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MiR-130a exerts neuroprotective effects against ischemic stroke through PTEN/PI3K/AKT pathway

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ABSTRACT

Background: Ischemic stroke is significantly affected by the dysfunction of the miRNA network. Recent research has described that disordered expression of miR-130a is associated with ischemic stroke. Here, we aimed to investigate the possible mechanism of the miR-130a-mediated neuroprotection that follows ischemia-reperfusion (I/R) injury.

Method: This study was comprised of two models: oxygen-glucose deprivation/Reperfusion (OGDR) and middle cerebral artery occlusion (MCAO). RT-PCR and immunoblotting were used to examine gene expression levels, and MTT assay and flow cytometric analysis were used to examine cell states. We also used 2, 3, 5-triphenyltetrazolium chloride (TTC) staining to assess the cerebral infarct volume. Then, we employed bioinformatics analysis and luciferase reporter assay to identify and validate the target molecule of miR-130a, PTEN.

Results: Our findings indicated that miR-130a expression was lower in PC12 cells after OGDR (oxygen-glucose deprivation/reperfusion) and in rats after MCAO (middle cerebral artery occlusion). Moreover, ectopic-expression of miR-130a can significantly improve cell survival rate and reduce cell apoptosis and ROS production in PC12 cells after OGDR. In addition, re-expression of miR-130a yielded an obvious reduction in MCAO-induced infarct volume and neurological deficits in rats. Bioinformatics analysis revealed that PTEN was a miR-130a target and could overturn the effect of miR-130a on cerebral ischemia, both *in vivo and in vitro*. Therefore, we set out to further investigate the PTEN-affected PI3K/AKT pathway and found that upregulation of miR-130a activated the PI3K/AKT pathway.

Conclusions: Our data demonstrated that miR-130a prevented cerebral I/R damage by mediating the PTEN/PI3K/AKT axis. These preliminarily findings furthered our understanding of this mechanism and identified new potential therapeutic targets for ischemic stroke.

1. Introduction

Stroke has been, and continues to be, an important cause of death and disability in many industrialized nations. In recent decades, much research has been devoted to the exploration of stroke pathology, with special focus on identifying novel targets for the development of potential new treatments. Among all stroke cases, greater than 80% are attributed to ischemic stroke, the cause for which is mainly stenosis or occlusion of the arteries that provide blood supply to the brain, the carotid artery and vertebral artery [1,2]. Cerebral ischemic injury is a complicated pathological and physiological process, involving energy failure, acidosis, calcium overload, excitatory amino acid toxicity, mitochondrial damage, oxidative stress, and the inflammatory response. These mechanisms of damage ultimately result in cell necrosis or apoptosis [3–5]. Based on clinical guidelines at home and abroad, the most effective treatment for acute cerebral ischemia is reperfusion with thrombolysis in the appropriate time window. However, this treatment can aggravate the inflammation or glutamate excitotoxicity that is

Abbreviations: I/R, ischemia-reperfusion; OGDR, Oxygen-glucose deprivation/Reperfusion; MCAO, middle cerebral artery occlusion; PTEN, phosphatase and tensin homologous protein; ATCC, American Type Culture Collection; RT-PCR, real-time polymerase chain reaction; SD, Sprague-Dawley; ECA, external carotid artery; ICA, internal carotid artery; CCA, common carotid artery; MCA, middle cerebral artery; mNSS, modified Neurological Severity Score; NC, normoxic control; PI, Propidium Iodide

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induced by cerebral ischemia, which can result in cerebral ischemia-reperfusion (I/R) injury [6,7]. Current research efforts center on improving patient outcomes and neurological function through the reduction of infarct volume, apoptosis, and inflammatory responses following ischemic injury. Therefore, it is an urgent task to reveal the mechanisms of cell death and neurological dysfunction that are induced by cerebral I/R injury, which required for advancing both our understanding of these processes as well as potential treatment options.

Previous studies have shown that miRNAs are closely related to cell growth and development, and to date many miRNAs have been clinically used as therapeutic guides for patients with metabolic diseases and cancers [8–10]. Recently, a number of clinical studies have shown that miRNAs, such as miR-125 and miR-143 [11–13]. In addition, it was reported that knockdown of miR-15a attenuated ischemic brain injury, providing a novel therapeutic target for ischemic stroke [14]. In one study, miR-130a in particular was downregulated in the plasma of acute ischemic stroke patients and served as a promising biomarker [15]. Similarly, Wang Y et al showed that miR-130a regulated the permeability of the blood-brain barrier induced by cerebral ischemia [16]. Despite these efforts, the molecular mechanism by which miR-130a exerts its neuroprotective effect after cerebral I/R injury remains unknown.

As we all know, phosphatase and tensin homologous protein (PTEN) negatively regulates the PI3K/AKT pathway, and importantly, PI3K/AKT/PTEN signaling is directly associated with tumor cell survival and apoptosis [17]. Previous study had showed that PTEN showed distinct functions in response to stress and might be involved in different mechanisms of neuroprotection [18]. Moreover, PTEN signaling pathway was involved in the anti-apoptotic effect of electroacupuncture following ischemic stroke in rats [19]. Furthermore, another report found that inhibition of PTEN protected against OGDR (Oxygen-glucose deprivation/ reperfusion)-induced cell death by PI3K/AKT signaling pathway [20]. However, whether or not miR-130a attenuates cerebral I/R injury by PTEN/PI3K/AKT pathway has not yet been investigated.

In this study, we explore the function that miR-130a exerts in the neuroprotection that follows cerebral I/R injury and to uncover the underlying mechanisms. This study has the potential to provide novel therapeutic targets for cerebral I/R injury.

2. Materials and methods

2.1. Cell culture

PC12 cells were acquired from American Type Culture Collection (ATCC, VA, USA) and cultured under conditions specified by the manufacturer. Concretely, DMEM/HIGH GLUCOSE medium (HyClone, Logan, Utah, USA), supplemented with 10% FBS fetal bovine serum (Gibco, USA), was used.

2.2. OGDR model

In order to generate I/R-like conditions by OGDR *in vitro*, PC12 cells were placed in a 37 °C anaerobic chamber (0.2% O_2, 5% CO_2, 95% N_2) and cultured in glucose-free medium for 3 h. After the oxygen-glucose deprivation, the cells were placed in glucose-containing DMEM with 10% FBS and then incubated under normoxic conditions for 24 h in order to affect reperfusion. Controls samples were taken from cells cultured under normal conditions.

2.3. Cell transfection

Vectors with miR-130a mimic/inhibitor, negative control, and PTEN over-expression were obtained from Genechem (Shanghai, China). After inoculation at the appropriate density, Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, USA) was used to transfect the cells. After 48 h, cells were subjected to OGDR treatment and harvested for the next study.

2.4. MTT assay

Cell viability of PC12 cells treated with OGDR under different transfection was evaluated by MTT assay First, PC12 cells were seeded in 48-well microplates at a density of 10^4 cells/well. After 24 h, 20 μL MTT solution was added to each well for another 4 h at 37 °C. Next, the crystals were melted fully with 150 μL DMSO then shaken for 10 min. Subsequently, absorbance measurements were done with a microplate reader (Thermo, MA, USA) at 490 nm to count survival cells.

2.5. Quantitative PCR

Isolation of total RNA was carried out in PC12 cells using TRIzol (Invitrogen, Shanghai, China). RT-PCR and data collection were performed with Reverse Transcriptase XL (AMV) (Takara, Dalian, China) and TB Green Premix EX Taq (Takara, Dalian, China). The primer sequences were miR-130a-F: 5′-ACACTCCAGCTGGGGCTCTTTTCACAT TGT-3′; miR-130a-R: 5′-CTCAACT GGTGTCGTGGAGTCGGCAATTCAG TTGAGAGTAGCAC-3′; PTEN-F: GTGCA GATAATGACAAG; PTEN-R: GATTTGACGGCTCCTCT; U6-F: CTCGCTTCGGC AGCAC; U6-R: ACGC TTCACGAATTTGC; GAPDH-F: 5′-GGAGCGAGATCCCT CCAAAAT-3′; GAPDH-R: 5′-GGCTGTTGTC ATACTTCTCATGG-3′. The levels of gene expression or miR-130a expression were normalized to GAPDH or U6 controls.

2.6. Immunoblotting

The cells or samples were gathered and homogenized in icy buffer. Afterwards, we collected the supernatant containing the total protein and used the BCA method to detect the protein concentration. Using lysis buffer, the total protein concentration of each group was adjusted to to contain equal amounts of protein. Next, we performed SDS-PAGE electrophoresis (8–15%) followed by transfer to polyvinylidene difluoride membranes. The blots were then incubated with primary antibody. Primary antibodies used are as follows: rabbit anti-PTEN (1:1000, ab32199, abcam), AKT (1:500, ab8805, abcam), p-AKT (1:1000, ab38449, abcam), GSK3β (1:5000, ab32391, abcam), p-GSK3β (1:1000, ab75745, abcam), p-c-Raf (1:1000, 9421, CST), p-BAD (1:1000, 5248, CST). Next, corresponding IgG-HRP secondary antibodies (1:2000, sc-2004, Santa cruz) were added and incubated for 1 h. Finally, chemiluminescence was measured using a GelDoc-2000 Imagine System in order to visualize the signal bands and perform the quantification. The amount of protein was normalized to the GAPDH value.

2.7. Flow cytometric analysis

Detection of apoptosis in PC12 cells was carried out using the Annexin V-FITC apoptosis detection kit (Beyotime, Shanghai, China). First, PC12 cells were washed twice with ice-cold 1 × PBS, after which the experiment was suspended using 100 μL 1 × Binding Buffer. After staining with Annexin V-EGFP and Propidium Iodide (PI), cells were detected using CytoFLEX flow cytometer (BD, NJ, USA). Two different tubes were analyzed for each treatment, and the experiment itself was performed at least in triplicate.

The oxidation-sensitive probe DCFH-DA was used for measurement of ROS formation in PC12 cells after OGDR by flow cytometric. Cells that underwent different treatments were immediately detached with trypsin and then incubated with 10 μM DCFH-DA for 30 min.. Finally, FAC Scan flow cytometer (BD, NJ, USA) was used to measure the cellular fluorescence intensity (ex490 nm, em 526 nm).

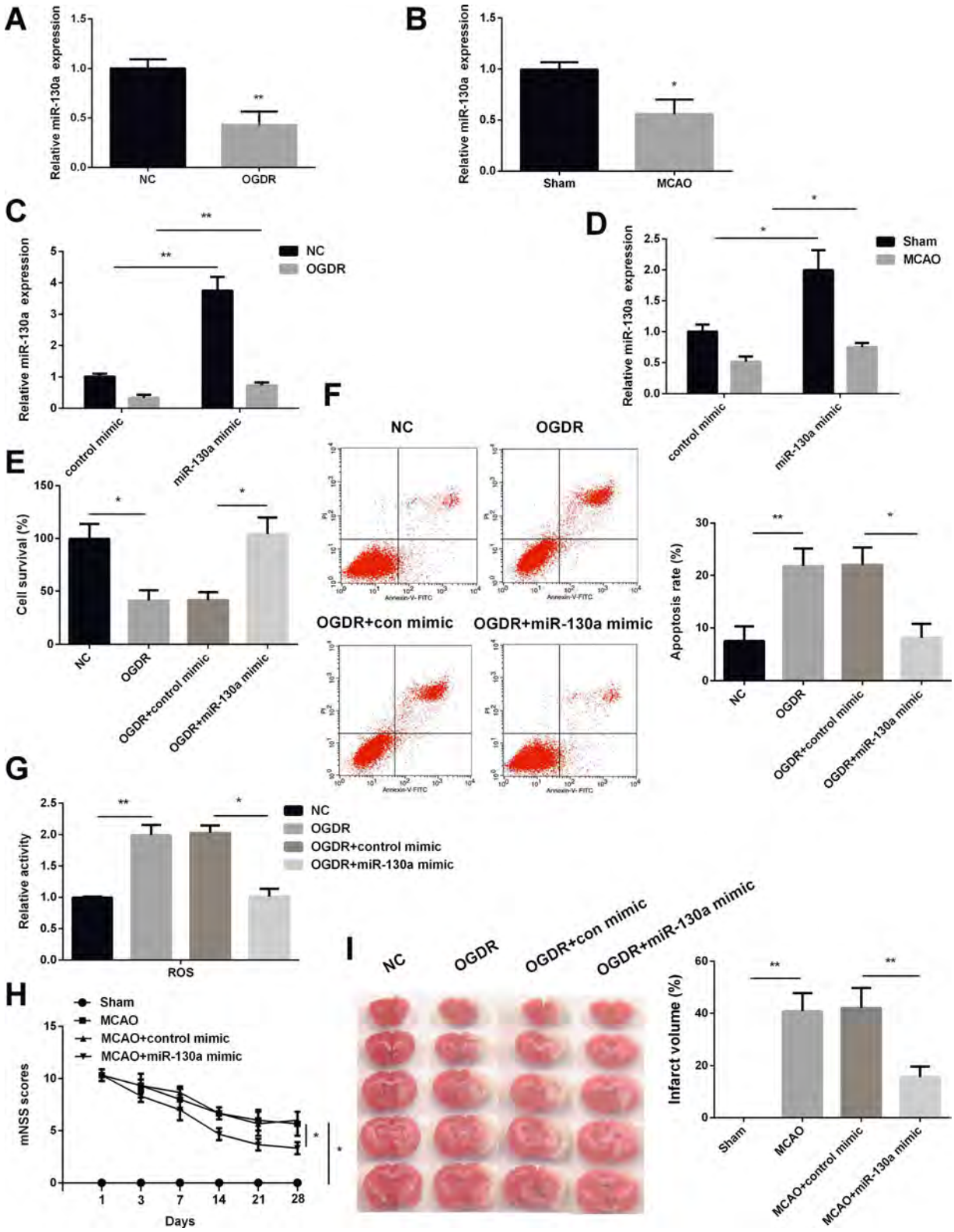

Fig. 1. The neuroprotective effect of miR-130a on cerebral I/R injury *in vivo and in vitro* (A) Measurement of miR-130a expression in OGDR-induced PC12 cells and MCAO rats (B) by RT-PCR. (C) The expression of miR-130a in PC12 cells transfected with miR-130a mimic as detected by RT-PCR. (D) The expression of miR-130a in SD rats injected with miR-130a mimic as detected by RT-PCR. (E) Survival of OGDR-simulated PC12 cells with miR-130a-expressing plasmid as detected by MTT assay. (F) Apoptosis of OGDR-simulated PC12 cells after transfection with miR-130a mimic as detected and quantified by flow cytometry assay. (G) The production of ROS detected in OGDR-simulated PC12 cells with miR-130a-expressing plasmid by flow cytometry assay. (H) The mNSS scores assessed in MCAO rats injected with miR-130a mimic. (I) The infarct volume stained and quantified in MCAO rats injected with miR-130a mimic. $^{*}P < 0.05$, $^{**}P < 0.01$.

2.8. Luciferase assay

The 3′ UTRs of wild and mutated PTEN (PTEN 3′ UTR-WT or 3′ UTR-MUT) were severally inserted downstream of the pGL3 reporter vector (Promega, Madison, USA). Then, vectors with miR-130a mimic/inhibitor and the PTEN-pGL3 reporter were co-transfected into cells. Two days post transfection, luciferase activities were assessed using a Dual-Luciferase Reporter System (Promega, Madison, USA) on a Gen5 Microplate Reader (BioTek).

2.9. Rat model

Adult male Sprague-Dawley rats weighing 260–280 g were obtained from Sparford Biology Co., Ltd. (Beijing, China) and fed on standard pellet chow and water. Prior to the implementation of the cerebral ischemia model, rates were fasted for 12 h without water deficiency. This experiment was approved by the Institutional Animal Care and Use Committee of Peking University Shenzhen Hospital. Anesthetized rats were treated with middle cerebral artery occlusion (MCAO) for 30 min in order to produce the transient focal cerebral ischemia model. Next, the right external and internal carotid artery and the common carotid artery (CCA) were isolated. Then, the right external and common carotid artery were ligated distally and proximally, respectively. A nylon filament (0.26 mm, rounded tip) was inserted into the ICA from the ECA until it reached the middle cerebral artery (MCA). At this time, the blood supply of the MCA was blocked, resulting in middle cerebral artery occlusion. After 30 min of ischemia, the filament was withdrawn by inches to accomplish the reperfusion. The sham operations were performed without nylon filaments. The rectal temperature was kept at 37 ± 0.5 °C following the MCAO operation. The anesthetic rats were placed on a temperature control carpet before waking up. Intraventricular injection of miR-130a mimic/inhibitor or control was performed two days prior to MCAO.

Neurological score The degree of neurological deficits in rats after MCAO was assessed at 1, 3, 7, 14, 21, and 28 days using a modified Neurological Severity Score (mNSS) test. Before the operation, the researchers conducted a three-day continuous training on the rats. The scores recorded on the day before surgery were taken as a baseline. Neurological scores reflected the total score of motor, sensory, reflex, and balance disorders. The score obtained from each rat was equal to the sum of all test scores ranging from 0 (normal score) to 18 (maximum defect score).

2.10. The assessment of infarct volume

Infarct volume was assessed using 2, 3, 5-triphenyltetrazolium chloride (TTC) staining. First, the rat brain was cut into 6 coronal slices at 2 mm thick. Next, 2% TTC was applied to stain coronal slices, and then fixed in 10% formalin. After the image is collected, the infarct volume was measured using Image-Pro Plus6.0. Calculation of the infarct volumes was performed with the following formula: 100 × (lesion volume/total brain volume). Researchers performing this analysis were blinded to the experimental group.

2.11. Statistical analysis

All of the statistical analyses were performed using SPSS 17.0. Each experiment was carried out more than 3 times. Differences between the groups were analyzed using ANOVA followed by Tukey's multiple range post-hoc test. A value for $P < 0.05$ was considered as to be a significant difference.

3. Results

3.1. MiR-130a suppresses cerebral I/R injury

In order to examine the neuroprotective effect of miR-130a in cerebral ischemic injury, we first observed the level of miR-130a in ischemic stroke both *in vivo and vitro*. PC12 cells were treated with OGDR for 3 h and then allowed to recover for 24 h to mimic I/R-like conditions *in vitro*. Analysis of RT-PCR data revealed the downregulation of miR-130a in OGDR PC12 cells when compared with normoxic control (NC) (Fig. 1A). Additionally, we employed an *in vivo* model for cerebral I/R injury where SD rats were subjected to MCAO for 30 min and then re-perfused for 24 h. The results displayed that miR-130a expression in the rats treated with MCAO was lower than that in the sham group (Fig. 1B). Next, we investigated whether miR-130a exerted a protective effect on cerebral I/R injury. To do this we transfected untreated PC12 cells with miR-130a mimic or inhibitor before intraventricular injection into rats prior to MCAO. Fig. 1C and D show that miR-130a was increased in the presence of its mimic and decreased in the presence of its inhibitor, both *in vivo and vitro*. Next, we used the MTT assay to determine PC12 cell survival after OGDR. The results revealed that OGDR treatment significantly inhibited the survival of PC12 cells when compared with the normoxic control, while overexpression of miR-130a resulted in an obvious increase in PC12 cell survival when compared with control mimic group (Fig. 1E). However, the results of cell apoptosis analysis indicated that the apoptosis rate of PC12 cells increased in the OGDR group, while there was a decrease in the rate of apoptosis of PC12 cells in the miR-130a mimic group (Fig. 1F). Furthermore, we detected ROS production in PC12 cells following OGDR. Compared with the normoxic control, ROS production increased in the OGDR group, whereas it decreased after expressing miR-130a mimic. Moreover, we assessed the neurological outcome of SD rats using the mNSS scores and values for infarct volume after MCAO. We found that, in the SD rats, the MCAO-operated group exhibited an increase in mNSS score, while the upregulation of miR-130a resulted in a decrease in the mNSS scores of MCAO rats, as shown in Fig. 1H. Results of TTC staining demonstrated that ischemic injury correlated with a larger the infarct volume than compared to the sham group, whereas miR-130a mimic effectively reduced the infarct volume in comparison with the control mimic group (Fig. 1I). Collectively, these results provide solid support for the neuroprotective effect that miR-130a exhibited on cerebral I/R injury.

3.2. PTEN levels were reduced by miR-130a after I/R injury

To investigate the molecular mechanism of miR-130a's neuroprotection after cerebral I/R injury, TargetScan (miRNA target analysis tool) was applied to predict targets of miR-130a in PC12 cells. The results showed that PTEN was a potential target for miR-130a (Fig. 2A). To verify the above prediction and to further confirm whether PTEN interacts with miR-130a, we used the luciferase reporter assay. We found that overexpression of miR-130a decreased the luciferase activity of WT-PTEN, but that silencing of miR-130a elevated it (Fig. 2B). However, there was no change in the MUT-PTEN luciferase activity. This finding suggested a direct interaction between PTEN and miR-130a. To further our understanding of this mechanism, we investigated whether or not miR-130a regulated PTEN expression. We found that miR-130a mimic was able to inhibit PTEN expression in PC12 cells induced by OGDR, while miR-130a inhibitor elevated PTEN expression by western blot and RT-PCR (Fig. 2C and D). We then detected the expression of PTEN in 4 MCAO-operated rats injected with miR-130a mimic. Our results showed that miR-130a mimic decreased PTEN protein level *in vivo* (Fig. 2E and F). Briefly, the above data stated that miR-130a reduced PTEN expression by binding to its 3′-UTR *in vivo* and *in vitro*.

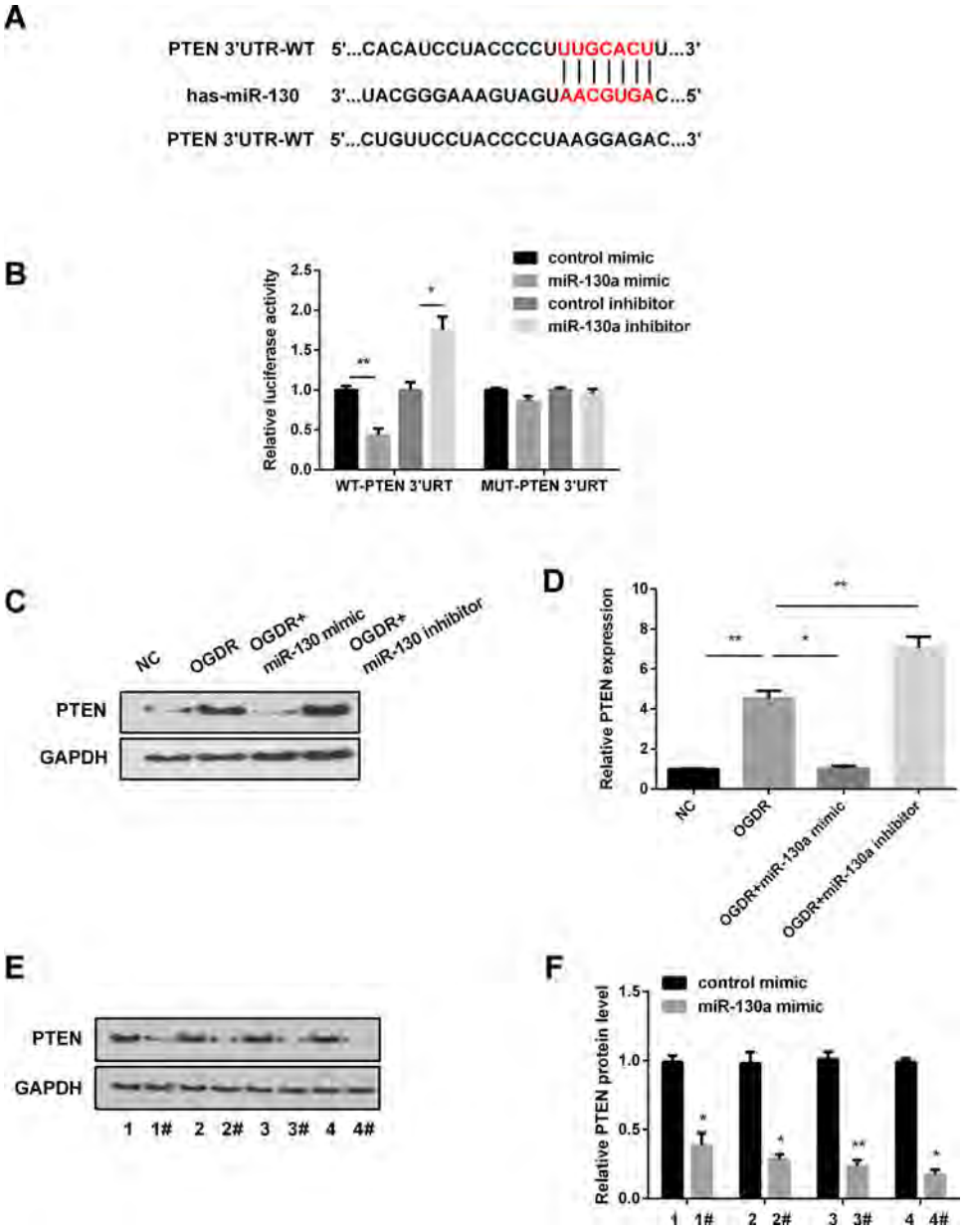

Fig. 2. (A) The predicted binding sites between miR-130a and PTEN. (B) The luciferase activity of PTEN 3′UTR-WT or -MuT detected in PC12 cells with miR-130a-expressing/inhibiting plasmid as measured by Dual-luciferase Reporter Assay. (C) Measurement of PTEN protein levels in OGDR-simulated PC12 cells with miR-130a-expressing/inhibiting plasmid by western blot. (D) Measurement of PTEN mRAN expression in OGDR-simulated PC12 cells with miR-130a-expressing/inhibiting plasmid by RT-PCR. (E and F) Western blot analysis of PTEN levels in the brains of MCAO rats injected with miR-130a-expressing/inhibiting plasmid. $^{*}P < 0.05$, $^{**}P < 0.01$.

3.3. PTEN attenuated miR-130a neuroprotection in cerebral I/R injury

Here, we investigated the function of PTEN in the miR-130a-mediated protective effect on cerebral I/R injury. Prior to OGDR, PTEN vector was transfected into PC12 cells or SD rats containing control mimic or miR-130a mimic. First, miR-130a mimic attenuates the OGDR-induced apoptosis rate of PC12 cell, while overexpression of PTEN reversed the inhibitory effect of miR-130a mimic on cell apoptosis (Fig. 3A). However, results of the MTT assay showed that miR-130a mimic apparently promote OGDR-induced PC12 cell survival, while over-expression of PTEN resulted in an obvious attenuation of the promoted effect of the miR-130a mimic on cell survival (Fig. 3B). In addition, miR-130a mimic inhibited ROS production, whereas PTEN vector reversed it, when compared with the OGDR group (Fig. 3C). Furthermore, when we assessed neurological outcome using the mNSS scores and infarct volumes of differently injected MCAO rats, it was found that miR-130a mimic decreased mNSS scores in the MCAO rats, whereas PTEN vector overturned the miR-130a effect (Fig. 3D). What's more, TTC staining results revealed that the increased level of miR-130a effectively attenuated the infarct volume of MCAO rats, while over-expression of PTEN increased the infarct volume (Fig. 3E). Together, these results indicated that PTEN reversed the neuroprotective effect of miR-130a on cerebral I/R injury.

3.4. The miR-130a/PTEN axis regulated the PI3K/AKT pathway

To clarify if the PI3K/AKT pathway takes part in the progression of cerebral I/R injury that is regulated by miR-130a/PTEN, downstream genes of the PI3K/AKT pathway were tested using western blots. As displayed in Fig. 4, in the OGDR group, the expression levels of p-AKT, p-GSK3β, p-c-Raf, and p-BAD were decreased significantly compared with normal control group. Upregulation of miR-130a resulted in an obvious increase in p-AKT expression compared to the OGDR group, while overexpression of PTEN showed the opposite effect (Fig. 4B). When compared with the OGDR group, the PTEN vector resulted in an obvious restoration of the decrease in p-GSK3β, p-c-Raf, and p-BAD expression that was induced by miR-130a mimic (Fig. 4C–E). The data also showed that there was no change in expression of AKT and GSK3β among the different treatments. These findings indicated that miR-130a promoted the PI3K/AKT pathway, which was inhibited by PTEN in

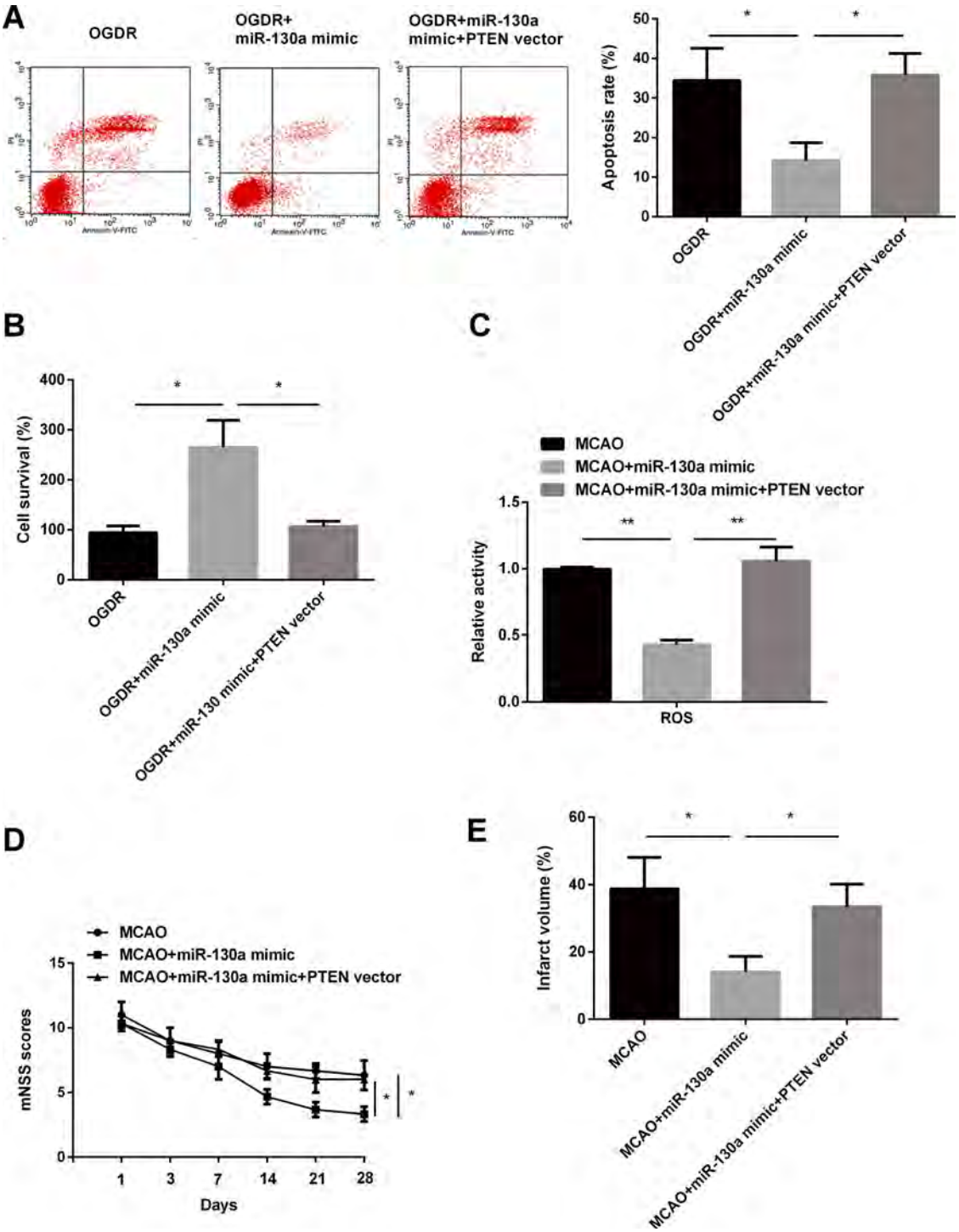

Fig. 3. Overexpression of PTEN relieved the protective effect of miR-130a mimic on I/R injury *in vivo and in vitro*. (A) Cell apoptosis was analyzed for OGDR-simulated PC12 cells after transfection with miR-130a mimic or combined with PTEN vector as detected and quantified by flow cytometry assay. (B) Survival of OGDR-simulated PC12 cells with miR-130a-expressing plasmid or combined with PTEN-expressing vector as detected by MTT assay. (C) The production of ROS detected in OGDR-simulated PC12 cells with miR-130a-expressing plasmid or combined with PTEN-expressing vector by flow cytometry assay. (H) The mNSS scores assessed in MCAO rats injected with miR-130a mimic or combined with PTEN vector. (I) The infarct volume of MCAO rats injected with miR-130a mimic or combined with PTEN vector as quantified by TTC staining. $^{*}P < 0.05$, $^{**}P < 0.01$.

cerebral I/R injury. It suggested that miR-130a activates the PI3K/AKT pathway through the inhibition of PTEN.

4. Discussion

In this study, we used both rat I/R model and OGDR-induced brain neural cells to examine the effect of miR-130a. We found that in both models, the OGDR-simulated PC12 cell *in vitro* model and the rat MCAO *in vivo* model, miR-130a was significantly downregulated. Meanwhile, we also found that re-expression of miR-130a protected against cerebral I/R injury through the PTEN/PI3K/AKT signaling pathway, which provided a novel idea for the diagnosis and therapy of acute cerebral I/R injury.

Emerging evidence has found the involvement of miR-130a in

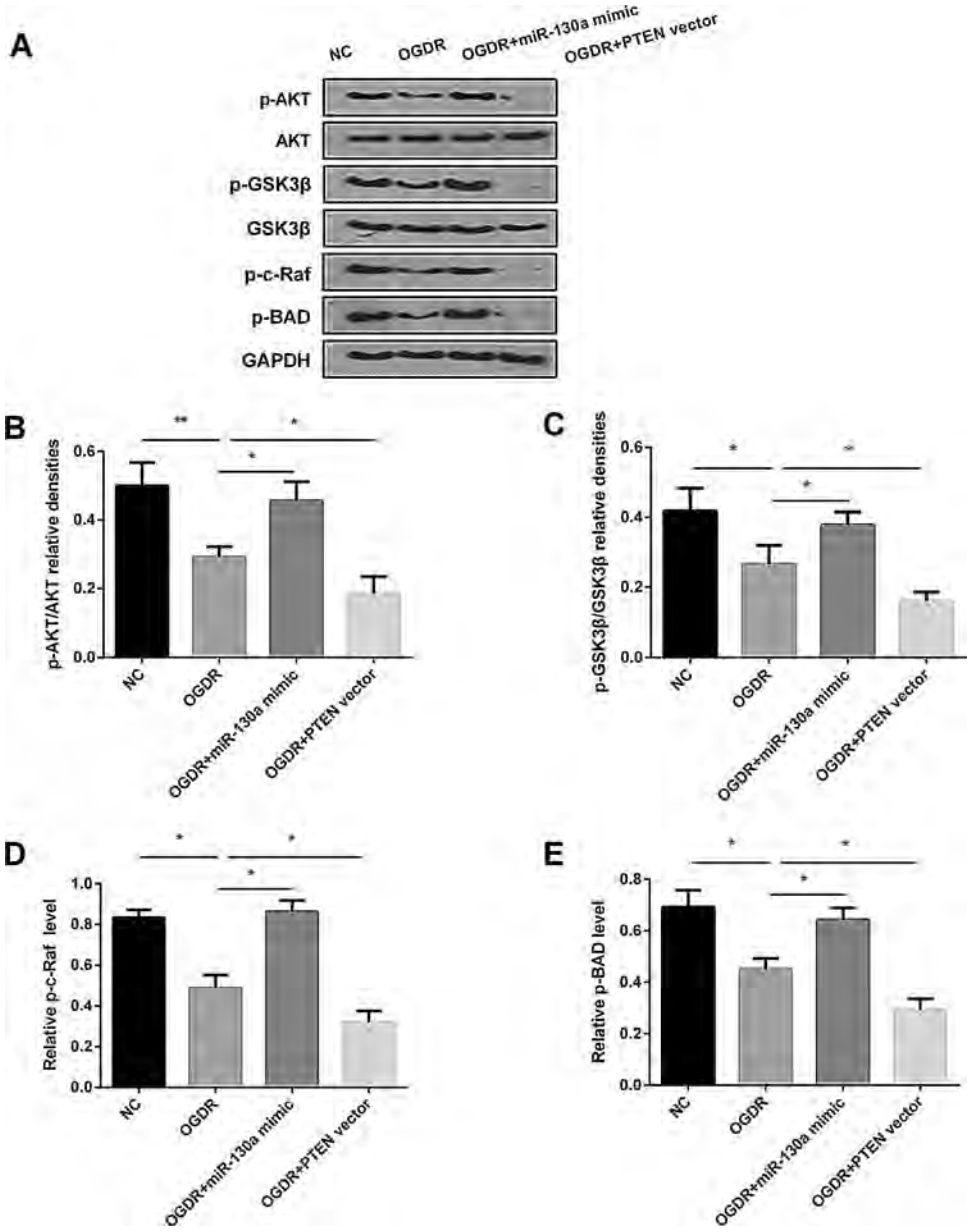

Fig. 4. Effect of miR-130a/PTEN on the PI3K/AKT axis. (A) Western blot detection of downstream genes of the PI3K/AKT signaling pathway in OGDR-simulated PC12 cells transfected with miR-130a mimic or PTEN vector, including AKT, p-AKT, GSK3β, p-GSK3β, p-c-Raf, and p-BAD. (B) Quantitation of the p-AKT/AKT relative densities in OGDR-simulated PC12 cells with miR-130a-expressing plasmid or PTEN-expressing vector. (C) Quantitation of the p-GSK3β/GSK3β relative densities in OGDR-simulated PC12 cells with miR-130a-expressing plasmid or PTEN-expressing vector. (D) Quantitation of the p-c-Raf level in OGDR-simulated PC12 cells with miR-130a-expressing plasmid or PTEN-expressing vector. (E) Quantitation of the p-BAD level in OGDR-simulated PC12 cells with miR-130a-expressing plasmid or PTEN-expressing vector. $^{*}P < 0.05$, $^{**}P < 0.01$.

multiple diseases, including tumors and cerebral ischemic injury. For instance, it was reported that miR-130a played an essential role in the tumor biology of esophageal squamous cell carcinoma [21]. Furthermore, it was discovered that miR-130a was upregulated in gastric cancer and promoted angiogenesis and cell growth [22]. In ischemic stroke, one study reported that the decrease level of miR-130a level was found in the plasma of patients and was negatively associated with disease risk [23]. This research displayed that the expression of miR-130a was decreased in cerebral ischemic injury. In our work, the upregulation of miR-130a in PC12 cells increased survival and decreased apoptosis and ROS production after OGDR. Moreover, in our *in vivo* model, miR-130a mimic reduced the mNSS scores and the infarct volume induced by MCAO.

It has been reported that suppression of PTEN increases activation of the PI3K/AKT axis. In recent years, many studies have been carried out for studying the neuroprotective effects of PTEN inhibitors in cerebral ischemic injury. Miao SY et al. stated that, in the OGDR and MCAO models, PTEN was overexpressed and also participated in the development of ischemic stroke *via* the PI3K/AKT pathway [19,24,25]. Likewise, we found that, in the current study, PTEN was upregulated in PC12 cells following OGDR and also in SD rats following MCAO. In addition, the overexpression of PTEN inhibited p-AKT activity. Studies have also found that PTEN has been reported to serve as a target of miR-21 and can participate in the protective effect against myocardial injury [26,27]. However, PTEN was originally proposed as a miR-130a target and overturned the neuroprotection of miR-130a in cerebral I/R injury. Besides that, miR-130a overexpression enhanced the activation of the PI3K/AKT axis through the inhibition of PTEN.

In conclusion, the evidence presented herein suggests that miR-130 can protect against cerebral ischemic injury. The overexpression of miR-130a enhanced the survival of PC12 cells, as well as suppressed their apoptosis and ROS production after OGDR. In rats, miR-130a also protected against neurological deficits following MCAO *via* activation of the PI3K/AKT axis and inhibition of PTEN. Because miR-130a protects against cerebral ischemic injury, it is a promising target for new treatments, although a clear and effective treatment window is still an important part of the next research. Clinically, the ability to reduce cerebral injury after ischemic stroke through the use of intravenous injection cannot be understated, and this research is expected to be translated to clinical application.

Funding

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Authors' contributions

Tingting Zheng carried out the research, performed the analysis, and participated in the writing of the manuscript. Yu Shi, Jun Zhang, Jiao Peng, Xue Zhang and Keke Chen performed the analysis and participated in the writing and reviewing of the manuscript. Yun Chen and Li Liu designed, supervised the research, and participated in the writing and reviewing of the manuscript. All authors have read and approved the final manuscript.

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