Overexpression of miR-582-5p Inhibits the Apoptosis of Neuronal Cells after Cerebral Ischemic Stroke Through Regulating PAR-1/Rho/Rho Axis

Hongsheng Ding, MD,* Shan Gao, MD,† Lei Wang, MM,* Yan Wei, MB,* and Meiyun Zhang, MD*

Objective: The purpose of this study was to explore the role of miR-582-5p/proteinase-activated receptors type I (PAR-1)/Rho/Rho in neuronal cell apoptosis after cerebral ischemic stroke (CIS). *Methods: In vivo* mouse model of CIS induced by middle cerebral artery occlusion and *in vitro* model induced by oxygen-glucose deprivation/reoxygenation (OGD/R) in N2A cells was established. The expressions of miR-582-5p, PAR-1, RhoA, and ROCKII in brain tissues and N2A cells were detected. Neuronal cell apoptosis was detected by flow cytometry. *Results:* We found that miR-582-5p expression was decreased and the expressions of PAR-1, RhoA, and ROCKII were increased in CIS mice and OGD/R model. Moreover, miR-582-5p negatively regulated PAR-1, and overexpression of miR-582-5p inhibited the activation of Rho/Rho pathway by downregulating PAR-1, thus reducing OGD/R-induced neuronal cell apoptosis. *Conclusions:* Our results suggested that miR-582-5p overexpression could regulate Rho/Rho-kinase signaling pathway via targeting PAR-1, thereby governing the apoptosis of neuronal cells after CIS.

Key Words: Cerebral ischemic stroke—neuronal cell apoptosis—miR-582-5p—PAR-1—Rho/Rho

Introduction

Cerebral ischemic stroke (CIS), also known as cerebral infarction, refers to the ischemic necrosis or softening of local cerebral caused by hypoxia and ischemia, which is seriously harmful to human health.[1] In recent years, the incidence and mortality of CIS is increasing and the diseased populations tend to be younger. Therefore, it is urgent to find effective treatments. Neuronal cell apoptosis is an important mechanism of neuronal injury in CIS.[2] Inhibiting neuronal cell apoptosis can improve neuronal injury following CIS.[3] Therefore, investigation of the molecular mechanism of neuronal cell apoptosis is conducive to explore the effective targets of preventing and treating the CIS-induced neuronal injury.

MicroRNAs (miRNAs) are a class of small noncoding RNAs, which have been confirmed to play a vital role in many biological processes, including cell migration, invasion, proliferation, and apoptosis.[4] Recent studies have found that miRNAs are closely related to the occurrence and development of CIS and can serve as potential targets for the diagnosis and treatment of CIS.[5,6] However, the underlying mechanisms of some miRNAs which abnormally expressed during the course of CIS have not been understood, such as miR-582-5p. MiR-582-5p has been identified as an important regulator in multiple cancers. For example, miR-582-5p could inhibit the development of colorectal carcinoma by targeting Rab27a.[7] In addition, miR-582-5p was demonstrated to decrease in traumatized cortex after traumatic brain injury, but the mechanism of miR-582-5p in neuronal cell apoptosis and neuroprotective in CIS has not been reported.

Rho/Rho-kinase signaling pathway is involved in the pathogenesis of various neurological diseases, including CIS[8]. Ras homolog family member A (RhoA), a member

From the *Department of Neurology, Tianjin Union Medical Center, Tianjin, China; and †Department of Neurology, ShangHai Jiaotong University affiliated the Sixth People Hospital, Shanghai, China.

Received August 7, 2018; revision received September 6, 2018; accepted September 14, 2018.

Funding: This research was supported by the Foundation of Tianjin Union Medical Center (Grant No.2016YJ040).

Address correspondence to Hongsheng Ding, Department of Neurology, Tianjin Union Medical Center. No.190, jieyuan Road, Hongqiao District, Tianjin 300121, China. E-mail: huansong789@163.com

1052-3057/$ - see front matter

https://doi.org/10.1016/j.jstrokecerebrovasdis.2018.09.023

of Rho family, plays an important role in neurite outgrowth, neural network formation and nervous system apoptosis. RhoA can exert the regulation on cellular biological behavior through its key downstream effector Rho-associated kinase (ROCK)[9]. ROCK is a kind of serine/threonine protein kinase that includes 2 subtypes (ROCKI and ROCKII), and the expression of ROCKII is widespread in the brain, muscle, cardio tissue and placenta.[10] Apoptosis is an essential cause of the neuronal damage that results from cerebral ischemia. Studies showed that when Rho/Rho-kinase was inhibited, the neurons apoptosis cerebral ischemia/reperfusion could be reduced.[11,12] Han et al[13] revealed that miR-431 could inhibit neurons apoptosis in a rat model of cerebral ischemia/reperfusion injury by negatively regulating the Rho/Rho-kinase signaling pathway.

The bioinformatics analysis (mirdb.org) showed that the binding sites of miR-582-5p exist in the 3′UTR (untranslated region) of proteinase-activated receptors type I (PAR-1), which has a significant role in inducing neuronal injury.[14] PAR-1, also called coagulation factor 2 receptor, is a receptor of thrombin and is overexpressed in brain tissues of CIS mice.[15] PAR-1 deficiency could protect against neuronal damage after cerebral hypoxia/ischemia.[16] Additionally, PAR-1 could activate Rho/Rho-kinase signaling pathway.[17] The above findings prompted the inference that miR-582-5p might participate in the pathogenesis of neuronal injury following CIS through regulating PAR-1/Rho/Rho.

In this study, we detected the expression of miR-582-5p and PAR-1 in CIS mice, explored the interaction between miR-582-5p and PAR-1 in neuronal cells, and further clarified the role of miR-582-5p/PAR-1/Rho/Rho in neuronal cell apoptosis.

Methods

Animal

C57BL/6 mice (8-week-old, 20-25 g) were purchased from Shanghai Lab. Animal research center, and housed in individual cages with free access to food and water. The study was approved by the Institutional Animal Care and Use Committee of Tianjin Union Medical Center.

Middle Cerebral Artery Occlusion Mouse Model

The cerebral artery occlusion (MCAO) model of CIS was established as described previously.[18] Briefly, mice were anesthetized using pentobarbital (40 mg/kg) by intraperitoneal injection. A blunt dissection was performed to expose the left common carotid artery (CCA) under a stereomicroscope. Then, the ipsilateral CCA proximal end and external carotid artery was ligated, and the internal carotid artery was clamped using an arterial clamp. After a small incision in the CCA between permanent and temporary sutures was made, a 5-0 nylon monofilament (silicone diameter, .23 mm) was inserted into the internal carotid artery approximately 18 mm beyond the carotid bifurcation, thus blocking the origin of the middle cerebral artery. After 1 hour of MCAO, the mice were allowed reperfusion for 6 hours (n = 6). Sham mice (n = 6) were subjected to the same procedure except for intraluminal introduction. Laser Doppler flowmetry (PeriFlux 5000, Perimed) was used to monitor the regional cerebral blood flow. A temperature-controlled heating pad (CMA 150, Carnegie Medicin, Sweden) was used to monitor the body temperature during surgery (Fig 1A). Twenty-four hours after surgery, mice were sacrificed and the brain of each mouse was obtained for subsequent experiments.

Thrombin Activity Assay

Thrombin activity was detected using a fluorometric assay as described previously.[19] Briefly, the left ischemic hemisphere of the brain from each mouse was taken, and then cut into coronal slices (1-mm thick). The coronal slices were placed into black microplate containing the substrate buffer. The thrombin activity levels that were measured using a microplate reader with excitation and emission filters of 360 ± 35 nm and 460 ± 35 nm, respectively.

Cell Culture and Treatment

The mouse neuroblastoma neuro 2A (N2A) cell line was purchased from American Type Culture Collection (Manassas, VA). N2A cells were cultured in Eagle's Minimum Essential Medium (EMEM; American Type Culture Collection) containing 10% fetal bovine serum (Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere of 5% CO_2 at 37°C. To establish in vitro oxygen-glucose deprivation/reoxygenation (OGD/R) model, N2A cells were cultured in serum/glucose-free EMEM medium in a humidified atmosphere of 1% O_2, 5% CO_2, and 95% N2 at 37°C. After 4 hours, the culture medium was replaced to normal EMEM medium for a 12-hour recovery in normoxic conditions. For the cell transfection experiments, N2A cells were pretreated with miR-582-5p inhibitor, miR-582-5p mimic, pcDNA-PAR-1, and their negative control for 24 hours.

Quantitative Real-Time Polymerase Chain Reaction

The expressions of miR-582-5p, miR-20-5p, miR-216a-5p, miR-326-3p, miR-212-3p, and PAR-1 messenger RNA in brain tissues or N2A cells were detected by quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was extracted from brain tissues or N2A cells using TRIzol reagent (Invitrogen, Waltham, MA) according to the manufacturer's instructions. For detection of miR-582-5p, miR-20-5p, miR-216a-5p, miR-326-3p, and miR-212-3p, the cDNA synthesis was performed using

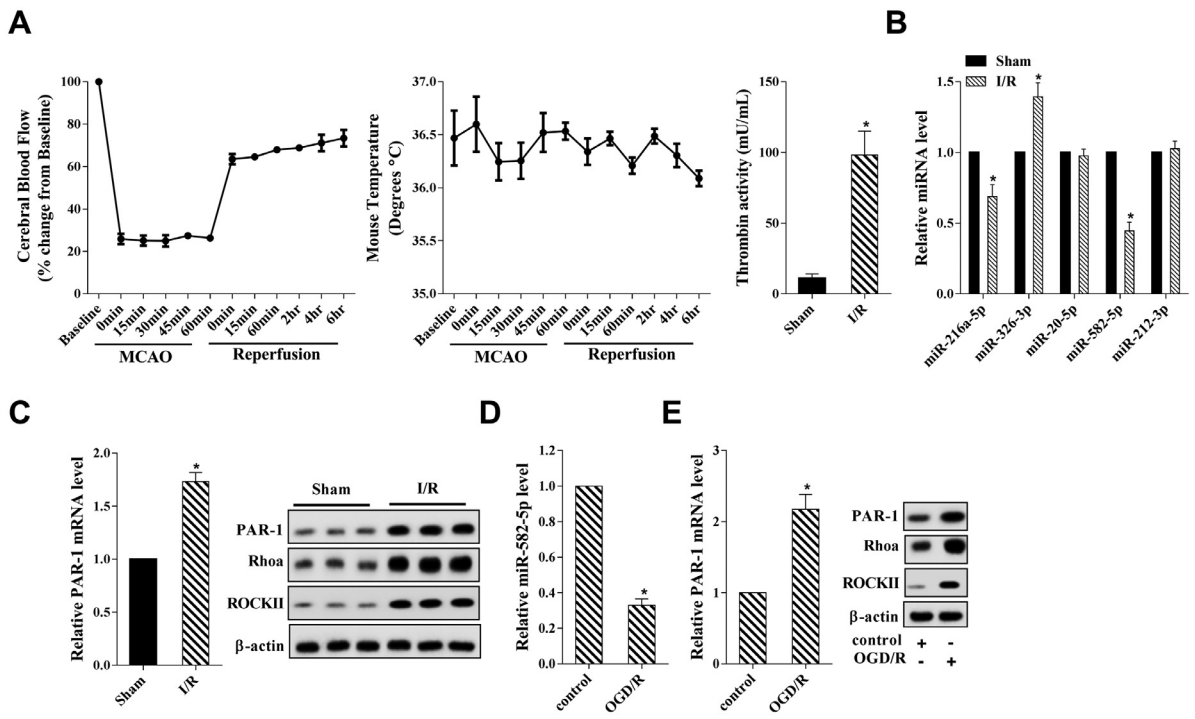

Figure 1. *The expression of miR-582-5p and PAR-1 in brain tissues of CIS mice and in vitro OGD/R model. MCAO mouse model was established. Twenty-four hours later, brain tissues of mice from sham group and I/R group were collected for thrombin activity assay, qRT-PCR, and western blot. OGD/R model was established using N2A cells. (A) CBF and body temperature monitoring, and thrombin activity. (B) The expression of miRNAs in brain tissues. (C) The expression of PAR-1, RhoA, and ROCKII in brain tissues. (D) MiR-582-5p expression in N2A cells. (E) The expression of PAR-1, RhoA, and ROCKII in N2A cells.* *P < .05 versus sham group or control group.

miRNA cDNA Synthesis Kit (TaKaRa, Dalian, China). For PAR-1 messenger RNA analysis, the cDNA synthesis was performed using SuperScript first-strand synthesis kit (Invitrogen). qRT-PCR was performed using SYBR Premix ExTaq II kit (Takara) according to the manufacturer's protocol.

Western Blot

Total protein was extracted from brain tissues or N2A cells using RIPA lysis buffer. The concentration of total protein was quantified using a BCA kit (Thermo Fisher Scientific, Waltham, MA). An equal amount of protein was separated by 12% SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and transferred to PVDF (polyvinylidene difluoride) membranes. After blocking with 5% skim milk for 2 hours at room temperature, the proteins on membranes were incubated with the primary antibodies-anti-PAR-1 (1:1000; Abcam), anti-RhoA (1:5000; Abcam), anti-ROCKII (1:1000; Abcam), anti-p-eNOS (1:1000; Cell Signaling Technology), anti-eNOS (1:500; Abcam), and anti-β-actin (1:5000; Abcam) at 4°C overnight. After washing with 1 × TBST (Tris-buffered saline Tween) 3 times, the proteins were incubated with horseradish peroxidase-conjugated secondary antibody for 1 hour at room temperature. Immunoblots were visualized by enhanced chemiluminescence (ECL kit, Santa Cruz Biotechnology). The β-actin band was served as a loading control.

Luciferase Report Gene Assay

The 3′-UTR of PAR-1 and its mutated form were synthesized and inserted into the luciferase reporter vector pmiRGLO by Genewiz (Beijing, China). 293T cells were cultured in 24-well plates overnight and co-transfected with miR-582-5p mimic or inhibitor and the luciferase reporter plasmids containing PAR-1 3′-UTR (PAR-1-WT) or its mutated form (PAR-1-MUT) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. After 24 hours of transfection, luciferase activity was detected using a luciferase reporter assay system (Promega) according to the manufacturer's instructions.

Cell Apoptosis Assay

The apoptosis of N2A cells was assessed using Annexin V-FITC Apoptosis Detection Kit (JingMei Biotech, Beijing, China). Cells were harvested and stained with Annexin V-FITC and PI according to the reagent's protocol. The ratio of apoptotic cells was analyzed by a flow cytometry (FACScan, BD Biosciences, Franklin Lakes, NJ).

Statistical Analysis

The data were analyzed by SPSS 18.0 software and expressed as the mean ± standard deviation. The difference between 2 groups was compared using Student's *t* test. The statistical significance between different groups was compared using One-way ANOVA followed by the Newman-Keuls post hoc test. $P < .05$ was considered statistically significant. All experiments were performed in triplicate.

Results

MiR-582-5p was Decreased and PAR-1 was Increased in CIS Mice and OGD/R Model

To test which miRNA was closely related to CIS, in vivo mouse model of CIS induced by MCAO was established. Twenty-four hours later, the brain tissues of mice from sham group and I/R group were collected for thrombin activity assay, qRT-PCR, and western blot. As shown in Figure 1A, the thrombin activity in I/R group was higher than that in the sham group. The results of qRT-PCR suggested that the expression of miR-582-5p and miR-216a-5p was significantly decreased in I/R group compared with the sham group, and miR-582-5p expression was lower than miR-216a-5p expression (Fig 1B). Thus, we chose miR-582-5p to carry out the future mechanism experiments. Results also showed that PAR-1 expression was markedly increased in brain tissues of mice from I/R group, and the protein expressions of RhoA and ROCKII were enhanced (Fig 1C).

To investigate the underlying mechanism of miR-582-5p in CIS, in vitro model induced by OGD/R in N2A cells was established. Figure 1D and E showed that treatment with OGD/R markedly reduced miR-582-5p expression compared with the control group, and upregulated the expression of PAR-1, RhoA and ROCKII.

MiR-582-5p Negatively Regulated PAR-1

According to the bind sites between miR-582-5p and PAR-1 that predicted by miRDB.org (Fig 2A), we performed luciferase report gene assay to explore the relationship between them. It was found that miR-582-5p inhibitor could enhance the luciferase activity of PAR-1 3′UTR-WT in 293T cells, while miR-582-5p mimic had an opposite effect (Fig 2B). To prove that miR-582-5p could regulate PAR-1 expression, miR-582-5p inhibitor or mimic were transfected into N2A cells to reduce or increase miR-582-5p expression. The results showed that inhibition of miR-582-5p upregulated PAR-1 expression, and overexpression of miR-582-5p downregulated PAR-1 expression (Fig 2C).

Overexpression of miR-582-5p Inhibited the Increased Expression of PAR-1 Induced by OGD/R

To investigate whether miR-582-5p regulated the changes of PAR-1 expression induced by OGD/R, N2A cells were divided into control group, OGD/R group, OGD/R+pre-NC group, and OGD/R+miR-582-5p mimic group. As shown in Figure 3, OGD/R induction reduced miR-582-5p expression and increased PAR-1expression, whereas miR-582-5p mimic changed the effect of OGD/R treatment.

Overexpression of miR-582-5p Inhibited OGD/R-Induced Neuronal Cell Apoptosis by Regulating PAR-1/Rho/Rho Axis

We first investigated whether miR-582-5p/PAR-1 regulated the activation of Rho/Rho-kinase signal pathway induced by OGD/R. N2A cells were divided into control group, OGD/R group, OGD/R+pre-NC group, OGD/R +miR-582-5p mimic group, OGD/R+miR-582-5p mimic +pcDNA group, and OGD/R+miR-582-5p mimic+pcDNA-PAR-1 group. The results revealed that miR-582-5p mimic could reverse the decreased expression of miR-582-5p and p-eNOS, as well as increased expression of PAR-1, RhoA, and ROCKII induced by OGD/R, while pcDNA-PAR-1 could change the effect of miR-582-5p (Fig 4A and B). These results indicated that overexpression of miR-582-5p could suppress the activation of Rho/Rho-kinase signal pathway induced by OGD/R via downregulating PAR-1.

To prove the role of miR-582-5p/PAR-1/Rho/Rho axis on OGD/R-induced neuronal cell apoptosis, N2A cells were pretreated with30 μmol/L of hydroxyfasudil (an inhibitor of Rho/Rho-kinase pathway). As shown in Figure 4C, treatment with OGD/R significantly promoted neuronal cell apoptosis, while miR-582-5p mimic inhibited the promotion effect. As expected, pcDNA-PAR-1 could increase neuronal cell apoptosis again, but hydroxyfasudil changed its effect.

Discussion

The role of noncoding RNAs (ncRNAs) in human diseases, including tumors, cardiovascular disease, and metabolic diseases, has attracted increasing attention in recent years. A number of papers have demonstrated ncRNAs are closely related to systemic or local inflammatory response, cell proliferation, apoptosis, and migration that are directly involved in the occurrence and development of human diseases. miRNAs, a class of ncRNAs, have been shown to participate in the pathogenesis of CIS.[13] For instance, miR-298 was downregulated in CIS, and upregulation of miR-298 could exacerbate ischemia/reperfusion injury following CIS.[20] In this study, we find a decrease in miR-582-5p expression in brain tissues of CIS mice. The abnormal miR-582-5p expression in CIS might be related to the neuronal injury after CIS. MiR-

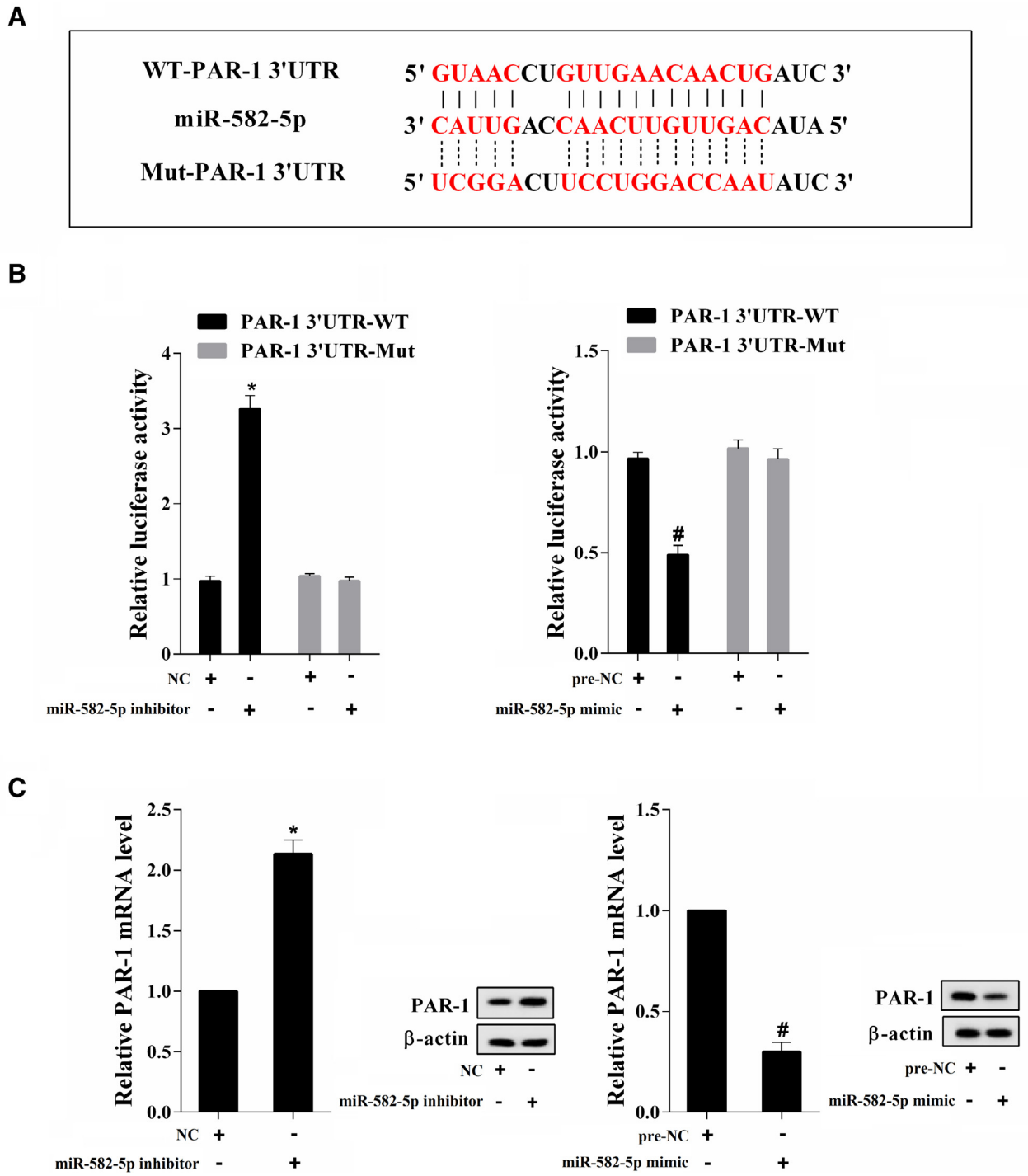

Figure 2. *Interaction between miR-582-5p and PAR-1. (A) Putative binding sites of miR-582-5p and PAR-1. (B) The interaction between miR-582-5p and PAR-1 3′UTR in 293T cells was explore using luciferase report gene assay. (C) MiR-582-5p inhibitor or mimic were transfected into N2A cells to reduce or increase miR-582-5p expression. Effects of miR-582-5p expression on PAR-1 mRNA and protein were analyzed.* *P < .05 versus NC; #P < .05 versus pre-NC.

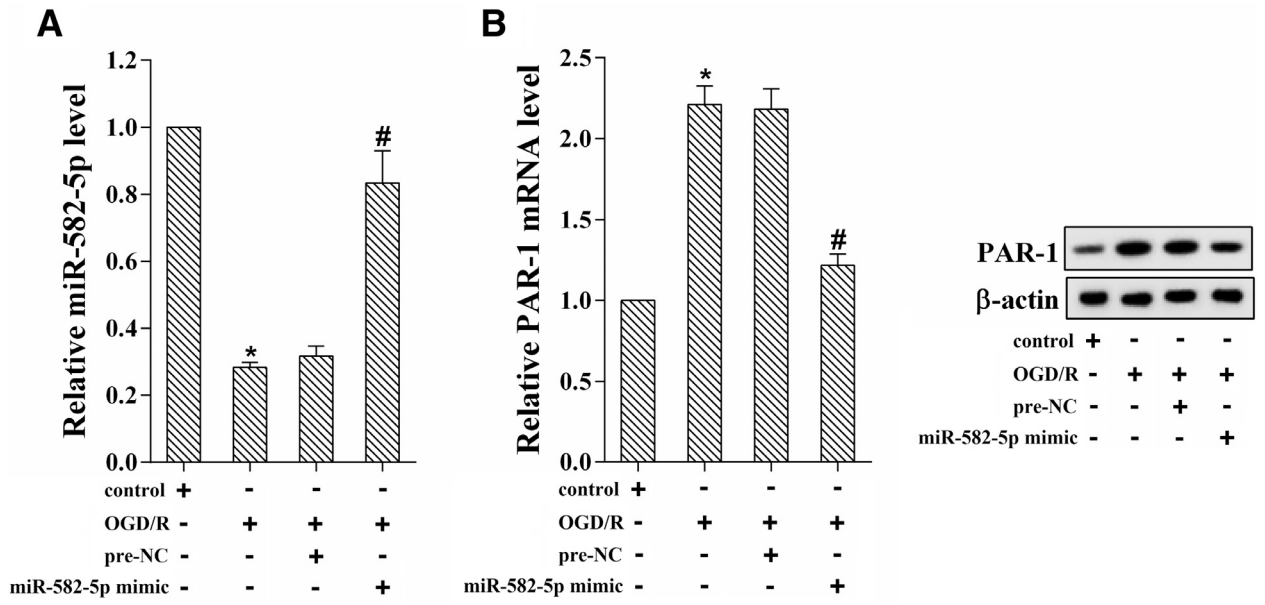

Figure 3. *Overexpression of miR-582-5p inhibited the increased expression of PAR-1 induced by OGD/R. N2A cells were divided into control group, OGD/R group, OGD/R+pre-NC group, and OGD/R+miR-582-5p mimic group. (A) MiR-582-5p expression in N2A cells. (B) PAR-1 expression in N2A cells.* *P < .05 *versus control;* #P < .05 *versus OGD/R+pre-NC.*

582-5p has been shown to be involved in many human diseases, including cancers and immune diseases.[21,22] In the current study, for the first time, we investigated the role of miR-582-5p in the neuronal injury after CIS and its underlying mechanism.

Thrombin plays a key role in neuronal injury after CIS.[14,23] Most of the effects of thrombin are mediated and regulated by PAR-1, which can aggravate neuronal injury after ischemia through various ways. For instance, Wang et al[24] showed that knockout of PAR-1 could inhibit neuronal death in mouse brain after cerebral ischemia. Xie et al[25] revealed that ginsenoside-Rg1 ameliorated the neurological injury induced by focal cerebral ischemia in rats by downregulating PAR-1. Moreover, the role of thrombin is also related to activation of Rho/Rho-kinase signaling pathway.[26] Previous study showed that thrombin increased endothelin-1 synthesis and secretion in retinal pigment epithelial cells via activating Rho/Rho-kinase.[27] It has been proved that the activation of Rho/Rho-kinase signaling pathway is closely regulated with neural development.[28] However, whether the role of PAR-1 in neuronal injury after CIS is related to the activation of Rho/Rho-kinase signaling pathway has not been reported. Therefore, the current study aimed to clarify the

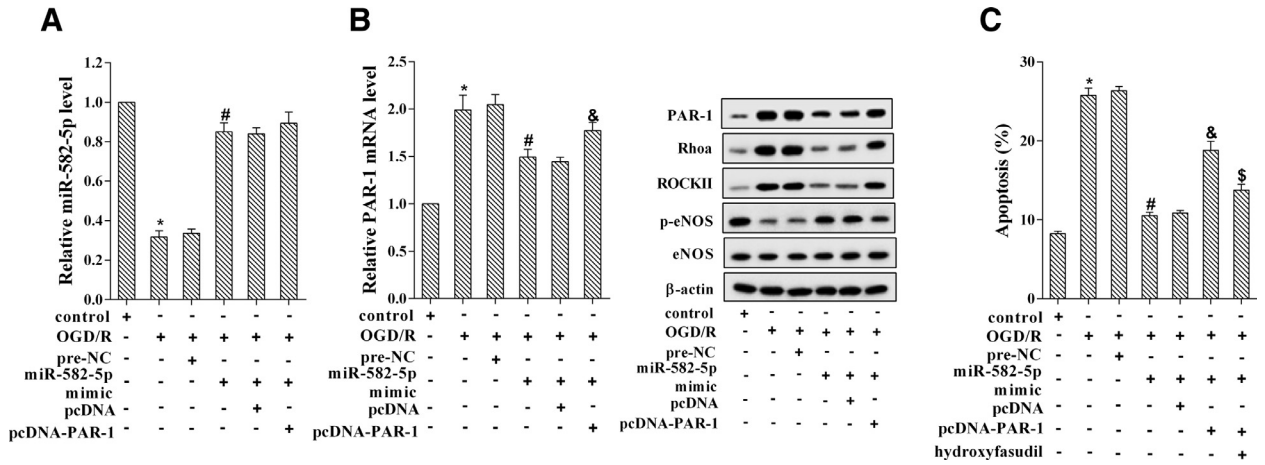

Figure 4. *Overexpression of miR-582-5p inhibited OGD/R-induced neuronal cell apoptosis by regulating PAR-1/Rho/Rho axis. N2A cells were divided into control group, OGD/R group, OGD/R+pre-NC group, OGD/R+miR-582-5p mimic group, OGD/R+miR-582-5p mimic+pcDNA group, OGD/R+miR-582-5p mimic+pcDNA-PAR-1 group, and OGD/R+miR-582-5p mimic+pcDNA-PAR-1+hydroxyfasudil group. (A) MiR-582-5p expression in N2A cells. (B) The expression of PAR-1, RhoA, ROCKII, p-eNOS, and eNOS in N2A cells. (C) The apoptosis of neuronal cells was analyzed by flow cytometry methods.* *P < .05 *versus control;* #P < .05 *versus OGD/R+pre-NC;* &P < .05 *versus OGD/R+miR-582-5p mimic+pcDNA;* $P < .05 *versus OGD/R+miR-582-5p mimic +pcDNA-PAR-1.*

molecular mechanism of miR-582-5p/PAR-1/Rho/Rho in the neuronal injury as a result of CIS. Herein, in this work, we analyzed and confirmed that PAR-1 was negatively regulated by miR-582-5p. Further experiment confirmed that overexpression of miR-582-5p mediated the inhibition of Rho/Rho-kinase signaling pathway activation via downregulating PAR-1, thereby exerted the inhibition effect on the apoptosis of neuronal cells.

Conclusions

In conclusions, our results suggested that miR-582-5p overexpression could reduce CIS-induced neuronal cell apoptosis by regulating Rho/Rho-kinase signaling pathway through targeting PAR-1. These findings have documented a potential role of miR-582-5p in the pathogenesis of neuronal injury after CIS. However, our study still had some shortcomings, such as, we did not verify the molecular mechanism in animal experiments. We will improve it in further experiments.

References

1. Margaritescu O, Mogoantă L, Pirici I, et al. Histopathological changes in acute ischemic stroke. Rom J Morphol Embryol 2009;50:327-339.
2. Radak D, Katsiki N, Resanovic I, et al. Apoptosis and Acute Brain Ischemia in Ischemic Stroke. Curr Vasc Pharmacol 2017;15:115-122.
3. Chen B, Wang G, Li W, et al. Memantine attenuates cell apoptosis by suppressing the calpain-caspase-3 pathway in an experimental model of ischemic stroke. Exp Cell Res 2017;351:163-172.
4. Ge W-q, Hao P, Huang Y-h, et al. miR-539 inhibits inflammation in renal transplant iscemia-reperfusion injury via blocking the MyD88/NF-κB pathway. Clin Surg Res Commun 2018;2:14-21.
5. Yao S, Tang B, Li G, et al. miR-455 inhibits neuronal cell death by targeting TRAF3 in cerebral ischemic stroke. Neuropsychiatr Dis Treat 2016;12:3083-3092.
6. Guo D, Ma J, Yan L, et al. Down-regulation of lncrna MALAT1 attenuates neuronal cell death through suppressing beclin1-dependent autophagy by regulating Mir-30a in cerebral ischemic stroke. Cell Physiol Biochem 2017;43:182-194.
7. Zhang X, Zhang Y, Yang J, et al. Upregulation of miR-582-5p inhibits cell proliferation, cell cycle progression and invasion by targeting Rab27a in human colorectal carcinoma. Cancer Gene Ther 2015;22:475-480.
8. Satoh S, Toshima Y, Hitomi A, et al. Wide therapeutic time window for Rho-kinase inhibition therapy in ischemic brain damage in a rat cerebral thrombosis model. Brain Res 2008;1193:102-108.
9. Yan YY, Wang XM, Jiang Y, et al. The role of Rho/Rho-kinase pathway and the neuroprotective effects of fasudil in chronic cerebral ischemia. Neural Regen Res 2015;10:1441-1449.
10. Tan HB, Zhong YS, Cheng Y, et al. Rho/ROCK pathway and neural regeneration: a potential therapeutic target for central nervous system and optic nerve damage. Int J Ophthalmol 2011;4:652-657.
11. Cui Q, Zhang Y, Chen H, et al. Rho kinase: a new target for treatment of cerebral ischemia/reperfusion injury. Neural Regen Res 2013;8:1180-1189.
12. Shintani N, Ishiyama T, Kotoda M, et al. The effects of Y-27632 on pial microvessels during global brain ischemia and reperfusion in rabbits. BMC Anesthesiol 2017;17:38.
13. Han XR, Wen X, Wang YJ, et al. Protective effects of microRNA-431 against cerebral ischemia-reperfusion injury in rats by targeting the Rho/Rho-kinase signaling pathway. J Cell Physiol 2018;233:5895-5907.
14. Chen B, Friedman B, Whitney MA, et al. Thrombin activity associated with neuronal damage during acute focal ischemia. J Neurosci 2012;32:7622-7631.
15. Stein ES, Itseksonhayosh Z, Aronovich A, et al. Thrombin induces ischemic LTP (iLTP): implications for synaptic plasticity in the acute phase of ischemic stroke. Sci Rep 2015;5:7912.
16. Olson EE, Lyuboslavsky P, Traynelis SF, et al. PAR-1 deficiency protects against neuronal damage and neurologic deficits after unilateral cerebral hypoxia/ischemia. J Cereb Blood Flow Metab 2004;24:964-971.
17. Pang L, Li JF, Su L, et al. ALEX1, a novel tumor suppressor gene, inhibits gastric cancer metastasis via the PAR-1/Rho GTPase signaling pathway. J Gastroenterol 2018;53:71-83.
18. Tian YS, Zhong D, Liu QQ, et al. Upregulation of miR-216a exerts neuroprotective effects against ischemic injury through negatively regulating JAK2/STAT3-involved apoptosis and inflammatory pathways. J Neurosurg 2018;1:1-12.
19. Bushi D, Stein ES, Golderman V, et al. A linear temporal increase in thrombin activity and loss of its receptor in mouse brain following ischemic stroke. Front Neurol 2017;8:138.
20. Sun H, Zhong D, Wang C, et al. MiR-298 Exacerbates Ischemia/Reperfusion Injury Following Ischemic Stroke by Targeting Act1. Cell Physiol Biochem 2018;48:528-539.
21. Maeno A, Terada N, Uegaki M, et al. Up-regulation of miR-582-5p regulates cellular proliferation of prostate cancer cells under androgen-deprived conditions. Prostate 2014;74:1604-1612.
22. Long X, Li Y, Qiu S, et al. MiR-582-5p/miR-590-5p targeted CREB1/CREB5-NF-kappaB signaling and caused opioid-induced immunosuppression in human monocytes. Transl Psychiatry 2016;6:e757.
23. Chen T, Wang J, Li C, et al. Nafamostat mesilate attenuates neuronal damage in a rat model of transient focal cerebral ischemia through thrombin inhibition. Sci Rep 2014;4:5531.
24. Wang J, Jin H, Hua Y, et al. Role of protease-activated receptor-1 in brain injury after experimental global cerebral ischemia. Stroke 2012;43:2476-2482.
25. Xie CL, Li J, Wang W, et al. Neuroprotective effect of ginsenoside-Rg1 on cerebral ischemia/reperfusion injury in rats by downregulating protease-activated receptor-1 expression. Life Sci 2015;121:145-151.
26. Rosenfeldt H, Castellone MD, Randazzo PA, et al. Rac inhibits thrombin-induced Rho activation: evidence of a Pak-dependent GTPase crosstalk. J Mol Signal 2006;1:8.
27. Narayan S, Prasanna G, Tchedre K, et al. Thrombin-induced endothelin-1 synthesis and secretion in retinal pigment epithelial cells is rho kinase dependent. J Ocul Pharmacol Ther 2010;26:389-397.
28. Kobayashi K, Sano H, Kato S, et al. Survival of corticostriatal neurons by Rho/Rho-kinase signaling pathway. Neurosci Lett 2016;630:45-52.