neurons from newborn rats. C: Protein abundance of Caap1 in primary hippocampal neurons from newborn rats. Experiments repeated three times (n = 3). No Mg^{2+}: magnesium-free medium. $^{**}p < 0.05$ vs Control group.

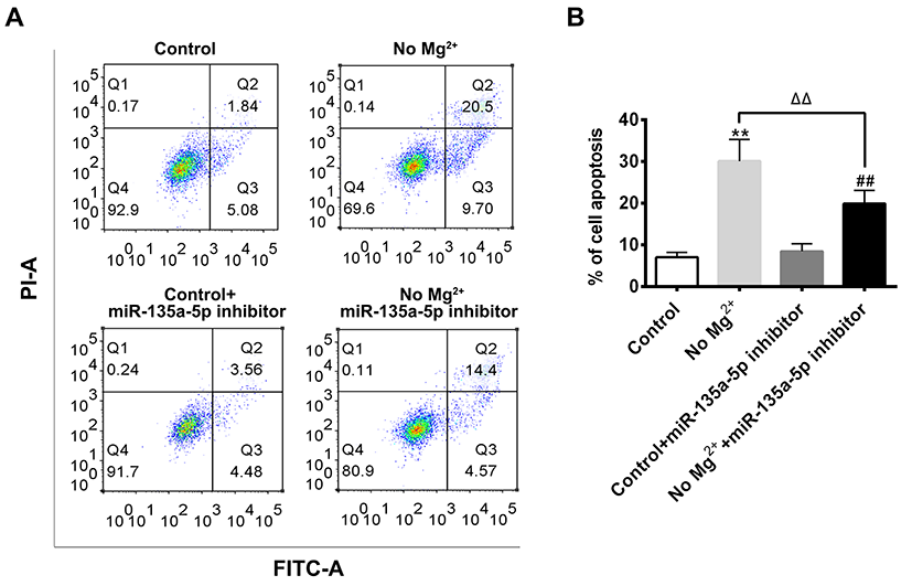

Figure 3. miR-135a-5p mediates the pro-apoptotic effect of TLE. A and B: FACS analysis of primary rat hippocampal cells for apoptosis and quantification of apoptotic cells per group. Cells treated with miR-135a-5p inhibitor for 24 hours before being cultured in magnesium-free medium. Annexin V labeled with FITC and PI were used to stain cells. No Mg^{2+}: magnesium-free medium. $^{**}p < 0.05$ vs. Control group; $^{\#\#}p < 0.05$ vs. Control+miR-135a-5p inhibitor group; $^{\Delta\Delta}p < 0.05$ vs No Mg^{2+} group.

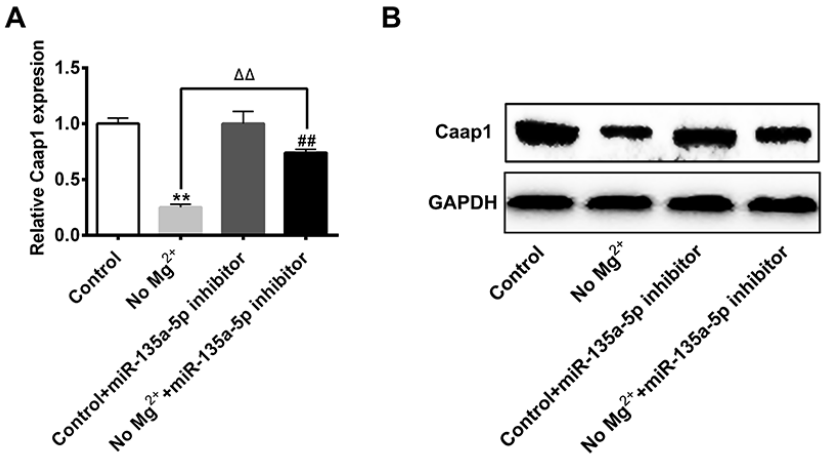

Figure 4. miR-135a-5p regulates Caap1 expression negatively in TLE model. A: mRNA level of Caap1 in primary rat hippocampus neurons from newborn rats treated with or without miR-135a-5p inhibitor. B: Protein abundance of Caap1 in hippocampus neurons from newborn rats. Experiments repeated three times (n = 3). No Mg^{2+}: magnesium-free medium. $^{**}p < 0.05$ vs. Control group; $^{\#\#}p < 0.05$ vs Control+miR-135a-5p inhibitor group; $^{\Delta\Delta}p < 0.05$ vs. No Mg^{2+} group.

This suggests that CAAP1 may be involved in cell death occurring in children with TLE.

Although *CAAP1* is described as a novel apoptosis inhibitor, it may play other key roles in children with TLE. Apart from reducing apoptosis, inhibition of miR-135a-5p also improves cultured rat hippocampal neuron survival after treatment in a magnesium-free medium while the expression of *Caap1* is restored. Previous study has indicated that *CAAP1* is able to interact with acetylase KAT7 (Stelzl et al., 2005), which is crucial for DNA replication (Iizuka et al., 2006). Several studies have shown that KAT7 interacts with proteins to positively regulate cell survival (Pardo et al., 2017; Santos et al., 2018; Newman et al., 2017). For example, KAT7 is essential for T-cell survival through maintaining global histone-H3 lysine 14 acetylation (H3K14ac) (Newman et al., 2017). Moreover, KAT7 promotes embryonic stem cell survival via interaction with a novel binding partner Niam (Pardo et al., 2017). In children with TLE, *Caap1* may act as a chaperon of KAT7 to modulate neuron cell survival.

To the best of the authors knowledge, this is the first investigation of the role of miR-135a-5p in TLE, which could be significant for the development of novel therapeutic treatments for those with this condition. However, pre-clinical animal experiments are required to further reveal the underlying mechanism of how *CAAP1* may suppress TLE-induced neuronal cell apoptosis.

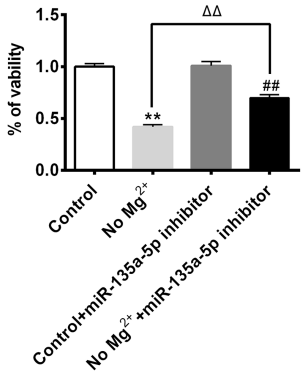

Figure 5. Inhibition of miR-135a-5p promotes TLE-reduced cell survival. Quantification of cell survival indicated by MTT assay in primary rat hippocampal cells. Cells treated with miR-135a-5p inhibitor for 24 hours before being cultured in magnesium-free medium. Experiments repeated three times (n = 3). No Mg^{2+}: magnesium-free medium. $^{**}p < 0.05$ vs. Control group; $^{\#\#}p < 0.05$ vs. Control+miR-135a-5p inhibitor group; $^{\Delta\Delta}p < 0.05$ vs. No Mg^{2+} group.

5. Summary

In summary, the expression of miR-135a-5p is increased in both children with TLE and TLE-like rat hippocampus neurons. Moreover, upregulation of miR-135a-5p initiates TLE-like hippocampus rat neuron cell apoptosis through suppression of *Caap1* expression *in vitro*, which implies it may have a similar effect on the survival of hippocampal neurons in children with TLE.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XJW and BQG designed the study. YJW, ZRS and SCR collected the data, WLY and YXD analyzed the data, CXT and YZY analyzed the results and drafted the manuscript.

Acknowledgments

Thanks to all the peer reviewers and editors for their opinions and suggestions.

Competing interests

The authors declare that they have no competing interests.

Submitted: November 9, 2018
Accepted: March 18, 2019
Published: March 30, 2019

References

Alsharafi, W. and Xiao, B. (2015) Dynamic expression of microRNAs (183, 135a, 125b, 128, 30c and 27a) in the rat pilocarpine model and temporal lobe epilepsy patients. *CNS & Neurological Disorders Drug Targets* **14**, 1096-1102.

Alsharafi, W. A., Xiao, B., Abuhamed, M. M. and Luo, Z. (2015) MiRNAs: biological and clinical determinants in epilepsy. *Frontiers in Molecular Neuroscience* **8**, 1-15.

Aronica, E., Fluiter, K., Iyer, A., Zurolo, E., Vreijling, J., van Vliet, E. A., Baayen, J. C. and Gorter, J. A. (2010) Expression pattern of miR-146a, an inflammation-associated microRNA, in experimental and human temporal lobe epilepsy. *The European Journal of Neuroscience* **31**, 1100-1107.

Ashhab, M., Omran, A., Gan, N., Kong, H., Peng, J. and Yin, F. (2013a) MicroRNA s (9, 138, 181A, 221, and 222) and mesial temporal lobe epilepsy in developing brains. *Translational Neuroscience* **4**, 357-362.

Ashhab, M. U., Omran, A., Kong, H., Gan, N., He, F., Peng, J. and Yin, F. (2013b) Expressions of tumor necrosis factor alpha and microRNA-155 in immature rat model of status epilepticus and children with mesial temporal lobe epilepsy. *Journal of Molecular Neuroscience: MN* **51**, 950-958.

Bartel, D. P. (2009) MicroRNAs: target recognition and regulatory functions. *Cell* **136**, 215-233.

Chen, Y., Huang, X., Chen, W., Wang, N. and Li, L. (2012) Tenuigenin promotes proliferation and differentiation of hippocampal neural stem cells. *Neurochemical Research* **37**, 771-777.

Danial, N. N. and Korsmeyer, S. J. (2004) Cell death: critical control points. *Cell* **116**, 205-219.

Gleissner, U., Sassen, R., Schramm, J., Elger, C. E. and Helmstaedter, C. (2005) Greater functional recovery after temporal lobe epilepsy surgery in children. *Brain : A Journal of Neurology* **128**, 2822-2829.

He, F., Liu, B., Meng, Q., Sun, Y., Wang, W. and Wang, C. (2016) Modulation of miR-146a/complement factor H-mediated inflammatory responses in a rat model of temporal lobe epilepsy. *Bioscience Reports* **36**, 1-12.

Henshall, D. C. (2014) MicroRNA and epilepsy: profiling, functions and potential clinical applications. *Current Opinion in Neurology* **27**, 199-205.

Henshall, D. C. (2007) Apoptosis signalling pathways in seizure-induced neuronal death and epilepsy. *Biochemical Society Transactions* **35**, 421-423.

Henshall, D. C. and Simon, R. P. (2005) Epilepsy and apoptosis pathways. *Journal of Cerebral Blood Flow and Metabolism* **25**, 1557-1572.

Iizuka, M., Matsui, T., Takisawa, H. and Smith, M. M. (2006) Regulation of replication licensing by acetyltransferase Hbo1. *Molecular and Cellular Biology* **26**, 1098-1108.

Kan, A. A., van Erp, S., Derijck, A. A., de Wit, M., Hessel, E. V., O'Duibhir, E., de Jager, W., Van Rijen, P. C., Gosselaar, P. H., de Graan, P. N. and Pasterkamp, R. J. (2012) Genome-wide microRNA profiling of human temporal lobe epilepsy identifies modulators of the immune response. *Cellular and Molecular Life Sciences* **69**, 3127-3145.

Lewis, D. V., Jones, L. S. and Mott, D. D. (1990) Hippocampal epileptiform activity induced by magnesium-free medium: differences between areas CA1 and CA2-3. *Epilepsy Research* **6**, 95-101.

Liu, N., Shi, Y. F., Diao, H. Y., Li, Y. X., Cui, Y., Song, X. J., Tian, X., Li, T. Y. and Liu, B. (2017) MicroRNA-135a regulates apoptosis induced by hydrogen peroxide in rat. *Cardiomyoblast Cells International Journal of Biological Sciences* **13**, 13-21.

McKiernan, R. C., Jimenez-Mateos, E. M., Bray, I., Engel, T., Brennan, G. P., Sano, T., Michalak, Z., Moran, C.,Delanty, N., Farrell, M., O'Brien, D., Meller, R., Simon, R. P., Stallings, R. L. and Henshall, D. C. (2012) Reduced mature microRNA levels in association with dicer loss in human temporal lobe epilepsy with hippocampal sclerosis. *PloS One* **7**, 1-9.

Navarro, A., Diaz, T., Martinez, A., Gaya, A., Pons, A., Gel, B., Codony, C., Ferrer, G., Martinez, C., Montserrat, E. and Monzo, M. (2009) Regulation of JAK2 by miR-135a: prognostic impact in classic. *Hodgkin Lymphoma Blood* **114**, 2945-2951.

Newman, D. M., Voss, A. K., Thomas, T. and Allan, R. S. (2017) Essential role for the histone acetyltransferase KAT7 in T cell development, fitness, and survival. *Journal of Leukocyte Biology* **101**, 887-892.

Nickels, K. C., Wong-Kisiel, L. C., Moseley, B. D. and Wirrell, E. C. (2011) Temporal lobe epilepsy in children. *Epilepsy Research*

and Treatment **2012**,657-668.
Nunez, J. (2008) Primary culture of hippocampal neurons from P0 newborn rats. *Journal of Visualized Experiments* **19**, 1-2.
Pardo, M., Yu, L., Shen, S., Tate, P., Bode, D., Letney, B. L., Quelle, D. E.,Skarnes, W. and Choudhary, J. S. (2017) Myst2/Kat7 histone acetyltransferase interaction proteomics reveals tumour-suppressor niam as a novel binding partner in embryonic stem cells. *Scientific Reports* **7**, 1-14.
Santos, P. K. F., de Souza Araujo, N., Francoso, E., Zuntini, A. R. and Arias, M. C. (2018) Diapause in a tropical oil-collecting bee: molecular basis unveiled by RNA-Seq. *BMC Genomics* **19**, 1-11.
Sombati, S. and Delorenzo, R. J. (1995) Recurrent spontaneous seizure activity in hippocampal neuronal networks in culture. *Journal of Neurophysiology* **73**, 1706-1711.
Stelzl, U., Worm, U., Lalowski, M., Haenig, C., Brembeck, F. H., Goehler, H., Stroedicke, M., Zenkner, M., Schoenherr, A., Koeppen, S., Timm, J., Mintzlaff, S., Abraham, C., Bock, N., Kietzmann, S., Goedde, A., Toksoz, E., Droege, A., Krobitsch, S., Korn, B., Birchmeier, W., Lehrach, H. and Wanker, E. E. (2005) A human protein-protein interaction network: a resource for annotating the proteome. *Cell* **122**, 957-968.
Tang, W. W., Wan, G. P., Wan, Y. C., Zhang, L. and Cheng, W. J. (2013) Effects of miR-135a on HOXA10 expression, proliferation and apoptosis of ovarian cancer cells. *Zhonghua Fu Chan Ke Za Zhi* **48**, 364-369. (In Chinses)
Thom, M. (2004) Recent advances in the neuropathology of focal lesions in epilepsy. *Expert Review of Neurotherapeutics* **4**, 973-984.
Yamamoto, A., Murphy, N., Schindler, C. K., So, N. K., Stohr, S., Taki, W., Prehn, J. H. and Henshall, D. C. (2006) Endoplasmic reticulum stress and apoptosis signaling in human temporal lobe epilepsy. *Journal of Neuropathology and Experimental Neurology* **65**, 217-225.
Wu, S., Lin, Y., Xu, D., Chen, J., Shu, M., Zhou, Y., Zhu, W., Su, X., Zhou, Y., Qiu, P. and Yan, G. (2012) MiR-135a functions as a selective killer of malignant glioma. *Oncogene* **31**, 3866-3874.
Zhang, T., Shao, Y., Chu, T. Y., Huang, H. S., Liou, Y. L., Li, Q. and Zhou, H. (2016) MiR-135a and MRP1 play pivotal roles in the selective lethality of phenethyl isothiocyanate to malignant glioma cells. *American Journal of Cancer Research* **6**, 957-972.
Zhang, Y., Johansson, E., Miller, M. L., Janicke, R. U., Ferguson, D. J., Plas, D., Meller, J. and Anderson, M. W. (2011) Identification of a conserved anti-apoptotic protein that modulates the mitochondrial apoptosis pathway. *PloS One* **6**, 1-14.
Zhao, J., Li, X., Zou, M., He, J., Han, Y., Wu, D., Yang, H. and Wu, J. (2014) miR-135a inhibition protects A549 cells from LPS-induced apoptosis by targeting Bcl-2. *Biochemical and Biophysical Research Communications* **452**, 951-957.
Zhong, J. X., Zhou, L., Li, Z., Wang, Y. and Gui, J. F. (2014) Zebrafish noxa promotes mitosis in early embryonic development and regulates apoptosis in subsequent embryogenesis. *Cell Death and Differentiation* **21**, 1013-1024.