European Review for Medical and Pharmacological Sciences 2019; 23: 7033-7041

MicroRNA-26a regulates cerebral ischemia injury through targeting PTEN

X.-L. GUO[1], H. LIANG[1], Y. SUN[1], C.-B. WANG[2]

[1]Department of Neurology, Yantai City Yantai Mountain Hospital, Yantai, China
[2]Department of Spine Surgery, Yantai City Yantai Mountain Hospital, Yantai, China

Abstract. – OBJECTIVE: MicroRNA-26a (miR-26a) exhibits diverse functions in different human disease. However, further research is needed to investigate the potential role of miR-26a in cerebral ischemia injury.

MATERIALS AND METHODS: The expressions of miR-26a and PTEN (phosphatase and tensin homolog) were detected *via* Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) assay. The protein expression of Bcl-2 and Bax was detected by Western blot assay. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to observe the cell viability of SH-SY5Y cells. The relationship between miR-26a and PTEN was confirmed by Dual-Luciferase assay and human SH-SY5Y cells were treated with oxygen-glucose deprivation (OGD)/reperfusion to mimic I/R injury.

RESULTS: The expression of miR-26a was increased in the OGDR model. Moreover, upregulation of miR-26a promoted cell viability and inhibited OGDR-induced apoptosis. PTEN was confirmed as a direct target gene for miR-26a. Under OGDR conditions, the expression of PTEN was significantly decreased. Moreover, overexpression of PTEN inhibited cell viability, promoted cell apoptosis and deepened the effect of the OGDR model.

CONCLUSIONS: MiR-26a promoted the viability of SH-SY5Y cells and suppressed apoptosis under OGDR conditions by targeting PTEN.

Key Words:
MiR-26a, Cerebral ischemia injury, PTEN, Proliferation, Apoptosis.

Introduction

Cerebral ischemia is a common cerebrovascular disease, and the mortality and morbidity of cerebral ischemia have gradually increased in recent years[1]. The pathological basis of cerebral ischemia is relatively transient stenosis of the cerebral vessels caused by specific causes, resulting in a temporary insufficiency of cerebral perfusion[2]. Next, the function of the corresponding brain tissue appeared a reversible functional injury. Cerebral ischemia usually occurs in the pathological process of many neurological diseases, such as cerebrovascular disease, brain tumor and systemic diseases[3]. The causes of cerebral ischemia are complex, such as hypertension, hyperlipidemia, heart disease, diabetes mellitus and cerebral arteriosclerosis[4]. Treatment should be based on the etiology, active treatment of risk factors and the application of anti-platelet aggregation drugs, which can be used to improve brain circulation.

As a research hotspot, the abnormal expression and function of microRNAs (miRNAs) are receiving more and more attention. In recent years, lots of miRNAs have had an important impact on the pathogenesis of the nervous system and cerebral ischemia injury[5]. For example, inhibition of miR-125b decreased cerebral ischemia/reperfusion injury by targeting CK2α/NADPH oxidase signaling[6]. Liang et al[7] reported that miR-320 facilitated brain parenchyma injury *via* regulating insulin-like growth factor 1 (IGF-1) during cerebral I/R injury in mice. Among them, the complex role of miR-26a in different human organs has attracted our attention. Leeper et al[8] proposed that miR-26a was a novel regulator of vascular smooth muscle cell function. In addition, miR-26a has a specific function in human cancers. MiR-26a had been found to enhance the sensitivity of gastric cancer cells to cisplatin by targeting NRAS and E2F2[9]. Moreover, miR-26a contributed to the malignant behavior of lung adenocarcinoma by targeting EZH2[10]. Besides that, miR-26a alleviated lower extremity ischemia-reperfusion injury to induce vascular endothelial cell injury through the adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) pathway[11]. However, the regulatory mechanism of miR-26a in cerebral ischemia injury remains to be investigated.

PTEN (phosphatase and tensin homolog) is usually mutated in tumor cells and acts as a tumor suppressor[12]. In particular, it had been reported that

Corresponding Author: Chuanbao Wang, MD; e-mail: htopqy@163.com

cell migration, survival and apoptosis in human cancers were regulated by PTEN, including neurons[13]. Lee et al[14] demonstrated that cilostazol prevented focal cerebral ischemic injury by enhancing CK2 phosphorylation and inhibiting PTEN phosphorylation in rats. Moreover, PTEN inhibition enhanced angiogenesis in an ischemic injury model *in vitro* by promoting protein kinase B (AKT) phosphorylation[15]. The purpose of this study was to examine the function of miR-26a and PTEN in the progression of cerebral ischemia injury. The interaction between miR-26a and PTEN was also testified. The results will further elucidate the molecular mechanism of cerebral ischemia injury.

Materials and Methods

Cells Culture

Human SH-SY5Y cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were then seeded in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Grand Island, NY, USA) with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA). SH-SY5Y cells were cultured in an incubator at 37°C with 5% CO_2.

OGDR Model

SH-SY5Y cells were cultured in a glucose-free Hanks' balanced salt solution (Invitrogen, Carlsbad, CA, USA) under hypoxic conditions (1% O_2 /94% N_2/5% CO_2) at 37°C for 4 h to build an OGD model. Then, the cells were placed in a glucose medium with 10% FBS, and cultured under normoxic conditions (95% air/5% CO_2) for 48 h for reperfusion. SH-SY5Y cells cultured under normoxic conditions were used as controls.

Cell Transfection

MiR-26a mimics and inhibitor were obtained from GenePharma (Shanghai, China). PTEN overexpression plasmid and small interfering RNA (siRNA) were constructed by GenePharma (Shanghai, China). They were then transferred to SH-SY5Y cells, which were treated with OGDR followed by reperfusion.

Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR) Analysis

Total RNA in SH-SY5Y cells was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The synthesis of complementary deoxyribose nucleic acid (cDNA) was performed using PrimeScript RT Master Mix (TaKaRa, Dalian, China). We performed Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR) using the SYBR PrimeScript miRNA RT-PCR Kit (TaKaRa, Dalian, China) on ABI 7500 Fast system (Applied Biosystems, Foster City, CA, USA). U6 or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a control for miR-26a or PTEN and their expressions were calculated using the $2^{-\Delta\Delta ct}$ method. The RT primers were as follows: miR-26a forward 5'-CGCGTTCAAGTAATCCAGGA-3', reverse: 5'-AGTGCAGGGTCCGAGGTATT-3'; PTEN forward 5'-AGGGACGAACTGGTGTAATGA-3', reverse 5'-CTGGTCCTTACTTCCCCATAGAA-3'; GAPDH forward, 5'-CGG AGTCAACGGATTTGGTCGTAT-3', reverse, 5'-AGCCTTCTCCATGGTGGTGAAGAC-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3'; reverse, 5'-AACGCTTCACGAATTTGCGT-3'.

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) Assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Sigma-Aldrich, St. Louis, MO, USA) was performed to assess cell viability. SH-SY5Y cells with transfection and OGDR treatment were incubated for 48 h in 96-well plates at a density of 4×10^3 cells. Next, the cells were incubated for 4 h at 37°C in serum-free medium with 20 μL of MTT. After removing the supernatant, they were dissolved in 100 μL of dimethyl sulfoxide, and the absorbance was read at 490 nm on a microplate spectrophotometer (Thermo Labsystems, Vantaa, Finland).

Dual-Luciferase Reporter Assay

The 3'-untranslated region (3'-UTR) of wild or mutant PTEN was inserted into the pcDNA3.1 plasmid (Promega, Madison, WI, USA) for Luciferase reporter assays. Next, the above plasmid and miR-26a mimics were transfected into HEK-293 cells. Finally, the Luciferase activity was measured by a Dual-Luciferase assay system (Promega, Madison, WI, USA).

Western Blot Analysis

Protein samples were obtained using radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China). Proteins were then separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were blocked with 5% skim milk and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) at

room temperature. Next, we incubated the membranes with anti-PTEN, anti-Bcl-2, anti-Bax, and anti-GAPDH primary antibodies overnight at 4°C. After washing, they were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 2 h at room temperature. Finally, the protein was visualized by electrochemiluminescence (ECL) (Pierce, Waltham, MA, USA).

Statistical Analysis

Data were shown as mean ± SD (Standard Deviation). Statistical Product and Service Solutions (SPSS) 19.0 (SPSS Inc., Chicago, IL, USA) or GraphPad Prism 6 (La Jolla, CA, USA) were used to analyze these data. Comparisons between groups were performed using One-way analysis of variance (ANOVA) test followed by Post-Hoc Test (Least Significant Difference). A significant difference was defined at $p<0.05$.

Results

The Expression of MiR-26a was Increased in the OGDR Model

First, miR-26a expression was detected in simulated cerebral ischemia/reperfusion injury. The expression of miR-26a was markedly upregulated in SH-SY5Y cells treated with OGDR compared to the control group ($p<0.01$, Figure 1A), indicating that cerebral ischemia/reperfusion could enhance the expression level of miR-26a. Next, miR-26 mimics or inhibitors were transfected into SH-SY5Y cells cultured under OGDR conditions. Compared to the OGDR group, miR-26a mimics promoted its expression, whereas miR-26a inhibitor reduced its expression in SH-SY5Y cells ($p<0.01$, Figure 1B), suggesting that miR-26a expression was successfully regulated artificially.

MiR-26a Regulated Cell Viability and Apoptosis in SH-SY5Y Cells

MTT assay was then performed to examine the effect of miR-26a on cell viability. The results showed that OGDR significantly inhibited the viability of SH-SY5Y cells ($p<0.05$, Figure 2A). Moreover, miR-26a mimics promoted proliferation of SH-SY5Y cells under hypoxic conditions compared to the OGDR group ($p<0.01$, Figure 2B). In contrast, miR-26a inhibitors suppressed cell proliferation in SH-SY5Y cells under hypoxic conditions ($p<0.05$, Figure 2B). Hence, miR-26a was considered to reverse the effects of OGDR and prolong cell survival.

Next, miR-26a-regulated apoptosis was investigated after cerebral ischemia injury. We observed the expression of apoptosis-related genes (Bax and Bcl-2) in SH-SY5Y cells. First, the expression level of Bax was improved by OGDR treatment. OGDR treatment reduced Bcl-2 expression ($p<0.05$ or 0.01, Figure 2C). In contrast, the above alterations of Bax and Bcl-2 expressions were reversed by overexpression of miR-26a. Upregulation of miR-26a dramatically reduced Bax expression and promoted survival gene Bcl-2 expression compared to the OGDR group ($p<0.01$,

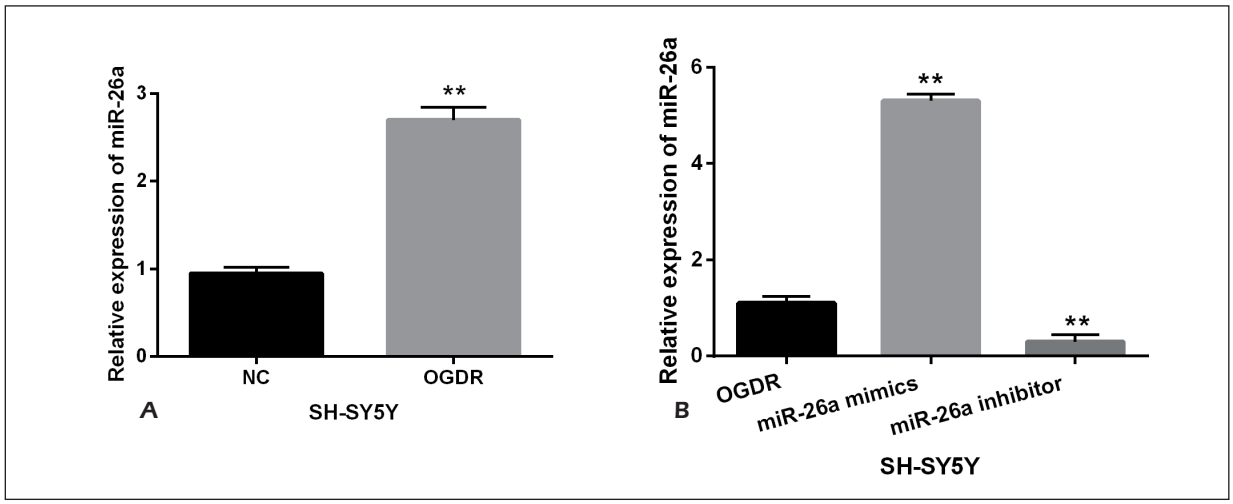

Figure 1. The expression of miR-26a was increased in the OGDR model. ***A***, The expressions of miR-26a in SH-SY5Y cells with OGDR treatment. ***B***, The expression of miR-26a was ex-amined in SH-SY5Y cells with miR-26a mimics or inhibitor under OGDR conditions. $^{**}p<0.01$.

Figure 2D). MiR-26a silencing had the same effect on Bax and Bcl-2 expressions as OGDR treatment, which promoted Bax expression and inhibited Bcl-2 expression (p<0.05 or 0.01, Figure 2D). Based on these results, overexpression of miR-26a promoted the viability of SH-SY5Y cells and inhibited apoptosis under OGDR conditions.

PTEN Was a Direct Target Gene of MiR-26a

Furthermore, the molecular mechanism by which miR-26a inhibits OGDR was investigated. MiR-26a was found to have a binding site to the 3'-UTR of PTEN predicted by TargetScan (http://www.targetscan.org, Figure 3A). To confirm the interaction

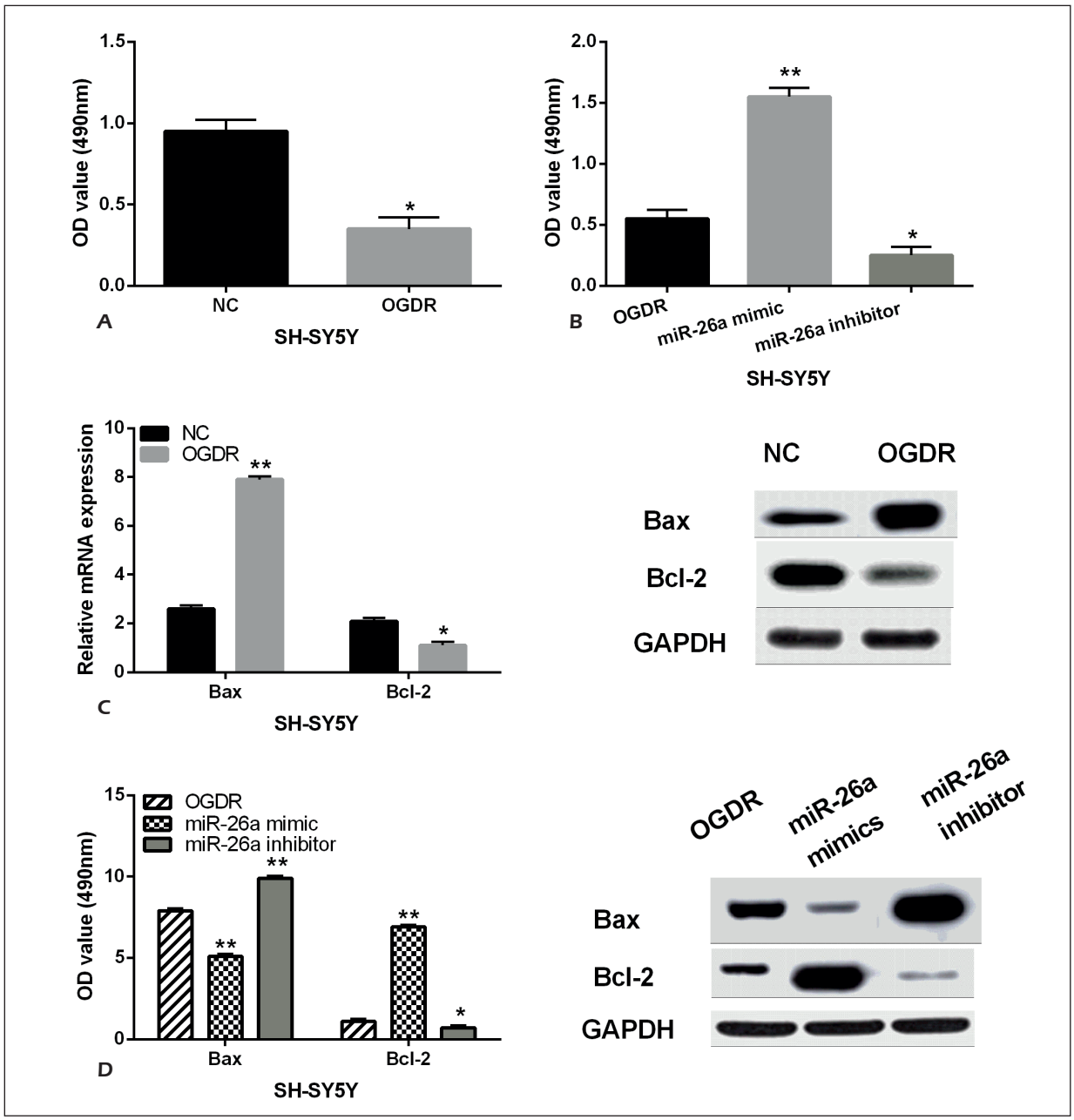

Figure 2. MiR-26a regulated cell viability and apoptosis in SH-SY5Y cells. ***A,*** Cell prolifera-tion was measured in SH-SY5Y cells with OGDR treatment. ***B,*** Cell proliferation was meas-ured in SH-SY5Y cells with miR-26a mimics or inhibitor under OGDR conditions. ***C,*** The expression of apoptosis-related genes (Bax and Bcl-2) in SH-SY5Y cells with OGDR treat-ment. D, The expression of apoptosis-related genes (Bax and Bcl-2) in SH-SY5Y cells with miR-26a mimics or inhibitor under OGDR conditions. *p<0.05, **p<0.01.

between miR-26a and PTEN, a Dual-Luciferase reporter assay was performed in HEK-293. The results showed that upregulation of miR-26a significantly suppressed the Luciferase activity of wild-type PTEN, but had no effect on mutant PTEN ($p<0.01$, Figure 3B). It was demonstrated that miR-26a directly targeted PTEN. Besides that, the expression of PTEN was markedly decreased under OGDR conditions compared to the OGDR-free group ($p<0.01$, Figure 3C). Interestingly, miR-26a mimics further reduced the expression of PTEN in SH-SY5Y cells treated with OGDR. However, miR-26a inhibitor enhanced the expression level of PTEN ($p<0.05$, Figure 3D). Therefore, miR-26a can further inhibit PTEN expression to regulate cerebral ischemia injury.

PTEN Expression Was Associated with Cell Viability and Apoptosis

To investigate the role of PTEN in cerebral ischemia injury, PTEN plasmid or siRNA was transfected into SH-SY5Y cells under OGDR conditions. We found that PTEN was upregulated by plasmids transfection and downregulated by siRNA transfection ($p<0.01$, Figure 4A). Next, the effects of upregulation and downregulation of PTEN on cell viability was investigated. MTT assay showed that overexpression of PTEN reduced cell viability compared to the OGDR group ($p<0.05$, Figure 4B). In contrast, PTEN silencing promoted cell viability in SH-SY5Y cells under OGDR conditions ($p<0.01$,

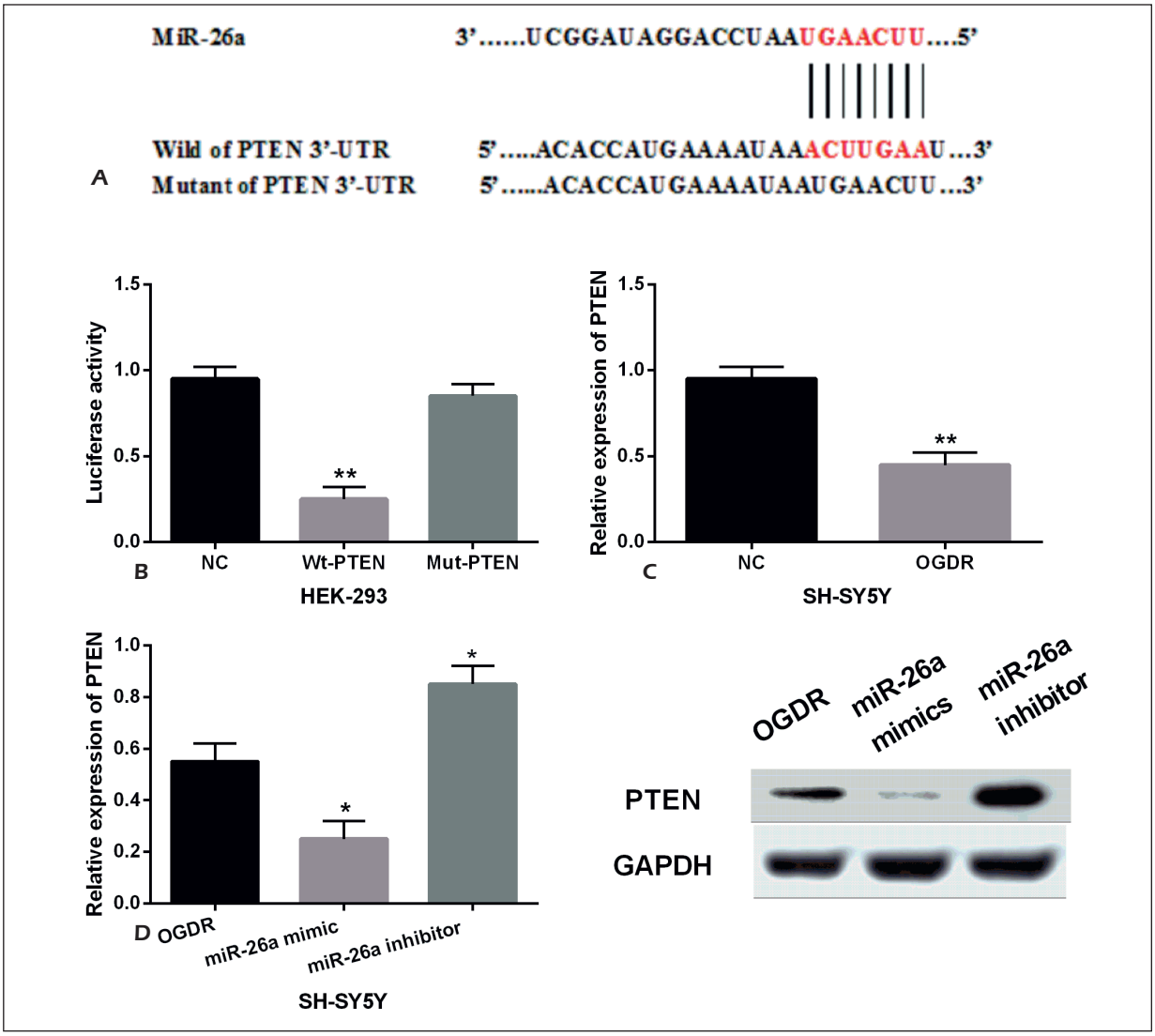

Figure 3. PTEN was a direct target gene of miR-26a. ***A,*** The binding sites between PTEN and miR-26a. ***B,*** Luciferase reporter assay. ***C,*** The expressions of PTEN in SH-SY5Y cells with OGDR treatment. ***D,*** The expression of PTEN was observed in SH-SY5Y cells with miR-26a mimics or inhibitor under OGDR conditions. *$p<0.05$, **$p<0.01$.

Figure 4B). Based on the above results, PTEN was considered to inhibit cell survival in cerebral ischemia injury, thereby deepening the effect of OGDR treatment.

Similarly, the effect of abnormal PTEN expression on apoptosis of SH-SY5Y cells under OGDR conditions was investigated by measuring expressions of Bax and Bcl-2. The results showed that overexpression of PTEN apparently promoted Bax expression, but blocked the expression of Bcl-2 ($p<0.01$, Figure 4C). These results implied that overexpression of PTEN could accelerate apoptosis, which was the same as OGDR treatment. In contrast, knockdown of PTEN remarkably inhibited Bax expression and promoted Bcl-2 expression under OGDR conditions ($p<0.01$, Figure 4D). Therefore, the downregulation of PTEN was elucidated for the first time to promote the viability of SH-SY5Y cells and inhibit apoptosis under OGDR conditions.

MiR-26 Regulated Cerebral Ischemia Injury by Targeting PTEN

Finally, miR-26a mimics and PTEN plasmid were co-transfected into SH-SY5Y cells under OGDR conditions to further detect their interaction in cerebral ischemia injury. The results of the RT-qPCR assay showed that the decreased expression of PTEN induced by miR-26a mimics was recovered by PTEN plasmid ($p<0.01$, Figure 5A). More importantly, PTEN plasmid prevented the acceleration of miR-26a on the proliferation of SH-SY5Y cells under OGDR conditions ($p<0.01$, Figure 5B). Similarly, the inhibitory effect of miR-26a on cell apoptosis was also reversed by the PTEN plasmid ($p<0.01$, Figure 5C). In combination with these results, miR-26a was considered to promote the viability of SH-SY5Y cells and suppress apoptosis by targeting PTEN under OGDR conditions.

Discussion

Previous studies have reported that many miRNAs are involved in the development of cerebral ischemia injury, such as miR-3473b[16], miR-130a[17] and miR-155[18]. In the current work, we focused on cerebral ischemia injury and subsequent apoptosis. To simulate cerebral ischemia injury, an OGDR model was constructed in SH-SY5Y cells. The function of miR-26a was investigated due to its close association with cerebral ischemia[19]. PTEN had been identified as s direct target gene for miR-26a, which played a key role in cerebral ischemia injury reported by Zhang et al[20]. First, the expression of miR-26a was significantly increased in SH-SY5Y cells under OGDR conditions. Moreover, overexpression of miR-26a promoted cell viability and inhibited apoptosis of SH-SY5Y cells. Consistently, Liang et al[21] had demonstrated that miR-26a promoted cell viability and inhibited apoptosis in a rat model of cerebral infarction. It was then found that miR-26 could negatively regulate cerebral ischemia injury by promoting cell survival and inhibiting apoptosis.

In particular, apoptosis is an important biological process of cerebral ischemia injury and is a prominent type of cell death[22]. Apoptosis-related proteins such as Bax and Bcl-2 can be modulated to affect apoptosis[23]. However, Bax and Bcl-2 have opposite effects. Bcl-2 inhibits cell apoptosis, and Bax promotes apoptosis[24]. Here, we found that Bax expression was improved by OGDR treatment that reduced Bcl-2 expression and upregulation of miR-26a sharply reduced Bax expression and promoted Bcl-2 expression. Thus, overexpression of miR-26a inhibited apoptosis induced by OGDR treatment. Similarly, miR-26a had been reported[21] to inhibit apoptosis in a rat model of cerebral infarction. Therefore, miR-26a negatively regulated cerebral ischemia injury by inhibiting apoptosis.

Furthermore, several target genes for miR-26a had been identified in human disease, including EZH2, HMGA1 and PLOD2[25-27]. In the current study, PTEN was identified as a direct target of miR-26a, which was associated with cell viability and apoptosis in cerebral ischemia injury. Recently, the protective effect of PTEN inhibition on cerebral ischemia damage had become the focus of attention. It was previously reported[28] that PTEN degradation was detected after ischemic stroke, which was a double-edged sword. We also found that the expression of PTEN was reduced under OGDR conditions in SH-SY5Y cells. In addition, PTEN inhibition had been shown to prevent cortical neuron injury in the rat following Hypoxia-Ischemia[29]. Consistently, downregulation of PTEN protected SH-SY5Y cells against cerebral ischemia injury-induced cell death in this study. Finally, it was observed that miR-26a prevented cerebral ischemia injury by targeting PTEN.

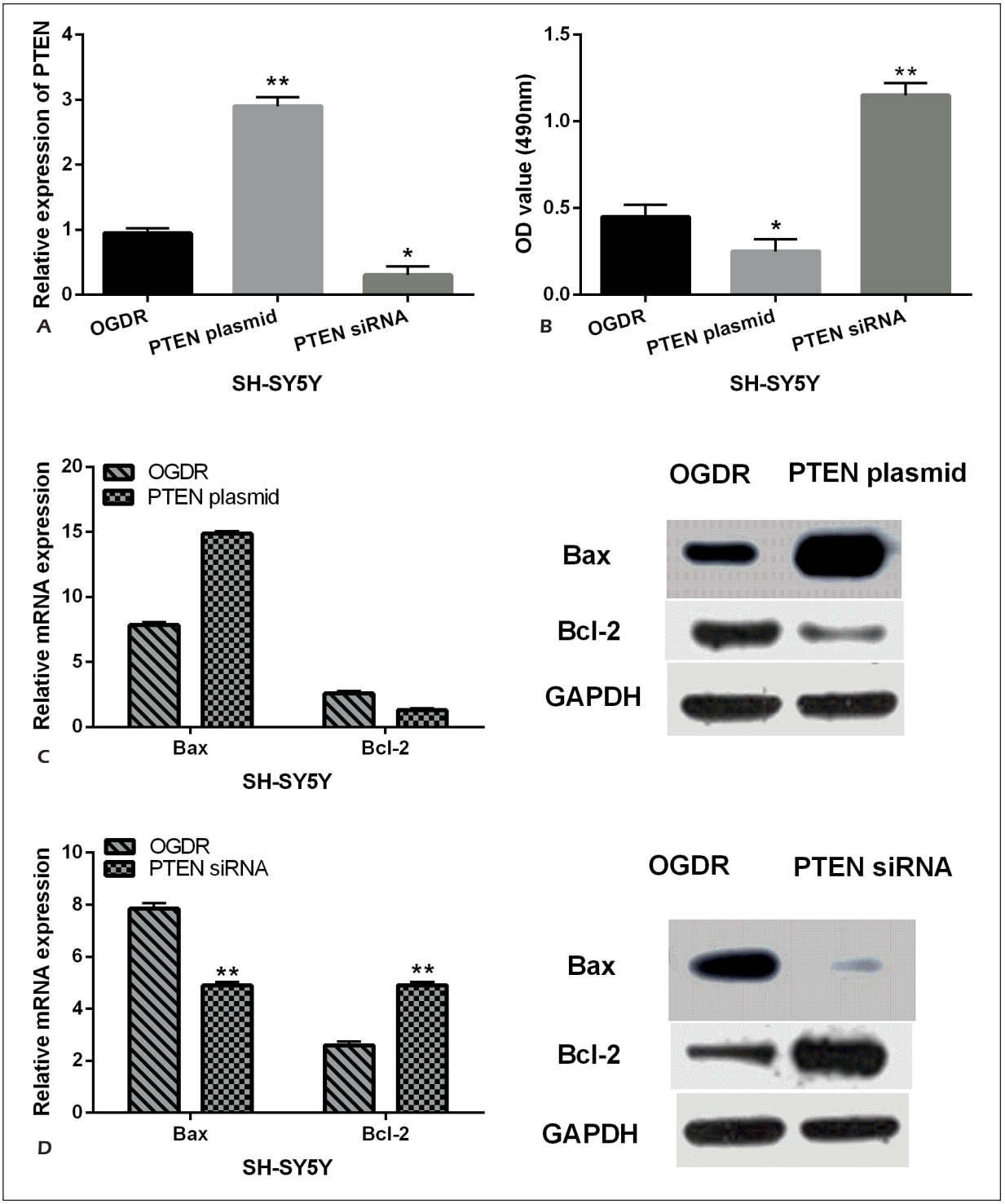

Figure 4. PTEN expression was associated with cell viability and apoptosis. ***A,*** The expression of PTEN was examined in SH-SY5Y cells with PTEN plasmid or siRNA under OGDR conditions. ***B,*** Cell proliferation was measured in SH-SY5Y cells with PTEN plasmid or siRNA under OGDR conditions. ***C,*** The expression of apoptosis-related genes (Bax and Bcl-2) in SH-SY5Y cells with PTEN plasmid under OGDR conditions. ***D,*** The expression of apoptosis-related genes (Bax and Bcl-2) in SH-SY5Y cells with PTEN siRNA under OGDR conditions. *p<0.05, **p<0.01.

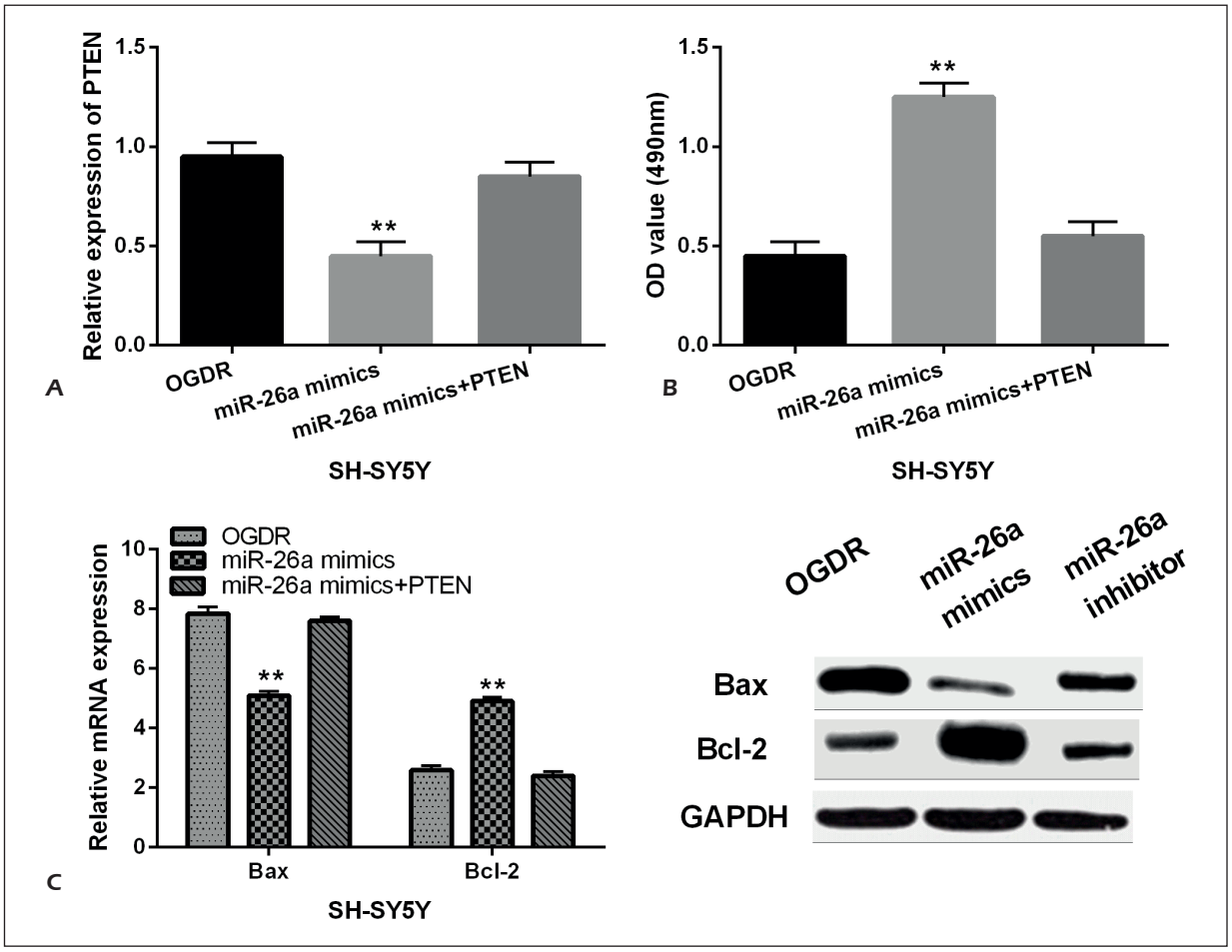

Figure 5. MiR-26 regulated cerebral ischemia injury by targeting PTEN. ***A,*** The expression of PTEN was measured in SH-SY5Y cells with PTEN plasmid and miR-26a mimics under OGDR conditions. ***B,*** Cell proliferation was measured in SH-SY5Y cells with PTEN plasmid and miR-26a mimics under OGDR conditions. ***C,*** The expression of apoptosis-related genes (Bax and Bcl-2) in SH-SY5Y cells with PTEN plasmid and miR-26a mimics under OGDR conditions.

Conclusions

This study mainly elucidated that miR-26a promoted cell viability and inhibited apoptosis by targeting PETN, thereby negatively regulating cerebral ischemic injury. Even so, the current research still has some drawbacks, such as the lack of animal experiment. Therefore, we will further explore the roles of miR-26a and PTEN in cerebral ischemia injury *in vivo*. Our findings will help us develop novel uses of miR-26a as a therapeutic target for cerebral ischemia injury.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. Circulation 2012; 125: e2-e220.
2) Xu W, Zheng J, Gao L, Li T, Zhang J, Shao A. Neuroprotective effects of stem cells in ischemic stroke. Stem Cells Int 2017; 2017: 4653936.
3) Khoshnam SE, Winlow W, Farzaneh M, Farbood Y, Moghaddam HF. Pathogenic mechanisms following ischemic stroke. Neurol Sci 2017; 38: 1167-1186.

4) Eichenbaum H. Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron 2004; 44: 109-120.
5) Martinez B, Peplow PV. Blood microRNAs as potential diagnostic and prognostic markers in cerebral ischemic injury. Neural Regen Res 2016; 11: 1375-1378.
6) Liang Y, Xu J, Wang Y, Tang JY, Yang SL, Xiang HG, Wu SX, Li XJ. Inhibition of miRNA-125b decreases cerebral ischemia/reperfusion injury by targeting CK2alpha/NADPH oxidase signaling. Cell Physiol Biochem 2018; 45: 1818-1826.
7) Liang L, Wang J, Yuan Y, Zhang Y, Liu H, Wu C, Yan Y. MicRNA-320 facilitates the brain parenchyma injury via regulating IGF-1 during cerebral I/R injury in mice. Biomed Pharmacother 2018; 102: 86-93.
8) Leeper NJ, Raiesdana A, Kojima Y, Chun HJ, Azuma J, Maegdefessel L, Kundu RK, Quertermous T, Tsao PS, Spin JM. MicroRNA-26a is a novel regulator of vascular smooth muscle cell function. J Cell Physiol 2011; 226: 1035-1043.
9) Wen L, Cheng F, Zhou Y, Yin C. MiR-26a enhances the sensitivity of gastric cancer cells to cisplatin by targeting NRAS and E2F2. Saudi J Gastroenterol 2015; 21: 313-319.
10) Chen J, Xu Y, Tao L, Pan Y, Zhang K, Wang R, Chen LB, Chu X. MiRNA-26a contributes to the acquisition of malignant behaviors of doctaxel-resistant lung adenocarcinoma cells through targeting EZH2. Cell Physiol Biochem 2017; 41: 583-597.
11) Wu Y, Zhang MH, Xue Y, Zhang T, Wu N, Guo W, Du X, Xu YL. Effect of microRNA-26a on vascular endothelial cell injury caused by lower extremity ischemia-reperfusion injury through the AMPK pathway by targeting PFKFB3. J Cell Physiol 2019; 234: 2916-2928.
12) Mutter GL. Pten, a protean tumor suppressor. Am J Pathol 2001; 158: 1895-1898.
13) Li L, Liu F, Ross AH. PTEN regulation of neural development and CNS stem cells. J Cell Biochem 2003; 88: 24-28.
14) Lee JH, Kim KY, Lee YK, Park SY, Kim CD, Lee WS, Rhim BY, Hong KW. Cilostazol prevents focal cerebral ischemic injury by enhancing casein kinase 2 phosphorylation and suppression of phosphatase and tensin homolog deleted from chromosome 10 phosphorylation in rats. J Pharmacol Exp Ther 2004; 308: 896-903.
15) Xue L, Huang J, Zhang T, Wang X, Fu J, Geng Z, Zhao Y, Chen H. PTEN inhibition enhances angiogenesis in an in vitro model of ischemic injury by promoting Akt phosphorylation and subsequent hypoxia inducible factor-1alpha upregulation. Metab Brain Dis 2018; 33: 1679-1688.
16) Wang X, Chen S, Ni J, Cheng J, Jia J, Zhen X. miRNA-3473b contributes to neuroinflammation following cerebral ischemia. Cell Death Dis 2018; 9: 11.
17) Wang Y, Wang MD, Xia YP, Gao Y, Zhu YY, Chen SC, Mao L, He QW, Yue ZY, Hu B. MicroRNA-130a regulates cerebral ischemia-induced blood-brain barrier permeability by targeting Homeobox A5. FASEB J 2018; 32: 935-944.
18) Yang Y, Zhang N, Wang S, Wen Y. MicroRNA-155 regulates inflammatory response in ischemic cerebral tissues through autophagy. Curr Neurovasc Res 2018; 15: 103-110.
19) Park H, Park H, Mun D, Kang J, Kim H, Kim M, Cui S, Lee SH, Joung B. Extracellular vesicles derived from hypoxic human mesenchymal stem cells attenuate GSK3beta expression via miRNA-26a in an ischemia-reperfusion Injury model. Yonsei Med J 2018; 59: 736-745.
20) Zhang QG, Wu DN, Han D, Zhang GY. Critical role of PTEN in the coupling between PI3K/Akt and JNK1/2 signaling in ischemic brain injury. FEBS Lett 2007; 581: 495-505.
21) Liang Z, Chi YJ, Lin GQ, Luo SH, Jiang QY, Chen YK. MiRNA-26a promotes angiogenesis in a rat model of cerebral infarction via PI3K/AKT and MAPK/ERK pathway. Eur Rev Med Pharmacol Sci 2018; 22: 3485-3492.
22) Nakka VP, Gusain A, Mehta SL, Raghubir R. Molecular mechanisms of apoptosis in cerebral ischemia: multiple neuroprotective opportunities. Mol Neurobiol 2008; 37: 7-38.
23) Shamas-Din A, Kale J, Leber B, Andrews DW. Mechanisms of action of Bcl-2 family proteins. Cold Spring Harb Perspect Biol 2013; 5: a8714.
24) Chen HC, Kanai M, Inoue-Yamauchi A, Tu HC, Huang Y, Ren D, Kim H, Takeda S, Reyna DE, Chan PM, Ganesan YT, Liao CP, Gavathiotis E, Hsieh JJ, Cheng EH. An interconnected hierarchical model of cell death regulation by the BCL-2 family. Nat Cell Biol 2015; 17: 1270-1281.
25) Wong CF, Tellam RL. MicroRNA-26a targets the histone methyltransferase enhancer of Zeste homolog 2 during myogenesis. J Biol Chem 2008; 283: 9836-9843.
26) Liu J, Mi B, Wang Y, Shi C, Mi X, Lu Y, Yu P. MiR-26a suppresses osteosarcoma migration and invasion by directly targeting HMGA1. Oncol Lett 2018; 15: 8303-8310.
27) Miyamoto K, Seki N, Matsushita R, Yonemori M, Yoshino H, Nakagawa M, Enokida H. Tumour-suppressive miRNA-26a-5p and miR-26b-5p inhibit cell aggressiveness by regulating PLOD2 in bladder cancer. Br J Cancer 2016; 115: 354-363.
28) Li W, Huang R, Chen Z, Yan LJ, Simpkins JW, Yang SH. PTEN degradation after ischemic stroke: a double-edged sword. Neuroscience 2014; 274: 153-161.
29) Zhao J, Qu Y, Wu J, Cao M, Ferriero DM, Zhang L, Mu D. PTEN inhibition prevents rat cortical neuron injury after hypoxia-ischemia. Neuroscience 2013; 238: 242-251.