ORIGINAL ARTICLE

# MiR-194-5p serves as a potential biomarker and regulates the proliferation and apoptosis of hippocampus neuron in children with temporal lobe epilepsy

**Xia Niu[a], Hai-Ling Zhu[a,*], Qian Liu[a], Jing-Fen Yan[b], Mei-Lian Li[c]**

*[a]Department of Pediatric, Affiliated Hospital of Weifang Medical University, Shandong, China; [b]Department of Rehabilitation, Affiliated Hospital of Weifang Medical University, Shandong, China; [c]Department of Orthopedics Rehabilitation, Weifang Hospital of Traditional Chinese Medicine, Shandong, China*

## Abstract

**Background:** The aim of the present study is to explore the expression level and the clinical significance of miR-194-5p to the children with temporal lobe epilepsy, and investigate its functions in regulating cell behaviors of hippocampal neurons.

**Methods:** The expression level of miR-194-5p was detected in the serum of 59 temporal lobe epilepsy (TLE) children and 63 healthy children. To further study the role of miR-194-5p in the development of TLE in children, the epileptiform discharge model was established in rat hippocampal neurons to mimic TLE conditions in children. Receiver operator characteristic (ROC) curves and area under the ROC curve were established to evaluate the diagnostic value of serum microRNAs to the differentiation of the TLE group and healthy group. The influence of miR-194-5p on the proliferation and apoptosis of hippocampus neurons was examined by using MTT and flow cytometric apoptosis assay. Luciferase reporter assay was performed to confirm the target gene of miR-194-5p.

**Results:** The result demonstrated that miR-194-5p was significantly dysregulated in plasma of TLE patients. Analysis of ROCs showed that the miR-194-5p had high specificity and sensitivity in the diagnosis of the TLE in children. The expression of miR-194-5p was found to increase in the hippocampal cells cultured in the magnesium-free medium through quantitative real-time polymerase chain reaction. Hyper-expressed of miR-194-5p reversed TLE-induced reduction for the cell viability, and inhibited the cell apoptosis induced by TLE. Insulin-like growth factor 1 receptor (IGF1R) was proved to be a direct target gene of miR-194-5p.

**Conclusion:** MiR-194-5p is a likely potential biomarker and treatment target of TLE in children. IGF1R might be involved in the regulatory role of miR-194-5p in hippocampus neuron apoptosis.

**Keywords:** Apoptosis; Children; Hippocampus neuron; MiR-194-5p; Proliferation; Temporal lobe epilepsy

## 1. INTRODUCTION

Temporal lobe epilepsy (TLE), a frequently found neurological disorder, is defined by typical characteristic clinical features and a typical seizure semiology.[1] Related morphologic brain changes might be associated with recurrent seizure, propagation, and deafferentation of brain regions connected to the hippocampus.[2] TLE in children is different from that in adults in terms of the etiology, the type, and the clinical manifestations, and there is a paucity of data in children with TLE. Children's brains are in a rapidly developing stage and are vulnerable to damage.[3] Some studies have demonstrated very favorable surgical results based on the temporal lobectomy in children, but performing presurgical evaluations on younger children is much more complex than that on the older patients.[4] The complex, multifactorial nature and its limited diagnostic rate of TLE could lead to misdiagnosis or delayed diagnosis, which imposes adverse influence on the patients and negative impact on healthcare resources.[5,6] Additionally, several researches showed that the detection of microRNAs in biofluids such as blood could help support the diagnosis of a brain condition.[5] Researchers show strong interest in the discovering of molecular biomarkers in the blood, as it could provide a fast, inexpensive, and noninvasive method for TLE diagnosis.[7] Meanwhile, because the brain tissue is not readily accessible, the investigations into the pathophysiology of TLE in children and its potential biomarkers have increasingly depended on the blood-based expression levels of microRNAs (miRNAs).[8]

MiRNAs represent a small noncoding regulatory RNA family that has been found to play an important role for miRNAs in major biological processes such as development differentiation, growth, and metabolism in the previous studies.[9] Based on the excellent stability of miRNAs in the biologic fluids, they guarantee a rapid and reliable detection at ultra-low levels using polymerase chain reaction (PCR) and other techniques.[5,10,11]

** Address correspondence. Dr. Hai-Ling Zhu, Department of Pediatric, Affiliated Hospital of Weifang Medical University, 2428, Yuhe Road, Weifang 261041, Shandong, China. E-mail address: zhu_hailing01@163.com (H.-L. Zhu).*

*Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.*

*Journal of Chinese Medical Association. (2021) 84: 510-516.*

*Received May 26, 2020; accepted January 21, 2021.*

*doi: 10.1097/JCMA.0000000000000518.*

*Copyright © 2021, the Chinese Medical Association. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)*

Meanwhile, because many miRNAs were tissue-specific and could be quantified reliably in blood samples, their latent capacity use as biomarkers has been increasingly recognized, especially its role in the pathogenesis of the disease is fast expanding. Interestingly, accumulating evidence suggested that miRNAs played the role of biomarkers in the psychiatric and neurodegenerative diseases, such as anxiety, ischemia, and Alzheimer's disease.[12–15] Furthermore, a number of miRNAs have been reported to be dysregulated in brain tissue from the patients with epilepsy, and the experimental manipulation of specific miRNAs is shown to alter seizure thresholds in animals.[16–18] Previous studies showed that miR-194-5p was significantly down-regulated in adult patients with epilepsy, and there were relatively few studies in children.[19–21] Considering the remarkable change of miR-194-5p expression in adults with TLE, its clinical significance to the diagnosis of children with TLE is worth noting.

In the present study, first, we found that miR-194-5p expression was abnormally downregulated in children with TLE and the prediction of serum-based miRNA biomarkers supports the TLE diagnosis in the children. We also explored the relationship between the expression level of the miR-194-5p and the hippocampal neuron proliferation and apoptosis.

## 2. METHODS

### 2.1. Study population and sample collection

This study included 59 TLE children and 63 healthy children. The TLE children had the mean age of 9.32 ± 3.27 years old, and the healthy individuals (as control group) had the mean age of 10.00 ± 3.44 years old. The diagnosis of patients was according to the clinical history, physical, clinical seizure semiology, and high-resolution magnetic resonance imaging and video-EEG monitoring outcomes. Subjects over 18 years of age were excluded from the study. Our study also excluded patients with nonepileptic disorders, such as metabolic disorders, electrolyte disturbances, acute encephalopathy or trauma, and nonepileptic paroxysmal events mimicking epilepsy. Blood samples (5 mL) from TLE patients and healthy controls were collected in EDTA-containing tubes. Whole-blood samples were centrifuged immediately after blood collection. Then serum frozen in aliquots was stored at −80°C until further analysis.

The protocol of this study was approved by the Ethics Committee of Affiliated Hospital of Weifang Medical University, and written informed consent was collected from each participant.

### 2.2. Primary culture of hippocampal neurons from newborn rats

Wistar rats were obtained from the Shanghai Animal Laboratory Center and raised under standard laboratory conditions. All animals were bred following the Guide for the Care and Use of Laboratory Animals of the Institute for Laboratory Animal Research. Primary cultures of hippocampal neurons were collected from P0 newborn rats <24 hours old as previous research described with some modifications.[22] To be brief, the hippocampus was collected and cut into small pieces after anesthesia and sterilization, followed by 15 minutes of digestion in 0.25% trypsin (Thermo Fisher Scientific, Inc., Waltham, MA) at 37°C. Single-cell suspension was prepared by vigorous washing with Hank's buffered saline solution. Cells were seeded on glass coverslips with plating medium after treatment with DNase I and kept at 37°C and 5% $CO_2$ in a humidified incubator. After attaching, cells were maintained in Neurobasal medium (Invitrogen; Thermo Fisher Scientific, Inc.). Replace one-third of the medium with fresh Neurobasal medium once a week. Then these cells were ready for further study. To confirm the purity of primary hippocampal neurons, cells were stained by NeuN (neuron), glial fibrillary acidic protein (GFAP, astrocytes), and Iba-1 (microglia); and quantification of the cells was calculated.

### 2.3. Establishment of epileptiform discharge model in rat hippocampal neurons

The epileptiform discharge model was set up using a conventional method to mimic TLE conditions in children. After 9 days of treatment with the nutrient medium, hippocampal neuron cells were cultured in a magnesium-free medium (10 mmol HEPES, 145 mmol NaCl, 2.5 mmol KCl, 2 mmol $CaCl_2$, 10 mmol glucose, 0.002 mmol glycine, pH 7.2) for 3 hours. Then the hippocampal epileptiform activity was observed within 12–24 hours using patch-clamp recordings and can last for the life of the neurons in culture. This hippocampal neuronal culture model of TLE has been well characterized as a useful in vitro model of TLE.[23] After that, cells were incubated in the normal culture medium (containing 1 mmol $MgCl_2$) for the other 6 hours.[24]

### 2.4. Cell transfection

MiR-194-5p mimic, miR-194-5p mimic negative control (miR-NC) were provided by GenePharma (Shanghai, China). Rat primary hippocampal neuron cells were seeded into a 96-well plate. Considering the different transfection steps, cells were divided into the following groups: untreated group as a control group, No $Mg^{2+}$ (cells were cultured in a magnesium-free medium), No $Mg^{2+}$ + miR-NC (cells were transfected with miR-NC, 24 hours posttransfection cells were cultured in a magnesium-free medium for 3 hours), No $Mg^{2+}$ + miR-194-5p mimic (cells were transfected with miR-194-5p mimic, 24 hours post-transfection cells were cultured in a magnesium-free medium for 3 hours). MiRNAs were transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. After treating in a magnesium-free medium, the TLE model in vitro was established.

### 2.5. RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was extracted using a TRIzol LS (Invitrogen) following the manufacturer's recommendations. For RNA isolation from serum, 250 μL of serum was homogenized in 750 μL of Trizol LS (Invitrogen). Then 200 μL of chloroform was added to the sample and the mixed solution was centrifugated. After an additional chloroform extraction and precipitation with isopropanol, the pellet was washed twice by centrifugation with 70% ethanol. The RNA pellet was dried for 10 minutes at room temperature and dissolved in 30 μL of diethylpyrocarbonate-treated water. Reverse transcription was conducted according to the instructions of TaqMan miRNA reverse transcription kit (Applied Biosystems, Foster City, CA). The reverse transcription primer sequences were as follows: miR-194-5p, 5′-CTCAACTGGTGTCGTGGAGT CGGCAATTCAGTTGAGTCCACATG; U6:5′-GTCGTATCCA GTGGCAGGG TCCGAGGTATTCGCACTGGATA CGACAA AAATA-3′. The reverse transcription conditions were as follows: 37°C for 15 minutes, followed by 5 seconds at 85°C for RT inactivation. Then, the miRNA expression levels were tested using quantitative real-time PCR (qRT-PCR) with a SYBR Premix Ex Taq II commercial kit (Takara, Dalian, China). The following thermocycling conditions were used for the PCR: Initial denaturation at 95°C for 5 minutes; 30 cycles of 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 20 seconds; and a final extension at 72°C for 10 minutes. The relative expression of the microRNA was analyzed using the $2^{-\Delta\Delta Ct}$ method. U6 snRNA levels were used as the internal reference.

The primer sequences used were as follows: miR-194-5p: forward, 5′-GCGGCGGTGTAA CAGCAACTC C-3′ and reverse, 5′-CTCAACTGGTGTCGTGGA-3′; U6-forward: 5′-CTCGCTTCGGCAGCACA-3′, and reverse, 5′-AACGCTT CACGAATTTGCGT-3′.

### 2.6. MTT assay

Cell viability was performed by the MTT assay.[25] Hippocampal neuron cells were plated in 96-well culture plates ($1 \times 10^5$ cells per well) and following cultured for 3 days before treatment. Fifty microliters of MTT (Sigma-Aldrich; Merck, Darmstadt, Germany) was added to each well and incubated (37°C) for 4 hours. Then dimethyl sulfoxide (Sigma-Aldrich; Merck) was added to dissolve the formazan crystals. Optical density was measured at 490 nm using a microplate reader to determine the cell proliferation.

### 2.7. Flow cytometric apoptosis assay

A flow cytometry analysis was performed to assess the apoptotic activity of cells by using an Annexin V-FITC Apoptosis Detection kit (Keygen Biotechnology, Nanjing, China). Hippocampal neuron cells were seeded at $2 \times 10^5$ cells/well in six well plates. After incubation, cells were harvested by trypsinization and blew to give a single cell suspension. Subsequently, cells mixed with 5 μL Annexin V-FITC and PI staining solution. Cells were then incubated at room temperature for 10 minutes in the dark and then assayed by flow cytometry. Each sample was run in triplicates. The apoptotic rates were analyzed using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA).

### 2.8. Luciferase reporter assay

The TargetScan Release 7.0 (http://targetscan.org/) analysis shows that miR-194-5p contains binding sites for insulin-like growth factor 1 receptor (IGF1R). The cells were cotransfected with miR-194-5p mimic or miR-NC, and the wide-type or mutant seed region of miR-194-5p in the 3′-UTR of IGF1R. The cell transfection was performed by using Lipofectamine 2000. Renilla luciferase was used for normalization. Then relative luciferase activity was detected with a microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA). Each sample was repeated three times.

### 2.9. Statistical analysis

Statistical analyses were performed using the SPSS 24.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 7.04 software (GraphPad Software, Inc., La Jolla, CA, USA). Measurement data were expressed as mean ± SD ($x \pm s$). An independent-samples *t*-test or a chi-squared test was used in the demographic data analysis of two groups. One-way analysis of variance or student's *t*-test was used to analyses significant differences between two groups. Receiver operating characteristic (ROC) curve analysis was used to determine the specificity and sensitivity of miR-194-5p levels about the diagnosis of TLE. All experiments were carried out at least three repetitions. $p < 0.05$ was considered to be statistically significant.

## 3. RESULTS

### 3.1. Demographic data

As shown in Table 1, the demographic and clinical characteristics of both patients and healthy groups were recorded and summarized. The age of the subjects ranged from 4 to 16 years old in the healthy controls group, and 4–14 years in the TLE patient group. Patients with TLE did not differ significantly from the healthy groups in terms of the age and gender distribution ($p > 0.05$, Table 1).

**Table 1**

**Clinical characteristics of the patients with TLE and the healthy controls**

| Parameters | Healthy controls (n = 63) | TLE patients (n = 59) | *p* |
|---|---|---|---|
| Age, mean ± SD (y) | 10.00 ± 3.44 | 9.32 ± 3.27 | 0.267 |
| Gender | | | |
| Male | 33 (52.4%) | 34 (57.6%) | 0.561 |
| Female | 30 (47.6%) | 25 (42.4%) | |
| Interictal EEG | | | |
| Unilateral temporal | NA | 45 (76.3%) | |
| Bilateral temporal | NA | 14 (24.7%) | |
| Epilepsy type | | | |
| Simple partial | NA | NA | |
| Complex partial | NA | 59 (100%) | |
| Brain MRI finding | | | |
| No abnormality | NA | 49 (83.0%) | |
| Mesial sclerosis | NA | 10 (17.0%) | |

EEG = electroencephalography; MRI = magnetic resonance imaging; TLE = temporal lobe epilepsy.

### 3.2. MiR-194-5p expression level

First, the relative expression of miR-194-5p was detected in 63 normal blood samples and 59 blood samples in children with TLE. The result showed that the expression of miR-194-5p in children with TLE was significantly down-regulated ($p < 0.001$) compared to the control group (Fig. 1). The expression levels of the miR-194-5p were detected by qRT-PCR assay.

### 3.3. The diagnostic value analysis of serum miR-194-5p for TLE

To explore the clinical diagnostic value of miR-194-5p, a ROC curve was established according to the expression level of miR-194-5p in children with or without TLE. As illustrated in **Fig. 2**, the area under the curve (AUC) for miR-194-5p was 0.896, with a sensitivity of 81.0% and specificity of 88.1% at the cutoff value of 0.859. These observations highlighted the clinical diagnostic value of miR-194-5p to the clinical diagnosis of the children with TLE, and miR-194-5p might be a sensitive biomarker for identifying TLE children.

### 3.4. Expression level of miR-194-5p in TLE cell model

To further confirm the significance of miR-194-5p to TLE, the TLE cell mode is established and the expression level of miR-194-5p

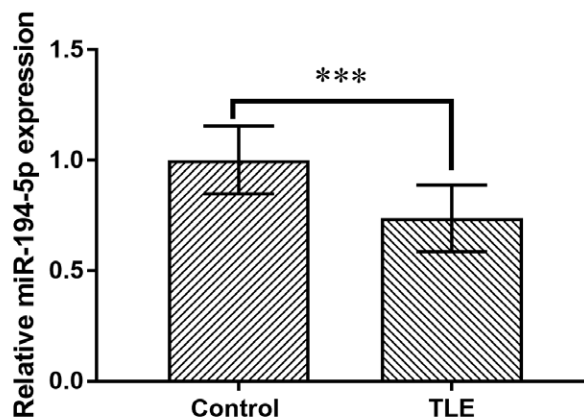


**Fig. 1** The expression level of miR-194-5p in the serum of children with temporal lobe epilepsy (TLE) and healthy controls. The expression level of miR-194-5p was down-regulated significantly in children with TLE. ***$p < 0.001$.

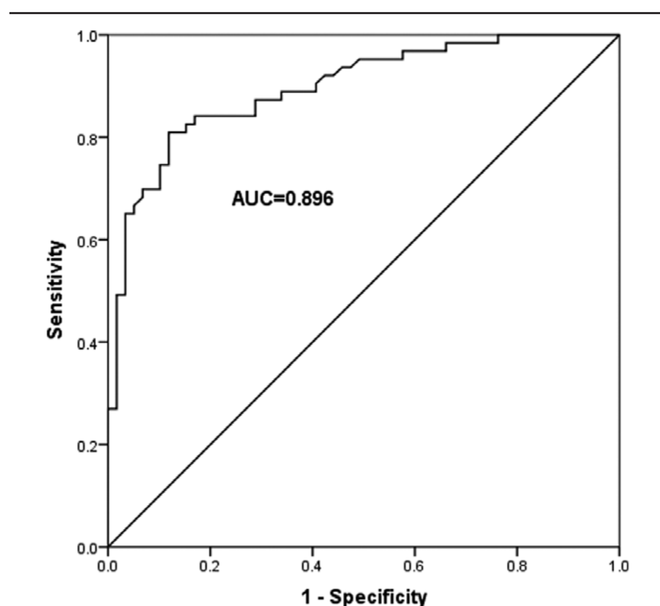


**Fig. 2** Receiver operating characteristic curve (ROC) was performed to analyze the diagnostic value of miR-194-5p for TLE in children. The area under the curve (AUC) for miR-194-5p was 0.896, with a sensitivity of 81.0% and specificity of 88.1% at the cutoff value of 0.859. TLE = temporal lobe epilepsy.

in vitro is explored. Primary hippocampal neurons from P0 newborn rats were cultured in the magnesium-free medium for 3 hours to mimic TLE condition in children. To confirm the purity of primary hippocampal neurons, cells were stained by NeuN, GFAP, and Iba-1. It was found that >96% of cells were neurons (Fig. 3A). As shown in Fig. 3B, the expression level of miR-194-5p was down-regulated clearly in the hippocampal cells which have been treated in a magnesium-free medium ($p < 0.001$).

### 3.5. Effects of miR-194-5p on cell proliferation and apoptosis

The expression level of miR-194-5p in hippocampal cells was regulated by the cell transfection to study the influence of miR-194-5p on the cell proliferation and apoptosis in vitro. The expression of miR-194-5p in cells was determined by using qRT-PCR, and the results demonstrated that the transfection of miR-194-5p mimic led to a remarkable increase in the expression level of miR-194-5p (Fig. 4A, $p< 0.001$). It was found that the viability of hippocampal cells treated with the magnesium-free medium was significantly reduced compared with the control group, while the cell apoptosis was remarkably increased ($p < 0.001$). As shown in Fig. 4B, transfection with miR-194-5p mimic significantly attenuated the TLE-induced reduction for the cell viability ($p < 0.001$). Moreover, overexpression of miR-194-5p also alleviated the TLE-induced cell apoptosis (Fig. 4C, $p < 0.001$).

### 3.6. IFG1R is a direct target of miR-194-5p

Bioinformatics analysis indicated that miR-194-5p contains binding sites for IGF1R (Fig. 5A). Furthermore, the data of luciferase reporter assay proved that the overexpression of miR-194-5p reduced the luciferase activity of cells transfected with Wt-3′-UTR of IGF1R (Fig. 5B). However, miR-194-5p mimic transfection did not affect the luciferase activity of cells that are transfected with Mut-3′-UTR of IGF1R. The results show that IFG1R is a direct target of miR-194-5p.

## 4. DISCUSSION

In recent years, miRNAs are gaining more and more attention and are considered as novel biomarkers for the diagnosis of several diseases including some mental sickness. Children with TLE often need to take two or more drugs for clinical treatment due to single antiseizure drug resistance. Meanwhile, now more and more attention has been paid to children with TLE.[26–28] So far, although a lot of research work has been done in this respect, there is no reliable biomarker for the diagnosis and treatment of TLE in children. To overcome the limitations with the current treatments for TLE in children, innovation is of great importance to the diagnosis and the efficacy of targeted therapy effects.

A lot of supportive evidence shows that miR-194-5p plays different roles in different types of human diseases. For instance,

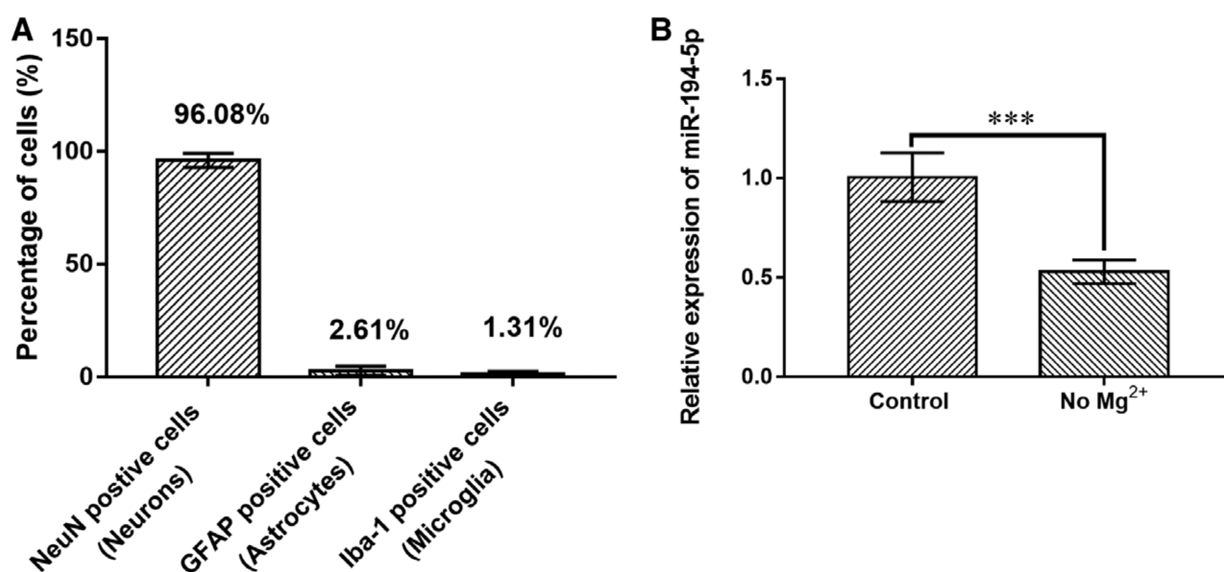


**Fig. 3** To establish TLE cell model, primary hippocampal cells were collected from newborn rats, and cultured in the magnesium-free medium for 3h. A, Quantification of the hippocampal cells stained by NeuN (neuron), GFAP (astrocytes), and Iba-1 (microglia). B, The expression level of miR-194-5p was decreased significantly in magnesium-free medium. ***$p < 0.001$. TLE = temporal lobe epilepsy.

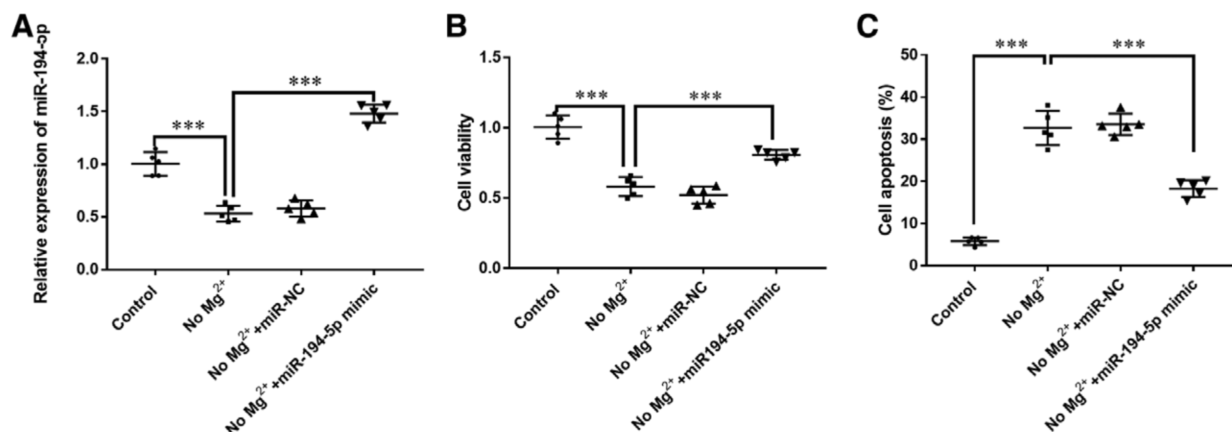


**Fig. 4** The influence of miR-194-5p on the proliferation and apoptosis of hippocampal neurons. A, The hippocampal neuron cells transfected by miR-194-5p mimic showed significantly high expression of miR-194-5p. B, The viability of hippocampal cells treated with the magnesium-free medium was significantly decreased, which was reversed by miR-194-5p mimic transfection. C, The cell apoptosis of hippocampal cells cultured in the magnesium-free medium was significantly increased, which upregulated miR-194-5p mitigated TLE-induced cell apoptosis. ***$p < 0.001$. TLE = temporal lobe epilepsy.

D'Angelo et al[29] demonstrated that miR-194-5p was significantly upregulated in patients responding to treatment and could serve as a predictive biomarker of responsiveness to the neoadjuvant chemoradiotherapy in patients with locally advanced rectal adenocarcinoma. As Mondanizadeh et al[30] reported, the plasma level of miR-194-5p is likely a potential biomarker for the diagnosis of intestinal-type gastric cancer in humans. Ran et al[31] proved that the downregulated expression of miR-194 was a predictor of poor prognosis of hepatocellular carcinoma patients. Moreover, miR-194-5p, which regulates the MAP4K4/c-Jun/MDM2 signaling pathway, might serve as a tumor suppressor and act as a potential target for CRC prevention and therapy.[32]

Moreover, the functional change of miR-194-5p has made significant influence on the development of hippocampal neurons. Wang et al found that miR-194-5p was highly expressed in the sensory patch of inner ear and the neurons in mice embryos.[33] Then they further found that miR-194-5p regulated the development and differentiation of the sensory patches and the ganglion of the inner ear.[34] In previous studies, the expression level of miR-194-5p in the serum was determined by quantitative RT-PCR, and the data showed that miR-194-5p was significantly decreased in adult patients with epilepsy compared to the healthy control groups.[19–21] In this study, primary hippocampal cells were collected from the newborn rats. To mimic TLE condition in children, the expression level of miR-194-5p in children with TLE was assessed after treating with the magnesium-free medium for 3 hours. The result was consistent with the condition in TLE children. Furthermore, analysis of ROC showed that miR-194-5pis of great significance to the diagnosis of TLE children and yielded AUC of 0.896 with 81.0% sensitivity and 88.1% specificity, and based on the research results, children patients with TLE can be distinguished from those without TLE. This result is obtained by referring to the results of a previous

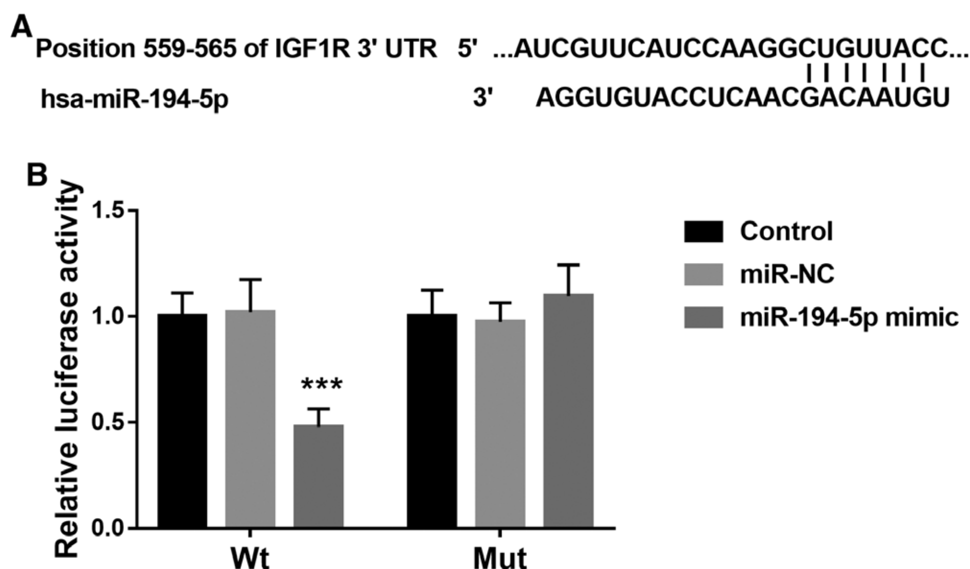


**Fig. 5** IFG1R is a direct target of miR-194-5p. A, Bioinformatics analysis indicated that miR-194-5p contains binding sites for IGF1R. B, Overexpression of miR-194-5p reduced the luciferase activity of cells transfected with Wt-3′-UTR of IGF1R. But miR-194-5p mimic transfection did not affect the luciferase activity of cells transfected with Mut-3′-UTR of IGF1R. ***$p < 0.001$. IGF1R = insulin-like growth factor 1 receptor.

study which found that the expression of miR-194-5p in plasma was decreased in epilepsy patients.[35] However, another study in the adult with TLE showed that miR-194-5p yielded AUC of 0.740 with 77.3% sensitivity and 60.0% specificity, which might be associated with different pathogenesis in adults and children. Additionally, several other diagnostic biomarkers have been identified in TLE children in previous studies, such as miR-134, S100 calcium-binding protein B, and matrix metallopeptidase 9.[36,37] In the future, it will be interesting and important to assess the diagnose value of combined multi-markers in TLE children.

In the current study, the influence of miR-194-5p on the cell proliferation and apoptosis of hippocampal cells was further studied. The expression level of miR-194-5p in hippocampal cells was regulated through cell transfection. The results showed that upregulated of miR-194-5p attenuated the TLE-induced abnormal behavior of hippocampal cells, and the neuroprotection role is possibly a potential mechanism of the involvement of miR-194-5p in the progress of children TLE. Furthermore, IGF1R was proved to be a direct target gene of miR-194-5p. IGF1R is the IGF1 receptor, which is distributed in the cytomembranes of neurons, astrocytes, and microglia.[38] Recent research has shown that IGF1R is upregulated in human epileptogenic tissue, while the inhibition of IGF1R is known to ameliorate the epileptiform activities in the pilocarpine and pentylenetetrazole rat models, which suggests that IGF1R is a promising target for the epilepsy therapy.[39] In the light of these findings, we proposed that the overexpression of miR-194-5p might alleviate the development of TLE by targeting IGF1R.

There are still some limitations with the present study. First, in the current study, only the targe relationship between miR-194-5p and IGF1R was confirmed. The concrete role of IGF1R in TLE should be further investigated by future studies. In addition, for qRT-PCR, U6 was used as the internal reference for the normalization of serum miR-194-5p levels in TLE. At the same time, it was found that the ct value of U6 in controls and TLE patients showed no significant difference. However, as reported by previous researches reported, miRNA or other RNA is not widely accepted as a good calibrator for exosomes or serum. According to the present results, it is presumptuous to say that U6 is an appropriate internal reference for the normalization of serum miRNA levels in TLE, because the population involved in the present study is relatively small, and the accurate level of U6 was not analyzed in the serum of samples from the control group and patients group. In future studies, it will be necessary and interesting to detect the accurate level of U6 in the serum of TLE patients through the performance of the digital PCR method, and find out whether U6 is an appropriate internal reference for normalization of serum miRNA levels in TLE.

In conclusion, we demonstrated that miR-194-5p was significantly downregulated in children with TLE, and its overexpression is associated with the proliferation and the apoptosis of hippocampal cells. Furthermore, this study suggested that the decrease in the expression of miR-194-5p could be a potential noninvasive biomarker that can be used in the diagnosis of TLE in children, and IGF1R was the target gene of miR-194-5p. Our results develop a new avenue for miRNA biology in children with TLE. Meanwhile, the upregulation of miR-194-5p may have the potential in improving the treatment of TLE. Further research is needed in the field of the function and molecular mechanism of miR-194-5p in children with TLE. In the future, we plan to study the expressions of miR-194-5p in epilepsy in much more children patients and explore its possible function and molecular mechanism for further evaluation.

## REFERENCES


1. Wu X, Wang Y, Sun Z, Ren S, Yang W, Deng Y, et al. Molecular expression and functional analysis of genes in children with temporal lobe epilepsy. *J Integr Neurosci* 2019;**18**:71–7.
2. Vanicek T, Hahn A, Traub-Weidinger T, Hilger E, Spies M, Wadsak W, et al. Insights into intrinsic brain networks based on graph theory and PET in right- compared to left-sided temporal lobe epilepsy. *Sci Rep* 2016;**6**:28513.
3. Bowler RM, Kornblith ES, Gocheva VV, Colledge MA, Bollweg G, Kim Y, et al. Environmental exposure to manganese in air: associations with cognitive functions. *Neurotoxicology* 2015;**49**:139–48.
4. Lee YJ, Kang HC, Bae SJ, Kim HD, Kim JT, Lee BI, et al. Comparison of temporal lobectomies of children and adults with intractable temporal lobe epilepsy. *Childs Nerv Syst* 2010;**26**:177–83.
5. Raoof R, Bauer S, El Naggar H, Connolly NMC, Brennan GP, Brindley E, et al. Dual-center, dual-platform microRNA profiling identifies potential plasma biomarkers of adult temporal lobe epilepsy. *Ebiomedicine* 2018;**38**:127–41.
6. Chowdhury FA, Nashef L, Elwes RD. Misdiagnosis in epilepsy: a review and recognition of diagnostic uncertainty. *Eur J Neurol* 2008;**15**:1034–42.
7. Pitkänen A, Löscher W, Vezzani A, Becker AJ, Simonato M, Lukasiuk K, et al. Advances in the development of biomarkers for epilepsy. *Lancet Neurol* 2016;**15**:843–56.
8. Ma J, Shang S, Wang J, Zhang T, Nie F, Song X, et al. Identification of miR-22-3p, miR-92a-3p, and miR-137 in peripheral blood as biomarker for schizophrenia. *Psychiatry Res* 2018;**265**:70–6.
9. Mahmoudi E, Cairns MJ. MiR-137: an important player in neural development and neoplastic transformation. *Mol Psychiatry* 2017;**22**:44–55.
10. Fabris L, Ceder Y, Chinnaiyan AM, Jenster GW, Sorensen KD, Tomlins S, et al. The potential of MicroRNAs as prostate cancer biomarkers. *Eur Urol* 2016;**70**:312–22.
11. Hunt EA, Broyles D, Head T, Deo SK. MicroRNA detection: current technology and research strategies. *Annu Rev Anal Chem (Palo Alto Calif)* 2015;**8**:217–37.
12. Sun Y, Wang X, Wang Z, Zhang Y, Che N, Luo X, et al. Expression of microRNA-129-2-3p and microRNA-935 in plasma and brain tissue of human refractory epilepsy. *Epilepsy Res* 2016;**127**:276–83.
13. Malan-Müller S, Hemmings SM, Seedat S. Big effects of small RNAs: a review of microRNAs in anxiety. *Mol Neurobiol* 2013;**47**:726–39.
14. Wang W, Kwon EJ, Tsai LH. MicroRNAs in learning, memory, and neurological diseases. *Learn Mem* 2012;**19**:359–68.
15. Saugstad JA. MicroRNAs as effectors of brain function with roles in ischemia and injury, neuroprotection, and neurodegeneration. *J Cereb Blood Flow Metab* 2010;**30**:1564–76.
16. Henshall DC, Hamer HM, Pasterkamp RJ, Goldstein DB, Kjems J, Prehn JHM, et al. MicroRNAs in epilepsy: pathophysiology and clinical utility. *Lancet Neurol* 2016;**15**:1368–76.
17. Korotkov A, Mills JD, Gorter JA, van Vliet EA, Aronica E. Systematic review and meta-analysis of differentially expressed miRNAs in experimental and human temporal lobe epilepsy. *Sci Rep* 2017;**7**:11592.
18. Mooney C, Becker BA, Raoof R, Henshall DC. EpimiRBase: a comprehensive database of microRNA-epilepsy associations. *Bioinformatics* 2016;**32**:1436–8.
19. Wang J, Yu JT, Tan L, Tian Y, Ma J, Tan CC, et al. Genome-wide circulating microRNA expression profiling indicates biomarkers for epilepsy. *Sci Rep* 2015;**5**:9522.
20. An N, Zhao W, Liu Y, Yang X, Chen P. Elevated serum miR-106b and miR-146a in patients with focal and generalized epilepsy. *Epilepsy Res* 2016;**127**:311–6.
21. Wang J, Tan L, Tan L, Tian Y, Ma J, Tan CC, et al. Circulating microRNAs are promising novel biomarkers for drug-resistant epilepsy. *Sci Rep* 2015;**5**:10201.
22. Nunez J. Primary culture of hippocampal neurons from P0 newborn rats. *J Vis Exp* 2008; **29**: 895.
23. Yuan J, Huang H, Zhou X, Liu X, Ou S, Xu T, et al. MicroRNA-132 interact with p250GAP/Cdc42 pathway in the hippocampal neuronal culture model of acquired epilepsy and associated with epileptogenesis process. *Neural Plast* 2016;**2016**:5108489.
24. Wang SQ, Li XJ, Zhou S, Sun DX, Wang H, Cheng PF, et al. Intervention effects of *Ganoderma lucidum* spores on epileptiform discharge hippocampal neurons and expression of neurotrophin-4 and N-cadherin. *PLoS One* 2013;**8**:e61687.

25. Zang C, Liu X, Li B, He Y, Jing S, He Y, et al. IL-6/STAT3/TWIST inhibition reverses ionizing radiation-induced EMT and radioresistance in esophageal squamous carcinoma. *Oncotarget* 2017;**8**:11228–38.
26. Nickels KC, Wong-Kisiel LC, Moseley BD, Wirrell EC. Temporal lobe epilepsy in children. *Epilepsy Res Treat* 2012;**2012**:849540.
27. Radhakrishnan A, Menon R, Abraham M, Vilanilam G, Sharma S, Thomas B, et al. Predictors of outcome after surgery in 134 children with drug-resistant TLE. *Epilepsy Res* 2018;**139**:150–6.
28. Gascoigne MB, Smith ML, Barton B, Webster R, Gill D, Lah S. Attention deficits in children with epilepsy: preliminary findings. *Epilepsy Behav* 2017;**67**:7–12.
29. D'Angelo E, Zanon C, Sensi F, Digito M, Rugge M, Fassan M, et al. miR-194 as predictive biomarker of responsiveness to neoadjuvant chemoradiotherapy in patients with locally advanced rectal adenocarcinoma. *J Clin Pathol* 2018;**71**:344–50.
30. Parvaee P, Sarmadian H, Khansarinejad B, Amini M, Mondanizadeh M. Plasma level of microRNAs, miR-107, miR-194 and miR-210 as potential biomarkers for diagnosis intestinal-type gastric cancer in human. *Asian Pac J Cancer Prev* 2019; **20**: 1421–6.
31. Ran RZ, Chen J, Cui LJ, Lin XL, Fan MM, Cong ZZ, et al. miR-194 inhibits liver cancer stem cell expansion by regulating RAC1 pathway. *Exp Cell Res* 2019;**378**:66–75.
32. Wang B, Shen ZL, Gao ZD, Zhao G, Wang CY, Yang Y, et al. MiR-194, commonly repressed in colorectal cancer, suppresses tumor growth by regulating the MAP4K4/c-Jun/MDM2 signaling pathway. *Cell Cycle* 2015;**14**:1046–58.
33. Wang XR, Zhang XM, Zhen J, Zhang PX, Xu G, Jiang H. MicroRNA expression in the embryonic mouse inner ear. *Neuroreport* 2010;**21**:611–7.
34. Cao H, Shi J, Du J, Chen K, Dong C, Jiang D, et al. MicroRNA-194 regulates the development and differentiation of sensory patches and statoacoustic ganglion of inner ear by Fgf4. *Med Sci Monit* 2018;**24**:1712–23.
35. Ma Y. The challenge of microRNA as a biomarker of epilepsy. *Curr Neuropharmacol* 2018;**16**:37–42.
36. Avansini SH, de Sousa Lima BP, Secolin R, Santos ML, Coan AC, Vieira AS, et al. MicroRNA hsa-miR-134 is a circulating biomarker for mesial temporal lobe epilepsy. *PLoS One* 2017;**12**:e0173060.
37. Meguid NA, Samir H, Bjørklund G, Anwar M, Hashish A, Koura F, et al. Altered S100 calcium-binding protein B and matrix metallopeptidase 9 as biomarkers of mesial temporal lobe epilepsy with hippocampus sclerosis. *J Mol Neurosci* 2018;**66**:482–91.
38. Li J, Choi E, Yu H, Bai XC. Structural basis of the activation of type 1 insulin-like growth factor receptor. *Nat Commun* 2019;**10**:4567.
39. Fu P, Yuan Q, Sun Y, Wu X, Du Z, Li Z, et al. Baicalein ameliorates epilepsy symptoms in a pilocarpine-induced rat model by regulation of IGF1R. *Neurochem Res* 2020;**45**:3021–33.