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MiR-207/352 REGULATE LYSOSOMAL-ASSOCIATED MEMBRANE PROTEINS AND ENZYMES FOLLOWING ISCHEMIC STROKE

J. TAO,[a†] W. LIU,[a†] G. SHANG,[b] Y. ZHENG,[b] J. HUANG,[c] R. LIN[b] AND L. CHEN[a*]

[a] *College of Rehabilitation Medicine & TCM Rehabilitation Research Center Of SATCM, Fujian University of Traditional Chinese Medicine, Fuzhou 350122, PR China*

[b] *Fujian Rehabilitation Tech Co-innovation Center (2011 Project), Fujian University of Traditional Chinese Medicine, Fuzhou 350122, PR China*

[c] *Fujian Rehabilitation Engineering Research Center & Fujian Key Lab of Motor Function Rehabilitation, Fujian University of Traditional Chinese Medicine, Fuzhou 350122, PR China*

Abstract—The role of microRNAs (miRNAs) in lysosome-mediated neuronal death and survival following ischemic stroke remains unknown. Herein, using miRNA and mRNA gene expression profiling microarrays, we identified the differentially expressed 24 miRNAs and 494 genes in the cortical peri-infarct area, respectively. Integrating the miRNA targets and mRNA expression profiles, we found 47 genes of miRNA targets, including lysosomal-associated membrane protein 2 (LAMP2), Hexb, Bcl2, etc. MiR-207 and miR-352 were mainly downregulated after ischemic stroke, followed by a slight return to baseline during post-middle cerebral artery occlusion (MCAO) 1 d to 7 d. Furthermore, the luciferase reporter assay demonstrated that LAMP2 and Hexb were the direct targets of miR-207 and miR-352, respectively. After lateral ventricle injection with miR-207 agonist mimics, the neurological deficit scores and infarct volumes were attenuated, and the structure of mitochondria ridges was improved. In addition, miR-207 mimics could reduce the number of cellular lysosome and autophagosome, whereas increase the number of autophagic vacuoles, indicating miR-207 might affect the latter part of lysosomal-autophagy pathway and mitochondria-induced apoptosis. These results suggested that miR-207 and miR-352 were involved in lysosomal pathway for mediating ischemic injury and spontaneous recovery. MiR-207 mimics as potential target drugs could protect against autophagic cell death after ischemic stroke.

Key words: miR-207, miR-352, lysosome, autophagy, stroke.

INTRODUCTION

In the acute phase of ischemic stroke, there is increasing evidence that lysosome may be involved in mediating neuronal death and survival, for regulating homeostasis of intracellular damaged proteins and organelles (Luzio et al., 2007). Autophagy is a catabolic process that is important for bulk removal of cellular proteins and organelles (Klionsky and Emr, 2000). For autophagic degradation, proteins are targeted and transported to membrane-enclosed vesicles. The vesicles are fused with lysosomes and their contents are degraded by lysosomal enzymes, cathepsins B, Hexb, et al. (Punnonen et al., 1993; Kurz et al., 2008). Lysosomes are ubiquitous in all animal cells, as an acidic compartment with limiting membranes and contain various typos of hydrolytic enzymes with acidic pH optima. Lysosomal proteolytic enzymes normally reside in lysosomes and are responsible for protein breakdown. Regulation of intracellular protein metabolism might cause a pathological proteolysis of cytosolic proteins and membranes leading to further cellular damage when released into the cytoplasm. In the ischemic stroke, studies have identified that targeting prodeath signaling upstream of mitochondria damage-cathepsin B in ischemic astrocytes contributes to neuroprotection against cerebral ischemia (Gu et al., 2013). In recent years, Terman et al. proposed a theory of lysosomal-mitochondrial axis for cell death and survival (Terman et al., 2006). Cerebral ischemia could induce lysosomal membrane permeabilization and releasing of lysosomal hydrolytic enzymes, causing neuronal death mediating mitochondrial autophagy (Lipton, 2013).

MicroRNAs (miRNAs) are an endogenous non-coding RNAs, comprising approximately 18–24 nucleotides of small regulatory molecules that cause post-transcriptional gene silencing by base pairing with messenger RNA (mRNA) for degradation and/or translational repression (Bartel, 2009). In recent years, there is an increasing amount of evidence that miRNAs play an important role in neural pathological and physiological changes (Sun and Hevner, 2014). The miRNA expression profile in cerebral ischemia was first reported by Jeyaseelan et al., which revealed 114 novel miRNAs in the brain tissue and peripheral blood of rats (Jeyaseelan et al., 2008). Subsequently, studies have demonstrated that miRNAs play an important pathogenetic role in neuronal apoptosis, inflammation response, and ionic homeostasis following stroke (Dharap et al., 2009; Gubern et al., 2013). However,

*Corresponding author. Tel/fax: +86-591-22861985.
E-mail address: fjtcm1958@sina.com (L. Chen).
[†] These authors contributed equally to this work.

Abbreviation: CBF, cerebral blood flow; FUTCM, Fujian University of Traditional Chinese Medicine; LAMP2, lysosomal-associated membrane protein 2; MCAO, middle cerebral artery occlusion; mNSS, Modified Neurological Severity Score; TTC, 2,3,5-triphenyltetrazolium chloride monohydrate.

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few reports have revealed that miRNAs regulate lysosomal function or autophagy in ischemic stroke.

Nowadays it is noteworthy that miRNA function was defined by bioinformatics analysis, which predicted their potential targets (Lhakhang and Chaudhry, 2012). However, predicted targets source from the Mirbase, MicroCosm, Miranda, Targetscan, Mirdb and Pictar database, many of which are presumed to be false, which frequently lead to the failure of the functional verification-related experiments. In order to enhance their reliability, researchers integrated the miRNA and mRNA expression profiles for understanding their potential function (Cava et al., 2014). However, integrated analysis of the miRNA and mRNA expression profiles in early ischemic stroke remains less.

Therefore, we aimed to analysis of miRNA and mRNA expression profiles highlights alterations in modulation of the neuronal death and survive in the cortical peri-infarct area of middle cerebral artery occlusion (MCAO) rats using high-throughput technologies. An integrative method was developed to identify lysosomal-autophagy pathway-related molecules which were involved in ischemic stroke. The findings of the current studies provided a novel insight into the molecular mechanisms following ischemic stroke.

EXPERIMENTAL PROCEDURES

Ethics statement

All experimental procedures were approved by the Committee on Animal Care and Usage of Fujian University of Traditional Chinese Medicine (FUTCM) in which all the principles followed the Chinese Specifications for the Production, Care and Use of the Laboratory Animals. All animal experiments were permitted under License SYXK (Min) 2012-007 for performing animal experiments. The rats were handled according to the guidelines of the Council for International Organization of Medical Sciences on Animal Experimentation (World Health Organization, Geneva, Switzerland) and the FUTCM.

MCAO rat model for focal cerebral ischemia

Adult male Sprague–Dawley rats ($n = 60$) weighting 230–250 g were housed in an environmentally controlled room at FUTCM (22 ± 2 °C with a 12-h/12-h light/dark cycle). The rats were supplied with standard rat chow and water *ad libitum*. Focal cerebral ischemia was induced by MCAO, as described previously (Tao et al., 2010) with slight modifications. Briefly, left MCAO was performed using an occluding suture (diameter, 0.26 mm) for 2 h for MCAO-evoked ischemia, and then the suture was slowly drawn back to allow reperfusion. The ipsilateral cerebral blood flow (CBF) was measured by Laser Doppler Flowmetry (BIOPAC Systems, Goleta, CA, USA). The MCAO model was considered successful (inclusion) only when cerebral blood flow dropped equal or more than 80% of baseline during occlusion. Furthermore, this blood flow rate was maintained for at least one hour (except for 0-h time point). Although the ipsilateral CBF was measured by Laser Doppler Flowmetry in MCAO surgery, the lack of monitoring of blood gases and cerebral blood flow which were normal for stroke research.

Rat groups

First experiment: Rats were randomly divided into the following four groups ($n = 16$ rats per group): Sham group (Sham), three groups of MCAO model groups with 2 h of ischemia followed by reperfusion for 1, 3 and 7 days, respectively. Rats in Sham group received the same surgical procedures as those in the model group MCAO, but the suture was not advanced beyond the internal carotid bifurcation.

Second experiment: Rats were randomly divided into the following three groups ($n = 12$ rats per group): (1) MCAO model with 3 days group (abbreviation: MCAO + 3d), (2) MCAO with injection of miR-207 mimics (abbreviation: MCAO + miR-207mimics), (3) MCAO with injection of miR-207 mimics negative control (abbreviation: MCAO + Vehicle). Lateral ventricle administration was performed in MCAO + miR-207 mimics and MCAO + Vehicles groups at 30 min before MCAO. Animals were anesthetized with 10% chloral hydrate, and then rats were placed in stereotaxic apparatus (RWD Life Science Co., Ltd., 68001). Subsequently, the miR-207 mimics (100 μM in saline) (GeneCopoeia Inc., Guangzhou, China) were respectively injected into the left brain lateral ventricle with 20 min in total volume of 7 μl (Zhao et al., 2013). Stereotactic coordinates were as follows: anteroposterior, 0.8 mm; mediolateral, 1.5 mm; depth, 3.5 mm. The ipsilateral CBF was measured by Laser Doppler Flowmetry (BIOPAC Systems, Goleta, CA, USA). The mimics or negative mimics were considered successful (injection) only when cerebral blood flow dropped equal baseline during injection. Furthermore, this blood flow rate was maintained for at least 20 min.

Assessment of neurological deficit scores and infarct volume

At post-reperfusion, neurological function was assessed in sixteen animals by an observer who was blinded to the experimental conditions. The evaluation of neurological function was performed using the Modified Neurological Severity Score (mNSS) testing approach (-Hernández-Jiménez et al., 2013). The mNSS is composed of a series of motor tests (i.e., muscle status and abnormal movements), sensory tests (i.e., visual, tactile, and proprioceptive assessment), balance tests, and reflex tests, and is graded on a composite scale of 0–18. The higher the mNSS is, and the more severe the injury is.

After assessment of infarct volume, rats ($n = 4$ per group) were narcotized by an intraperitoneal (i.p.) injection of 10% chloral hydrate (100 g/0.3 ml) and sacrificed by decapitation at times after reperfusion. Frozen brains were coronally sectioned into slices of 1-mm thickness, and stained with 2% 2,3,5-triphenyltetrazolium chloride monohydrate (TTC, Sigma-Al-drich, St. Louis, MO, USA) as described previously