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MiR-125a-5p silencing inhibits cerebral ischemia-induced injury through targeting IGFBP3

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Abstract

Dysregulated microRNAs (miRNAs) are crucial regulators of cerebrovascular conditions, including ischemic stroke. Circulating miR-125a-5p is associated with ischemic stroke and may have clinical utility as an early diagnostic biomarker. This study conducted a series of experiments that were designed to elucidate the regulatory action of miR-125a-5p in ischemic brain injury and its underlying mechanisms. The results of this study found that the expression of miR-125a-5p was increased in BV2 microglial cells under oxygen-glucose deprivation/reoxygenation (OGD/R) setting, as well as in rat brain tissue after middle cerebral artery occlusion (MCAO). OGD/R triggered BV2 microglial cell apoptosis, whereas downregulation of miRNA-125a-5p suppressed the apoptosis rate in OGD/R-induced BV2 microglial cells. Subsequently, insulin-like growth factor binding protein 3 (IGFBP3) is a molecular target of miR-125a-5p, and IGFBP3 knockdown reversed the effects of miR-125a-5p inhibitor on BV2 microglial cells in vitro. These data supported the fact that miR-125a-5p silencing protects against cerebral ischemia-induced injury by targeting IGFBP3.

Key words: *miR-125a-5p, IGFBP3, apoptosis, ischemic stroke.*

Introduction

Ischemic stroke presents a significant risk to public health worldwide. To explore effective therapeutic strategies, extensive efforts have been made to investigate the intrinsic molecular mechanisms underlying ischemic cerebral damage [19]. The multiple pathological processes underlying cerebral ischemia-reperfusion injury are sophisticated and are influenced by numerous factors.

MicroRNAs (miRNAs) are single-stranded, non-coding RNAs that post-transcriptionally modulate gene expression by specifically pairing with the 3′-untranslated region (3′-UTR) of mRNAs [22]. Previous studies have established a connection between miRNAs and multiple inflammatory factors [4,21,25]. Recently, RNA-Seq analysis revealed that circulating miR-125b-5p, miR-143-3p, and miR-125a-5p (also known as miR-125a) can serve as latent biomarkers in ischemic stroke [6]. The level of miR-125b-5p is elevated in the MCAO rat model and miR-125b-5p is associated with OGD-initiated PC-12 cellular injury *via* regulation of CBS/H_2S [18]. Early reports have investigated the protective function of miR-125a-5p in oxidized low-density lipoprotein (ox-LDL)-evoked functional alterations in brain microvascular endothelial cells (HBMECs) [16].

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Oxidized LDL receptor-1 (LOX-1) contributes to plaque instability and the development of acute coronary syndromes, as demonstrated by LOX-1 deletion, which reduces proinflammatory signalling and attenuates atherogenesis [14]. MiR-125a-5p has also been shown to be upregulated in macrophages following oxidized LDL exposure and inhibition of miRNA-125a-5p by a complimentary binding miRNA "antagomiR" increases lipid uptake by macrophages and enhances the expression of LOX-1 [3]. MiR-125a-3p is involved in behavioural disorders in animal models of MCAO by regulating cell adhesion molecule 2 (Cadm2) [13]. Bioinformatics analyses unanimously suggest that miR-125a-5p levels are elevated in acute ischemic stroke samples and healthy controls [23,27].

Microglia become activated and injured under ischemic and hypoxic conditions [10]. Activated microglia intensively release inflammatory mediators, including tumour necrosis factor α (TNF-α), interleukin (IL)-1β, IL-6, monocyte chemoattractant protein-1 (MCP-1), prostaglandin E2 (PGE2), and nitric oxide (NO) [5]. These uncontrolled inflammatory responses are involved in microglial dysfunction and death in various brain diseases, including ischemic stroke [11]. The inflammatory or apoptotic mediators produced by over-activated microglia may cause and exacerbate microglial apoptosis [24]. Altogether, microglial cells exhibit changes in proliferation, apoptosis and releasing of cytokines in response to cerebral ischemia. Nevertheless, the regulatory role of miR-125a-5p in cerebral ischemia has not yet been elucidated.

This study revealed that miR-125a-5p was upregulated in rats following MCAO treatment. Furthermore, IGFBP3 was targeted by miR-125a-5p, and silencing of miR-125a-5p restrains cerebral ischemia-induced BV2 microglial cells apoptosis in part by indirectly targeting IGFBP3.

Material and methods

Cell culture and OGD/R model

BV2 microglial cells were purchased from GuangZhou Jennio Biotech Co., Ltd (GuangZhou, China) and maintained in DMEM (Thermo Fisher Scientific, Waltham, MA, USA) and 10% fetal bovine serum (FBS) supplemented with 1% penicillin/streptomycin (Sigma-Aldrich, Shanghai, China) in a 5% CO_2 incubator at 37°C. BV2 cells were washed twice with phosphate-buffered saline (PBS) and re-suspended in pre-warmed DMEM without glucose. During model construction, cells were grown in a hypoxia chamber (1% O_2, 5% CO_2, and 94% N_2, Thermo Fisher Scientific) with compact oxygen for 6 h. Thereafter, cells were cultured in normal DMEM supplemented with glucose (4.5 g/l) and incubated under an atmosphere of 5% CO_2 at 37°C for 24 h.

Cell transfection

miRNA corresponding control (miR-Ctrl) and miR-125a-5p mimic, or miR-125a-5p inhibitor (anti-miR-125a-5p) and its negative control (anti-miR-Ctrl) were obtained from GeneCopoeia (Guangzhou, China). IGFBP3 shRNA (sh-IGFBP3) and its negative control (sh-Ctrl) were obtained from GeneCopoeia. BV2 cells were transfected with miRNA or shRNA using Lipofectamine 2000 (Thermo Fisher Scientific) for 24 h.

Cell proliferation and apoptosis

Cells (3×10^3) were inoculated in 96-well culture plates overnight. After 1, 2, 3, or 4 days of incubation, 10 µl of cell counting kit-8 (CCK-8, Beyotime Biotechnology, Nanjing, China) reagents were plated into the culture medium. After 2 h, the optical density (OD) was determined at 450 nm using a microplate reader. For the cell apoptosis assay, BV2 cells were dyed with 5 µl of Annexin V-FITC and 5 µl of propidium iodide (PI) solution (Beyotime Biotechnology) for 15 min in an incubator. BV2 cell apoptosis was analysed using flow cytometry.

Rat model of transient focal cerebral ischemia

Sprague-Dawley rats were purchased from Shanghai Slake Laboratory Animal Co., Ltd. (Shanghai, China). Rats were anesthetized using xylazine and ketamine, and an artificial midline incision was made on the neck. Subsequently, the left common carotid artery was exposed, and its branches were electrocoagulated. To block the occlusion of the middle cerebral artery (MCA), a 6-0 surgical nylon filament with a blunt tip was advanced from the external carotid artery to the internal carotid artery. One hour after MCA occlusion, the filament was withdrawn for reperfusion for 24 h. Sham-operated rats underwent identical operations, except for the insertion of the nylon filament. After reperfusion, the rats were sacrificed, and brain tissues were collected for infarct determination. The protocol for this model

was approved by the Animal Ethics Committee of Qingdao Central Hospital. For triphenyl tetrazolium chloride (TTC) staining, 2 mm-coronal thin slices of brain tissue were incubated with 2% TTC dye solution in the dark at 37°C for 20 min. Subsequently, staining was completed after the slices were washed with PBS. The stained slices were fixed in 4% paraformaldehyde for 2 h. Unstained areas were defined as the infarct regions. For the miR-125a-5p antagomir assay, rats were injected with miR-125a-5p antagomirs (30 pmol/g, Suzhou GenePharma Co., Ltd, China) or miR-Ctrl antagomir (30 pmol/g) through the tail vein 3 days prior to MCAO. A rat model of MCAO injury was established as described previously.

Reverse transcription quantitative PCR

The total RNA was extracted using a RNEasy Purification Kit (Qiagen), while miRNAs was extracted using a mirVana miRNA Isolation Kit (Thermo Fisher Scientific). The cDNA was reverse transcribed using a PrimeScript RT Reagent Kit (Takara, Shiga, Japan). Reverse transcription quantitative PCR (qRT-PCR) was performed using SYBR Premix Ex Taq (Takara) or All-in-One™ miRNA qPCR Detection Kit (GeneCopoeia, Guangzhou, China) on an ABI PRISM 7500 Sequence Detection system (Applied Biosystems, USA). The primers used were as follows: miR-125a-5p: forward, 5′-TATTCGCACTGGATACGACACAAAC-3′ and reverse, 5′-GCCCGTGGAGTGTGACAATGGT-3′; U6: forward, 5′-AAAGCAAATCATCGGACGACC-3′ and reverse, 5′-GTACAACACATTGTTTCCTCGGA-3′; IGFBP3: forward, 5′-AGAGCACAGATACCCAGAACT-3′ and reverse, 5′-GGTGATTCAGTGTGTCTTCCATT-3′; GAPDH, forward, 5′-TGGATTTGGACGCATTGGTC-3′ and reverse, 5′-TTTGCACTGGTACGTGTTGAT-3′. Relative gene expression was measured using the $2^{-\Delta\Delta Cq}$ method. U6 was used as a control for miR-125a-5p, and GADPH was used as a control for IGFBP3.

Luciferase reporter assay

The wild-type (wt) or mutant type (mut) 3'-UTR of IGFBP3 was inserted into the pGL3M vector (Promega, Madison, WI, USA). BV2 microglial cells were seeded in 96-well plates and co-transfected with the pGL3M vector and miR-125a-5p mimic using Lipofectamine 2000 (Thermo Fisher Scientific). After 48 h post transfection, the relative luciferase activity in cells was assessed using the Dual-Luciferase Reporter Assay System (Promega).

Immunoblotting analysis

Total protein was extracted using RIPA lysis buffer (Beyotime, Nanjing, Jiangsu, China). Thirty micrograms of protein were separated using 8% SDS-PAGE and electrically transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Braunschweig, Germany). Membranes were incubated with antibodies against IGFBP3, Bcl-2, Bax, or β-tubulin (1 : 1,000; Abcam, Cambridge, UK) at 4°C overnight. HRP-conjugated anti-rabbit IgG (1 : 10,000; Cell Signaling Technology) was used as the secondary antibody. The bands were detected using an electrochemical luminescence (ECL) kit.

Statistical analysis

Data are shown as the mean ± SD. Differences were statistically analysed using Student's *t*-test or one-way ANOVA followed by Tukey's post hoc test. Statistical significance was set at $p < 0.05$.

Results

MiR-125a-5p is upregulated in the MCAO model

To probe the implication of miR-125a-5p in cerebral ischemia, MCAO was successfully established in a rat model. Brain tissue samples were collected from rats subjected to MCAO. As shown in Figure 1A, TTC staining showed that, compared with the sham group, the infarct volume in the MCAO group was evidently augmented. The cortex was damaged. Nuclear pyknosis, gliocyte swelling, and interstitial oedema in the cortex were observed (Fig. 1B). The qRT-PCR analysis indicated that the miR-125a-5p level was higher in the cerebral ischemia rats than in the sham group (Fig. 1C). To determine the role of miR-125a-5p in BV2 microglial cell injury, an OGD/R model was constructed using BV2 cells *in vitro*. The level of miR-125a-5p was upregulated in the OGD/R group compared to that in the normoxic condition (Fig. 1D). To summarize these findings, miR-125a-5p is associated with the molecular pathogenesis of cerebral ischemia.

Effects of miR-125a-5p mimic on OGD/R-caused BV2 cells injury

Thereafter, the effect of miR-125a-5p on BV2 cell viability was uncovered after treatment with OGD/R. MiR-125a-5p mimic or anti-miR-125a-5p was separately transfected into BV2 cells (Fig. 2A). BV2 cell growth

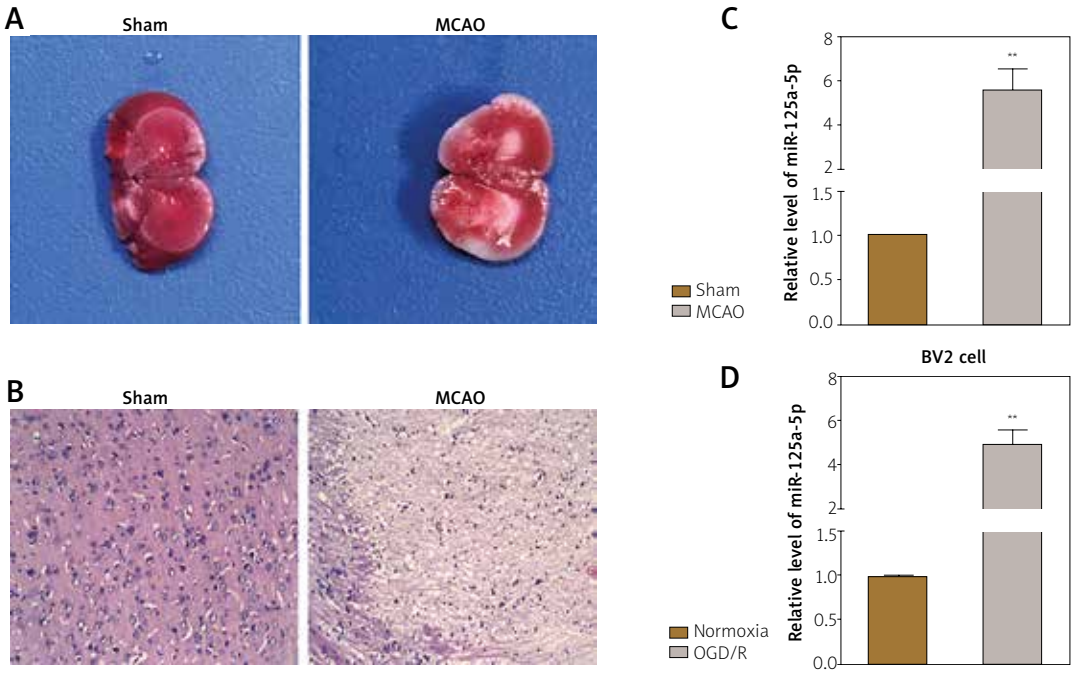

Fig. 1. Expression of miR-125a-5p in cerebral ischemia. **A**) The infarct volume was stained with TTC. **B**) H&E staining of cortex. **C**) The levels of miR-125a-5p in sham and MCAO were detected using qRT-PCR assay. $^{**}p < 0.01$ vs. sham group. **D**) The levels of miR-125a-5p in normoxia or OGD/R treated BV2 cell were detected using qRT-PCR assay. $^{**}p < 0.01$ vs. normoxia group.

was distinctly lower in the OGD/R group than in the normoxia group. However, BV2 cell proliferation in the miR-125a-5p treated group was substantially lower than that in the miR-Ctrl group (Fig. 2B). When compared to the anti-miR-Ctrl group, cell viability was rescued in the anti-miR-125a-5p group (Fig. 2C). Furthermore, the apoptosis induced by OGD/R injury was aggravated by miR-125a-5p, although decreased by anti-miR-125a-5p (Fig. 2D). In addition, a distinct elevation in the pro-apoptotic gene Bax and a reduction in the anti-apoptotic gene Bcl-2 were found in the MCAO group. A large degree of reduction in Bax expression and elevation in Bcl-2 expression was observed in BV2 cells upon anti-miR-125a-5p transfection. However, it was observed that Bax expression was increased and Bcl-2 was reduced in miR-125a-5p overexpressing BV2 cells compared to the miR-Ctrl group (Fig. 2E). These results indicated that anti-miR-125a-5p relieved OGD/R-induced BV2 cell injury.

miR-125a-5p regulates IGFBP3 in BV2

RNA22 tool (http://www.mybiosoftware.com/rna22-v2-microrna-target-detection.html) was selected to search for the targets of miR-125a-5p. IGFBP3 was predicted as the probable target of miR-125a-5p (Fig. 3A), and we focused on IGFBP3, whose expression level was decreased in OGD/R-treated cells and the MCAO rat model (Fig. 3B). This binding action was verified using a luciferase reporter assay. The IGFBP3-3′-UTR plasmid carrying wild-type (wt) or mutant-type (mut) miR-125a-5p binding site was inserted into the pGL3M vector. BV2 cells were then co-transfected with plasmids and miR-125a-5p mimic or miR-Ctrl. Different from the IGFBP3-wt + miR-Ctrl group. The relative luciferase activity was strikingly lower in the IGFBP3-wt + miR-125a-5p group (Fig. 3C). To substantiate the inhibitory effect of miR-125a-5p on IGFBP3 *in vitro*, IGFBP3 mRNA and protein levels in miR-125a-5p mimic-treated BV2 cells were assessed by qRT-PCR and immunoblotting. As shown in Figure 3D, miR-125a-5p diminished the level of IGFBP3. Nevertheless, anti-miR-125a-5p treatment markedly increased IGFBP3 levels in BV2 cells (Fig. 3E). The aforementioned data show that miR-125a-5p suppresses IGFBP3 expression in BV2 cells.

Downregulation of IGFBP3 decreases the protection effects of anti-miR-125a-5p in cerebral ischemia

To verify the action of IGFBP3 in cerebral ischemia, shRNA targeting IGFBP3 (sh-IGFBP3) was utilized to reduce IGFBP3 levels. BV2 cells were transfected

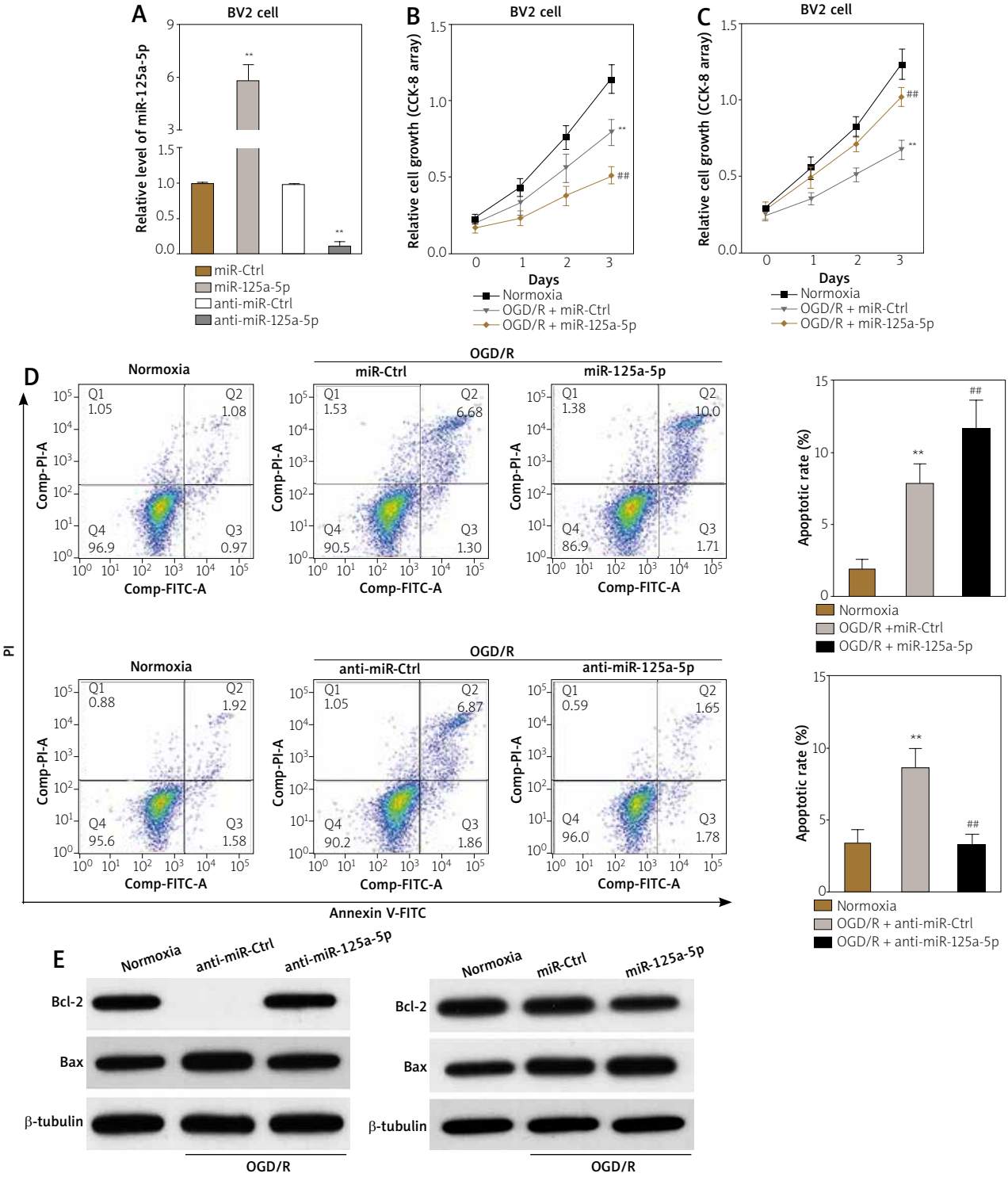

Fig. 2. Effect of miR-125a-5p on the cell proliferation and apoptosis of BV2 cell. **A**) BV2 cell was transfected with anti-miR-125a-5p or miR-125a-5p mimics. The level of miR-125a-5p was detected using qRT-PCR assay. **B, C**) BV2 cell was transfected with miR-125a-5p mimics or anti-miR-125a-5p. After transfection, cell was subjected for OGD/R treatment. The cell proliferation was detected using CCK-8 assay. **D**) After transfection, cell was subjected for OGD/R treatment. The apoptosis of BV2 cell was analyzed using flow cytometry assay. **E**) The expressions of Bcl-2 and Bax were analyzed using western blotting assay. $^{**}p < 0.01$ vs. normoxia group, $^{\#\#}p < 0.01$ vs. OGD/R + anti-miR-Ctrl or OGD/R + miR-Ctrl group.

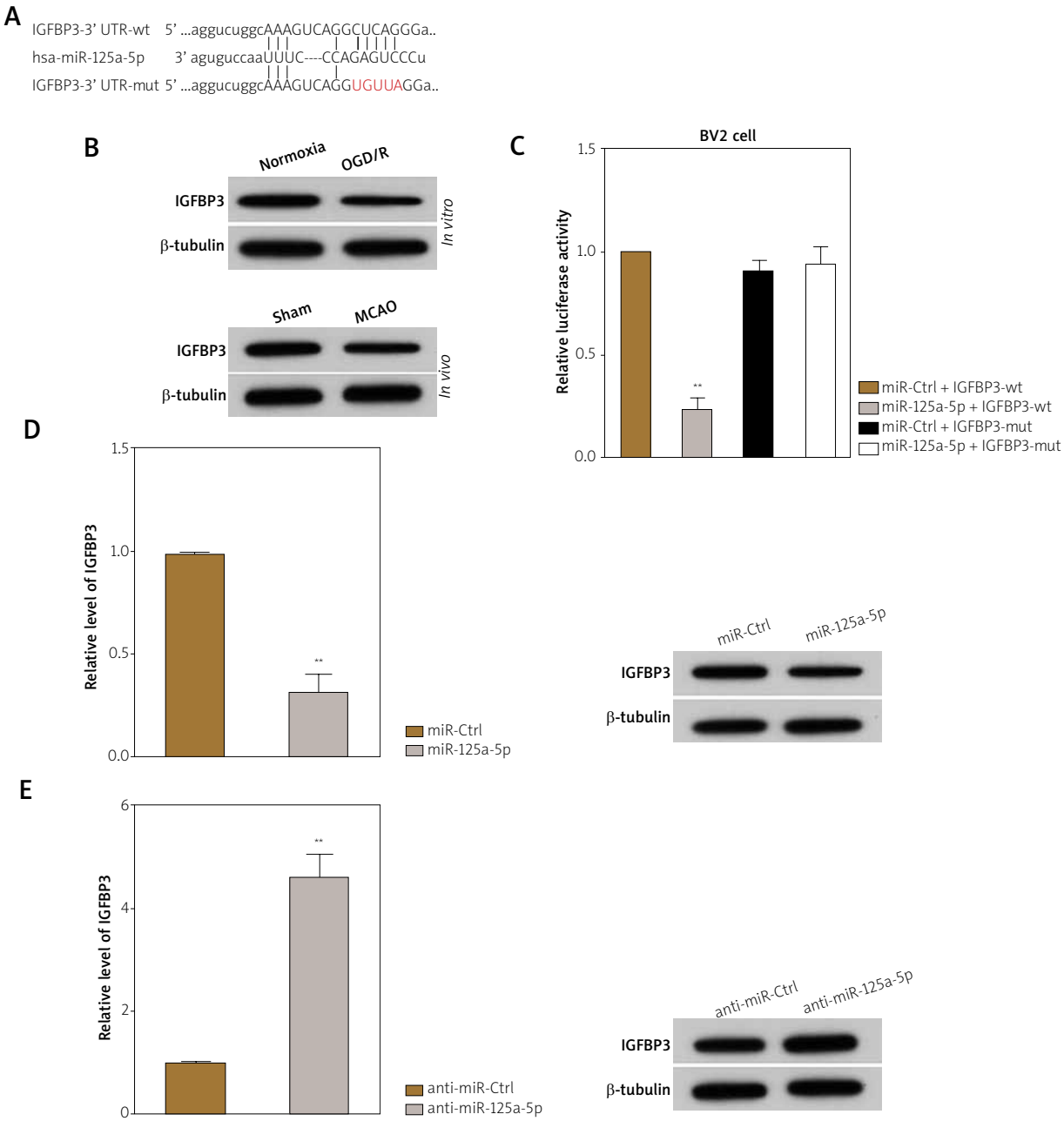

Fig. 3. miR-125a-5p binds with IGFBP3. **A**) The potential miR-125a-5p binding sites in the 3'-UTR of IGFBP3. **B**) The level of IGFBP3 in sham and MCAO was detected using western blot assay (upper panel). The level of IGFBP3 in normoxia or OGD/R treated BV2 cell were detected using western blot assay (lower panel). **C**) Luciferase reporter activity in BV2 cell following miR-125a-5p overexpression. **D, E**) qRT-PCR and western blot analysis of IGFBP3 levels in BV2 cell transfected with miR-125a-5p or anti-miR-125a-5p. $^{**}p < 0.01$ vs. anti-miR-Ctrl or miR-Ctrl group.

with anti-miR-125a-5p or co-transfected with anti-miR-125a-5p and sh-IGFBP3. Upon anti-miR-125a-5p treatment, the expression of IGFBP3 was increased, whereas that of IGFBP3 was decreased in cells co-transfected with sh-IGFBP3 and anti-miR-125a-5p (Fig. 4A). Meanwhile, the viability was increased, and BV2 cell apoptosis was decreased in anti-miR-125a-5p transfected BV2 cells following treatment with OGD/R. However, co-transfection with anti-miR-125a-5p plus sh-IGFBP3 significantly boosted the apopto-

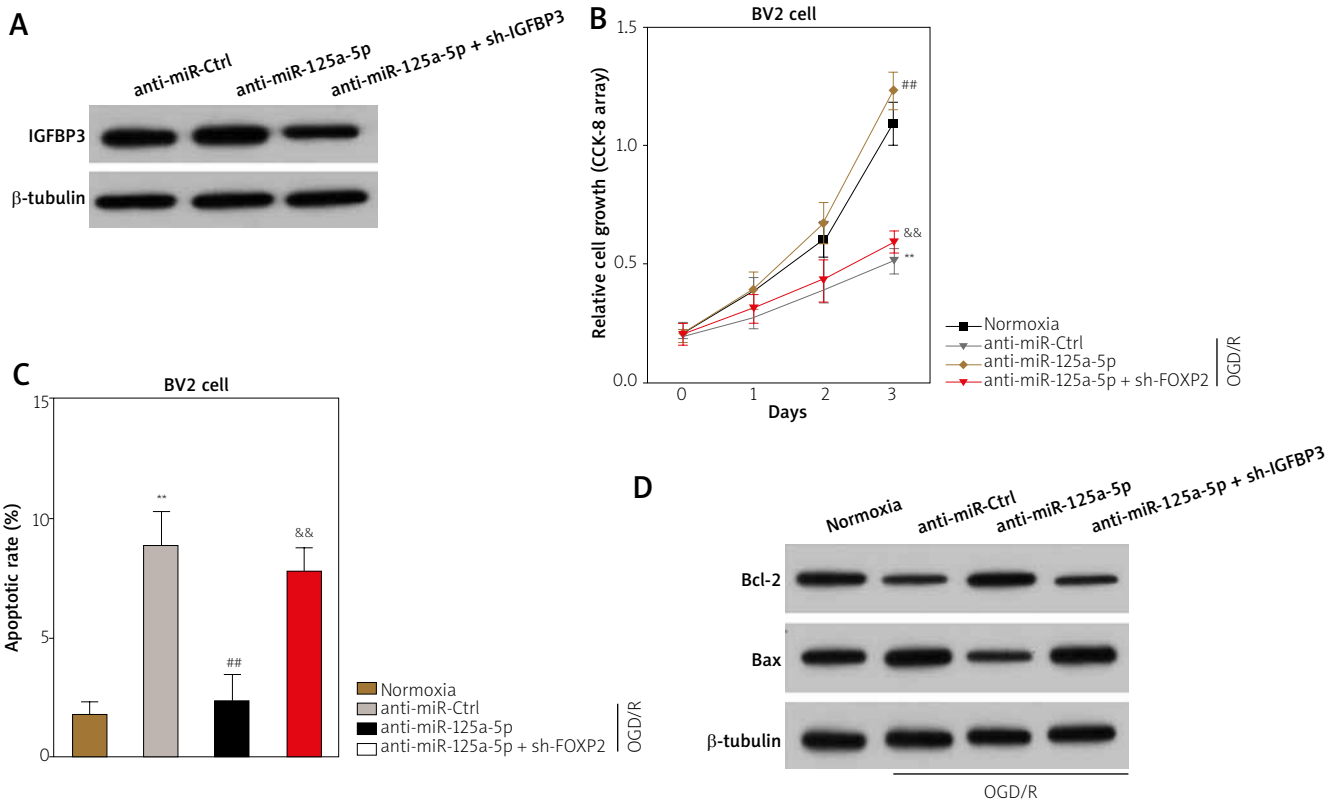

Fig. 4. IGFBP3 silencing attenuates the protective effects of anti-miR-125a-5p on ischemic injury. **A**) BV2 cell was transfected with anti-miR-125a-5p or cotransfected with anti-miR-125a-5p and sh-IGFBP3, and the IGFBP3 protein expression was determined by western blot. **B**) BV2 cell was transfected with anti-miR-125a-5p or cotransfected with anti-miR-125a-5p and sh-IGFBP3 before exposure to OGD/R. The cell proliferation was detected using CCK-8 assay. **C**) The apoptosis of BV2 cell was analyzed using flow cytometry assay. **D**) Bac-2 and Bax protein expressions were determined by western blotting assay. $^{**}p < 0.01$ vs. normoxia group, $^{\#\#}p < 0.01$ vs. OGD/R + anti-miR-Ctrl, $^{\&\&}p < 0.01$ vs. OGD/R + anti-miR-125a-5p.

sis of BV2 cells and decreased cell viability compared with the anti-miR-125a-5p alone group (Fig. 4B, C). The levels of Bcl-2 increased by anti-miR-125a-5p in BV2 cells following treatment with OGD/R was inhibited by sh-IGHBP3. However, co-transfection with anti-miR-125a-5p and sh-IGFBP3 significantly restored the protein expression of Bax compared to the anti-miR-125a-5p group (Fig. 4D). These data collectively indicate that anti-miR-125a-5p regulates BV2 cell apoptosis partly by altering IGFBP3 levels.

Pretreatment with miR-125a-5p antagomir lessens infarct damage following MCAO

Finally, the role of miR-125a-5p in infarct damage *in vivo* was investigated. Rats were treated with either miR-125a-5p antagonist or miR-Ctrl antagomir 3 days prior to MCAO (Fig. 5A). As illustrated in Figure 5B, rats treated with miR-125a-5p antagomir exhibited smaller infarct volumes than rats treated with miR-Ctrl antagomir. Compared with the miR-Ctrl antagomir, miR-125a-5p antagomir decreased the level of miR-125a-5p in brain tissue, as confirmed by qRT-PCR (Fig. 5C). To investigate the potential mechanisms of miR-125a-5p in the regulation of ischemic brain injury, the expression levels of Bcl-2/Bax and IGFBP3 were examined using western blotting. miR-125a-5p antagomir effectively enhanced Bcl-2 and IGFBP3 protein levels and reduced the expression of Bax in rats after focal cerebral ischemia (Fig. 5D). These data suggest that the miR-125a-5p antagonist weakens infarct damage following MCAO.

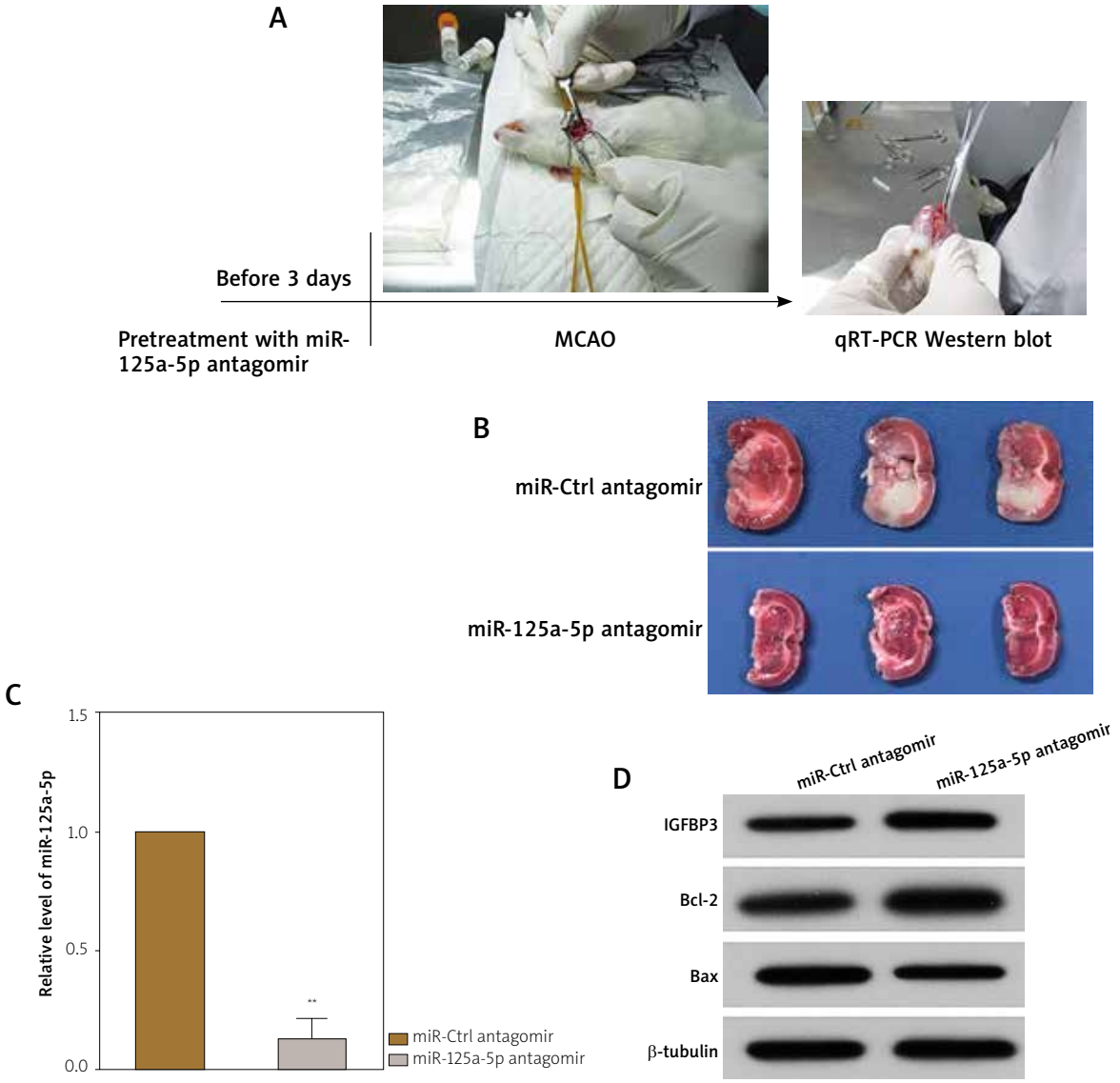

Fig. 5. The effect of miR-125a-5p antagomir on ischemic brain infarct. **A**) Rat was treated either miR-125a-5p antagomir (30 pmol/g) or miR-Ctrl antagomir (30 pmol/g) 3 days prior to MCAO. **B**) The infarct volumes in sham and MCAO group were stained with TTC. **C**) qRT-PCR data demonstrates that miR-125a-5p expression was significantly inhibited in the brain tissue from miR-125a-5p antagomir group. **D**) The expressions of Bcl-2, Bax and IGFBP3 were measured by western blotting assay. $^{**}p < 0.01$ vs. miR-Ctrl antagomir.

Discussion

Stroke is one of the main causes of death and disability worldwide [15]. Significant improvements have been made in therapeutic options for ischemic stroke. Noncoding RNAs consist of several major classes, including miRNAs and lncRNAs. miRNAs have been implicated in many diseases, including cancer, asthma, and uraemia. Furthermore, miRNAs have been found to be abundantly expressed in the central nervous system and play critical roles in cerebral diseases, such as ischemic stroke, traumatic brain injury and Alzheimer's disease [2,20].

Recently, several miRNAs have been identified as candidate biomarkers for brain damage and prognosis in ischemic stroke [20,23]. The evidence implies that miRNAs play critical roles in several cellular changes following ischemic stroke, including oxidative stress, mitochondrial dysfunction, cytokine-me-

diated cytotoxicity, and inflammatory responses [28]. Whole blood and plasma can both be adopted as sources of circulatory miRNAs. Microarray analysis of blood specimens from healthy individuals and individuals diagnosed with cerebral ischemia has revealed that circulating miRNAs in plasma could serve as biomarkers for diagnosing and treating stroke [26]. In addition, other studies evaluated the expression profiles of miRNAs in the cerebrospinal fluid (CSF) after stroke. miRNA profiles in CSF are physiologically contiguous with profiles in the brain extracellular fluid [8]. Manipulating the levels of miRNAs using either mimic sequences or inhibitory sequences (antagomirs) has been shown to mediate neuroprotection in stroke models [7,19]. Abe *et al*. identified five miRNAs whose expression levels were altered with the onset and treatment of cerebral infarction, suggesting their potential use for predicting symptoms of cerebral infarction [1]. These findings will promote the development of miRNA-based clinical applications and provide a new guiding strategy for miRNA-based stroke treatment.

In this study, miR-125a-5p-5p levels were distinctly elevated in brain tissue samples collected from rats receiving MCAO. Consequently, this study identified the influence of miR-125a-5p on OGD/R-derived BV2 cell injury *in vitro*. Cell viability was significantly increased by the miR-125a-5p inhibitor, although significantly decreased by the miR-125a-5p mimics. Apoptosis was greatly increased in the pathological process of cerebral infarction, resulting in cerebral infarction. The results of this study showed that OGD/R induced significant apoptosis in BV2 cells. However, OGD/R-induced cell apoptosis was relieved by the miR-125a-5p inhibitor and was significantly aggravated by the miR-125a-5p mimic, which suggests that miR-125a-5p silencing has a neuroprotective effect. Many molecules are associated with apoptosis, such as Bax (pro-apoptotic) and Bcl-2 (anti-apoptotic) genes. Bcl-2 has been validated to exert the neuroprotective effects of neurons in cerebral infarction. In this study, miR-125a-5p downregulation increased Bcl-2 expression and decreased Bax expression in BV2 cells and rat MCAO models.

To explore the mechanisms underlying the protective role of the miR-125a-5p inhibitor, IGFBP3 was bioinformatically predicted to be the target of miR-125a-5p. We hypothesized that the protective role of miR-125a-5p inhibitor depends on IGFBP3. First, the interaction between IGFBP3 and miR-125a-5p was verified by luciferase reporter gene assay and western blotting. Particularly, the expression of IGFBP-3 in patients with acute cerebral ischemia was markedly lower than that in healthy individuals [9,17]. These findings suggest that IGFBP-3 may exert neuroprotective effects against I/R injury. The observations from the *in vitro* OGD/R BV2 cell model showed that IGFBP3 expression was markedly lowered, which was opposite to the miR-125a-5p level. Furthermore, it was found that knockdown of IGFBP3 reversed the protective effects of miR-125a-5p downregulation against OGD/R-triggered BV2 cell damage, which proves that IGFBP3 serves as the downstream effector in miR-125a-5p-mediated BV2 cell survival when exposed to OGD/R. Pretreatment with miR-125a-5p antagomir reduced infarct damage following MCAO in a rat model. In our current study, we mainly focus on that silencing of miR-125a-5p restrains OGD/R-induced BV2 microglial cell apoptosis by indirectly targeting IGFBP3 *in vitro* and pretreatment with miR-125a-5p antagomir lessens infarct damage following MCAO *in vivo*. This study is only a preliminary study of the role of miR-125a-5p in cerebral ischemia-induced injury. More studies using primary neuron culture or hippocampal slice culture would be useful to understand the role of miR-125a-5p in cerebral infarction. For a better understanding of the mechanism, the use of hippocampal slice culture or primary cortical neuronal culture is planned. After a "pure" primary cortical neuronal cell line is obtained, the optimal miR-125a-5p mimic concentration for transfection should be determined.

In conclusion, this study provides novel insights into the protective action of miR-125a-5p downregulation in inhibiting microglial cell apoptosis following cerebral infarction by targeting IGFBP3.

Disclosure

The authors report no conflict of interest.

References

1. Abe A, Tanaka M, Yasuoka A, Saito Y, Okada S, Mishina M, Abe K, Kimura K, Asakura T. Changes in whole-blood microRNA profiles during the onset and treatment process of cerebral infarction: a human study. Int J Mol Sci 2020; 21: 3107.
2. Cao Y, Zhang H, Lu X, Wang J, Zhang X, Sun S, Bao Z, Tian W, Ning S, Wang L, Cui L. Overexpression of microRNA-9a-5p ameliorates NLRP1 inflammasome-mediated ischemic injury in rats following ischemic stroke. Neuroscience 2020; 444: 106-177.

3. Chen T, Huang Z, Wang L, Wang Y, Wu F, Meng S, Wang C. MicroRNA-125a-5p partly regulates the inflammatory response, lipid uptake, and ORP9 expression in oxLDL-stimulated monocyte/macrophages. Cardiovasc Res 2009; 83: 131-139.
4. Chen Y, Song Y, Huang J, Qu M, Zhang Y, Geng J, Zhang Z, Liu J, Yang GY. Increased circulating exosomal miRNA-223 Is associated with acute ischemic stroke. Front Neurol 2017; 8: 57.
5. Graeber MB, Streit WJ. Microglia: biology and pathology. Acta Neuropathol 2010; 119: 89-105.
6. Heydari E, Alishahi M, Ghaedrahmati F, Winlow W, Khoshnam SE, Anbiyaiee A. The role of non-coding RNAs in neuroprotection and angiogenesis following ischemic stroke. Metab Brain Dis 2020; 35: 31-43.
7. Huang L, Ma Q, Li Y, Li B, Zhang L. Inhibition of microRNA-210 suppresses pro-inflammatory response and reduces acute brain injury of ischemic stroke in mice. Exp Neurol 2018; 300: 41-50.
8. Iwuchukwu I, Nguyen D, Sulaiman W. MicroRNA profile in cerebrospinal fluid and plasma of patients with spontaneous intracerebral hemorrhage. CNS Neurosci Ther 2016; 22: 1015-1018.
9. Johnsen SP, Hundborg HH, Sorensen HT, Orskov H, Tjonneland A, Overvad K, Jorgensen JO. Insulin-like growth factor (IGF) I, -II, and IGF binding protein-3 and risk of ischemic stroke. J Clin Endocrinol Metab 2005; 90: 5937-5941.
10. Kaur C, Rathnasamy G, Ling EA. Roles of activated microglia in hypoxia induced neuroinflammation in the developing brain and the retina. J Neuroimmune Pharmacol 2013; 8: 66-78.
11. Kim SJ, Li J. Caspase blockade induces RIP3-mediated programmed necrosis in Toll-like receptor-activated microglia. Cell Death Dis 2013; 4: e716.
12. Liu X, Feng Z, Du L, Huang Y, Ge J, Deng Y, Mei Z. The potential role of microRNA-124 in cerebral ischemia injury. Int J Mol Sci 2019; 21: 120.
13. Liu Y, Li Y, Ren Z, Si W, Li Y, Wei G, Zhao W, Zhou J, Tian Y, Chen D. MicroRNA-125a-3p is involved in early behavioral disorders in stroke-afflicted rats through the regulation of Cadm2. Int J Mol Med 2017; 40: 1851-1859.
14. Mehta JL, Sanada N, Hu CP, Chen J, Dandapat A, Sugawara F, Satoh H, Inoue K, Kawase Y, Jishage K, Suzuki H, Takeya M, Schnackenberg L, Beger R, Hermonat PL, Thomas M, Sawamura T. Deletion of LOX-1 reduces atherogenesis in LDLR knockout mice fed high cholesterol diet. Circ Res 2007; 100: 1634-1642.
15. Meng XL, Zhang DL, Sui SH. Acute remote ischemic preconditioning alleviates free radical injury and inflammatory response in cerebral ischemia/reperfusion rats. Exp Ther Med 2019; 18: 1953-1960.
16. Pan Q, Liao X, Liu H, Wang Y, Chen Y, Zhao B, Lazartigues E, Yang Y, Ma X. MicroRNA-125a-5p alleviates the deleterious effects of ox-LDL on multiple functions of human brain microvessel endothelial cells. Am J Physiol Cell Physiol 2017; 312: C119-C130.
17. Schwab S, Spranger M, Krempien S, Hacke W, Bettendorf M. Plasma insulin-like growth factor I and IGF binding protein 3 levels in patients with acute cerebral ischemic injury. Stroke 1997; 28: 1744-1748.
18. Shen Y, Shen Z, Guo L, Zhang Q, Wang Z, Miao L, Wang M, Wu J, Guo W, Zhu Y. MiR-125b-5p is involved in oxygen and glucose deprivation injury in PC-12 cells via CBS/H2S pathway. Nitric Oxide 2018; 78: 11-21.
19. Sohrabji F, Selvamani A. Sex differences in miRNA as therapies for ischemic stroke. Neurochem Int 2019; 127: 56-63.
20. Sonoda T, Matsuzaki J, Yamamoto Y, Sakurai T, Aoki Y, Takizawa S, Niida S, Ochiya T. Serum microRNA-based risk prediction for stroke. Stroke 2019; 50: 1510-1518.
21. Sorensen SS, Nygaard AB, Nielsen MY, Jensen K, Christensen T. miRNA expression profiles in cerebrospinal fluid and blood of patients with acute ischemic stroke. Transl Stroke Res 2014; 5: 711-718.
22. Takahashi RU, Miyazaki, H, Takeshita, F, Yamamoto, Y, Minoura, K, Ono, M, Kodaira M, Tamura K, Mori M, Ochiya, T. Loss of microRNA-27b contributes to breast cancer stem cell generation by activating ENPP1. Nat Commun 2015; 6: 7318.
23. Tiedt S, Prestel M, Malik R, Schieferdecker N, Duering M, Kautzky V, Stoycheva I, Bock J, Northoff BH, Klein M, Dorn F, Krohn K, Teupser D, Liesz A, Plesnila N, Holdt LM, Dichgans M. RNA-Seq identifies circulating miR-125a-5p, miR-125b-5p, and miR-143-3p as potential biomarkers for acute ischemic stroke. Circ Res 2017; 121: 970-980.
24. Xiang HF, Cao DH, Yang YQ, Wang HQ, Zhu LJ, Ruan BH, Du J, Wang MC. Isoflurane protects against injury caused by deprivation of oxygen and glucose in microglia through regulation of the Toll-like receptor 4 pathway. J Mol Neurosci 2014; 54: 664-670.
25. Xiao S, Ma Y, Zhu H, Sun H, Yin Y, Feng G. miRNA functional synergistic network analysis of mice with ischemic stroke. Neurol Sci 2015; 36: 143-148.
26. Xu W, Gao L, Zheng J, Li T, Shao A, Reis C, Chen S, Zhang J. The roles of microRNAs in stroke: possible therapeutic targets. Cell Transplant 2018; 27: 1778-1788.
27. Zhang L, Liu B, Han J, Wang T, Han L. Competing endogenous RNA network analysis for screening inflammationrelated long noncoding RNAs for acute ischemic stroke. Mol Med Rep 2020; 22: 3081-3094.
28. Zuo X, Lu J, Manaenko A, Qi X, Tang J, Mei Q, Xia Y, Hu Q. MicroRNA-132 attenuates cerebral injury by protecting blood-brain-barrier in MCAO mice. Exp Neurol 2019; 316: 12-19.