MicroRNA-124–Mediated Regulation of Inhibitory Member of Apoptosis-Stimulating Protein of p53 Family in Experimental Stroke

Xiangrong Liu, MD, PhD; Fang Li, MS; Shangfeng Zhao, MD, PhD; Yumin Luo, MD, PhD; Jun Kang, MD, PhD; Haiping Zhao, PhD; Feng Yan, MS; Sijie Li, MS; Xunming Ji, MD, PhD

Background and Purpose—p53-mediated neuronal death is a central pathway of stroke pathophysiology, but its mechanistic details remain unclear. Here, we identified a novel microRNA mechanism that downregulates inhibitory member of the apoptosis-stimulating proteins of p53 family (iASPP) by the brain-specific microRNA-124 (miR-124) promotes neuronal death after cerebral ischemia.

Methods—In a mouse model of focal permanent cerebral ischemia, the expression of iASPP and miR-124 was quantified by reverse transcription quantitative real-time polymerase chain reaction, immunofluorescence staining, and Western blot. Luciferase reporter assay was used to validate whether miR-124 can directly bind to the 3′-untranslated region of iASPP mRNA. To evaluate the role of miR-124, miR-124 mimic and its inhibitor were transfected into Neuro-2a cells and C57 mice.

Results—There was no change in the iASPP mRNA level in cerebral ischemia. However, iASPP protein was remarkably decreased, with a concurrent elevation in miR-124 level. Furthermore, miR-124 can bind to the 3′-untranslated region of iASPP in 293T cells and downregulate its protein levels in Neuro-2a cells. In vivo, infusion of miR-124 decreased brain levels of iASPP, whereas inhibition of miR-124 enhanced iASPP levels and significantly reduced infarction in mouse focal cerebral ischemia.

Conclusions—These data demonstrate that p53-mediated neuronal cell death after stroke can be nontranscriptionally regulated by a novel mechanism involving suppression of endogenous cell death inhibitors by miR-124. Further dissection of microRNA regulatory mechanisms may lead to new therapeutic opportunities for preventing neuronal death after stroke. (Stroke. 2013;44:1973-1980.)

Key Words: apoptosis ● ASPP ● cerebral ischemia ● iASPP ● microRNAs

Ischemic stroke is one of the leading causes of death and disability worldwide. The pathophysiological mechanisms involve a complex cascade of deleterious biochemical events that ultimately lead to the death of brain cells. p53 is a central regulator of cellular stress responses, which mediates neuronal apoptosis after stroke. However, molecular detail of this critically important pathway remains to be fully defined.

p53-mediated cell death is known to involve the apoptosis-stimulating proteins of p53 (ASPP) family that consists of 3 members: ASPP1, ASPP2, and inhibitory member of the ASPP family (iASPP). All 3 members exhibit a high degree of homology at their C terminus, which each contains 4 ankyrin repeats, an SH3 domain, and a proline-rich region. ASPP1 and ASPP2 enhance apoptosis mediated by p53, whereas iASPP, as the most evolutionarily conserved member of the ASPP family, competes with ASPP1 and ASPP2, and thereby inhibits p53-mediated apoptosis. The roles of ASPP family in tumor biology have been extensively investigated, but their physiological functions in cerebral ischemia remain unclear.

MicroRNAs (miRNAs) are short noncoding RNA molecules that negatively modulate gene expression by either degradation or translational repression of target mRNA. Although miRNAs control several conditions and diseases, only a few miRNAs have been described in neuronal death.

In this study, we mapped the expression profiles of ASPP family members in mouse models under focal cerebral ischemia and showed that the brain-specific miRNA-124 (miR-124) can reduce iASPP protein levels to suppress this endogenous prosurvival pathway and promote neuronal death. Our findings support the existence of a novel miRNA-mediated regulation of inhibitory member of ASPP family in experimental stroke.
regulatory pathway by which p53-mediated neuronal death can be regulated in a posttranscriptional way.

Materials and Methods

Animal Model

All animal experiments were approved by the Institutional Animal Care and Use Committee of Capital Medical University and are in accordance with the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Permanent focal ischemia was induced in male C57 mice weighing 21 to 23 g using the intraluminal vascular occlusion method as previously described. The mice were randomly assigned to 2 groups: sham-operated group and middle cerebral artery occlusion (MCAO) group (mice with permanent MCAO). To ensure the occurrence of ischemia by MCAO, regional cerebral blood flow was monitored using laser Doppler flowmetry (PeriFlux System 5000. Perimed, Stockholm, Sweden). Rectal temperature was controlled at 37.0°C during and after surgery with a temperature-regulated heating pad. Blood pressure was monitored through a measurement (MP100A-CE, BIOPAC Systems, Inc, CA). All animals were maintained in an air-conditioned room at 25±1°C after recovering from anesthesia. Infarct volume was determined by 2,3,5-triphenyltetrazolium chloride as previously described. Penumbra and ischemic core were identified and isolated as previously described.

Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction for mRNA and miRNA Quantification

Total RNA was extracted from cerebral tissues or cells using Trizol (Invitrogen, Carlsbad, CA) according to the protocol supplied with the reagent and then reverse transcribed into cDNA using Superscript III reverse transcriptase kit (Invitrogen). The resulting cDNA was used for polymerase chain reaction (PCR) using SYBR(G)er (green 1 Nuclease A (Invitrogen) in triplicates. Primers for quantitative real-time PCR were as follows: iASPP forward primer: 5′-CGCAACTACCTTCGGGCTCTT-3′; iASPP reverse primer: 5′-TTAGCTAGCTGGATCGGGCA-3′ (the PCR product was 135 bp); ASPP1 forward primer: 5′-AGTGGGAGCCGCTATCCTT-3′; ASPP1 reverse primer: 5′-ACGGTGCCCTTGGTCATC-3′ (the PCR product was 146 bp); ASPP2 forward primer: 5′-GTGATGACCCAAGCTACCA-3′; ASPP2 reverse primer: 5′-GGCGTCCATCCATCACTA-3′ (the PCR product was 137 bp); GAPDH forward primer: 5′-GCACTCCTGGCAGTGGAC-3′; GAPDH reverse primer: 5′-CAGCTGATCCCTGGATCTA-3′ (the PCR product was 137 bp); GAPDH forward primer: 5′-GCACTCCTGGCAGTGGAC-3′; GAPDH reverse primer: 5′-GTCATACGGAAGATGGCCTGACA-3′ (the PCR product was 83 bp); PCR and data collection were performed on 7500 fast real-time PCR system (Applied Biosystems, Alameda, CA). All quantifications were normalized to an endogenous GAPDH control. The mature form of miR-124 was detected using the miRNA RT kit (Ambion) and TaqMan Universal PCR Master Mix (ABI) according to the manufacturer’s instructions. U6 was used as an internal control. The relative quantification value for each target gene was performed using the comparative cycle threshold method.

Western Blot Analysis

The cerebral tissues were sampled at 1 hour, 4 hours, and 24 hours after ischemia. Cells were collected at 0 hours, 24 hours, 48 hours, and 72 hours after transfection. The lysis buffer was used as previously described. The tissues and cells were homogenized and centrifuged at 12,000 rpm for 30 minutes at 4°C, and then the supernatant was collected. Protein concentration was determined by a bichinonic acid protein assay kit (Pierce, Rockford, IL) using BSA as the standard. Protein samples (100 μg per lane) were separated on an 8% SDS-PAGE and then transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA). After blocking in 5% skimmed milk for 2 hours at room temperature, the membranes were incubated overnight at 4°C with the following antibodies: rabbit anti-iASPP1 polyclonal antibody (1:200 dilution; Abbiotec, San Diego, CA), rabbit anti-ASPP2 polyclonal antibody (1:200 dilution; Abbiotec), rabbit anti-iASPP polyclonal antibody (1:1000 dilution; Abcam, Hong Kong, China), and rabbit anti-actin polyclonal antibody (1:2000 dilution; Santa Cruz Biotechnology Inc, Santa Cruz, CA). Anti-actin antibody was used as an internal loading control. Membranes were then incubated with peroxidase-conjugated goat anti-rabbit IgG (1:2000 dilution, Santa Cruz Biotechnology Inc) in blocking solution for 1 hour. The blots were visualized by chemiluminescence (Millipore, Billerica, MA). Protein levels were normalized to β-actin as a loading control. The relative optical density of protein bands was measured after subtracting the film background.

Immunofluorescence Staining

Formalin-fixed/paraffin-embedded sections (4 μm) were deparaffinized in dimethyl benzene and rehydrated through graded alcohols. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes. Sections were incubated in sodium citrate buffer (0.01 mol/L; pH 6.0) for 15 minutes in a microwave oven. After cooling to room temperature, slides were washed in PBS and immersed in normal horse blocking serum (Maxin, Fuzhou, China) for 30 minutes. For double staining, slides were incubated with rabbit polyclonal anti-ASPP1 antibody (diluted 1:100; Abbiotec), rabbit polyclonal anti-ASPP2 antibody (diluted 1:100; Abbiotec), rabbit polyclonal anti-PP antibody (diluted 1:100; Abcam), and mouse monoclonal anti-neuron-specific nuclear protein (NeuN) antibody (diluted 1:100; Millipore, Billerica, MA) in a humidified chamber at 4°C overnight. Goat anti-rabbit secondary antibody (tetramethyl rhodamin isothiocyanate conjugated; diluted 1:200; Jackson Immuno Research Laboratories Inc, West Grove, PA) and goat anti-mouse secondary antibody (fluorescein isothiocyanate conjugated; diluted 1:200; Jackson Immuno Research Laboratories Inc) were added for 30 minutes. The nuclei of cells were stained by 4′,6-diamidino-2-phenylindole before taking the images. Sections were observed under the fluorescence microscope (Carl Zeiss, Jena, Germany).

Terminal Transferase–Mediated dUTP Nick End Labeling Staining

Terminal transferase–mediated dUTP nick end labeling (TUNEL) staining was performed using an in situ cell death detection kit according to the manufacturer’s instructions (Roche Applied Science, South San Francisco, CA). The paraffin-embedded coronal sections were deparaffinized and rehydrated and then treated with proteinase K (10 μg/mL) for 30 minutes at room temperature. Slides were incubated with rabbit polyclonal anti-ASPP1 antibody (diluted 1:100; Abbiotec), rabbit polyclonal anti-ASPP2 antibody (diluted 1:100; Abbiotec), and rabbit polyclonal anti-iASPP antibody (diluted 1:100; Abcam), respectively, in a humidified chamber at 4°C overnight and then goat anti-rabbit secondary antibody (fluorescein isothiocyanate conjugated; diluted 1:200; Jackson Immuno Research Laboratories Inc, West Grove, PA). After washing 3× in PBS, sections were incubated with reaction buffer containing enzyme and at 37°C for 30 minutes in the dark. The nuclei were stained with 4′,6-diamidino-2-phenylindole. Sections were observed under the fluorescence microscope (Carl Zeiss).

Cells Culture and Oligonucleotide Transfection

Human embryonic kidney cells and Neuro-2a cells were incubated in DMEM (Gibico, Invitrogen) with 10% fetal bovine serum (Gibico, Invitrogen) and 100 U/mL penicillin/streptomycin (Invitrogen). Cells were maintained at 37°C and 5% CO2, MiR-124 mimic, miR-124 inhibitor, and relevant controls were purchased from Invitrogen. The sequence of miR-124 mimic is 5′-UAAGGCAACCGGUGAAGCCG-3′, and the sequence of its negative control is 5′-UUGUACUACACAAAGUACUG-3′. The sequence of inhibitor is 5′-CGUGUUCACAGCGGACCUUGAU-3′, and the sequence of its negative control is 5′-CAGUACUUUUGUGUGAUCA-3′. Neuro-2a cells were inoculated into 6-well plates at a density of 4.0×104 per
well and were transiently transfected using Lipofectamine RNAiMAX (Invitrogen) when they reached 60% confluence. Cells were collected at 24 hours, 48 hours, and 72 hours after transfection, and 3 independent repeats were performed for all experiments.

Luciferase Reporter Assay

The 3′-untranslated region (UTR) of the mouse iASPP was synthesized (including 2 predicted binding sites for miR-124 on iASPP, carrying the XbaI restriction sites) from Invitrogen. The PCR product was cloned downstream of the renilla luciferase stop codon in pMIR report vector (Ambion, USA), named p3′UTR-iASPP. We constructed 2 single-mutant luciferase reporter vectors (p3′UTR mut1-iASPP and p3′UTR mut2-iASPP) and 1 double-mutant luciferase reporter vector (p3′UTR 2mut-iASPP) by site-directed mutagenesis. In 6-well plates, 2.0×10^5 293T cells per well were plated. When the cells were 50% confluent, they were transfected by Lipofectamine 2000 (Invitrogen) according to the manufacturer’s handbook. p3′UTR-iASPP, p3′UTR mut1-iASPP, p3′UTR mut2-iASPP, or p3′UTR 2mut-iASPP luciferase reporter vector was cotransfected into 293T cells, along with the miR-124 mimic or control. A renilla luciferase vector (pRL-TK) was used to normalize differences in transfection efficiency. Cells were harvested 36 hours after transfection and assayed using Luciferase Assay System (Promega, Madison, WI) according to the manufacturer’s handbook. All assays were performed at least in triplicate. Each experiment was independently repeated ≥3×.

Intracerebroventricular Injection

After permanent MCAO, mice were randomly divided into miRNA and control miRNA mimic groups. Adult male CB57/B6 mice were anesthetized with 2% isoflurane in 70% N2O balance O2 by face-mask and placed in a stereotaxic frame with a mouse head holder. Intracerebroventricular injection was performed as previously described.14 The miRNA mimic or control miRNA mixture (miRNA or control miRNA 4.2 μL, lipofectamine 2000 1.2 μL, and ddH2O 0.6 μL) was immediately stereotaxically delivered into the ipsilateral lateral ventricle for >10 minutes. The bone wound was closed with bone wax.

Statistical Analysis

All data were reported as mean±SD. Statistical analysis was performed using SPSS version 11.0 (SPSS, Chicago, IL). The significance of difference was assessed by Student t test (single comparisons) or by 1-way ANOVA and post hoc Scheffe tests. The effects were considered statistically significant with P<0.05.
Results
Ischemic Expression of ASPP1, ASPP2, and iASPP in Mouse Focal Cerebral Ischemia

To evaluate the expression of ASPP1, ASPP2, and iASPP, quantitative real-time PCR, Western blot, and immunohistochemistry were performed in brain samples from sham-operated controls, and mice were subjected to focal cerebral ischemia. In comparison with samples from sham-operated mice, mRNA level of both ASPP1 and ASPP2 significantly increased at 4 hours after ischemia, in both the penumbra and ischemic core (Figure 1A; \( P<0.05 \)). However, no detectable changes were observed for iASPP mRNA level (Figure 1A).

We further quantified the protein levels of ASPP1, ASPP2, and iASPP in these samples. Our results showed that ASPP1 and ASPP2 protein levels were remarkably elevated at 4 hours after ischemia in penumbra and ischemic core, which were well consistent with their mRNA levels (Figure 1B and 1C). Interestingly, the iASPP protein level in penumbra of MCAO mice significantly dropped at 24 hours after ischemia (Figure 1B and 1C). In ischemic core, compared with these from sham-operated mice, the iASPP protein demonstrated an even more immediate decrease at only 1 hour after ischemia (Figure 1B and 1C). The inconsistency between mRNA and protein levels of iASPP suggests that perhaps there is a post-transcriptional modulation of iASPP protein level under ischemic condition.

Based on our previous Western blot results, we further examined the cellular distribution and the level of ASPP family proteins from both sham-operated and MCAO mice after 4 hours (for ASPP1 and ASPP2) or 24 hours (for iASPP) of ischemia by immunofluorescence staining. It is well known that NeuN is a nuclear marker for neurons. But the immunohistochemical staining of NeuN, which was used in our study, is primarily localized in the nucleus of neurons, with lighter staining in the cytoplasm (http://www.millipore.com/catalogue/item/mab377#). Thus, 4′,6-diamidino-2-phenylindole, a nuclear marker, was used to further position the neuron nuclei with NeuN. In brain slices from sham-operated mice, ASPP1, ASPP2, and iASPP all localized primarily in the neuronal cytoplasm, which merged well with NeuN but not with 4′,6-diamidino-2-phenylindole (Figure 2A–2C). However, in samples from ischemic brains, ASPP1 and ASPP2 protein significantly increased and also translocated into the neuronal nucleus (Figure 2A–2C). Consistent with the Western blot results (Figure 1B and 1C), the iASPP protein was strongly...
stained in slices of sham-operated mice but almost undetectable in those from MCAO mice (Figure 2C). However, Western blot results showed ≈50% reduction in iASPP expression from MCAO mice (Figure 1B and 1C), which indicated that Western blot might be more sensitive than immunohistochemical staining.

Colocalization of ASPP Proteins and Apoptosis in Mouse Focal Cerebral Ischemia
To evaluate the role of ASPP proteins in ischemia-induced neuronal cell death, we also examined apoptotic cells in brain of MCAO mice using TUNEL assay and immunofluorescence staining. Based on our previous Western blot results, we chose the samples at 4 hours (for ASPP1 and ASPP2) or 24 hours (for iASPP) after ischemia. Our results showed that apoptotic cells were widely detected in ischemic brain, and TUNEL-positive signals colocalized well with ASPP1- and ASPP2-positive cells (Figure 3A and 3B). On the contrary, there was no colocalization between TUNEL-positive cells and iASPP-positive cells in ischemic brain tissues (Figure 3C). All these results suggest that ASPP proteins perhaps play an important role in mediating ischemia-induced neuronal cell apoptosis.

Elevation of miR-124 in Mouse Focal Cerebral Ischemia
Inspired by the inconsistency between mRNA and protein level of iASPP, we hypothesized that post-transcriptional regulation may exist to suppress its translation process. Through Targetscan database (http://www.targetscan.org/), we identified an miRNA, miR-124, that may suppress iASPP mRNA translation. To further confirm the finding, we examined the miR-124 level in brain samples from sham-operated and ischemic mice using quantitative real-time PCR. The brain tissues were taken at 1 hour, 4 hours, and 24 hours after ischemia. Our results demonstrated that miR-124 level was significantly increased in the penumbra at 24 hours after ischemia (Figure 4). However, miR-124 level in ischemic core did not show remarkable changes at 24 hours after ischemia.

Regulation of iASPP by miR-124
To study whether miR-124 can directly recognize the 3′-UTR of iASPP mRNA, we constructed a luciferase reporter vector containing the 3′-UTR of mouse iASPP (NM_006663). The Targetscan database (http://www.targetscan.org/) predicted 2 potential target sites of miR-124 in the iASPP 3′-UTR. Therefore, we also generated 3 vectors carrying mutations in either one or both binding sites (p3′-UTR Mut1, p3′-UTR Mut2, and p3′-UTR 2Mut; Figure 5A). The vectors were cotransfected with either miR-124 mimic or control into 293T cells. As shown in Figure 5B, the luciferase activity assay showed that miR-124 mimic lead to a significant reduction in luciferase expression in 293T cells as compared with p3′-UTR or p3′-UTR Mut1. However, miR-124 mimic has little effects on luciferase activity of 293T cells carrying mutation at binding site 2 or both. These data indicate that miR-124 directly regulates iASPP by binding to the second target site in the 3′-UTR of iASPP.

Downregulation of iASPP Protein by miR-124 in Neuro-2a Cells
To further assess the effects of miR-124 in downregulating iASPP protein level, we used Neuro-2a cell line, which has been extensively used to study neuronal signaling pathways.15 Our results clearly indicated that miR-124 strongly suppressed the iASPP protein level in Neuro-2a cells, whereas the control had no obvious effect (Figure 5C). However, the miR-124 inhibitor did not show detectable inhibitory effects (Figure 5D), which may result from the low miR-124 level.
and high iASPP mRNA level in Neuro-2a cell line (data not shown).

Effect of miR-124 Overexpression and Inhibition on Permanent Focal Cerebral Ischemic Injury

To evaluate the role of miR-124 in ischemic brain injury, we injected either miR-124 mimic or miR-124 inhibitor into the ventricles of mice and then analyzed their effect on iASPP protein level, as well as infarct volume of brain tissues after ischemia. All samples were taken from the penumbra of mice at 24 hours after cerebral ischemia. Western blot results showed that miR-124 mimic can efficiently suppress iASPP protein level in brain tissue (Figure 6A), whereas miR-124 inhibitor had an opposite effect as expected (Figure 6B). Finally, we examined the effects of miR-124 manipulation on infarct volume of brain samples from mice of focal cerebral ischemia. miR-124 mimic slightly enhances the infarct volume of brain samples at 24 hours after ischemia, although it did not show a statistical difference with those from control mice (Figure 6C). However, inhibition of miR-124 effectively attenuated ischemic injury by reducing the infarct volume (Figure 6D). Our results suggested that upregulation of iASPP level by miR-124 inhibitor in brain is a potential novel therapeutic intervention to prevent ischemia-induced neuronal cell death.

Figure 4. Expression of microRNA-124 (miR-124) after focal ischemia (middle cerebral artery occlusion) in mice. The brain tissues were sampled at 1 hour, 4 hours, and 24 hours after ischemia, and miR-124 level was measured by quantitative real-time polymerase chain reaction. U6 was used as internal control for quantification of microRNAs. Error bars indicate SD (n=5, *P<0.05).

Figure 5. MicroRNA-124 (miR-124) targets and inhibits inhibitory member of the apoptosis-stimulating proteins of p53 family (iASPP) expression. A, The 2 putative binding sites from the Targetscan database and the 2 mutated binding sites for miR-124 in the 3′-untranslated region (UTR) of the iASPP mRNA. B, miR-124 inhibits the expression of iASPP through putative binding site 2. p3′UTR-iASPP, p3′UTR mut1-iASPP, p3′UTR mut2-iASPP, or p3′UTR 2mut-iASPP luciferase vector was cotransfected into 293T cells, along with miR-124 mimic or control. C, miR-124 leads to downregulation of iASPP protein level in Neuro-2a cells. Intracellular iASPP protein was examined by Western blot after treatment with miRNA mimic or control for 24 hours, 48 hours, and 72 hours. β-actin served as loading control. Right, Relative expression of iASPP protein was determined by densitometry. D, Effect of miR-124 inhibitor on iASPP protein level of Neuro-2a cells. Data were presented as mean±SD in right. *P<0.05.
Discussion and Conclusion

Neuronal cell death is a critical part of stroke pathophysiology, and as a central regulator of cellular stress, p53 plays a key role. Previous studies showed that the ASPP family of proteins comprises a central set of apoptosis-regulating proteins for the p53 pathway in cancer. But the role of ASPP proteins in neuronal death is unknown. To the best of our knowledge, this is the first report that describes the role of ASPP proteins in cerebral ischemia. Our data demonstrate that prodeath ASPP1 and ASPP2 are upregulated, whereas the prosurvival iASPP is decreased. Importantly, a key observation was the opposite effects on mRNA versus protein levels of iASPP in cerebral ischemia, suggesting that some form of miRNA pathway might contribute to its transcription-independent regulation.

As a specific brain miRNA, miR-124 is conserved between invertebrates and vertebrates and is the most abundant miRNA in adult and embryonic brain. However, the precise mechanism of miR-124 in cerebral ischemia is unknown. miR-124 is thought to be involved in the transformation from the neuronal stem cells to mature neurons. Recently, plasma miR-124 was found to be elevated in rats after cerebral ischemia, suggesting its potential role as biomarker for cerebral infarction. miR-124 can target a large number of mRNAs, such as cyclin-dependent kinase 6, laminin γ1, Lhx2, and Sox9. Hence, miR-124 may have multiple and complex roles in cerebral ischemia. A major finding of this report is that the antiapoptotic protein iASPP, as a potential target of miR-124, is reduced after cerebral ischemia via a transcriptionally independent pathway. Hence, in the context of ischemic stroke, miR-124 amplifies neuronal death. Recent studies confirmed that miR-124 can inhibit cell proliferation and promote cell differentiation. Downregulation of iASPP expression inhibits cell proliferation and induces apoptosis, and iASPP overexpression antagonizes apoptosis in cancer cells.

Our study indicates that miR-124 may contribute to cell apoptosis by targeting iASPP in early stage of cerebral ischemia. But some caveats exist. First, it will be useful to show in future studies whether miR-124 can directly influence neuronal cell death in culture systems. Second, in ischemic core, we did not see any change in miR-124 until 24 hours after ischemia, whereas the iASPP protein level decreased from 1 hour to 24 hours, which may be the result of other unknown mechanisms that regulate miR-124 and iASPP expression during the early ischemic injury. The time course of miR-124 and iASPP interactions should be more fully defined from the acute to delayed stages after ischemia. Finally, it is likely that beyond iASPP, miR-124 may have other targets in stroke. No significant neuronal injury was observed in miR-124–overexpressing mice, suggesting that parallel compensatory signals may exist. Further mechanistic studies are warranted to dissect the complex pathways carefully for miR-124 in stroke and neuronal death.

In conclusion, our current study indicates that the prosurvival p53 signaling protein iASPP is reduced in the early stages of focal cerebral ischemia by a novel miR-124–mediated mechanism. Further studies to dissect this molecular pathway may lead us to new therapeutic opportunities to promote neuronal survival in stroke and brain injury.

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Disclosures

None.

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