MicroRNA-424 Protects Against Focal Cerebral Ischemia and Reperfusion Injury in Mice by Suppressing Oxidative Stress

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Background and Purpose—We previously showed that the microRNA miR-424 protects against permanent cerebral ischemic injury in mice by suppressing microglia activation. This study investigated the role of miR-424 in transient cerebral ischemia in mice with a focus on oxidative stress–induced neuronal injury.

Methods—Transient cerebral ischemia was induced in C57/BL6 mice by middle cerebral artery occlusion for 1 hour followed by reperfusion (ischemia/reperfusion). The miR-424 level in the peri-infarct cortex was quantified. Mice were also administered miR-424 angomir by intracerebroventricular injection. Cerebral infarct volume, neuronal apoptosis, and levels of oxidative stress markers and antioxidants were evaluated. In an in vitro experiment, primary cortical neurons were exposed to H_2O_2 and treated with miR-424 angomir, nuclear factor erythroid 2-related factor 2 siRNA, and superoxide dismutase (SOD) inhibitor; cell activity, lactate dehydrogenase release, malondialdehyde level, and manganese (Mn)SOD activity were then evaluated.

Results—MiR-424 levels in the peri-infarct cortex increased at 1 and 4 hours then decreased 24 hours after reperfusion. Treatment with miR-424 decreased infarct volume and inhibited neuronal apoptosis after ischemia/reperfusion, reduced reactive oxygen species and malondialdehyde levels in the cortex, and increased the expression and activation of MnSOD as well as the expression of extracellular SOD and the redox-sensitive transcription factor nuclear factor erythroid 2-related factor. In neuronal cultures, miR-424 treatment abrogated H_2O_2-induced injury, as evidenced by decreased lactate dehydrogenase leakage and malondialdehyde level and increased cell viability and MnSOD activity; the protective effects of miR-424 against oxidative stress were reversed by nuclear factor erythroid 2-related factor knockdown and SOD inhibitor treatment.

Conclusions—MiR-424 protects against transient cerebral ischemia/reperfusion injury by inhibiting oxidative stress. **(*Stroke*. 2015;46:513-519. DOI: 10.1161/STROKEAHA.114.007482.)**

Key Words: ischemia-reperfusion injury ■ MiR-424 ■ nuclear factor erythroid 2-related factor 2 ■ oxidative stress ■ superoxide dismutase

Ischemic stroke is one of the leading causes of death and disability in the world, resulting from the disruption of blood supply to the brain. Intervention requires the restoration of blood flow, which can lead to reperfusion injury. Oxidative stress is thought to be the primary event during this process[1] because reperfusion stimulates an overproduction of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), which leads to the oxidation of proteins, lipids, and DNA and can induce cell proliferation, growth arrest, apoptosis, and necrosis.[2] Meanwhile, the dysfunction of superoxide dismutase (SOD) and glutathione peroxidase can compromise endogenous antioxidant defense mechanisms and further exacerbate oxidative stress and ischemic/reperfusion (I/R) injury.[3,4] Nuclear factor erythroid 2-related factor (Nrf)2 activates the transcription of antioxidant stress genes whose products act concertedly to remove ROS through sequential enzymatic reactions.[5] Studies have uncovered the potential for Nrf2-mediated transcription to protect from neurodegeneration resulting from mechanisms involving oxidative stress. For this reason, Nrf2 is considered a valuable therapeutic target for free radical damage in brain after ischemia and reperfusion.

MicroRNAs (miRs) are small (≈22 nt), noncoding, single-stranded RNA molecules that regulate gene expression at the posttranscriptional level by inhibiting translation or by cleaving RNA transcripts in a sequence-specific manner.[6] MiR-424 is a tumor marker that is involved in cancer cell proliferation,

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migration, and invasion, with a demonstrated role in cell cycle regulation.[7,8] It can also promote monoblastic cell differentiation,[9,10] modulate vascular endothelial and smooth muscle cell phenotype and angiogenesis,[11,12] and improve pulmonary hypertension in experimental models.[13] With respect to cerebrovascular disease, studies have shown that the miR-424 level is decreased in the blood and brain tissue of ischemic stroke patients and experimental animals.[14,15] We have recently reported that miR-424 exerts neuroprotective effects in a permanent cerebral ischemia model by suppressing microglia activation via translational suppression of key activators of the G1/S phase transition.[15] However, its influence on cerebral I/R remains unclear, and there is presently no direct evidence of the effect of miR-424 on neuronal injury under oxidative stress conditions. As recommended by Stroke Therapy Academic Industry Roundtable for stroke preclinical study, permanent occlusion model should be studied first, followed by transient models.[16] Therefore, the present study investigated the role of miR-424 in transient cerebral I/R injury in mice and mechanisms of neuroprotection and whether miR-424 protects neurons from oxidative stress via an Nrf2-independent pathway.

Methods

Mouse Model of Focal Cerebral I/R

Animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Capital Medical University. Male C57BL/6J mice, weighing 22 to 25 g, were purchased from Vital River Laboratory Animal Technology Co. Ltd (Beijing, China). Middle cerebral artery occlusion (MCAO)[17] was performed to induce focal ischemia after intracerebroventricular injection of miR-424 angomir or vehicle solution. Reperfusion was established after 1 hour. Regional cerebral blood flow was monitored by laser Doppler flowmetry (PeriFlux System 5000; Perimed, Järfälla, Sweden) to confirm the occurrence of ischemia (regional cerebral blood flow <80% of baseline) and reperfusion (regional cerebral blood flow >70% of baseline). Body temperature was maintained at 37.0°C±0.5°C during surgery using a temperature-controlled heating pad (CMA 150; Carnegie Medicin, Stockholm, Sweden). Infarct volumes were measured by staining brain sections with 2, 3, 5-triphenyltetrazolium chloride (1.5%) 24 hour after reperfusion. Ischemic core and peri-infarct areas were identified and isolated as previously described.[18] Animals were randomly divided into 5 groups: sham (n=15), ischemia 1 hour/reperfusion 1 hour (I/R 1 hour; n=3), ischemia 1 hour/reperfusion 4 hours (I/R 4 hours; n=3), ischemia 1 hour/reperfusion 24 hours (I/R 24 hours; n=25), and ischemia 1 hour/reperfusion 24 hour+miR-424 angomir treatment (I/R+miR424; n = 22).

MiR-424 Angomir Intracerebroventricular Injection

MiR-424 angomir and the negative control were purchased from GenePharma (Shanghai, China) and administered as previously described[15]: 9 μL of miR-424 angomir or the control (100 μM) were mixed with 2.5 μL Lipofectamine RNAiMAX Transfection Reagent (Invitrogen, Carlsbad, CA) and incubated at 37°C for 30 minutes; 7 μL of the mixture was administered immediately by right intracerebroventricular injection,[19] and the needle was kept in place for 10 minutes before MCAO.

Real-Time PCR

MiR-424 level in the peri-infarct cortex[18] after I/R injury was measured by real-time PCR. Total RNA was extracted from 30 mg brain tissue with TRIzol reagent (Invitrogen), and 500 ng were reverse-transcribed into cDNA using SuperScript III reverse transcriptase (Invitrogen) and miR-424 primers (GenePharma, Shanghai, China) with the following sequences: 5′-CCA GCA GTT CAA AAC ATG AAT TG-3′ and 5′-TAT GGT TGT TCT CGA CTC CTT GAC-3′. The expression of mature miR-424 was measured using Maxima SYBR Green qPCR Master Mix (Fermentas, Burlington, Canada) and the StepOne detection system (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions, with U6 used as an internal control.

Primary Cortical Neuronal Culture and Treatment

Primary cortical neurons were obtained from 1-day-old male C57BL/6 J mice and cultured in neurobasal medium (Invitrogen) supplemented with 2% B27 (Invitrogen), 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C in a 5% CO_2/air environment. The miR-424 or control angomir and Nrf2 short interfering (si)RNA (GenePharma) at a final concentration of 62.5 nmol/L (determined by a concentration gradient test; data not shown) were transfected into primary cortical neurons for 48 hours with Lipofectamine RNAiMAX Transfection Reagent according to manufacturer's protocol. Sequence of Nrf2 siRNA: sense, 5′-CGA GAA GUG UUU GAC UUU ATT-3′ and antisense 5′-UAA AGU CAA ACA CUU CUC GTT-3′. Primary cortical neurons were exposed to 200 μmol/L H_2O_2; 1 hour later, the SOD inhibitor sodium diethyldithiocarbamate (12.5 μmol/l; Sigma-Aldrich, St. Louis, MO) was added for 5 hours, and neurons and supernatant were collected for measurements.

Measurement of Cell Activity and Lactate Dehydrogenase Release

Cell activity was evaluated using the CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI) and intracellular lactate dehydrogenase (LDH) release was determined using the QuantiChrom Lactate Dehydrogenase Kit (BioAssay Systems, Hayward, CA) according to the manufacturers' protocols.

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling

Neural apoptosis was assessed in 20-μm frozen coronal sections using the In Situ Cell Death Detection Kit-POD (Roche, San Francisco, CA) following the manufacturer's instructions.

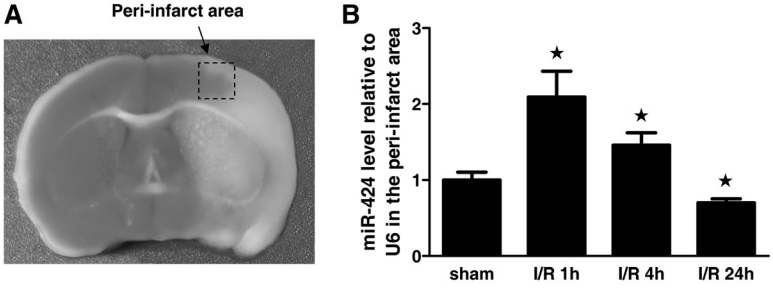

Figure 1. MiR-424 expression in the cortex of mice after transient focal cerebral ischemia/reperfusion (I/R) injury. **A**, Representative image of the peri-infarct cortex. **B**, MiR-424 levels at different time points after I/R were determined by real-time PCR, with U6 used as internal control. I/R 1 h, I/R 4 h, and I/R 24 h denote mice were subjected to middle cerebral artery occlusion (MCAO) for 1 h followed by reperfusion for 1, 4, and 24 h, respectively. Values represent mean±SEM (n=8 per group). **P*<0.05 vs. sham group.

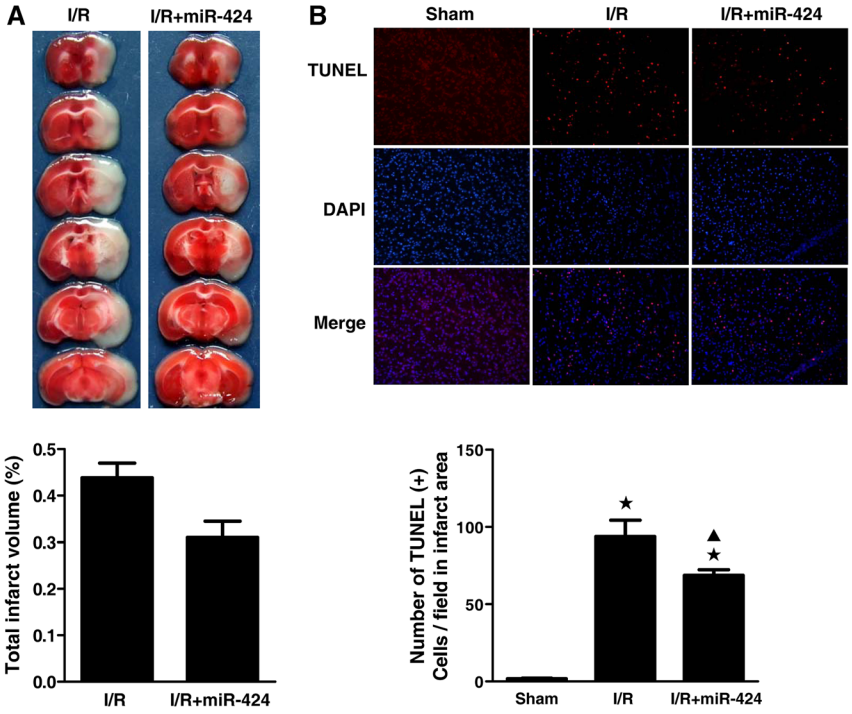

Figure 2. MiR-424 protects against ischemia/reperfusion (I/R) injury in mice. **A**, Cerebral infarct volume evaluated by 2, 3, 5-triphenyltetrazolium chloride (TTC) staining of coronal brain sections (n=10). **B**, Neuronal apoptosis in the ipsilateral cortex as detected by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and DAPI double staining (n=3). I/R denotes mice were subjected to middle cerebral artery occlusion (MCAO) for 1 h followed by reperfusion for 24 h; I/R+miR424 denotes mice were subjected to MCAO for 1 h followed by reperfusion for 24 h, with miR-424 angomir administered by intracerebroventricular injection immediately before ischemia. Values represent mean±SEM. *P<0.05 vs sham group; ▲P<0.05 vs I/R group.

Measurement of Intracellular ROS

The fluorescent probe dihydroethidium (Vigorous Biotechnology Beijing Co, Ltd, Beijing, China) was used to measure ROS production. Frozen coronal brain sections (20 μm) were fixed with acetone and incubated for 2 hours in a blocking solution containing 5% bovine serum albumin followed by incubation for 60 minutes with 2 μmol/L dihydroethidium in the dark. After washing 3 times with phosphate-buffered saline, sections were counterstained with DAPI to visualize nuclei and then examined by fluorescence microscopy (Carl Zeiss, Jena, Germany). The experiment was performed in triplicate.

Western Blotting

The peri-infarct cortex was dissected 24 hours after reperfusion. Samples were processed for western blot analysis as previously described.[19] Primary antibodies were as follows: rabbit monoclonal anti-manganese (Mn)SOD (1:800), mouse monoclonal antiextracellular (Ec) SOD (1:500), and rabbit polyclonal anti-Nrf2 (1:800; all from Abcam, Cambridge, UK) and rabbit polyclonal antiβ-actin (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA), which was used as a loading control. Bands were detected using horseradish peroxidase-conjugated secondary

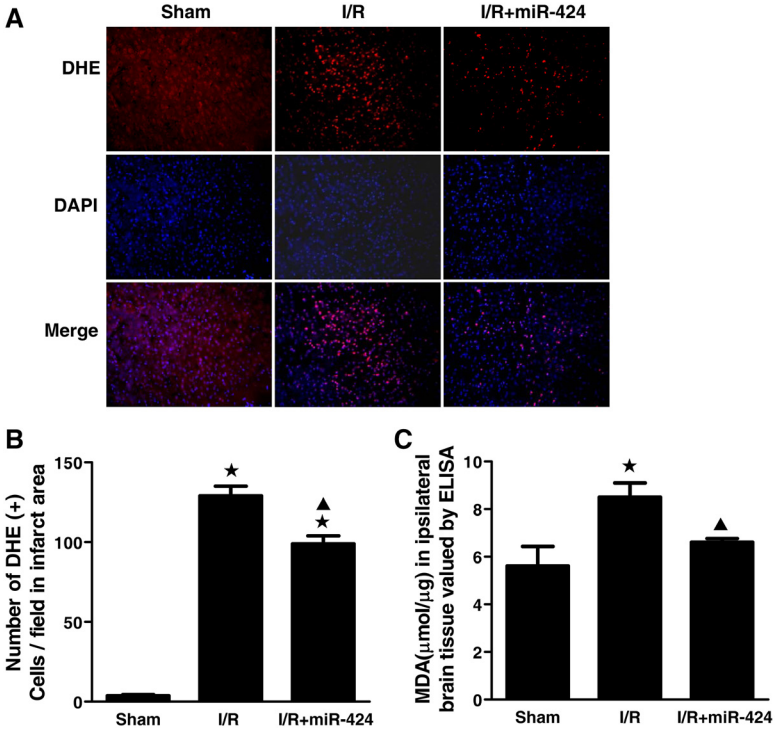

Figure 3. MiR-424 reduces oxidative stress after ischemia/reperfusion (I/R) in mice. **A**, Reactive oxygen species (ROS) level in the peri-infarct cortex was detected by dihydroethidium (DHE) and DAPI double staining (n=3). **B**, Malondialdehyde (MDA) content in the peri-infarct cortex (n=6). I/R denotes mice were subjected to middle cerebral artery occlusion (MCAO) for 1 h followed by reperfusion for 24 h; I/R+miR424 denotes mice were subjected to MCAO for 1 h followed by reperfusion for 24 h, with miR-424 angomir administered by intracerebroventricular injection immediately before ischemia. Values represent mean±SEM, *P<0.05 vs sham group; ▲P<0.05 vs I/R group.

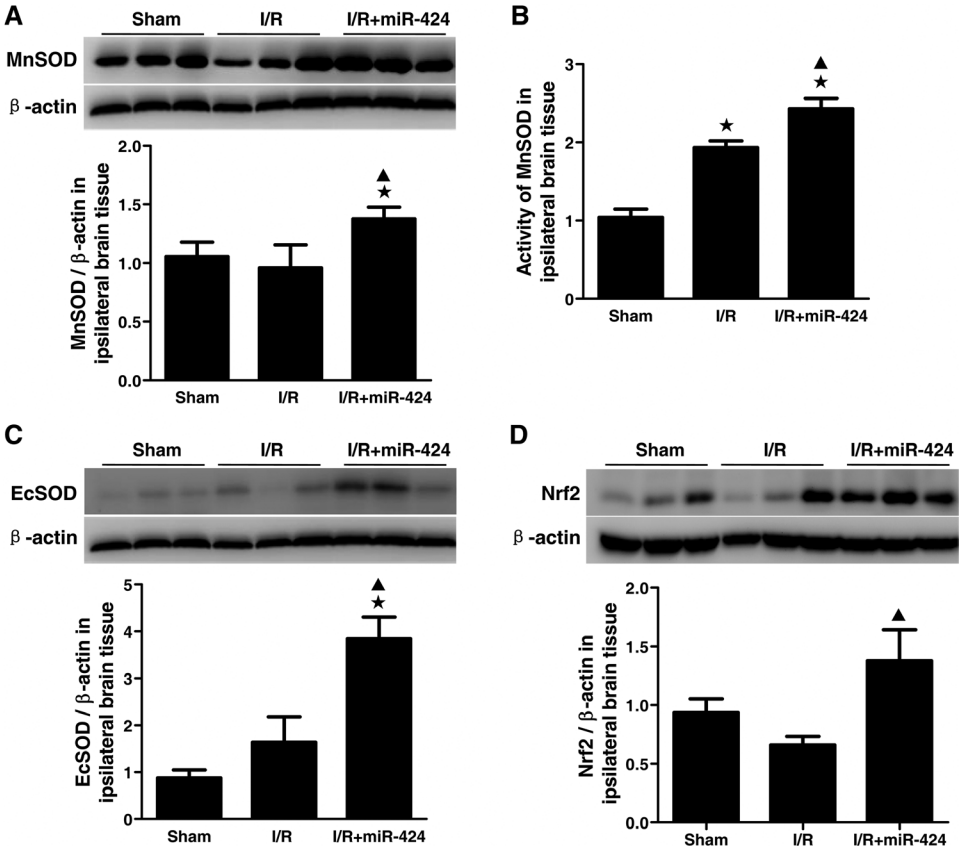

Figure 4. MiR-424 upregulates antioxidant expression and activity in the cortex after ischemia/reperfusion (I/R) injury. **A**, MnSOD protein level in peri-infarct cortex, as determined by western blotting (n=3). **B**, Effect of miR-424 on MnSOD activity (n=6). EcSOD (**C**) and Nrf2 protein levels (**D**) in peri-infarct cortex as determined by western blotting (n=3 each). I/R denotes mice were subjected to middle cerebral artery occlusion (MCAO) for 1 h followed by reperfusion for 24 h; I/R+miR-424 denotes mice were subjected to MCAO for 1 h followed by reperfusion for 24 h, with miR-424 angomir administered by injection immediately after ischemia. Values represent mean±SEM. **P*<0.05 vs sham group, ▲*P*<0.05 vs I/R group.

antibody (1:2000; Santa Cruz Biotechnology), and immunoreactivity was visualized using an enhanced chemiluminescence kit.

Biochemical Methods

Peri-infarct cortex homogenates (10% wt/vol) and primary cortical neurons were resuspended in cold phosphate-buffered saline. MnSOD activity and malondialdehyde (MDA) levels were assessed using the MnSOD assay kit with WST-1 (Beyotime Institute of Biotechnology, Jiangsu, China) and MDA assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), respectively, according to the manufacturers' protocols.

Statistical Analysis

Data are shown as mean±SEM and were analyzed with the *t* test and by 2-way analysis of variance followed by Newman–Keuel's test. *P*<0.05 was considered statistically significant.

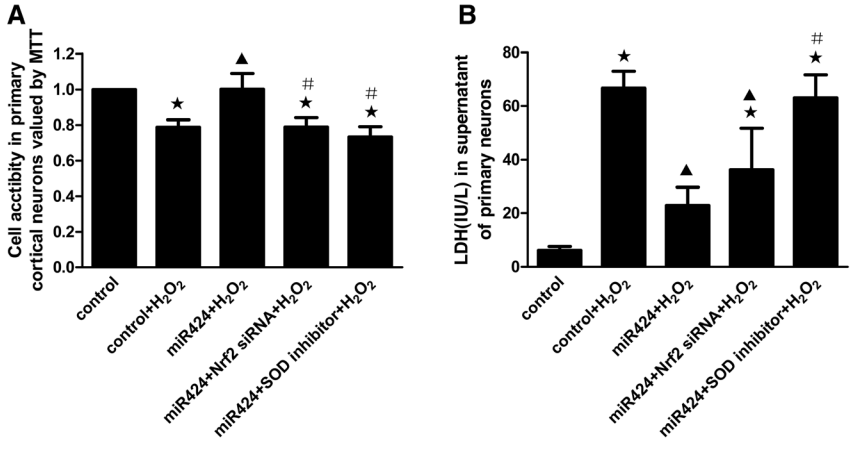

Figure 5. MiR-424 increases cell viability of primary cortical neurons exposed to oxidative stress. **A**, Viability of neurons was determined by the MTT assay. **B**, Lactate dehydrogenase (LDH) release in neurons after H_2O_2 treatment. Control: neurons transfected with scrambled oligonucleotide without H_2O_2 exposure; control+H_2O_2: neurons transfected with scrambled oligonucleotide and exposed to H_2O_2 for 6 h; miR424+H_2O_2: neurons transfected with miR-424 angomir and exposed to H_2O_2 for 6 h; miR424+Nrf2 siRNA+H_2O_2: neurons transfected with miR-424 angomir and Nrf2 siRNA, then exposed to H_2O_2 for 6 h; miR424+SOD inhibitor+H_2O_2: neurons transfected with miR-424 angomir, exposed to H_2O_2 for 1 h, with SOD inhibitor then added for 5 h. Values represent mean±SEM (n=8 per group). **P*<0.05 vs control; ▲*P*<0.05 vs control+H_2O_2 group; #*P*<0.05 vs miR424+H_2O_2 group.

Results

MiR-424 Expression Is Upregulated After Transient Cerebral I/R

We previously reported that the miR-424 level on the ipsilateral side of the brain decreased in a time-dependent manner during permanent focal cerebral ischemia.[15] To assess the effects of miR-424 on transient cerebral I/R injury, miR-424 expression in the peri-infarct cortex was investigated by real time PCR at 1, 4, and 24 hours after reperfusion (Figure 1A). In contrast to changes that occur during permanent ischemia,[15] miR-424 levels in the peri-infarct cortex increased at 1 and 4 hours and decreased at 24 hours relative to sham-operated mice (Figure 1B; $P<0.05$).

MiR-424 Protects the Brain Against I/R Injury

To evaluate the role of miR-424 in cerebral I/R injury, the effect of miR-424 angomir delivered via intracerebroventricular injection was examined by measuring infarct volume and neuronal apoptosis after 1 hour of ischemia and 24 hours of reperfusion. 2, 3, 5-Triphenyltetrazolium chloride staining showed that miR-424 angomir reduced cerebral infarct volume relative to the I/R group (Figure 2A; $P<0.05$). Few TUNEL-positive cells were detected in the cortex of sham-operated mice; in contrast, I/R increased the number of TUNEL-positive cells in the cortex, an effect that was abrogated by miR-424 angomir treatment (Figure 2B; $P<0.05$). These results demonstrate that miR-424 attenuates cerebral I/R injury in the cortex.

MiR-424 Suppresses I/R-Induced Oxidative Stress in the Brain

To assess whether miR-424 treatment can attenuate oxidative stress induced by cerebral I/R in vivo, ROS production and the level of the lipid peroxidation marker MDA were evaluated. Dihydroethidium staining showed that ROS production increased after reperfusion; this increase was abrogated by miR-424 angomir treatment (Figure 3A; $P<0.05$). I/R similarly increased MDA content in the peri-infarct cortex relative to the sham group, but the level was suppressed by miR-424 angomir treatment (Figure 3B and 3C; $P<0.05$). These data indicate that miR-424 inhibits ROS production and can protect the brain against oxidative damage induced by I/R.

MiR-424 Induces the Upregulation in MnSOD, EcSOD, and Nrf2 Expression After I/R

MnSOD and EcSOD have antioxidant activity that reduces ROS, whereas Nrf2 is a redox-sensitive transcription factor that activates SOD expression, thereby protecting cells against oxidative stress.[20–22] To investigate the effect of miR-424 on antioxidant enzyme expression and activity in vivo, MnSOD and EcSOD expression and MnSOD activity in the peri-infarct cortex were assessed by western blot and biochemical analyses. MnSOD protein levels were similar in the I/R and sham-operated groups, but were upregulated by miR-424 angomir treatment (Figure 4A; $P<0.05$). MnSOD activity was increased on I/R as compared with the sham-operated animals and was further increased by miR424 treatment (Figure 4B; $P<0.05$). Similar changes were observed for EcSOD expression, which was markedly elevated in the miR-424 angomir-treated group as compared with the sham-operated and I/R groups (Figure 4C; $P<0.05$). In addition, miR-424 angomir treatment also induced an upregulation in Nrf2 expression relative to the I/R group (Figure 4D; $P<0.05$).

MiR-424 Blocks H_2O_2-Induced Oxidative Damage in Primary Cortical Neurons

To directly assess the antioxidative effects of miR-424, primary cortical neurons transfected with miR-424 or control angomir were treated with H_2O_2, and cell viability and LDH release were evaluated. H_2O_2 exposure reduced the viability of neurons and increased LDH level. On the other hand, miR-424 angomir treatment increased the cell viability (Figure 5A; $P<0.05$) and suppressed LDH release (Figure 5B; $P<0.05$). The protective function of miR-424 on cell viability was blocked by siRNA-mediated knockdown of Nrf2 expression and SOD inhibitor treatment; LDH release was partly inhibited by Nrf2 depletion and suppressed by the SOD inhibitor (Figure 5B; $P<0.05$).

To confirm the positive effects of miR-424 on antioxidants in vitro, MnSOD activity and MDA content were examined. The biochemical analysis revealed that MnSOD activity in

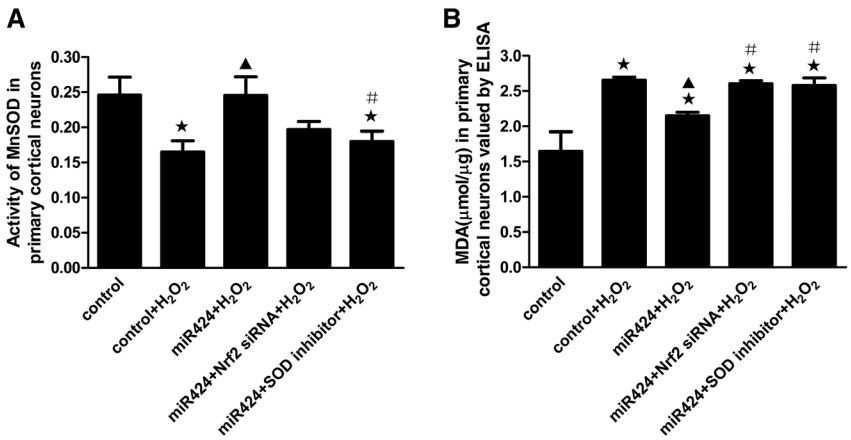

Figure 6. MiR-424 protects primary cortical neurons against oxidative stress damage. Effect of miR-424 on MnSOD activity (**A**) and MDA content in neurons (**B**). Control: neurons transfected with scrambled oligonucleotide without H_2O_2 exposure; control+H_2O_2: neurons transfected with scrambled oligonucleotide and exposed to H_2O_2 for 6 h; miR424+H_2O_2: neurons transfected with miR-424 angomir and exposed to H_2O_2 for 6 h; miR424+Nrf2 siRNA+H_2O_2: neurons transfected with miR-424 angomir and Nrf2 siRNA, then exposed to H_2O_2 for 6 h; miR424+SOD inhibitor+H_2O_2: neurons transfected with miR-424 angomir, exposed to H_2O_2 for 1 h, with SOD inhibitor then added for 5 h. Values represent mean±SEM (n=6 per group). *$P<0.05$ vs control; ▲$P<0.05$ vs control+H_2O_2 group; #$P<0.05$ vs miR424 + H_2O_2 group.

primary cortical neurons, which was reduced on exposure to H_2O_2, was elevated in the miR-424 angomir-treated group (Figure 6A; $P<0.05$). The stimulation of MnSOD activity by miR-424 was partially inhibited by siRNA-mediated knockdown of Nrf2 and suppressed by SOD inhibitor treatment (Figure 6A; $P<0.05$). Additionally, the increase in MDA level induced by H_2O_2 was blocked by miR-424 angomir treatment (Figure 6B; $P<0.05$); this effect was abrogated by both Nrf2 knockdown and SOD inhibitor (Figure 6A; $P<0.05$). These results suggest that miR-424 positively regulates the expression and activity of antioxidant enzymes, thereby suppressing H_2O_2-induced oxidative injury in primary cortical neurons by a mechanism that involves the activation of MnSOD or upregulation of Nrf2.

Discussion

The present study investigated the role of miR-424 in mice with cerebral I/R injury and in primary cortical neurons subjected to oxidative stress induced by H_2O_2. MiR-424 levels first increased and then decreased in the peri-infarct cortex over a 24-hour period after I/R. MiR-424 angomir administered immediately after ischemia reduced cerebral infarct size, neural cell apoptosis, and oxidative stress in the brain. These effects were accompanied by increased SOD and Nrf2 expression and SOD activity, demonstrating that miR-424 protects against transient focal I/R injury by stimulating antioxidant responses in neurons.

Excessive ROS formation is a deleterious effect of cerebral I/R injury, leading to oxidative stress and ultimately to neuronal death.[23] The present study showed that miR-424 abrogated the increases in ROS and MDA levels in the peri-infarct cortex after I/R and H_2O_2-induced oxidative stress in primary cortical neurons. MiR-424 also blocked the increase in LDH leakage and MDA content caused by H_2O_2 and improved cell viability and MnSOD activity of primary cortical neurons; these protective effects were inhibited by Nrf2 knockdown and SOD inhibitor treatment. Taken together, these results demonstrate that miR-424 reduces oxidative stress both in vivo and in vitro.

MiRNAs are implicated in the cellular response to oxidative stress via several different mechanisms[24–27]; of these, the antioxidant defense system is responsible for preventing ROS accumulation during cerebral I/R. Some miRNAs directly target antioxidant/oxidant genes; for instance, miR-34a induces apoptosis in the human glioma cell line via enhanced nicotinamide adenine dinucleotide phosphate oxidase 2 expression and ROS production,[24] whereas miR-155 modulates ROS level by targeting SH2-domain-containing inositol 5-phosphatases in macrophages.[25] Additionally, miRNAs prevent oxidative stress-induced cell apoptosis; for example, miR-214 protects cardiac myocytes against H_2O_2-induced injury by targeting phosphatase and tensin homolog,[26] whereas miR-92a inhibits H_2O_2-induced vascular smooth muscle cell apoptosis by targeting the mitogen-activated protein kinase kinase 4-c-Jun N-terminal kinase 1 pathway.[27]

The present study investigated the effect of miR-424 on the expression and activity of the endogenous antioxidants MnSOD and EcSOD, 2 enzymes that help the brain recover from I/R injury.[28,29] The results provide the first demonstration of miR-424 acting as a modulator of antioxidants after I/R by increasing the levels of MnSOD and EcSOD and stimulating MnSOD activity. Although SOD expression in the peri-infarct cortex was unaltered after 24 hours of reperfusion, MnSOD activity increased; in primary cortical neurons, MnSOD activity decreased after 6 hours of exposure to H_2O_2. In both cases, miR-424 induced an upregulation in SOD level. MnSOD expression in response to oxidative stress differs depending on the model used; however, miR-424 can upregulate SOD expression regardless of the experimental conditions. Moreover, increasing Nrf2 level leads to the upregulation of MnSOD and EcSOD.[30–34] In this study, Nrf2 expression was induced by miR-424, suggesting that it is a target of miR-424 regulation; in addition, the protective function of miR-424 on neuron was inhibited by Nrf2 depletion and SOD inhibition. These results demonstrate that miR-424 positively regulates the endogenous antioxidant system in neurons, providing protection against oxidative damage during transient cerebral I/R. However, further studies are required to elucidate the detailed mechanisms underlying the regulation of Nrf2 and SOD by miR-424.

Conclusions

The present study demonstrated that miR-424 overexpression protects against transient focal cerebral I/R injury in vivo and suppresses H_2O_2-induced oxidative stress injury in vitro via activation of the cellular antioxidant response. These findings substantiate the results of our previous study, which showed that a high level of miR-424 is associated with a better outcome in ischemic stroke patients.[15] MiR-424 is therefore a promising diagnostic and therapeutic tool for ischemic stroke. However, it is far away from the clinical application because of some severe problems waiting to solve; one problem is how to bring the miRNA into the brain. Obviously, direct injection of the miRNA-mimic into the brain would be limited in the patients for the secondary injury of the surgery and the expensive fees. Intravenous delivery is easy and safe, but the disadvantage is the poor distribution in the brain because of blood brain barrier. Recently, intranasal delivery of miRNA to the brain comes up.[35] The efficacy of miR-424 through intravenous injection or intranasal delivery in a mouse model of stroke will be the subject of future investigations.

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Disclosures

None.

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