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Study on the Mechanism of miR-124-3p Regulating Cerebral Ischemia-Reperfusion Injury

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Abstract: *Objective:* To investigate the role of miR-124-3p in ischemia-reperfusion injury following ischemic stroke and to evaluate the neuroprotective effects of miR-124-3p inhibition on neurological outcomes, infarct size, and apoptosis. *Methods:* A middle cerebral artery occlusion (MCAO) model was used to induce ischemia-reperfusion injury in rats. Experimental groups included a sham surgery group, an MCAO group, an MCAO + miR-124-3p NC group, and an MCAO + miR-124-3p antagomir group. Neurological deficits were assessed at 2, 8, and 24 hours post-ischemia. Infarct size and apoptosis levels were measured using brain tissue weight analysis and TUNEL assay, respectively, to evaluate the extent of ischemic damage and cell death. *Results:* The MCAO group showed progressively worsening neurological deficits, increased infarct size, and extensive apoptosis. In contrast, miR-124-3p inhibition significantly reduced neurological deficits, with lower scores at all time points. The MCAO + miR-124-3p inhibitor group demonstrated a significant reduction in infarct size compared to the MCAO group and the NC group, suggesting tissue preservation. Additionally, the miR-124-3p inhibitor group showed markedly reduced apoptosis, as evidenced by decreased TUNEL-positive signals, indicating a reduction in cell death following ischemia-reperfusion injury. *Conclusion:* Inhibition of miR-124-3p plays a neuroprotective role in ischemia-reperfusion injury by mitigating neurological deficits, reducing infarct size, and lowering apoptosis levels. These findings suggest that miR-124-3p inhibition could be a promising therapeutic target for minimizing brain damage and improving recovery in ischemic stroke patients.

Keywords: Ischemic stroke; Ischemic-reperfusion injury; miR-124-3p; Neurological function; Infarct volume

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1. Introduction

Stroke is the second leading cause of mortality [1], with ischemic stroke being the most frequent type accounting for 87% of all stroke cases [2]. An ischemic stroke occurs when the blood supply to part of the brain is obstructed, often caused by blood clots or fatty deposits, resulting in significant neurological impairment [3]. Reperfusion following ischemia can lead to exacerbated neuronal damage due to mechanisms such as cell

damage (apoptosis, necrosis, and ferroptosis), oxidative stress, inflammatory response, and more [4], therefore regulating these processes is crucial to mitigating ischemic-reperfusion injury. Recent studies have identified miRNAs, particularly miR-124-3p, as crucial mediators in the pathogenesis of ischemic stroke [5]. miR-124-3p has shown potential as a biomarker for ischemic brain injury [5] and may play a neuroprotective role through pathways such as PI3K/Akt/mTOR [6], which regulates cell survival and apoptosis. This project aims to investigate the protective effects of miR-124-3p in brain ischemia-reperfusion injury by studying its impact on neurological function and infarct volume. The results could provide new insights into therapeutic targets for stroke treatment.

2. Methods

2.1. Animals and grouping

Sixty male Sprague-Dawley rats, 8 weeks old, weighing 220–280 g, were used. All rats were acclimatized to the experimental environment for one week before the study, with controlled room temperature (21 ± 2°C), humidity (50 ± 5%), and a 12 h/12 h light-dark cycle, with free access to food and water. The animal experiments were conducted in strict accordance with the regulations of the hospital's ethics committee. The rats were randomly divided into four groups: sham surgery group, middle cerebral artery occlusion (MCAO) group, MCAO + miR-124-3p NC group, and MCAO + miR-124-3p antagomir group.

2.2. Model establishment

The Zea Longa suture method was used to establish the MCAO model in rats. After 2 hours of ischemia, the suture was gently removed to achieve ischemia-reperfusion, and the model's success was evaluated based on Zea Longa standard scoring. Stereotactic injections of miR-124-3p mimic/antagomir were performed 4–6 hours post-operation, and the rats were sacrificed 24 hours after successful modeling.

2.3. Stereotactic injection

At 4–6 hours post-surgery, rats were anesthetized via intraperitoneal injection of 10% chloral hydrate (3 mL/kg). The rats were then fixed in a stereotaxic apparatus for brain operations. The head hair was shaved, and after disinfecting with povidone-iodine, a midline incision of about 25 mm was made on the rat's scalp. The muscle and fascia were carefully separated until the skull plate was exposed. Using the bregma as the reference point (AP = 0 mm, LL = 0 mm, V = 0 mm), the injection site will be located (0.2 mm posterior to the bregma, 1.4 mm lateral, and 4.0 mm deep). A microinjector was used to slowly administer the drug. Fifty microliters of miR-124-3p mimic or antagomir were injected evenly over 30 minutes, and the needle was slowly withdrawn within 10 minutes. Fluorescent dye DiI (2 μL, 2 mg/mL) was injected to verify the accuracy of the lateral ventricle injection site. Penicillin powder was applied for local disinfection. After suturing the skin, the area was disinfected again, and the rats were returned to an isolation cage for recovery.

2.4. Measurement indicators

(1) Subarachnoid hemorrhage (SAH) severity evaluation and neurological function scoring: The neurological function of rats in each group (7 rats per group) was assessed using the Zea-Longa standard scoring system at 2, 8, and 24 hours after cerebral ischemia-reperfusion injury to evaluate the

success of the model. The scoring criteria are as follows: 0 points: no neurological deficits; 1 point: mild focal neurological deficit (incomplete forelimb extension); 2 points: moderate focal neurological deficit (circling to the unaffected side while walking); 3 points: severe neurological deficit (tilting to the unaffected side while walking); 4 points: inability to walk independently, decreased consciousness level.

(2) 2,3,5-Triphenyltetrazolium chloride (TTC) staining to detect infarct size in brain tissue. Each group has 2 rats.

(3) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay to detect apoptosis in brain tissue cells. Each group has 2 rats.

3. Results

3.1. SAH severity evaluation and neurological function scoring

Figure 1 shows the establishment of the rat MCAO model, while **Table 1** shows the SAH severity evaluation and neurological function scoring.

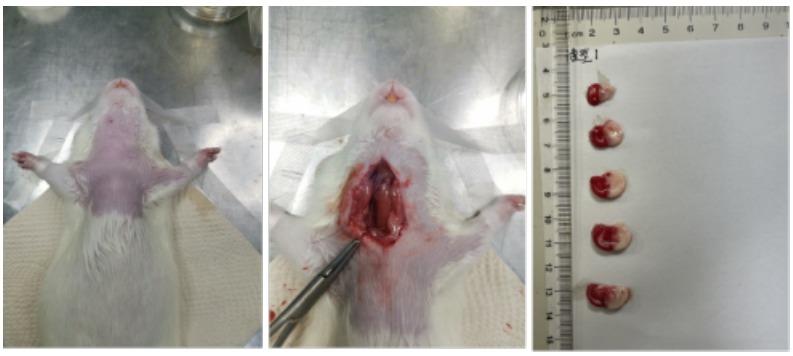

Figure 1. Rat MCAO model

Table 1. SAH severity evaluation and neurological function scoring of the four groups at 2 hours, 8 hours, and 24 hours after cerebral ischemia-reperfusion injury

ID/Time	Sham surgery group	MCAO group	MCAO + miR-124-3p NC group	MCAO + miR-124-3p antagomir group
1	0, 0, 0	1, 2, 4	1, 3, 4	1, 2, 3
2	0, 0, 0	1, 1, 2	1, 2, 3	0, 1, 2
3	0, 0, 0	0, 1, 2	0, 2, 3	0, 1, 2
4	0, 0, 0	0, 1, 1	1, 3, 4	0, 1, 2
5	0, 0, 0	1, 2, 3	1, 2, 3	0, 2, 2
6	0, 0, 0	2, 3, 4	1, 2, 3	0, 1, 2
7	0, 0, 0	1, 3, 4	1, 1, 2	1, 1, 2

Table 1 indicates that the sham surgery group consistently scored 0 at 2, 8, and 24 hours, showing no neurological deficits. In contrast, the MCAO group exhibited progressively worsening neurological function, with scores ranging from mild deficits (1–2 points) at 2 hours to severe deficits (3–4 points) by 24 hours. The MCAO + miR-124-3p NC group showed similar trends, with moderate to severe neurological deficits at 24

hours (3–4 points). However, the MCAO + miR-124-3p antagomir group demonstrated a notable improvement, with milder deficits (0–2 points) at each time point, suggesting that miR-124-3p antagomir treatment mitigated the severity of neurological impairments after ischemia-reperfusion injury.

3.2. TTC staining to detect brain infarct area

The rats were euthanized 24 hours after modeling, and TTC staining was performed to detect the infarct area in brain tissue. TTC staining is used to determine the viability of neuronal tissue and the infarct size caused by ischemia-reperfusion [7]. **Figure 2** and **Table 2** show the results of TTC staining.

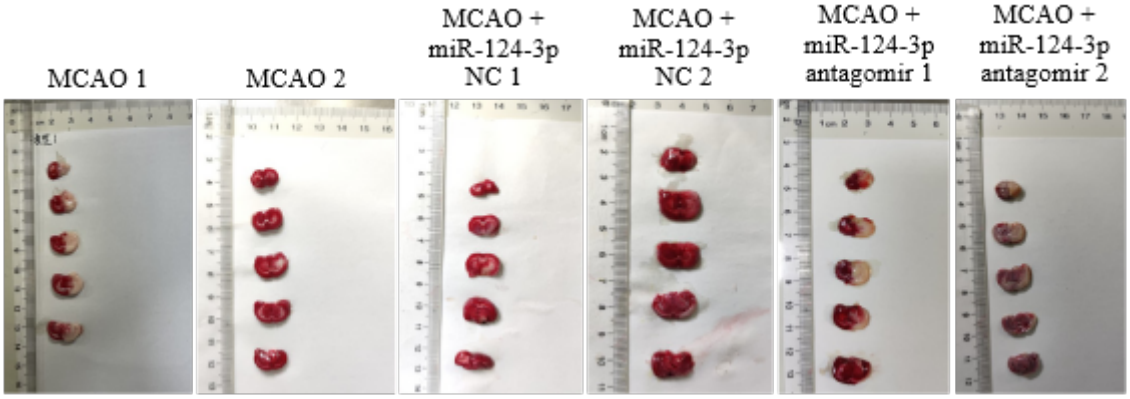

Figure 2. TTC staining for MCAO groups, MCAO + miR-124-3p NC groups, and MCAO + miR-124-3p antagomir groups

Table 2. Brain infarct area

Group	Non-infarct area (g)	Infarct area (g)
Sham surgery group 1	1.36	0
Sham surgery group 2	1.40	0
MCAO group 1	0.75	0.73
MCAO group 2	1.15	0.21
MCAO + miR-124-3p NC group 1	0.90	0.50
MCAO + miR-124-3p NC group 2	0.98	0.43
MCAO + miR-124-3p antagomir group 1	1.05	0.32
MCAO + miR-124-3p antagomir group 2	0.95	0.32

Figure 2 and **Table 2** show that the sham surgery group had no infarct area, with brain tissue weight in the non-infarct area remaining around 1.36–1.40 g. In the MCAO group, the infarct area was significantly larger, with infarct areas of 0.73 g and 0.21 g, and corresponding non-infarct areas of 0.75 g and 1.15 g. The MCAO + miR-124-3p NC group exhibited reduced infarct areas compared to the MCAO group, with infarct areas ranging from 0.43 g to 0.50 g. In the MCAO + miR-124-3p antagomir, the infarct area was further reduced to 0.32 g in both cases, indicating a protective effect of the miR-124-3p antagomir in reducing ischemic damage.

3.3. TUNEL assay to detect apoptosis in brain tissue cells

The rats were euthanized 24 hours after modeling, and a TUNEL assay was used to detect apoptosis in brain

tissue cells. TUNEL assay is a commonly performed test that uses a terminal deoxynucleotidyl transferase enzyme to incorporate fluorescent-labeled dUTPs in damaged nucleic acid regions found in apoptotic and necrotic cells [8]. The results are shown in **Figure 3**.

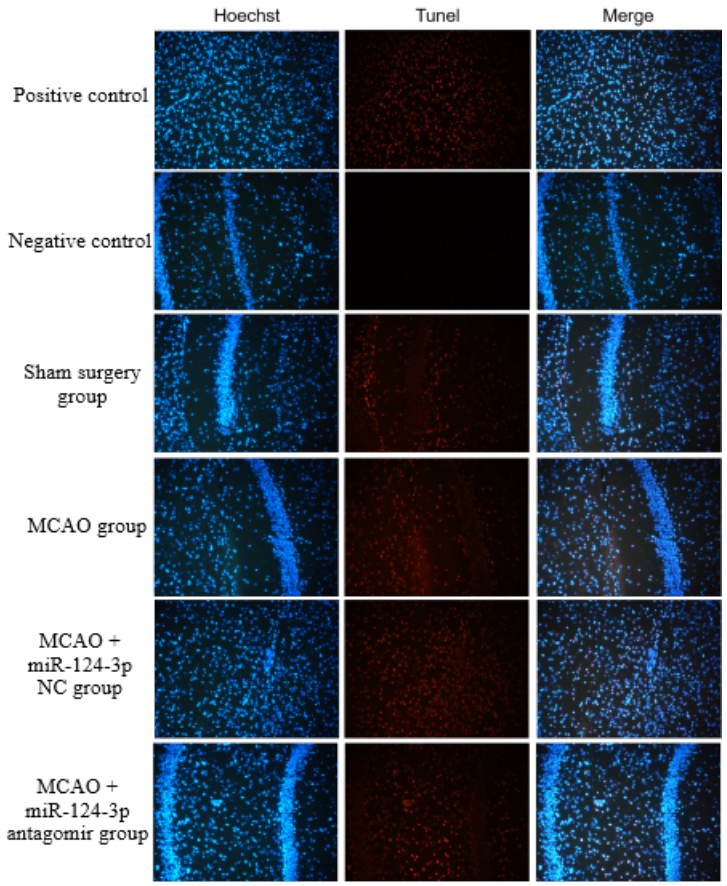

Figure 3. TUNEL assay to detect apoptosis in brain tissue cells

Figure 3 shows that the sham surgery group, MCAO group, MCAO + miR-124-3p NC group, and MCAO + miR-124-3p antagomir group show signals of apoptosis in brain tissue cells. The MCAO group showed the highest regions of red fluorescence, indicating that the group has the highest level of cell death. Both MCAO + miR-124-3p NC group and MCAO + miR-124-3p antagomir groups showed relatively lower regions of red fluorescence, especially the MCAO + miR-124-3p antagomir group, indicating that miR-124-3p antagomir plays a role in reducing apoptosis following ischemic-reperfusion.

4. Discussion

Ischemia-reperfusion injury is defined as the exacerbation of cellular dysfunction and death upon the restoration of blood flow to previously ischemic brain tissues following an ischemic stroke [9]. This study demonstrates that miR-124-3p plays a significant role in modulating neurological damage and apoptosis after ischemia-reperfusion injury. The results indicate that the MCAO group exhibited progressively worsening neurological deficits and extensive brain infarction, as expected in this ischemic stroke model. Importantly, miR-124-3p inhibition markedly reduced neurological deficits, infarct size, and apoptotic cell death, as evidenced by

improved behavioral scores, reduced infarct areas, and lower levels of apoptosis.

The findings from **Table 1** suggest that miR-124-3p inhibition provides a neuroprotective effect by mitigating the severity of neurological impairments post-ischemia. This is further supported by **Table 2**, where the infarct area was significantly reduced in the MCAO + miR-124-3p antagomir group compared to both the MCAO group and the NC group, indicating the potential of miR-124-3p inhibition to preserve brain tissue following ischemic damage. Moreover, **Figure 3** highlights the role of miR-124-3p in apoptosis regulation. The sham group showed minimal apoptotic signals, while the MCAO group exhibited extensive apoptosis, as indicated by increased red fluorescence. Notably, the MCAO + miR-124-3p antagomir group had a marked reduction in apoptosis compared to the MCAO group, further confirming the neuroprotective effects of miR-124-3p inhibition.

Previous studies have found that miR-124-3p plays a neuroprotective role in cerebral ischemic stroke. It has been shown that miR-124-3p regulates neuronal autophagy and apoptosis in cerebral ischemic stroke by targeting p38 MAPK [10]. Huang and colleagues also found that miR-124-3p may reduce cerebral ischemia-induced neuroaxonal damage by preventing Rnf38-mediated effects on the Nrep axis, thereby reducing apoptosis and increasing neuronal activity [11]. Other studies have found that miR-124-3p plays a neuroprotective role in other neurological diseases. Geng *et al.* [12] found that miR-124-3p attenuated MPP^+-induced injury in a Parkinson's disease model *in vitro* by suppressing neurotoxicity, neuronal apoptosis, neuroinflammation, and oxidative stress. Kang *et al.* [13] discovered that the downregulation of miR-124-3p promotes subventricular zone neural stem cell proliferation and improves motor function in rats with traumatic brain injury. However, the role of miR-124-3p in ischemia-reperfusion injury following stroke remains largely unknown and requires further investigation, particularly in terms of identifying specific molecular targets.

5. Conclusion

In conclusion, our findings demonstrate that miR-124-3p plays a crucial role in the pathogenesis of ischemia-reperfusion injury by promoting apoptosis and exacerbating neurological damage. Inhibition of miR-124-3p confers neuroprotection by reducing infarct size, mitigating apoptosis, and improving neurological outcomes, highlighting its potential as a therapeutic target for ischemic stroke. The reduction in apoptosis following miR-124-3p inhibition could provide a basis for developing treatments aimed at minimizing cell death and improving functional recovery in stroke patients.

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Disclosure statement

The authors declare no conflict of interest.

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