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# MiR-155-5p accelerates cerebral ischemia-reperfusion injury *via* targeting DUSP14 by regulating NF-κB and MAPKs signaling pathways

Y. SHI[1], K. LI[2], K. XU[3], Q.-H. LIU[4]

[1]Department of Neurology, Xuzhou Cancer Hospital, Xuzhou City, Jiangsu Province, China
[2]Department of Neurology, Affiliated Hospital of Xuzhou Medical University, Xuzhou City, Jiangsu Province, China
[3]Department of Neurology, Xuzhou Central Hospital, Xuzhou City, Jiangsu Province, China
[4]Department of Radiology, Xuzhou Central Hospital, Xuzhou City, Jiangsu Province, China

*Yu Shi* and *Ke Li* contributed equally to this work

**Abstract. – OBJECTIVE: This study aimed to explore the role of miR-155-5p in middle cerebral artery occlusion/reperfusion (MCAO/R) model in rats and oxygen-glucose deprivation/reoxygenation (OGD/R)-induced SH-SY5Y cells. In addition, this study also aimed to explore the underlying mechanisms to expect that miR-155-5p may be investigated as a new and effective diagnostic and therapeutic target for ischemic stroke.**

**MATERIALS AND METHODS: The *in vivo* MCAO/R rat model and *in vitro* OGD/R cell model were established. The miR-155-5p mRNA expression was detected by quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR). Dual specificity ATPase (DUSP) 14 was predicted to be a potential target of miR-155-5p by TargetScan. The targeting relationship was confirmed by Luciferase assay. The cell viability was determined using the Cell Counting Kit-8 (CCK-8). The expression level of inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) levels were detected by Enzyme-Linked Immunosorbent Assay (ELISA). Western blot was used to detect the protein expression of DUSP14, the apoptotic protein Cleaved cysteine-aspartic acid protease (caspase)-3, and Cleaved PARP, as well as nuclear factor kappa B (NF-κB) and MAPKs signaling pathways related proteins.**

**RESULTS: MiR-155-5p was upregulated in both MCAO/R rats and OGD/R-induced SH-SY5Y cells. MiR-155-5p knockdown inhibited OGD/R-induced cell injury and inflammation, as well as MCAO/R-induced brain injury. MiR-155-5p regulated the NF-κB and MAPKs signaling pathways by targeting DUSP14. DUSP14 knockdown partially reversed the protective effect of miR-155-5p knockdown on OGD/R-induced SH-SY5Y cell injury and inflammation.**

**CONCLUSIONS: MiR-155-5p accelerates cerebral I/R injury via targeting DUSP14 by regulating NF-κB and MAPKs signaling pathways. Inhibition of miR-155-5p significantly reduces apoptosis and brain injury. These results indicated that miR-155-5p plays a key role in cerebral I/R injury and has the potential to be explored as a new target for ischemic stroke.**

*Key Words:*
Cerebral ischemia-reperfusion injury, OGD/R, MiR-155-5p, DUSP14, NF-κB, MAPKs.

## Introduction

Ischemic stroke is one of cerebrovascular diseases with extremely complicated pathogenesis and high mortality rate. By 2030, the death toll from stroke is expected to rise to 7.8 million[1-4] and the incidences keep increasing in recent years[5]. The cerebral I/R injury is accompanied with apoptosis, oxidative stress, and the glutamate excitotoxicity in neurons, which seriously damages the nerve functions[6-8]. Currently, the treatment strategy mainly relies on pharmacology and/or mechanical thrombolysis[9]. However, the therapeutic effects are limited, and its underlying molecular mechanisms still remain unclear. Better understanding of the nature of cerebral I/R injury may offer new therapeutic strategies for ischemic insults.



*Corresponding Author:* Yu Shi, MD; e-mail: YuShisrt@163.com

miRNA is a non-coding single-stranded RNA with a length of 18-25 bp. It causes degradation of the target gene by binding to the 3'-UTR region of mRNA and inhibits the target gene expression[10]. Some studies have indicated that miRNAs are closely associated with neurologic dysfunctions, including stroke[11] and Alzheimer's diseases[12]. MiRNA was reported to regulate apoptosis and oxidative stress in ischemic stroke[13]. Among miRNAs, miR-181c was downregulated in the stroke plasma, which may exacerbate brain injury[14]. In contrast, Wang et al[15] showed that miR-29b is a target to alleviate the ischemic injury. However, the exact effects of miR-155-5p on cerebral I/R injury are still not clear.

MiR-155-5p is a by-product of the miR-155 precursor and is dysregulated in breast cancer, colon carcinoma, and other malignant tumors[16-18]. Recently, the study showed that inhibition of the miR-155-5p decreased the neuroinflammation after experimental traumatic brain injury in mice, and improved the functional recovery[19]. Downregulation of miR-155-5p mediated the anti-apoptotic effect of IL-17 in experimental autoimmune encephalomyelitis[20]. Furthermore, tMCAO significantly induced the increased expression of miR155-5p and iNOS in the ischemic cortex[21]. However, the impact of miR-155-5p on ischemic stroke has never been explored.

DUSP14, also known as MKP6[22], can dephosphorylate the MAPKs and regulate various cellular responses, such as stress response, proliferation, and immune defense[23,24]. Janrong et al[25] have shown that DUSP14 reduces inflammation and apoptosis by activating Nrf-2, thereby rescues cerebral ischemia reperfusion (IR) injury.

Here, the *in vivo* MCAO/R rat model and *in vitro* OGD/R cell model were established to explore the effect of miR-155-5p on cerebral I/R injury and the underlying mechanisms. Our results suggested that miR-155-5p is expected to be used in the diagnosis, prognosis, and therapeutic intervention of ischemic stroke.

## Materials and Methods

### Materials

Male Sprague-Dawley (SD) rats (SPF, 30, 270-320 g; Weitong Lihua, Beijing,China), TTC (2, 3, 5-triphenyltetrazolium chloride; Sigma-Aldrich, St. Louis, MO, USA, T8160), Cell Counting Kit-8 (CCK-8; Beyotime, Beijing, China, C0037), LDH Release Assay Kit (Beyotime, Beijing, China C0016), Lipid Peroxidation MDA Assay Kit (Beyotime, Beijing, China, S0131), the Dual-Luciferase assay kit (Promega, Madison, WI, USA), Human IL-6 Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Beyotime, Beijing, China, PI330), Human IL-1β ELISA Kit (Beyotime, Beijing, China, PI305), Human TNF-α ELISA Kit (Beyotime, Beijing, China, PT518). All experiment protocols were approved by the Ethics Committee of Xuzhou Cancer Hospital (Approval No. 2019-04-001).

### MCAO/R Model

The SD rats were randomly divided into 4 groups: Sham, ischemia for 30 min, and reperfusion for 12 h, 24 h, and 48 h, respectively (n = 6/group). The rats were anesthetized and fixed in supine position. The skin was cut to expose and separate the carotid artery. We closed the bifurcation of internal and external carotid arteries with artery clips, ligated the proximal and distal ends of external carotid arteries, and cut them in the middle. Nylon wire was inserted into the internal carotid artery from the free end of the external carotid artery at 18.5 ± 0.5 mm until resistance was felt. The end of nylon thread was allowed to pass through the beginning of the middle cerebral artery, and the internal carotid artery was ligated to fix the nylon thread and prevent bleeding. The end of the nylon thread was leaving 1 cm outside the skin. After 30 min, the nylon wire was pulled out and reperfusion was performed for 12 h, 24 h, and 48 h respectively. The sham group did not block the middle cerebral artery, but other operations are consistent with the MCAO/R group.

### Cell Culture

The SH-SY5Y cell line (Cell bank of the Chinese Academy of Sciences, Shanghai, China) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin, 10% fetal bovine serum (FBS) in 5% $CO_2$ at 37°C.

### OGD/R Model

OGD/R is established to simulate the I/R model *in vitro*. After OGD culture for 4 h, the cells were reoxygenated for 6 h, 12 h, and 24 h, respectively.

### Plasmids Transfection

When the cells confluence reached 60%-70%, the miR-155-5p and its negative control (miR-NC), as well as miR-155-5p inhibitor and the

negative control (inhibitor NC), were transfected by the Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instruction. Culture medium was changed 4-6 h after transfection.

### Western Blot Analysis

The total proteins from each sample were extracted and the concentration was determined by bicinchoninic acid (BCA) kit (Pierce, Rockford, IL, USA). Cell protein samples and loading buffer (5×) were mixed (4:1), then, boiled at 100° C for 5-10 min. 20 μL of loading samples were separated by 10%.

The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were then transferred to the polyvinylidene difluoride (PVDF) membrane, and then, the membranes were blocked with 5% Bovine Serum Albumin (BSA) in TBST for 1-2 h. The membranes were incubated with the corresponding primary antibodies at 4° C overnight. Then, the membranes were washed with TBST for 10 min × 3 times. Then, the membranes were incubated with the secondary antibodies at room temperature for 2 h. TBST was used to wash the membranes for 5 min × 3 times. Odyssey Infrared Imaging (LI-COR, Bad Homburg, Germany) was used to detect the protein expression.

### Quantitative Real Time Reverse-Transcription Polymerase Chain Reaction (qRT-PCR)

The total RNAs were extracted using TRIzol or miRNAs extraction kits according to the manufacturer's instructions. Specific miR-155-5p reverse transcription primers were used for reverse transcription reaction according to the RNA reverse transcription kit manual. Real Time-PCR amplification was performed using 2 μl cDNA as the template in the Bio-Rad quantitative PCR instrument (Hercules, CA, USA). The primer sequence is as follows: miR-155-5p (UUAAUGCUAAUUGUGAUAGGGGU); miR-423-5p (UGA GGGGCAGAGAGCGAGACUUUU); GAPDH (F: AACCTGCCAAGTATGATGAC, R: GGAGTTGCTGTTGAAGTCA); TNF-α (F: CATCTTCTCAAAATTCGAGTGACAA, R: TGGGAGTAGACAAGGTACAACCC); IL-1β (F: CAGAAGTACCTGAGCTCGCC, R: AGATTCGTAGCTGGATGCCG); IL-6 (F: TTCGGTCCAGTTGCCTTCTC, R: TCTTCTCCTGGGGGTACTGG); DUSP14 (F: GAGGCGTACAACTGGGTGAA, R: ATCAGTTGCCTCCAGAAGCC).

### TTC Staining

The rats were anesthetized and sacrificed and the brain tissue in each group were cut into 2.0 mm sections. Brain tissue was immersed in 2% TTC for 30 min and fixed overnight in 4% paraformaldehyde at 4° C. Then, Image J software (NIH, Bethesda, MD, USA) was used to calculate the cerebral infarction area and take the average value.

### Cell Counting Kit-8 assay (CCK-8)

Cell viability was detected by using the CCK-8 assay. The cells were digested with trypsin and inoculated into a 96-well plate, triplicate wells for each group. CCK-8 solution (10 μl) was then added and the 96-well plate were further incubated for 1 h at 37° C, 5% $CO_2$. The absorbance at 450 nm was evaluated by an automated microplate reader (Tecan Infinite M200, Männedorf, Switzerland).

### Assessment of LDH Levels

The LDH Cytotoxicity assay was detected following the manufacturer's protocol. SH-SY5Y cells ($4 \times 10^4$ cells/well) were seeded into 96-well plates and transfected the corresponding plasmids for 24 h. The LDH reagent was added (1:100) and incubated at 37° C for 1 h. Then, transfer 120 μl/well to a new 96-well plate and the absorbance value at 490 nm was detected by an automated microplate reader (Tecan Infinite M200, Männedorf, Switzerland).

### Assessment of MDA Levels

SH-SY5Y cells ($1 \times 10^5$ cells/well) were transfected the corresponding plasmids for 24 h. The MDA production were detected using the Lipid peroxidation MDA assay kit (Beyotime, Beijing, China) according to the manufacturer's instructions.

### ELISA

The production of TNF-α, IL-1β, and IL-6 in the cell supernatant were determined by Sandwich ELISA using commercial ELISA kits. The absorbance at 450 nm was detected by an automated microplate reader (Tecan Infinite M200, Männedorf, Switzerland) to achieve quantitative detection.

### Luciferase Assay

The full length of DUSP14 3'UTR containing miR-155-5p binding site was cloned into the Luciferase reporter plasmids after PCR ampli-

fication, named as DUSP14-WT and DUSP14 3'UTR mutation was named as DUSP14-MUT. The Luciferase reporter plasmids containing the DUSP14-WT and DUSP14-MUT were co-transfected into SH-SY5Y cells with negative control plasmids, and then, the Luciferase activity was detected using the Dual-Luciferase Reporter Assay following the manufacturer's protocols (Promega, Madison, WI, USA).

### Statistical Analysis

GraphPad Prism 6.05 (La Jolla, San Diego, CA, USA) was used for data analysis and the experimental data were represented as the mean value ± SD. One-way analysis of variance (ANOVA) and Student's *t*-test were used for comparative analysis of differences and $p<0.05$ was considered to be statistically significant.

## Results

### MiR-155-5p Was Upregulated in MCAO/R Rats

*In vivo* MCAO/R rat model was established, and the reperfusion was performed after ischemia for 30 min. The expression level of miR-155-5p was detected at 12 h, 24 h, and 48 h of reperfusion. qRT-PCR results indicated that the miR-155-5p level was significantly increased in a reperfusion time-dependent manner while no significant change was detected in the sham group (Figure 1A). Next, miR-155-5p antagomir was injected into MCAO/R rats and the miR-155-5p level and the brain injury were detected. qRT-PCR results illustrated that the expression level of miR-155-5p was significantly reduced by miR-155-5p antagomir (Figure 1B). Then, the TTC staining results showed that miR-155-5p antagomir decreased the cerebral infarction area (Figure 1C). miR-155-5p antagomir also significantly reduced LDH and MDA levels (Figure 1D), indicating that miR-155-5p might be a regulator of cerebral I/R injury.

### MiR-155-5p Knockdown Inhibited OGD/R-Induced SH-SY5Y Cell Injury

*In vitro* OGD/R cell model was established by deprivation of oxygen-glucose for 4 h and then reoxygenated for 6 h, 12 h, and 24 h, respectively. The qRT-PCR results indicated that the expression of miR-155-5p was significantly increased after OGD/R (Figure 2A). Then, miR-155-5p inhibitor was transfected into the cells to reduce the miR-155-5p mRNA levels (Figure 2B). CCK-8 assay showed that miR-155-5p inhibitor increased cell viability (Figure 2C) and reduced the LDH and MDA level (Figure 2D and Figure 2E). In addition, Western blot results indicated that miR-155-5p inhibitor decreased the cleaved caspase-3 and cleaved PARP expression (Figure 2F). These results indicated that inhibition of miR-155-5p inhibited OGD/R-induced SH-SY5Y cells injury.

### Inhibition of MiR-155-5p Decreased OGD/R-Induced Inflammation

The miR-155-5p inhibitor was transfected into OGD/R-induced cells. Then, the expression of the inflammatory cytokines was measured using qRT-PCR and ELISA methods, respectively. The qRT-PCR results showed that miR-155-5p inhibitor decreased the IL-6, IL-β, TNF-α mRNA expression (Figure 3A). The ELISA results indicated that miR-155-5p inhibitor reduced the IL-6, IL-β, TNF-α protein levels (Figure 3B). These results suggested that miR-155-5p inhibitor decreased OGD/R-induced inflammation.

### MiR-155-5p Inhibits DUSP14 Via Directly Binding to the 3'UTR

To find the downstream genes of miR-155-5p, TargetScan was used for the prediction. The predicted results showed that DUSP14 might be a potential target gene of miR-155-5p. Figure 4A showed the binding site of DUSP14 on miR-155-5p. Then, the AGCAUUA sequence was mutated to UCGUAAU, which is named as DUSP14-MUT. Then, miR-155-5p was over-expressed or knocked down in SH-SY5Y cells. The efficiency was confirmed by qRT-PCR. Figure 4B showed that the miR-155-5p levels were significantly increased by miR-155-5p mimics while decreased by miR-155-5p inhibitor. Luciferase assay results demonstrated that miR-155-5p over-expression reduced DUSP14-WT Luciferase activity while had no effect on DUSP14-MUT Luciferase activity (Figure 4C). In addition, miR-155-5p overexpression reduced DUSP14 expression whereas miR-155-5p knockdown increased DUSP14 level. These results suggested that miR-155-5p inhibited DUSP14 expression by directly binding to its 3'UTR (Figure 4D).

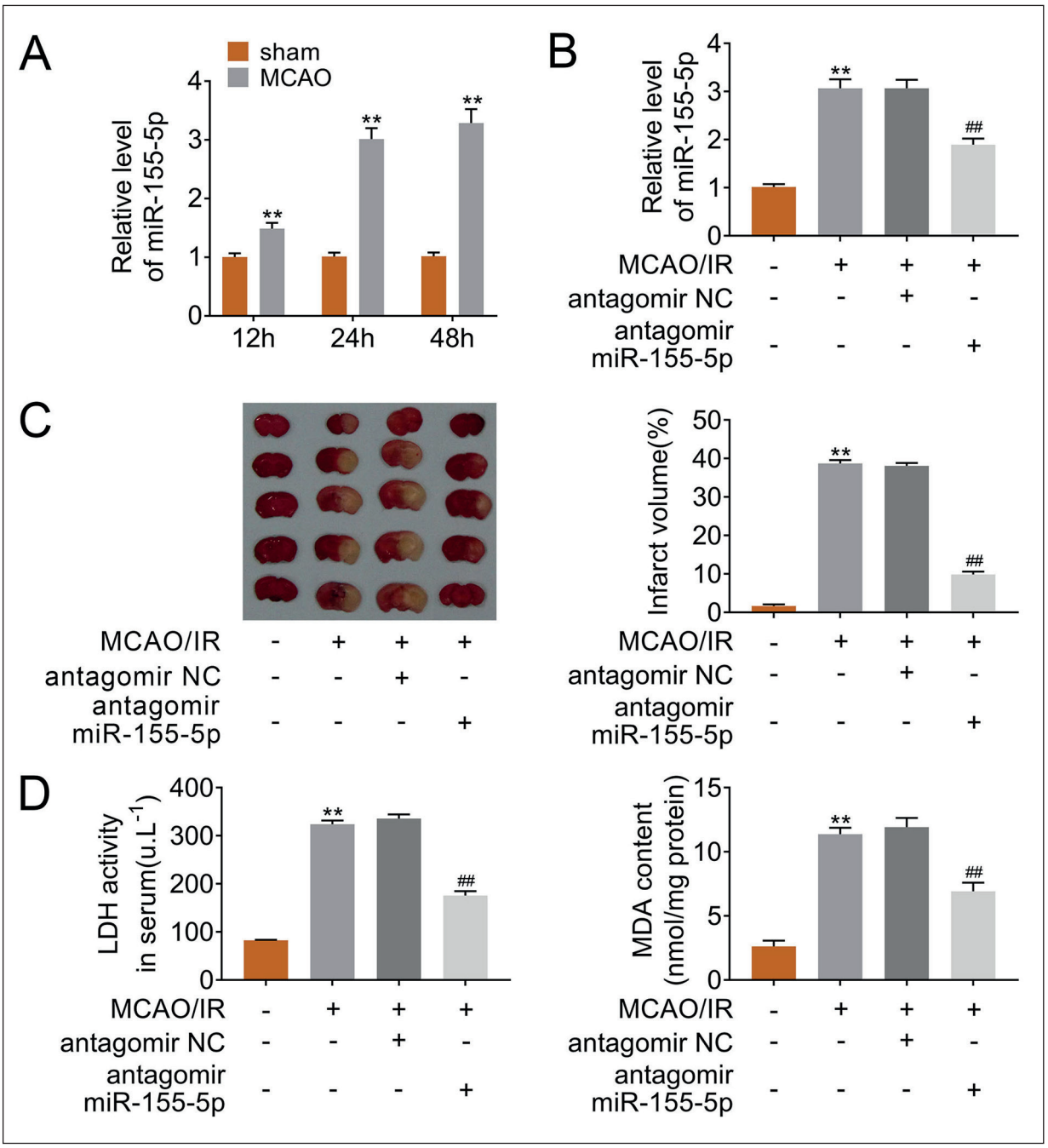


**Figure 1.** MiR-155-5p was increased in MCAO/R rats. **A,** MCAO/R model was established and miR-155-5p mRNA level was measured by qRT-PCR. **B,** Effect of miR-155-5p antagomir on miR-155-5p level in MCAO/R rats. **C,** The area of cerebral infarction was detected by TTC staining and the infarcted areas were in white and non-infarcted areas were in red. **D,** Effects of miR-155-5p antagomir on the LDH and MDA levels. $^{**}p<0.05$, $^{\#\#}p<0.05$.

### *MiR-155-5p Regulates the NF-κB and MAPKs Signaling Pathways by Targeting DUSP14*

It has been reported that DUSP14 negatively regulated the TCR signaling by inhibiting JNK and ERK pathways. Furthermore, NF-κB is activated and quickly enters into the nucleus, binding with specific sequences of DNA and promoting the expression of adhesion molecules, cytokines, resulting in inflammation

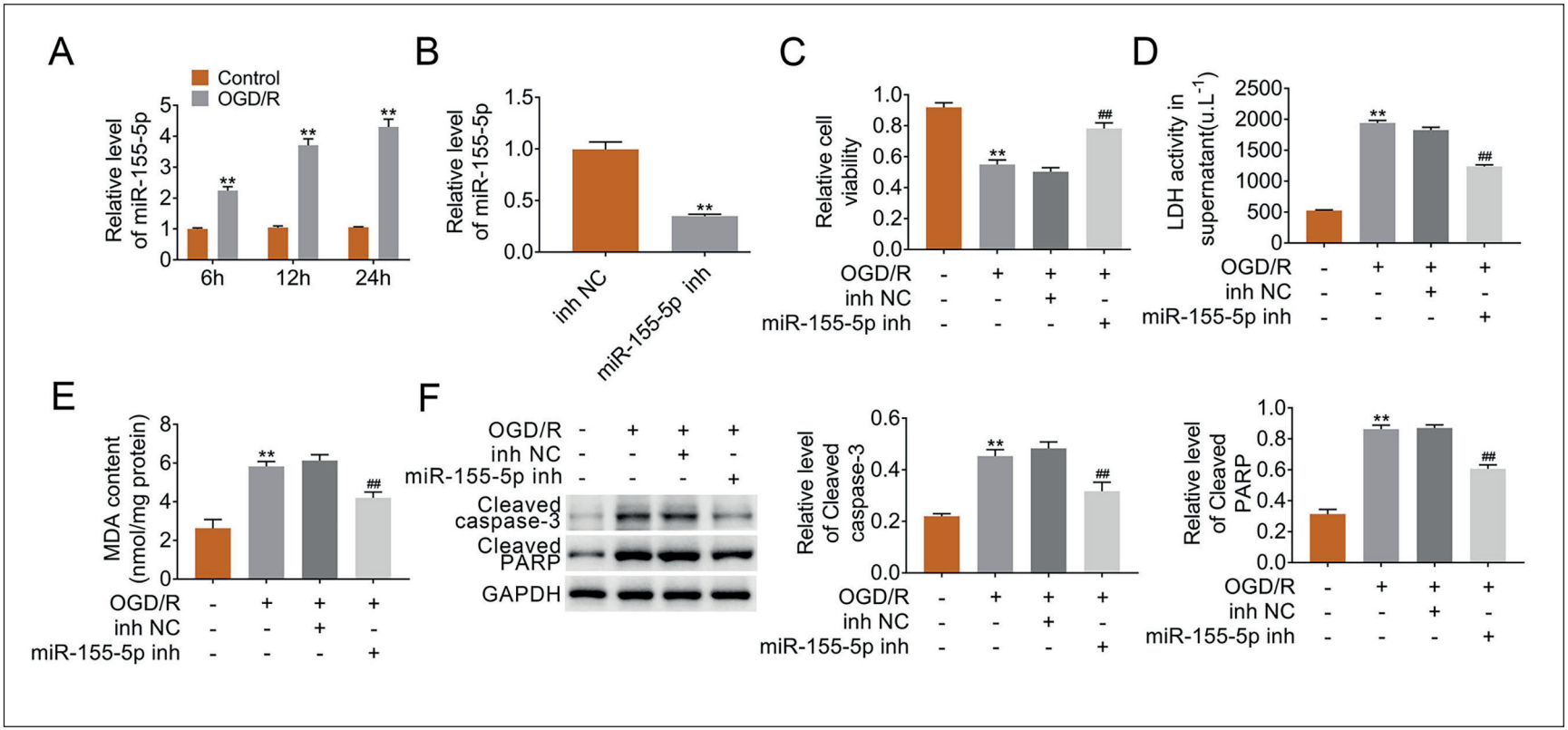


**Figure 2.** MiR-155-5p Knockdown Inhibited OGD/R-Induced Cells Injury. **A,** The miR-155-5p mRNA levels in OGD/R-induced cells injury. **B,** The efficiency of miR-155-5p inhibitor. **C,** The cell viability was measured by CCK-8 assay. **D,** and **E,** Effects of miR-155-5p inhibitor on the LDH and MDA levels. **F,** Effects of miR-155-5p inhibitor on the apoptotic protein cleaved caspase-3 and cleaved PARP. $^{**}p<0.05$, $^{\#\#}p<0.05$.

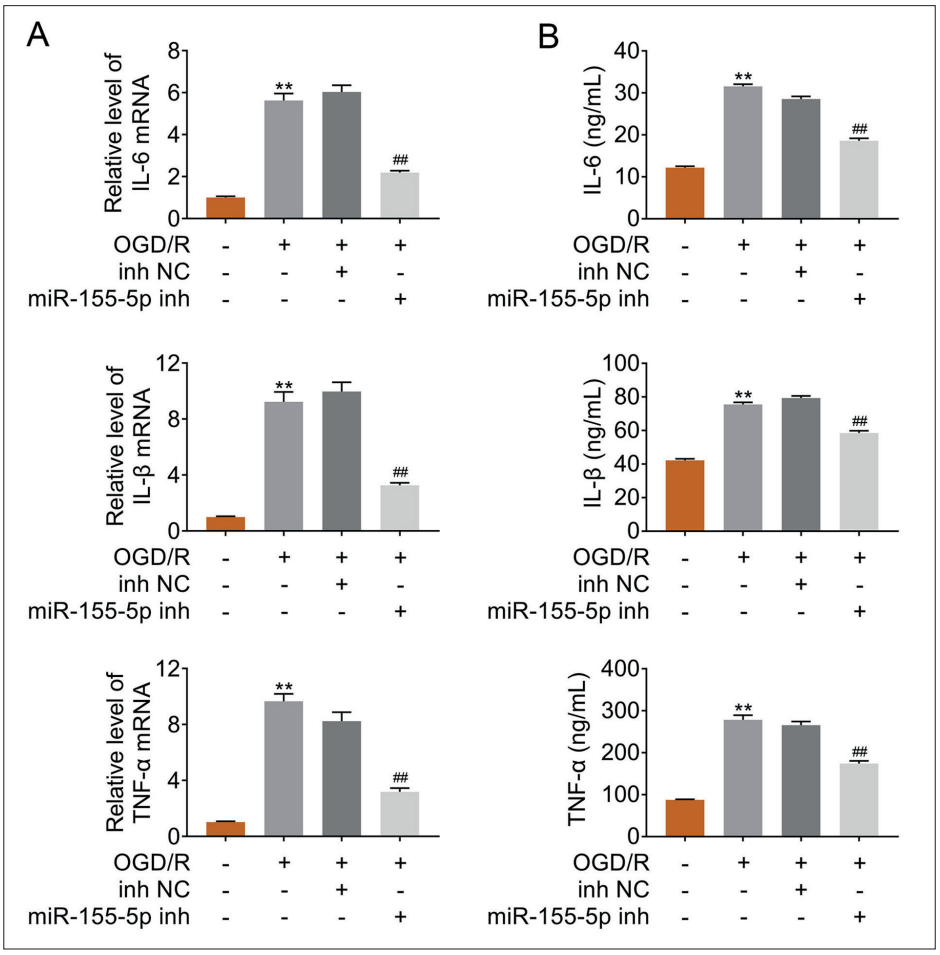


**Figure 3.** Inhibition of miR-155-5p decreased OGD/R-induced inflammation. **A,** Effects of miR-155-5p inhibitor on IL-6, IL-β, TNF-α mRNA levels were detected by qRT-PCR. **B,** Effects of miR-155-5p inhibitor on IL-6, IL-β, TNF-α contents were measured by ELISA. $^{**}p<0.05$, $^{\#\#}p<0.05$.

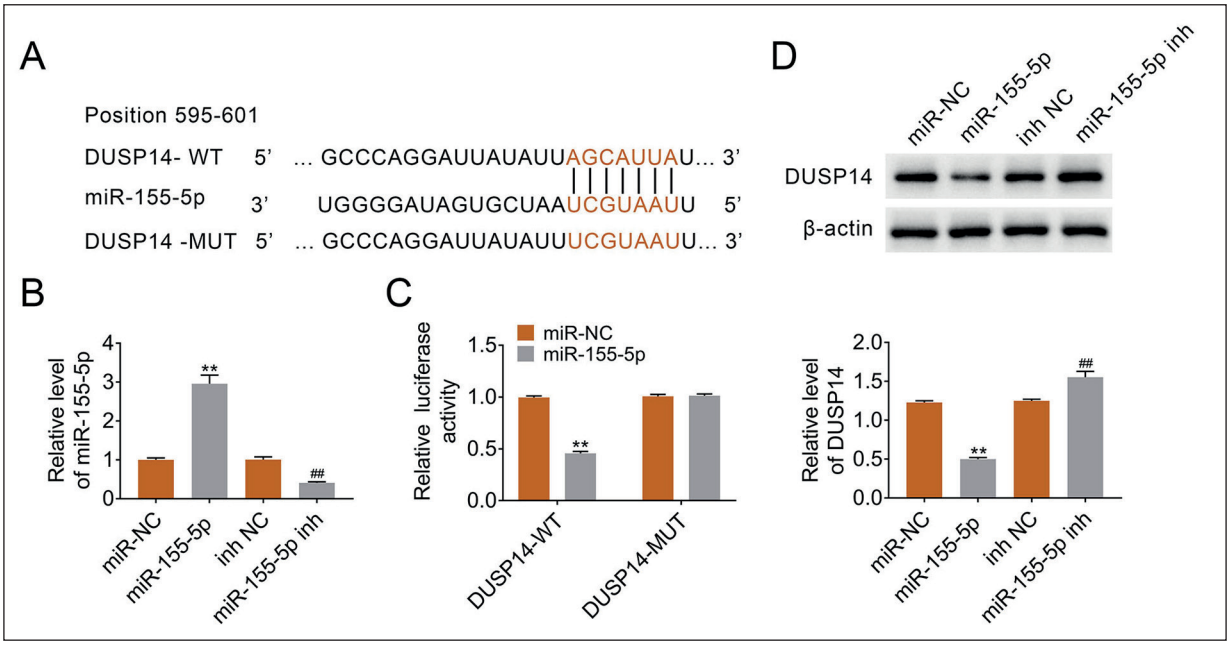


**Figure 4.** MiR-155-5p Inhibits DUSP14 by Directly Binding to the 3'UTR. **A,** The binding site of DUSP14 on miR-155-5p 3'UTR. **B,** The efficiency of miR-155-5p overexpression and knockdown. **C,** Effects of miR-155-5p overexpression on DUSP14 expression were detected by Luciferase assay. **D,** Effects of miR-155-5p on DUSP14 protein levels were detected by Western Blot. $^{**}p<0.05$, $^{\#\#}p<0.05$.

when the cerebral ischemia occurs. Therefore, we co-transfected miR-155-5p inhibitor and DUSP14 siRNA in OGD/R-induced SH-SY5Y cells to detect the expression of NF-κB and MAPKs pathways related proteins. First, the knockdown efficiency of DUSP14 siRNA was detected by qRT-PCR and the results showed that DUSP14 siRNA significantly reduced the DUSP14 mRNA level (Figure 5A). Then, the Western blot results indicated that miR-155-5p inhibitor significantly increased DUSP14 while decreased p-p38, p-ERK 1/2, p-JNK, and p-NF-κB expression. These effects were reversed by DUSP14 knockdown (Figure 5B and 5C). The above results suggested that miR-155-5p regulated NF-κB and MAPKs signaling pathways by inhibiting DUSP14.

### *DUSP14 Knockdown Partially Reversed the Protective Effect of MiR-155-5p Inhibitor on OGD/R-Induced Cell Injury*

Next, the cell injury was examined after co-transfected with miR-155-5p inhibitor and DUSP14 siRNA in OGD/R-induced SH-SY5Y cells. The CCK-8 results showed that the cell viability was significantly increased in miR-155-5p inhibitor treated cells while decreased by DUSP14 siRNA (Figure 6A). In addition, LDH and MDA levels were decreased in miR-155-5p inhibitor treated cells whereas reversed by DUSP14 siRNA (Figure 6B). Furthermore, ELISA and qRT-PCR results indicated that IL-6, IL-β, and TNF-α levels were decreased in miR-155-5p inhibitor treated cells while reversed by DUSP14 siRNA (Figure 6C and 6D). Western blot results showed that the cleaved caspase-3 and cleaved PARP protein

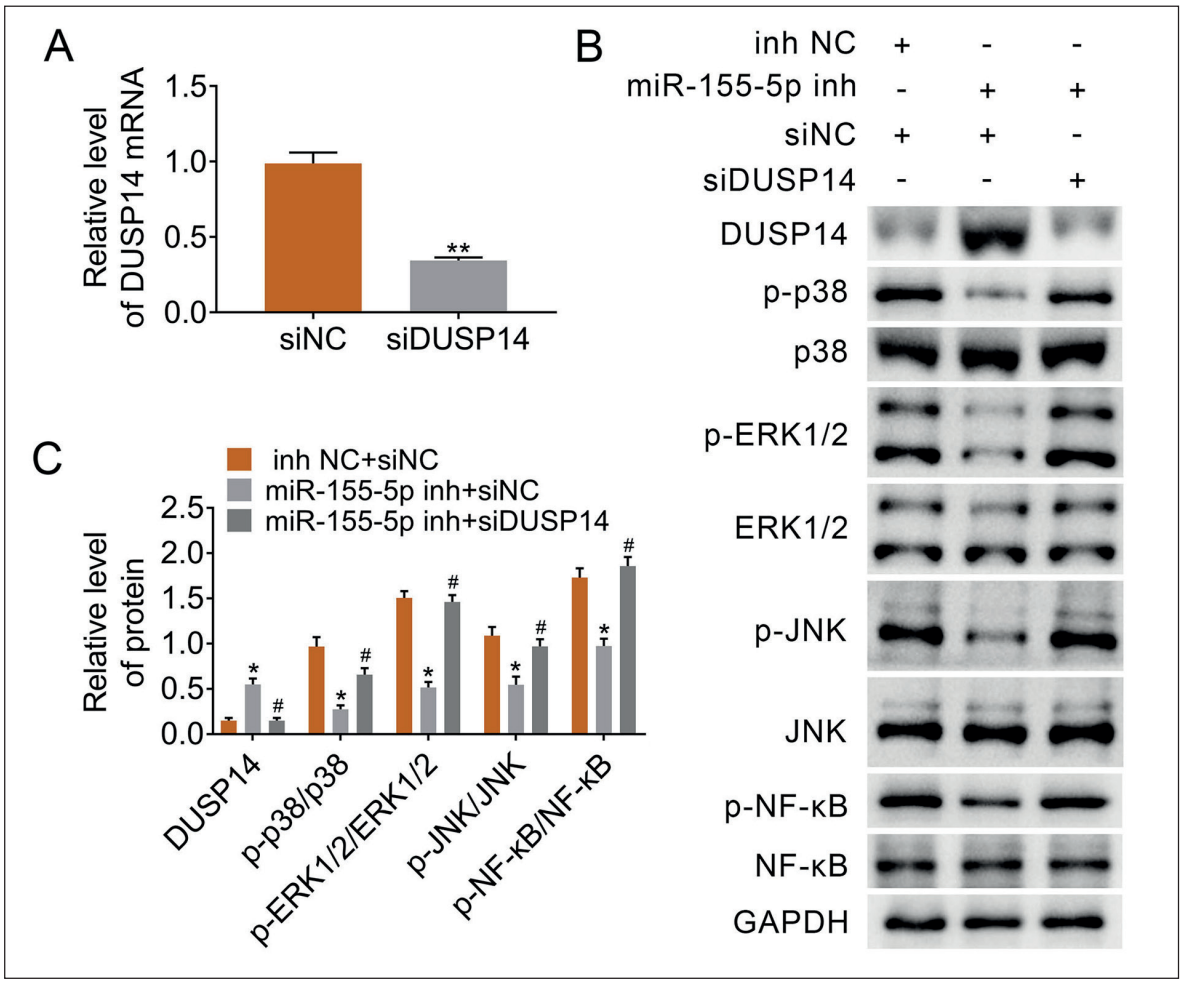


**Figure 5.** MiR-155-5p regulates the NF-κB and MAPKs signaling pathways by targeting DUSP14. **A,** The knockdown efficiency of DUSP14 siRNA was detected by qRT-PCR. **B,** Effects of miR-155-5p inhibitor on DUSP14 and the NF-κB and MAPKs pathways related proteins expression by Western blot. **C,** The protein quantitative bars. $^{**}p<0.05$, $^{\#\#}p<0.05$.

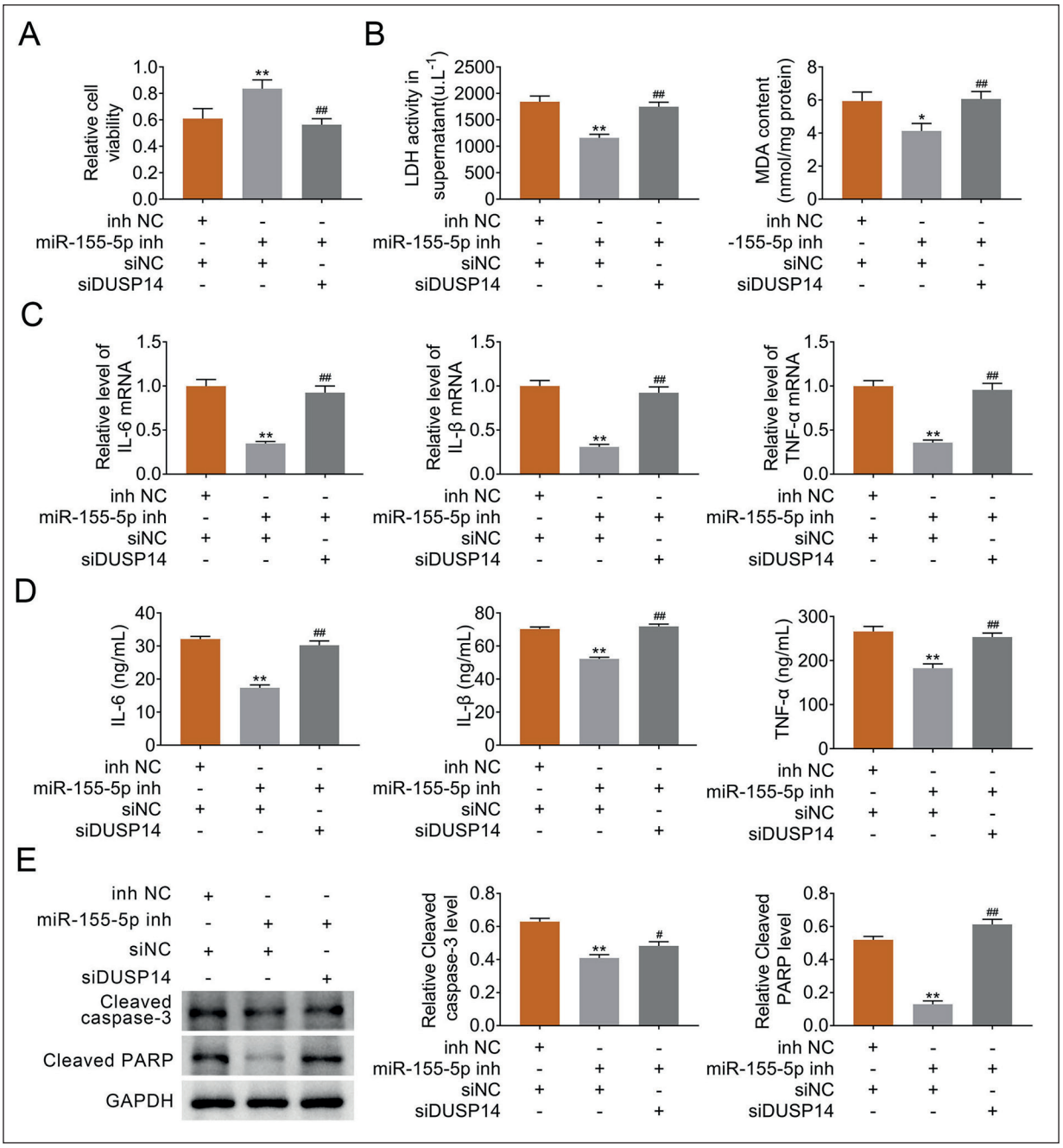


**Figure 6.** DUSP14 knockdown partially reversed the protective effect of MiR-155-5p inhibitor on OGD/R-induced cell injury. **A,** Cell viability was detected by CCK-8 assay. **B,** Effects of miR-155-5p inhibitor and DUSP14 siRNA on the LDH and MDA levels. **C,** Effects of miR-155-5p inhibitor and DUSP14 siRNA on the IL-6, IL-β and TNF-α mRNA levels. **D,** Effects of miR-155-5p inhibitor and DUSP14 siRNA on the IL-6, IL-β and TNF-α contents by ELISA. **E,** Effects of miR-155-5p inhibitor and DUSP14 siRNA on the IL-6, IL-β, and TNF-α protein levels by Western blot. $^{**}p<0.05$, $^{\#\#}p<0.05$.

levels were decreased after the cells treatment with miR-155-5p inhibitor, whereas these effects were reversed by DUSP14 siRNA (Figure 6E). These above results suggested that DUSP14 knockdown partially reversed the protective effect of miR-155-5p inhibitor on OGD/R-induced cell injury.

## Discussion

Ischemic stroke is characterized by high mortality and morbidity. The common cause is middle cerebral artery occlusion, which results in cell injury and apoptosis[26,27]. Increasing reports[28,29] suggest that

miRNAs play an important role in cerebral I/R injury. The present study indicated that miR-155-5p accelerates cerebral I/R injury *via* targeting DUSP14 by regulating NF-κB and MAPKs signaling pathways. The inhibition of miR-155-5p significantly reduces apoptosis and brain injury, indicating that miR-155-5p plays a key role in cerebral I/R injury. Therefore, miR-155-5p may be explored as a new target for the treatment of ischemic stroke.

Currently, the MCAO/R model is regarded as a classic model for cerebral I/R injury. In this study, the rat MCAO/R model was used to explore the effects of miR-155-5p on cerebral I/R injury *in vivo*. MiR-155-5p is up-regulated in MCAO/R model. Furthermore, miR-155-5p antagomir significantly reduced cerebral infarction areas indicated by TTC staining (Figure 1C). In addition, the OGD/R model was established in cells *in vitro*. The results showed that miR-155-5p knockdown inhibited OGD/R-induced cell injury and significantly increased cell viability (Figure 2A and 2C). Hence, these results suggested that miR-155-5p might be a regulator of cerebral I/R injury.

Cerebral I/R injury is associated with neuronal apoptosis and the caspase-3 activation and plays a critical role in triggering apoptosis[30]. In addition, the cleavage of PARP also serves as a marker of apoptosis[31]. In this study, both cleaved caspase-3 and cleaved PARP expression were detected. The results showed that miR-155-5p inhibitor significantly reduced the cleaved caspase-3 and cleaved PARP expression (Figure 2F), indicating that miR-155-5p knockdown inhibited OGD/R-induced neuronal apoptosis. Moreover, many studies have shown that apoptosis leads to the destruction of cell membrane structure and the enzymes release to the cytoplasm, including LDH. The LDH release is regarded as an important indicator of membrane integrity[32]. Furthermore, MDA is a natural product of lipid peroxidation, which was produced when cells undergo oxidative stress. It is widely used as an indicator of lipid oxidation and cell injury[33,34]. Here the miR-155-5p antagomir significantly reduced the LDH and MDA level in MCAO/R rats (Figure 1D). In addition, the miR-155-5p inhibitor also significantly reduced LDH and MDA level in OGD/R-induced cells (Figure 2D and Figure 2E). These results suggested that miR-155-5p knockdown inhibited OGD/R-induced cell injury. Numerous studies have shown that the inflammatory immune response is also an important pathogenetic factor of cerebral I/R injury. Under I/R injury, a variety of inflammatory cytokines are released, including IL-6, IL-β, and TNF-α, which can directly damage neurons to cause necrosis of ischemic tissue[35,36]. In this study, miR-155-5p inhibitor significantly reduced the IL-6, IL-β, and TNF-α levels (Figure 3A and 3B). These findings suggested that miR-155-5p inhibitor decreased OGD/R-induced inflammation.

The Dual-specificity phosphatases (DUSPs) family are reported to modulate MAPK signaling and play important functions in many biologic processes, including neuronal differentiation, cell motility, anti-obesity responses, and immune responses[23,37]. DUSP14, also known as MAP-kinase phosphatase 6, can negatively regulate the TCR signaling by inhibiting JNK and ERK[38]. It is also reported that the ERK signaling is activated in DUSP14 knockout T cells, which supports the finding that DUSP14 directly dephosphorylates ERK *in vitro*[38]. Furthermore, it has been reported that DUSP14 is also co-immunoprecipitated with TAK1 and TAB1[23]. In this study, our results indicate that DUSP14 might serve as a downstream gene of miR-155-5p by TargetScan. After mutating the binding site AGCAUUA to UCGUAAU, the DUSP14-MUT was obtained. MiR-155-5p overexpression reduced DUSP14-WT level while unchanged DUSP14-MUT level by Luciferase assay (Figure 4C). These results suggested that miR-155-5p inhibits DUSP14 by directly binding to the 3'UTR.

The mechanism of cerebral I/R injury is complex, including inflammatory reaction, apoptosis, mitochondrial injury, etc. When the cerebral ischemia occurs, NF-κB is activated. Then, it quickly enters into the nucleus, binding with specific sequences of DNA and promoting the expression of adhesion molecules, cytokines, resulting in inflammation. Therefore, NF-κB is considered as one of the initiating factors of the cerebrovascular endothelial cells, neurons or glial cells injury. In addition, MAPKs also play a key role in regulating cell growth and differentiation. In this study, miR-155-5p inhibitor significantly increased DUSP14 levels. While it decreased phosphorylated-p38, phosphorylated-ERK1/2, phosphorylated-JNK, and phosphorylated-NF-κB protein levels. These effects were reversed by DUSP14 knockdown (Figure 5B and 5C). These results suggested that miR-155-5p regulates NF-κB and MAPKs signaling pathways by inhibiting DUSP14.

## Conclusions

In summary, this study illustrated that miR-155-5p accelerated the ischemia reperfusion in-

jury and the inhibition of miR-155-5p effectively improved the ischemia reperfusion injury. The mechanistic studies showed that miR-155-59 targeted DUSP14 by regulating the NF-κB and MAPKs signaling pathways. This study may provide a new potential therapeutic target for ischemia reperfusion injury.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

### Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

### Authors' Contribution

YS conceived and designed the experiments, KL analyzed and interpreted the results of the experiments, KX and QHL performed the experiments

### Ethics Approval and Consent to Participate

The animal use protocol listed below has been reviewed and approved by the Animal Ethical and Welfare Committee. Approval No. 2019-04-001.

### Informed Consent

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article

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