Neurochemistry International 107 (2017) 182–190

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

MicroRNA-132 attenuates neurobehavioral and neuropathological changes associated with intracerebral hemorrhage in mice

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ARTICLE INFO

Article history:
Received 14 September 2016
Received in revised form
21 November 2016
Accepted 28 November 2016
Available online 9 December 2016

Keywords:
Stroke
Intracerebral hemorrhage
Inflammation
miR-132
AChE

ABSTRACT

Recent studies suggest that microRNA-132 (miR-132) potentiates the cholinergic blockade of inflammatory reactions by targeting acetylcholinesterase (AChE) and affords robust protection against ischemia-induced neuronal death. However, the role of miR-132 in intracerebral hemorrhage (ICH) remains unexplored. This study aimed to determine whether miR-132 participates in the process and launches an anti-inflammatory response in a mouse model of ICH. To establish a relationship between miR-132 and ICH-induced neuronal inflammation and death, we used unilateral stereotaxic injections to deliver lentiviruses encoding miR-132, anti-miR-132 or an empty lentiviral vector directly into the right caudate nuclei of 192 living male C57BL/6 mice. Fourteen days later, ICH was induced by injection of autologous blood into these three groups. Neurodeficits, brain edema, blood-brain barrier (BBB) integrity, inflammatory reactions, together with cell death were assessed after ICH. Compared with the control group, the mice overexpressing miR-132 in the brain responded with attenuated neurological deficits and brain edema. The counts of activated microglia and the expression of proinflammatory cytokines were also decreased in these mice. Additionally, BBB integrity improved, and the extent of neuronal death decreased in ICH mice injected with lentivirus encoding miR-132. On the contrary, a decrease of miR-132 expression aggravated the severity of inflammation and increased cell apoptosis. Overall, these findings support a protective role of miR-132 in a mouse model of ICH, providing new opportunities for therapeutic intervention.

1. Introduction

Intracerebral hemorrhage (ICH)-related strokes are characterized by hematoma expansion and inflammation leading to severe motor dysfunction and a high incidence of morbidity (Qureshi et al., 2009; Keep et al., 2012). Early in the course of ICH, blood constituents such as thrombin and hemoglobin typically trigger inflammation (Lee et al., 2006; Fujimoto et al., 2007; Felberg et al., 2002). However, effective treatments are rarely available to date (Keep et al., 2012). Accumulating evidence has demonstrated that inflammation contributes to the secondary brain injury of ICH, involving neuronal death and increased permeability of the blood-brain barrier (BBB) as well as release of inflammatory cytokines (Matsushita et al., 2012; Katsuki, 2010; Fu et al., 2015). Therefore, modulating the inflammatory microenvironment within the brain may be a promising therapeutic strategy for these patients.

MicroRNAs (miRNAs) are endogenous small RNAs that regulate multiple molecular pathways, including the cellular processes of differentiation, proliferation, apoptosis and patterning of the nervous system. Additionally, miRNAs have the capacity to modulate neuronal as well as immune processes (Ambros, 2004; Landgraf et al., 2007; Krek et al., 2005). By binding to their corresponding mRNA targets in specific locations of the latter's 3'untranslated region, miRNAs regulate the expression of mRNAs, specifically those that are translationally repressed and/or degraded (Krek

Abbreviations: ACh, Acetylcholine; AChE, Acetylcholinesterase; BBB, Blood-Brain Barrier; EAE, experimental autoimmune encephalomyelitis; EB, Evans Blue; FP, Forelimb Placing; GAPDH, Glyceraldehyde 3-phosphate Dehydrogenase; IBD, inflammatory bowel disease; ICH, Intracerebral Hemorrhage; IL, Interleukin; LV, Lentivirus; miR-132, microRNA-132; Min, minute; miRNAs, microRNAs; mNSS, modified Neurological Severity Score; OD, Optical Density; RIPA, Ristocetin-Induced Platelet Aggregation; RNA, Ribonucleic Acid; TNF, Tumor Necrosis Factor; TUNEL, TdT-mediated Biotin-dUTP Nick End labeling; ZO-1, Zonula Occluden-1.

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http://dx.doi.org/10.1016/j.neuint.2016.11.011
0197-0186/

et al., 2005; Filipowicz et al., 2008). Specifically, miRNA-132 has a well-known ability to potentiate the cholinergic anti-inflammatory pathway by targeting acetylcholinesterase (AChE), a hydrolytic enzyme for acetylcholine (ACh), thereby increasing ACh content. The result is a reduced release of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β (Shaked et al., 2009). In addition to its anti-inflammatory benefit, miR-132 has dual roles in immunological and neuronal functions and was, therefore, designated as "NeurimmiR" (Soreq and Wolf, 2011). As documented, miR-132 has positive, pivotal roles in brain development, dendritogenesis, synapse formation and synapse maturation (Siegel et al., 2011; Schratt, 2009; Hwang et al., 2013). The negation, i.e., repression, of miR-132 expression contributes to ischemia-induced neuronal death (Hwang et al., 2014). MiR-132 loss has been found in patients with Alzheimer's disease, and down-regulation of miR-132 in transgenic mice significantly aggravates the pathologic effects of this disease (Salta et al., 2016; Hernandez-Rapp et al., 2016). Recently, however, the anti-inflammatory role of miR-132 has gained increasing attention and was identified in several diseases, such as experimental autoimmune encephalomyelitis (EAE) (Hanieh and Alzahrani, 2013) and inflammatory bowel disease (IBD) (Maharshak et al., 2013). Nevertheless, the possible participation of miR-132 in the brain after ICH has not been investigated.

Therefore, we have now inquired whether miR-132 potentiates cholinergic anti-inflammation in brains undergoing ICH. To that end, we regulated the expression of miR-132 by injecting lentiviruses encoding miR-132, -miR-132 or an empty lentiviral vector into brains of mice in each group. The packaged lentiviruses were named LV-miR-132, LV-anti-miR-132 and LV-control, respectively. Our results demonstrated, for the first time that, in mice with experimentally induced ICH, miR-132 ameliorated neuronal damage by regulating AChE expression. We further showed that overexpression of miR-132 significantly impeded the production of pro-inflammatory mediators and improved the outcome of this disease.

2. Material and methods

2.1. Study design

All animal experiments were designed, performed, and reported according to the ARRIVE guidelines (Kilkenny et al., 2012; Schulz et al., 2010). Age-matched, male mice (C57BL/6J, 5–6 weeks of age, 18–20 g body weights, Charles River Laboratories, Beijing China) were housed in the animal care facility at Tianjin Medical University General Hospital (Tianjin, China). This study was conducted in accordance with the local authorities, and the use of animals in research and all protocols were approved by the institutional animal care and use committee. Animals were kept in a well-ventilated room under a 12-h light/dark cycle (lights on between 8:00AM and 8:00PM) with temperature maintained at 25 ± 3 °C and provided with normal amounts of food and water. Two weeks before the induction of ICH, 192 wild type mice were randomly assigned into three groups according to the first injection of three kinds of lentivirus:LV-miR-132 (n = 64), LV-anti-miR-132 (n = 64) and LV-control (n = 64). Then, all these mice underwent surgery for induction of ICH by injection of autologous blood. Mice deceased within 1 day were excluded. Data from each group of mice were collected and analyzed by at least two investigators who were blind to group assignment.

2.2. Infusion of lentivirus

The lentiviral vectors with miRNAs were constructed by the GeneChem Company (Shanghai, China). GV369 and GV280 received insertions of double-stranded oligonucleotides encoding miR-132, -miR-132 or a negative control (GeneChem), which were further confirmed by sequencing. Components were as follows: Ubi-MCS-SV40-EGFP-IRES-puromycin for LV-miR-132 and hU6-MCS-Ubiquitin-EGFP-IRES-puromycin for LV-anti-miR-132. Primers for amplification were the following: 5' - GAGGATCCCCGGGTACCGGCTGTGGGTTGCGGTGGGCGCAG - 3' (forward), 5' - CACACATTCCACAGGCTAGCCTCCTCTTGCTCTGTATCTG-3' (reverse) for LV-miR-132, 5' - TAACAGTCTACAGCCATGGTCG - 3' (forward), 5′-CGACCATGGCTGTAGACTGTTA - 3' (reverse) for LV-anti-miR-132. Lentiviruses were diluted to 5×10^8 Tu/ml with enhanced infection solution (Genechem, Shanghai, China). Before the surgery, mice were treated with an intraperitoneal injection of 50 mg/kg pentobarbital and then fixed on a stereotaxic frame (RWD Life-science, Shenzhen, China). A 1 mm burr hole was drilled, and a 10-gauge needle carrying 2 μl of lentivirus was inserted into the striatum (stereotaxic coordinates; 2.3 mm lateral to the midline, 0.2 mm anterior to the bregma, and 3.5 mm deep below the skull). Each mouse was injected with 2 μl of lentivirus at a rate of 0.5 μl/min through the infusion pump (Kd Scientific, USA). After the infusion was completed, the needle was held in place for 20 min and was then slowly withdrawn at a rate of 1 mm/min over the course of 3 extractions at 5 min intervals. Finally, the burr hole was sealed with bone wax (Johnson & Johnson, USA) and sterilized, and the wound was then sutured. After surgery, animals were placed in cages with free access to food and water. Warming lamps were used to provide thermal support throughout the procedure.

2.3. Mouse model of ICH

Two weeks later, mice were anesthetized and again fixed on the stereotactic frames. We chose the blood injection model to induce ICH as previously described (Rynkowski et al., 2008; Krafft et al., 2012). Briefly, blood was withdrawn from the angular vein and quickly transferred into a 50 μl μL syringe with a 26 G needle (Hanmilton Company, Shanghai, China). The first 5 μl was injected into each mouse to generate clotting along the needle track; after a 5min pause, the remaining 25 μl was injected at a rate of 1 μl/min. After completing the infusion, the needle was withdrawn as described above. Again mice were housed, fed and warmed as described above.

2.4. Evaluation of neurological function and survival

The motor, sensory, reflex and balance functions were evaluated at baseline, day 1 and day 3 after ICH induction. Tests chosen for that purpose were the Modified Neurological Severity Score (mNSS), Corner Turning Test and Forelimb Placing (FP) test performed with each mouse as previously described (Chen et al., 2001; Hua et al., 2002). The 14-day-survival indices were calculated for all three groups.

2.5. Assessment of brain water content

At day 3 after the induction of ICH, brains were isolated and divided into three parts: left hemisphere, right hemisphere and cerebellum. After being weighed on an electronic analytical balance, the wet weight of each part was obtained. The dry weights were then obtained after drying the tissue for 24 h at 100 °C. Weights were calculated as follows: (Wet Weight-Dry Weight)/Wet Weight x 100%.

2.6. Assessment of hematoma volume

At day 3 after the second surgery, brains were removed and