Acquired inhibition of microRNA-124 protects against spinal cord ischemia–reperfusion injury partially through a mitophagy-dependent pathway

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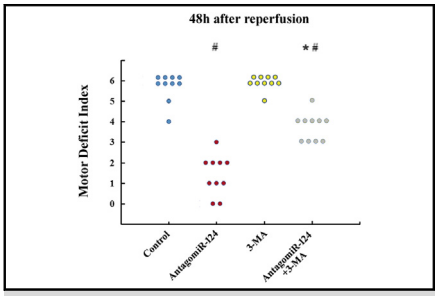
ABSTRACT

Objective: Mitophagy results in selective clearance of damaged mitochondria. We investigated whether mitophagy was involved in the neuroprotection by inhibiting microRNA (miRNA)-124 on ischemic spinal cords.

Methods: Inhibition of miRNA-124 was conducted by intrathecal injection of lentivirus vectors containing antagomiR-124. Spinal cord ischemia was induced in rats by crossclamping the descending aorta just distal to the left subclavian artery for 14 minutes. Hind-limb motor function was assessed with the motor deficit index (MDI). Lumbar spinal cords were harvested for ultrastructural, histologic examinations, and terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick-end labeling staining. Mitophagy was evaluated by expressions of beclin-1 and LC3-II in mitochondria. Expressions of inhibitory member of the apoptosis-stimulating proteins of p53 family, p53, beclin-1, LC3-II, and miRNA-124 were measured by Western blot and quantitative real-time polymerase chain reaction. Mitophagy was inhibited by the antagonist of 3-methyladenine.

Results: Compared with control animals, antagomiR-124 significantly inhibited expressions of miRNA-124 ($P < .01$) and p53 ($P < .05$) and enhanced expressions of inhibitory member of the apoptosis-stimulating proteins of p53 family, becline-1 and LC3-II ($P < .01$, respectively) in spinal cords. MDI at 6, 12, 24, and 48 hours after reperfusion were markedly lower in antagomiR-124 group ($P < .01$, vs control group, respectively). More motor neurons and less apoptotic cells were detected in lumbar spinal cords of antagomiR-124 group ($P < .01$ vs control group). Administration of 3-methyladenine completely abolished enhancements of mitochondrial becline-1 and LC3-II by antagomiR-124 ($P < .01$ vs antagomiR-124 group) and partially inhibited effects of antagomiR-124 on MDI, number of motor neurons, and apoptotic cells ($P < .01$ or $< .05$ vs control group and antagomiR-124 group, respectively).

Conclusions: Inhibition of miRNA-124 exerts neuroprotection on spinal cords against ischemia–reperfusion injury, possibly by induction of mitophagy and antiapoptotic effects. (J Thorac Cardiovasc Surg 2017;154:1498-508)



Neuroprotection that results from down-regulation of miR-124 is partially blocked by inhibiting mitophagy. *$P < .01$ compared with the antagomiR-124 group. #$P < .01$ compared with the control group.

Central Message

Inhibition of microRNA-124 results in neuroprotection against spinal cord ischemia-reperfusion injury, which is possibly mediated by induction of mitophagy and antiapoptotic effects.

Perspective

Inhibition of microRNA (miRNA)-124 provides a powerful neuroprotection on ischemic spinal cords, which is possibly mediated by both induction of mitophagy and antiapoptotic effects. To our knowledge, this is the first report that mitophagy serves as a novel mechanism of neuroprotection on ischemic spinal cords by targeting miRNAs. miRNAs and mitophagy may become potential therapeutic targets for ischemic spinal cords.

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Transient or permanent spinal cord ischemia remains the most devastating complication after open and endovascular thoracoabdominal aortic aneurysm repair, which is associated with a high incidence of paraplegia. Regardless of the refinements of surgical techniques and perioperative strategies, spinal cord ischemia is still

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Abbreviations and Acronyms

3-MA = 3-methyladenine
ASPP = apoptosis-stimulating proteins of p53
iASPP = inhibitory member of the apoptosis-stimulating proteins of p53 family
MDI = Motor deficit index
miRNA = microRNA
ROS = reactive oxygen species
TU = transfection units
TUNEL = terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick-end labeling

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understood incompletely and not entirely predictable or preventable.[1,2]

microRNAs (miRNAs) are 21- to 23-nucleotide nonprotein-coding RNA molecules that act as negative regulators of gene expression by either degrading the target mRNAs or arresting their translation.[3] miRNAs have been shown to be abundant in central nervous system and play an important role in the development of neurons, maintenance of the neuron phenotype, neurodegeneration, and neuroprotection against ischemia and injury.[4,5] Transient focal ischemia induces extensive temporal changes in rat cerebral microRNAome.[6] Expressions of a large set of miRNAs targeting components that are involved in the inflammation, oxidation, and apoptosis are altered after a spinal cord contusive injury.[7] Inhibition of miRNA-29c has been shown to protect the brain in a rat model of prolonged hypothermic circulatory arrest.[8] In our previous reports, inhibition of miRNA-320 and overexpression of miRNA-21 are indicated to induce neuroprotection on rat spinal cords against transient ischemia.[9,10] Collectively, available findings suggest that miRNAs may be an attractive therapeutic target for spinal cord ischemia–reperfusion injury.

Mitochondria serve as the powerhouse of cells, respond to cellular demands and stressors, and play an essential role in cell signaling, differentiation, and survival. In response to changes in the intracellular environment, mitochondria become producers of excessive reactive oxygen species (ROS) and release prodeath proteins, resulting in disrupted ATP synthesis and activation of cell death pathways.[11] Autophagy is a cellular housekeeping function responsible for the bulk degradation of large protein aggregates or damaged organelles.[12] Mitophagy, the mitochondrial autophagy, results in selective clearance of damaged mitochondria in cells before they cause activation of cell death.[11] Therefore, mitophagy is a crucial mechanism to control the quality of mitochondria by preventing the generation of ROS from dysfunctional mitochondria.[13] miRNAs can affect the mitochondrial metabolism, participate in the regulation of mitochondria-mediated apoptosis, and regulate mitochondrial morphology.[14] Furthermore, recent works have suggested a potential role of miRNAs in controlling autophagy, including miRNA-101,[15] miRNA-204,[16] and miRNA-30a.[17] However, it remains unknown whether miRNAs regulate mitophagy in the pathologic process of spinal cord ischemia–reperfusion injury.

miRNA-124 is one of the most abundant miRNAs in adult and embryonic brain[18] and is involved in the transformation from the neuronal stem cells to mature neurons.[19] Plasma miRNA-124 has been found to be increased in rats after cerebral ischemia, suggesting its potential role as a biomarker for cerebral infarction.[20] Knockdown or inhibition of cerebral miRNA-124 reduces cell death and infarct size and improves neurologic outcomes.[21] In the current study, we highlighted the possible neuroprotective effects of inhibition of miRNA-124 on spinal cords against transient ischemia and tried to explore the role of mitophagy in such neuroprotection. Inhibitory member of the apoptosis-stimulating proteins of p53 family (iASPP) was indicated to be a target protein of miRNA-124 with the help of a bioinformatics-based database (http://www.targetscan.org)[22] and published reports.[23] After inhibition of miRNA-124 and transient spinal cord ischemia, expression of iASPP and its downstream protein p53 were measured. To evaluate the role of mitophagy in spinal cords suffering from transient ischemia, mitochondrial expressions of beclin-1 and LC3-II (molecular markers of autophagy) were measured.

METHODS

Animals

Male Wistar rats weighing about 250 g were enrolled in the present study. The animal protocol was approved by the Ethics Review Committee for Animal Experimentation of China Medical University (Shenyang, People's Republic of China). It was conducted in accordance with the Guide for the Use and Care of Laboratory Animals (National Institutes of Health, Bethesda, Md).

Inhibition of miRNA-124 In Vivo

Chemically modified antisense oligonucleotides of rat miRNA-124 (antagomiR-124) lentivirus gene transfer vectors were constructed by Genechem (Shanghai, China). Lentivirus vectors without antagomiR-124 were used as control vectors. The lentivirus vector of antagomiR-124 and control vector were prepared and titered to 1×10^9 transfection units (TUs) $\cdot$ mL^{-1}, according to manufacturer guidelines.

Lentivirus vectors of antagomiR-124 or control vectors were transfected in vivo by means of intrathecal injection, as reported previously.[9,10] Animals were included in the following protocol only if they had normal hind-limb motor function 3 days after the intrathecal injection.