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Regular paper

miR-155-5p accelerates cerebral ischemia-reperfusion inflammation injury and cell pyroptosis *via* DUSP14/ TXNIP/ NLRP3 pathway

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Objective: Cerebral ischemia/reperfusion (I/R) injury is stimulated by blood restoration after ischemic stroke. Inflammatory response and inflammasome activation exerted vital functions in the development of cerebral I/R injury. miR-155-5p regulates inflammatory response in some diseases, while its role in inflammatory response and inflammasome activation of cerebral I/R injury development is unclear. Hence, the research focuses on investigating if miR-155-5p attenuate cerebral I/R injury *via* regulating inflammatory response and inflammasome activation and exploring the potential mechanism. **Methods:** The oxygen-glucose deprivation/reoxygenation (OGD/R) model and the middle cerebral artery occlusion (MCAO) model were constructed. Cell viability and cytotoxicity were reflected by CCK-8 assay and LDH activity. The inflammatory cytokines secretion was determined using ELISA assay. Brain tissue infarction was evaluated using TTC staining. **Results:** miR-155-5p, Thioredoxin Interacting Protein (TXNIP) and NLR Family Pyrin Domain Containing 3 (NLRP3) were highly expressed in OGD/R model and MCAO rats. Knockdown of miR-155-5p alleviated cell injury, cell inflammation, and cell pyroptosis stimulated by OGD/R. Besides, miR-155-5p regulated TXNIP/NLRP3 pathway through modulating Dual-Specificity Phosphatase 14 (DUSP14) expression. Furthermore, knockdown of miR-155-5p improved brain tissue infarction and inhibited inflammation response and cell pyroptosis of MCAO rats. **Conclusion:** Knockdown of miR-155-5p attenuated I/R inflammation and cell pyroptosis of cerebral *via* modulating DUSP14/TXNIP/NLRP3 pathway. These findings may provide a promising strategy to attenuate cerebral I/R injury.

Key words: miR-155-5p, cerebral I/R injury, pyroptosis, DUSP14, TXNIP, NLRP3

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Abbreviations: DUSP14, Dual-Specificity Phosphatase 14; I/R, Cerebral ischemia/reperfusion; MCAO, middle cerebral artery occlusion; NLRP3, NLR Family Pyrin Domain Containing 3; OGD/R, oxygen-glucose deprivation/reoxygenation; TXNIP, Thioredoxin Interacting Protein

INTRODUCTION

Ischemic stroke is the primary kind of stroke, accounting for approximately 87% of total strokes (Yousufuddin & Young, 2019). Ischemic stroke is usually caused by blocked blood circulation due to cerebral arteries occlusion (Hao *et al.*, 2021). At present, blood flow restoration is the primary treatment approach (Dong *et al.*, 2019). However, cerebral ischemia/reperfusion (I/R) injury may be occur following blood restoration, characterized by brain injury and dysfunction (Chai *et al.*, 2020; Chomova & Zitnanova, 2016). Hence, it is imperative to investigate the potential mechanisms and alleviate the I/R injury of cerebral.

The pathogenesis mechanism of cerebral I/R injury is confused, of which inflammatory response played an important role (Chen *et al.*, 2012; Düzeylerine *et al.*, 2003). It could aggravate damage and bleeding and hinder tissue repair (Cao *et al.*, 2016). During the inflammatory response cascade, the innate immune system, such as inflammasome, was activated (Cao *et al.*, 2016). Recent researches revealed that pyroptosis also exerted vital functions in the pathogenesis of cerebral I/R injury (Zhang *et al.*, 2021b; Zhao *et al.*, 2021). For instance, Zhao *et al.* found that Berberine acted as neuroprotective drug against cerebral I/R injury via suppressing pyroptosis (Zhao *et al.*, 2021). Pyroptosis was an inflammasome-mediated cell death form (Tan *et al.*, 2021). Once inflammasome was activated, it could recruit and activate caspase-1, and activated caspase-1 cleaved GSDMD, IL-1β, and IL-18 to mature forms, thereby inducing cell pyroptosis (Yu *et al.*, 2021). Studies revealed that inflammasome NLRP3 was started in cerebral I/R injury (Hong *et al.*, 2018). NLRP3 modulated the caspase-1 activity and regulated IL-1β release and finally triggered cell pyroptosis (Tong *et al.*, 2015). Besides, the initiation of the NLRP3 inflammasome required collaboration with Thioredoxin

Interacting Protein (TXNIP) which separated from the Thioredoxin1(Trx1)/TXNIP complex during oxidative pressure (Hou *et al.*, 2018; Zhou *et al.*, 2010a). Hou *et al.* found that Nrf2 suppressed NLRP3 activation via modulating Trx1/TXNIP complex in I/R injury of cerebral (Hou *et al.*, 2018). Currently, restraining NLRP3 activation and alleviating inflammatory damage was regarded as a prospective strategy to relieve I/R injury of cerebral.

MicroRNAs (miRNAs) were revealed to exert regulation functions in many diseases containing I/R injury in cerebral recently (Liu *et al.*, 2020; Min *et al.*, 2020; Ren *et al.*, 2020). Min *et al.* demonstrated the protective action

of miR-18b for I/R injury of cerebral (Min *et al*., 2020). Ren and others (Ren *et al*., 2020) found that inhibiting of miR-187-3p attenuated cerebral I/R injury *via* increasing Seipin expression to regulate autophagy. miR-155-5p, a miRNA discovered currently, is an inflammatory factor regulator and it regulated the inflammation-related disease pathology (Yuan *et al*., 2020; Zhu *et al*., 2020). The previous study revealed the beneficial effects of miR-155-5p in I/R injury of cerebral via targeting Dual-Specificity Phosphatase 14 (DUSP14) (Shi *et al*., 2020). DUSP14, also known as MKP6, played critical roles in immune regulation (Hijikata *et al*., 2016). Furthermore, the previous researches discovered that DUSP14 could inhibit NLRP3 inflammasome activation and inflammation response (Que *et al*., 2020). For example, Que and others (Que *et al*., 2020) proved that overexpressed DUSP14 protected against isoflurane-caused inflammatory response, pyroptosis and cognitive impairment in aged rats *via* restraining the NLRP3 inflammasome. At present, the action of miR-155-5p on inflammation injury and pyroptosis in cerebral I/R injury remained elusive. Based on the above evidence, we conjectured that miR-155-5p might regulate inflammation and pyroptosis in cerebral I/R injury *via* modulating

DUSP14.Hence, the current study focused on investigating if miR-155-5p alleviated cerebral I/R injury *via* regulating inflammatory response and inflammasome activation and exploring the potential mechanism.

MATERIALS AND METHODS

Establishment of oxygen-glucose deprivation/reoxygenation (OGD/R) model

SH-SY5Y cell line was acquired from American Type Culture Collection (ATCC). To establish the OGD/R model, SH-SY5Y cells was cultivated in Earles balanced salt solution (EBSS) and maintained for 2 h in the hypoxic atmosphere (94% N_2, 5% CO_2, and 1% O_2). Then, the cells were returned to an oxygen-containing incubator (95% air and 5% CO_2) for 24 h with the complete DMEM/F-12 medium plus 10% FBS.

Establishment of middle cerebral artery occlusion (MCAO) model

Sprague–Dawley adult male rats weighing 250–280 g were acquired from Beijing Laboratory Animal Research Center (Beijing, China). All rats were grouped as shown below (n=5 for each group): Sham group, MCAO group, MCAO+antagomir-NC group and MCAO+ antagomiR-155-5p group. The MCAO rat model was established according to the reported procedures (Song *et al*., 2019). Rats were administrated with 10% chloral hydrate intraperitoneally and fixed. The skin of rats at the midline of the neck was cut to disclose the carotid arteries. Subsequently, a 0.2 mm filament was utilized to occlude MCA for 90 min. After that, the filament was withdrawn to accomplish reperfusion. After 4 days, the rats were used to perform following experiments. In rats in sham group underwent all procedures except for the MCA occlusion. The rats in MCAO+antagomir-NC group and MCAO+ antagomiR-155-5p group were administered with antagomir-NC and antagomiR-155-5p respectively through intracerebroventricular injection 3 days before MCAO establishment. The procedures were carried out in compliance with the Guide for Care and Use of Laboratory Animals and authorized by the ethics committee of Xuzhou Tumor Hospital (2020-02-049-K01).

Table 1. Information of the qRT-PCR primer sequences.

Gene name	Forward (5′-3′)	Reverse (5′-3′)
miR-155-5p	GGTGCATTGTAGTTG-CATTGC	GTGCAGGGTCCGAG-GTATTC
U6	GCGCGTCGTGA-AGCGTTC	GTGCAGGGTCCGAGGT

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was separated utilizing TRIzol (Beyotime, Beijing, China). The first-strand cDNA was generated using TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA). Then, cDNA was applied to qPCR assay through employing SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) in compliance with the supplier's protocol. The primers of miR-155-5p and U6 were listed in Table 1. The relative miR-155-5p expression was obtained by the $2^{-\Delta\Delta Ct}$ method.

Western blot

The lysate was separated using the SDS-PAGE gels and electroblotted onto the PVDF membranes. After prevented non-specific binding, the PVDF membranes were probed with anti-TXNIP (1:1000), NLRP3 (1:500), cleaved Caspase-1 (1:500), IL-1β (1:1000), IL-18 (1:1000), DUSP14 (1:1000) and GAPDH (1:2500) antibodies overnight at 4°C and treated with secondary antibody for 2 h. Total antibodies were bought from Abcam (Abcam, Cambridge, MA, UK) except for the anti-DUSP14 antibody (Thermo Scientific, Waltham, MA, USA). The bands were shown utilizing ECL western blot analysis substrate (Beyotime, Beijing, China).

Cell transfection

miR-155-5p inhibitor (5'-ACCCCUAUCACGAUUAGCAUUAA-3'), negative control inhibitor (NC inhibitor, 5'-CAGUACUUUUGUGUAGUACAA-3'), shRNA against DUSP14 (sh-DUSP14, 5'-GCATTGTTAATGCTACCATTG-3') and shRNA negative control (sh-NC, 5'-CUUGAUCUUGUAAAUAUAUAA-3') were produced by GenePharma (Shanghai, China). miR-155-5p inhibitor or sh-DUSP14 were transfected to SH-SY5Y cells through employing Lipofectamine 3000 (Thermo Scientific, Waltham, MA, USA) in the manner of the supplier's procedures. After 48 h, the cells were applied to follow-up experiments.

Cell Counting Kit-8 (CCK-8) assay

After indicated treatment, SH-SY5Y cells were inoculated into 96-well plates (3×10^3 cells each well) and grown overnight. Subsequently, per well was added to 10 μL of CCK-8 (Abcam, Cambridge, MA, UK) and cultured for 2 h at 37°C. The optical density was detected utilizing the MRX II microplate reader (Dynex Technologies, Chantilly, USA) at 450 nm.

Lactate dehydrogenase (LDH) analysis

After indicated treatment, SH-SY5Y cells were collected to detect LDH activity. The activity of LDH was measured utilizing a commercial LDH Assay Kit (Ab-

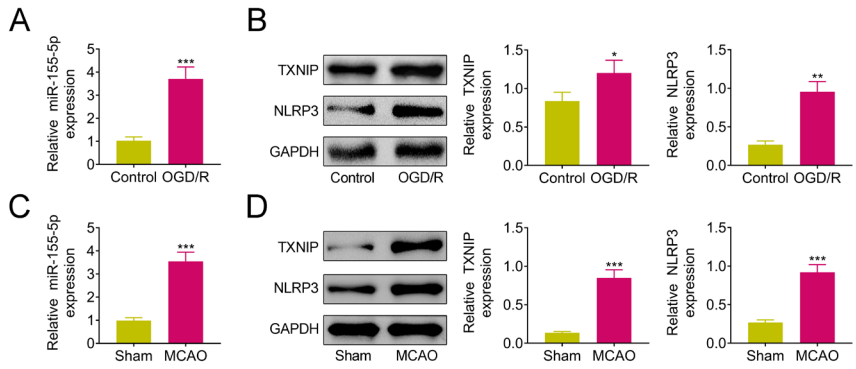

Figure 1. miR-155-5p and TXNIP/NLRP3 were highly expressed in cerebral I/R injury.
(**A**) The expression of miR-155-5p in SH-SY5Y cells with OGD/R treatment was detected using qRT-PCR. (**B**) The protein levels of TXNIP and NLRP3 in SH-SY5Y cells with OGD/R treatment were determined utilizing Western blot. (**C**) miR-155-5p expression in brain tissues of MCAO rats was measured using qRT-PCR. (**D**) The protein levels of TXNIP and NLRP3 in brain tissues of MCAO rats were detected using Western blot. **P*<0.05. ***P*<0.01. ****P*<0.001.

cam, Cambridge, MA, UK) in compliance with the supplier's procedures.

Enzyme-linked immunosorbent assay (ELISA)

SH-SY5Y cells and brain tissues were harvested to prepare supernatant and quantity the production of TNF-α, IL-1β, and IL-6 through employing corresponding ELISA assay kits (Abcam, Cambridge, MA, UK) following the supplier's procedures.

2,3,5-Triphenyltetrazolium chloride (TTC) staining

After rats sacrificed, the rat brains were quickly collected and transferred to –20°C and maintained for 20 min. After that, the tissues were prepared into 2 mm coronal sections. The sections were placed into 0.2% TTC solution for 30 min and immobilized using 4% formaldehyde. The infarction volume was analyzed utilizing ImageJ software.

Statistical analysis

Data were displayed as the mean ± standard deviations (S.D.) and statistical analysis was accomplished by SPSS Statistics 22.0 (SPSS, Chicago, IL, USA). Group differences analysis was completed using Student's *t*-test and one-way ANOVA. The *p*-value below 0.05 was regarded as statistical significance.

RESULTS

miR-155-5p and TXNIP/NLRP3 were highly expressed in cerebral I/R injury

To explore if miR-155-5p regulated inflammasome activation during I/R injury of cerebral, the OGD/R cell model and MCAO rat model were established. OGD/R stimulation promoted miR-155-5p expression (Fig. 1A). Besides, the levels of TXNIP and NLRP3 were increased after OGD/R treatment (Fig. 1B). Furthermore, miR-155-5p, TXNIP and NLRP3 were enhanced in MCAO rat (Figs. 1C and 1D). Therefore, miR-155-5p and TXNIP/NLRP3 were highly expressed in I/R injury of cerebral.

Knockdown of miR-155-5p attenuated OGD/R-induced cell injury

To explore the function of miR-155-5p on cerebral I/R injury in depth, the miR-155-5p inhibitor was transfected to OGD/R cell model. miR-155-5p inhibitor greatly suppressed the miR-155-5p expression (Fig. 2A). OGD/R stimulation weakened the SH-SY5Y cell viability, which was abrogated by the knockdown of miR-155-5p (Fig. 2B). Besides, OGD/R treatment elevated LDH activity but was suppressed by knockdown of miR-155-5p (Fig. 2C). Collectively, suppressing miR-155-5p alleviated the cell injury stimulated by OGD/R.

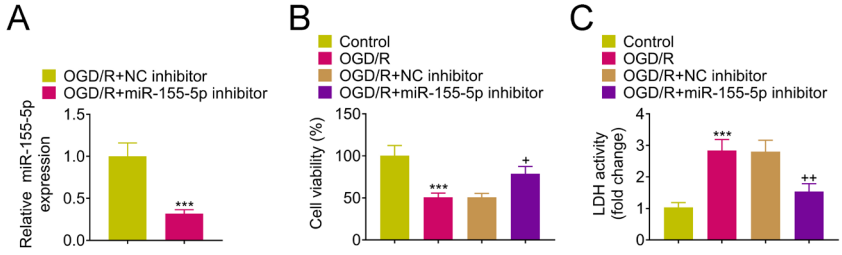

Figure 2. Knockdown of miR-155-5p alleviated OGD/R-induced cell injury.
(**A**) miR-155-5p expression in OGD/R-induced SH-SY5Y cells after transfected with miR-155-5p inhibitor was determined using qRT-PCR. (**B**) CCK-8 assay was used to detect the cell viability of OGD/R-induced SH-SY5Y cells after transfected with miR-155-5p inhibitor. (**C**) LDH activity in OGD/R-induced SH-SY5Y cells after transfected with miR-155-5p inhibitor was measured. **P*<0.05. ***P*<0.01. ****P*<0.001.

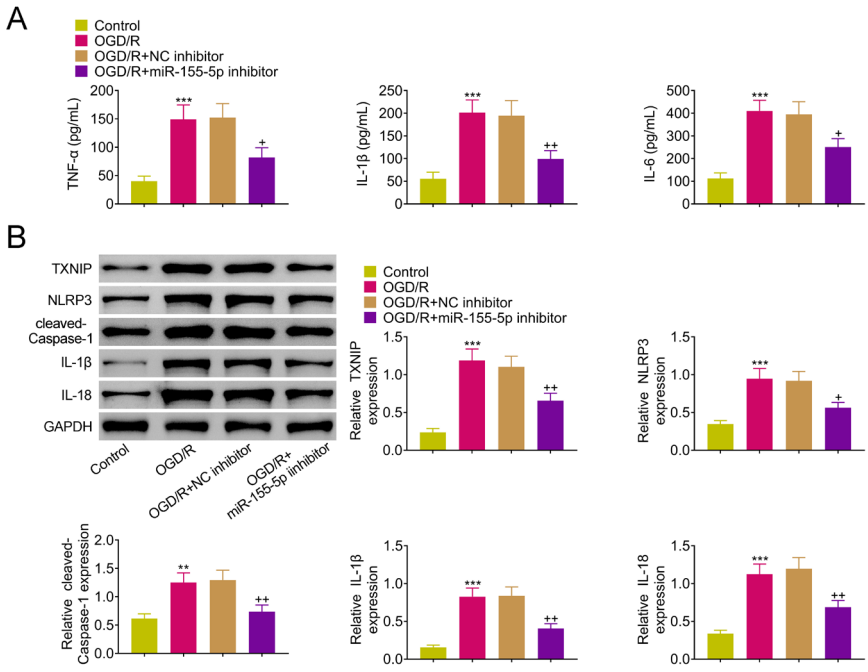

Figure 3. Knockdown of miR-155-5p inhibited OGD/R-induced cell inflammation and cell pyroptosis.
(**A**) **The production levels of TNF-α, IL-1β, and IL-6 in OGD/R-induced SH-SY5Y cells after transfected with miR-155-5p inhibitor were measured using ELISA. (B) Western blot was conducted to determine the protein levels of TXNIP, NLRP3, cleaved Caspase-1, IL-1β, and IL-18 in OGD/R-induced SH-SY5Y cells after transfected with miR-155-5p inhibitor. *P<0.05. **P<0.01. ***P<0.001.**

Knockdown of miR-155-5p suppressed OGD/R-induced cell inflammation and cell pyroptosis

To better interpret the influence of miR-155-5p on inflammation emerged in I/R injury of cerebral, the production of inflammatory cytokines in OGD/R model was determined after knockdown miR-155-5p. It was observed that OGD/R stimulation elevated the levels of TNF-α, IL-1β, and IL-6, while was decreased by the miR-155-5p inhibitor (Fig. 3A). Besides, the proteins related to cell pyroptosis such as TXNIP, NLRP3, cleaved Caspase-1, IL-1β, and IL-18 were up-regulated in OGD/R model, which were inhibited by the miR-155-5p inhibitor (Fig. 3B). Thus, down-regulated miR-155-5p repressed cell inflammation and cell pyroptosis stimulated by OGD/R.

miR-155-5p regulated TXNIP/NLRP3 pathway through modulating DUSP14 expression

To explicate the mechanism of miR-155-5p on inflammation response during I/R injury of cerebral, the

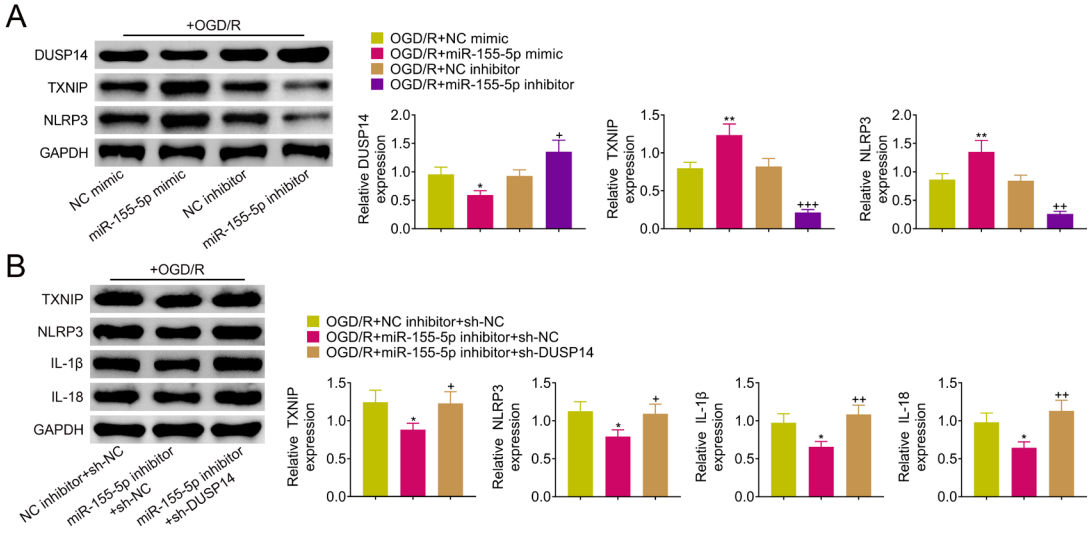

Figure 4. miR-155-5p regulated TXNIP/NLRP3 pathway through modulating DUSP14 expression.
(**A**) The protein levels of DUSP14, TXNIP, and NLRP3 in OGD/R-induced SH-SY5Y cells after transfected with miR-155-5p mimic or inhibitor were determined by Western blot. (**B**) The protein levels of TXNIP, NLRP3 IL-1β, and IL-6 in OGD/R-induced SH-SY5Y cells after transfected with miR-155-5p mimic or inhibitor were determined by Western blot. *P<0.05. **P<0.01.

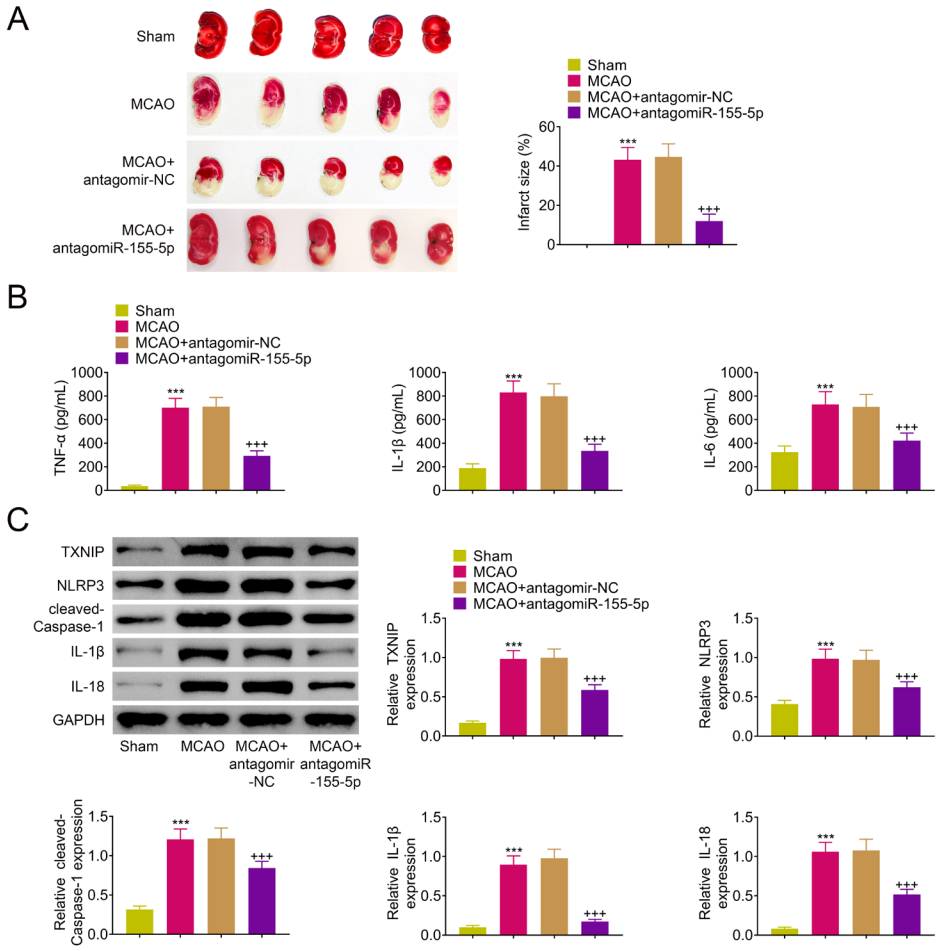

Figure 5. Knockdown of miR-155-5p suppressed MCAO-induced brain damage
(**A**) The brain tissue infarction in MCAO rats after antagomiR-155-5p treatment was assessed using TTC staining. (**B**) The levels of TNF-α, IL-1β, and IL-6 in MCAO rats after antagomiR-155-5p treatment were determined using ELISA. (**C**) The protein levels of TXNIP, NLRP3, cleaved Caspase-1, IL-1β, and IL-18 in MCAO rats after antagomiR-155-5p treatment were evaluated using Western blot. ***P<0.001.

DUSP14 expression in OGD/R model was detected after overexpression and knockdown of miR-155-5p. It was observed that enforced miR-155-5p expression significantly restrained DUSP14 expression (Fig. 4A). Conversely, down-regulated miR-155-5p potentiated DUSP14 level (Fig. 4A). In addition, miR-155-5p mimic enhanced the TXNIP and NLRP3 expressions (Fig. 4A), while miR-155-5p inhibitor repressed the TXNIP and NLRP3 expressions in OGD/R model (Fig. 4A). Furthermore, to understand whether DUSP14 mediated the favorable effect of miR-155-5p on cerebral I/R injury, miR-155-5p inhibitor and sh-DUSP14 were co-transfected to OGD/R model. Results found that miR-155-5p inhibitor suppressed the TXNIP, NLRP3, IL-1β, and IL-18 expressions caused by OGD/R, which was abrogated by sh-DUSP14 (Fig. 4B). Hence, miR-155-5p regulated TXNIP/NLRP3 pathway through modulating DUSP14 expression.

Knockdown of miR-155-5p suppressed MCAO-induced brain damage

To study the beneficial action of miR-155-5p on I/R injury of cerebral *in vivo*, the MCAO rat model was produced and treated with antagomiR-155-5p. TTC staining results showed that antagomiR-155-5p alleviated brain tissue infarction caused by MCAO (Fig. 5A). Besides, the secretion of TNF-α, IL-1β, and IL-6 was induced by MCAO but was inhibited by antagomiR-155-5p (Fig. 5B). Moreover, the TXNIP, NLRP3, cleaved Caspase-1, IL-1β, and IL-18 expressions were strengthened in the MCAO model, which was reversed by antagomiR-155-5p (Fig. 5C). Taken together, knockdown of miR-155-5p suppressed MCAO-induced brain damage.

DISCUSSION

Cerebral I/R injury is a disease caused by blood restoration after ischemic stroke (Chai *et al.*, 2020; Chomova & Zitnanova, 2016). Inflammatory response and inflammasome activation played vital roles during the development of cerebral I/R injury (Cao *et al.*, 2016; Düzeylerine, 2008; Hong *et al.*, 2018). Thus, inhibition of inflammasome activation and inflammatory damage was a promising strategy for cerebral I/R injury therapy. miR-155-5p exerted inflammatory regulation function in some diseases (Yuan *et al.*, 2020; Zhu *et al.*, 2020). Besides, miR-155-5p was proved to be a protective factor in I/R injury of cerebral (Shi *et al.*, 2020). However, whether miR-155-5p alleviated I/R injury of cerebral via regulat-

ing inflammatory response and inflammasome activation remained elusive.

To explain if miR-155-5p regulated inflammasome activation, the miR-155-5p, TXNIP, and NLRP3 expressions were firstly detected. It was found that miR-155-5p, TXNIP and NLRP3 were potentiated in cerebral I/R injury models. Similarly, Zhang and others (Zhang *et al.*, 2020a) reported the overexpression of miR-155-5p in OGD/R and MCAO models. In the study performed by Zhang and others (Zhang *et al.*, 2020a) the OGD model was constructed via maintaining SH-SY5Y cells in the hypoxic atmosphere for 6 h and the MCAO model was established *via* performing MCA occlusion for 60 min and reperfusion for 24 h, which were different from the methods of this study. Despite the differences in experimental methods of OGD and MCAO model establishment, their finding was consistent with our study. The results of TXNIP and NLRP3 in this study were also in keeping with the published researches (Hong *et al.*, 2018; Hou *et al.*, 2018). Hou *et al.* discovered that TXNIP and NLRP3 were increased in MCAO rats (Hou *et al.*, 2018). The overexpressed TXNIP and NLRP3 indicated that the NLRP3 inflammasome was activated.

To verify the beneficial role of miR-155-5p in I/R injury, miR-155-5p was knockdown in OGD/R model. Decreased miR-155-5p heightened cell viability and inhibited LDH activity of OGD/R model. In other words, the knockdown of miR-155-5p attenuated cell injury stimulated by OGD/R, which was consistent with the findings reported in the previous research (Zhang *et al.*, 2020a). Zhang and others (Zhang *et al.*, 2020a) revealed that down-regulation of miR-155-5p relieved I/R injury of cerebral through targeting MafB. Furthermore, to further investigate if miR-155-5p alleviated cerebral I/R injury *via* regulating inflammatory response, the pro-inflammatory cytokines, NLRP3 inflammasome, and cell pyroptosis were detected after knockdown miR-155-5p. The findings revealed knockdown of miR-155-5p inhibited inflammation and cell pyroptosis stimulated by OGD/R. The inhibition effect of miR-155-5p on inflammation was reported previously (Lin *et al.*, 2019; Zhou *et al.*, 2020a). Zhou and others (Zhou *et al.*, 2020a) found that knockdown of miR-155-5p attenuated acute seizures likely through restraining hippocampal inflammation. Nevertheless, the action of miR-155-5p on cell pyroptosis was contrary to the research performed by Xu and others (Xu *et al.*, 2021). Xu *et al.* reported that decreased miR-155-5p increased the inhibition action of cetuximab on triple-negative breast cancer though increasing cell apoptosis and pyroptosis (Xu *et al.*, 2021). This phenomenon may be caused by different types of diseases. Taken together, this study first revealed that low-expression of miR-155-5p abated cell inflammation and cell pyroptosis stimulated by OGD/R.

The previous study announced that miR-155-5p regulated cerebral I/R injury *via* binding to DUSP14 (Shi *et al.*, 2020). Recent studies revealed that DUSP14 could inhibit NLRP3 inflammasome activation and inflammation response (Lin *et al.*, 2018; Que *et al.*, 2020). Therefore, we inferred that DUSP14 might mediate the protective action of miR-155-5p on inflammation, NLRP3 inflammasome activation, and cell pyroptosis in cerebral I/R injury. miR-155-5p inhibitor and sh-DUSP14 were co-transfected to SH-SY5Y cells after treated with OGD/R to verify the hypothesis. It was suggested that the miR-155-5p controlled TXNIP/NLRP3 pathway through modulating DUSP14 expression. In other words, miR-155-5p regulated inflammation response, NLRP3 inflammasome activation, and cell pyroptosis through regulating DUSP14 expression. This signaling pathway about cell pyroptosis and inflammation in cerebral I/R injury was presented in Fig. 6.

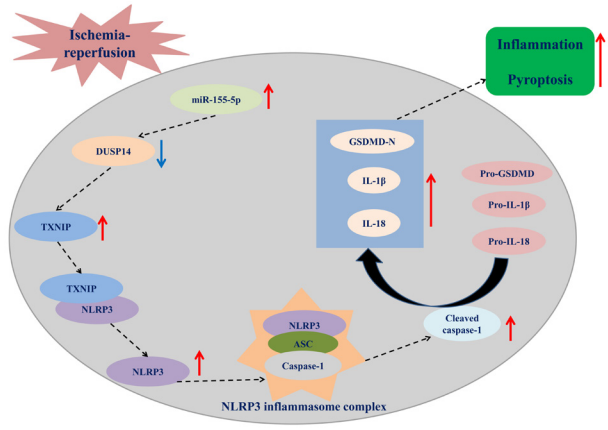

Figure 6. Proposed molecular mechanisms of miR-155-5p in regulation cell pyroptosis and inflammation in cerebral I/R injury. miR-155-5p regulated NLRP3 inflammasome activation, cell pyroptosis and inflammation response through modulating DUSP14 expression.

Finally, this study investigated the beneficial effect of miR-155-5p on cerebral I/R damage *in vivo*. Findings indicated that down-regulated miR-155-5p suppressed brain damage and inhibited inflammation response and cell pyroptosis of MCAO rats, suggesting that inhibition of miR-155-5p may be a valuable strategy for cerebral I/R injury therapy.

CONCLUSION

miR-155-5p was up-regulated in cerebral I/R injury. Knockdown of miR-155-5p attenuated cerebral I/R inflammation and cell pyroptosis *via* modulating DUSP14/TXNIP/NLRP3 pathway. These findings may provide a promising approach to attenuate cerebral I/R injury.

Declarfations

Availability of Data and Materials. All data generated or analyzed during this study are included in this published article.

Competing interests. The authors state that there are no conflicts of interest to disclose.

Ethics approval. Ethical approval was obtained from the Ethics Committee of Xuzhou Tumor Hospital (2020-02-049-K01).

Contribution of authors. Yu Shi and Zhendong Li designed the study, supervised the data collection, Ke Li analyzed the data, interpreted the data, Ke Xu prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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