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MiR-590-5p alleviates intracerebral hemorrhage-induced brain injury through targeting Peli1 gene expression

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ABSTRACT

Intracerebral haemorrhage (ICH) is a common and devastating cerebrovascular disease with high morbidity and mortality, and its pathophysiological mechanisms were complex and still unclear. Increasing researches reported that microRNAs (miRNAs) played an important role in ICH-induced brain injury and microglial activation. In this study, we investigated the biological function of miR-590-5p and explored its molecular mechanism in ICH mice. The results of qRT-PCR showed that miR-590-5p expression level was down-regulated in perihematomal brain samples of ICH mice compared with that of sham group. In LPS-induced microglia cells, miR-590-5p level was also down-regulated at 24 h post-LPS compared with that of control group. Moreover, miR-590-5p overexpression remarkably increased the cerebral water content and neurological severity scores compared with that of scramble group in ICH mice. The production of inflammatory cytokine including IL-6, interleukin (IL)-1β, and tumor necrosis factor (TNF)-α in ICH mice was notably inhibited by miR-590-5p overexpression. Furthermore, the results of dual-luciferase reporter assay indicated that Pellino-1 (Peli1) was a direct target of miR-590-5p. MiR-590-5p overexpression remarkably inhibited the Peli1 gene expression both mRNA and protein levels. In addition, Peli1 overexpression partly abrogated the inhibitory effect of miR-590-5p mimic. Taken together, these datas suggested that miR-590-5p attenuated brain injury in ICH mice through inhibiting Peli1 gene expression, indicating that miR-590-5p may be a promising molecular target for ICH treatment.

1. Introduction

Intracerebral haemorrhage (ICH) is a common and devastating cerebrovascular disease with high morbidity and mortality [1]. After ICH, a series of pathophysiological processes would occur in the brain, including hematoma formation, perihematomal edema, inflammatory responses and activation of microglia [2,3], these of which could lead to brain damage and severe neurological deficits or death [4]. The etiology of ICH is closely associated with hypertension, atherosclerosis, and vascular malformation, whereas its exact etiology remains unknown. Increasing researches have focused on finding the effective therapeutic strategies to mitigate brain haemorrhage after injury and improve its prognosis [5–7]. However, there is still a lack of effective treatment for ICH due to the complex of its pathophysiology. Therefore, further exploring its molecular basis in ICH will help to find the specific molecular target for ICH treatment.

MicroRNAs (miRNAs) are a class of small non-coding RNAs with the length of 20–22 nucleotides that regulates gene expression at the posttranscriptional level by binding to the 3′-untranslated region (3′UTR) of their target genes [8,9]. Previous reports have confirmed that miRNAs are closely associated with various aspects of biological functions and pathological processes, including differentiation, metabolism, aging, autophagy, cell proliferation, and apoptosis [10,11]. Recently, all kinds of dysregulated miRNAs were found in both brain and blood after ICH [7–12]. Yu A et al. reported that ICH up-regulated the mRNA expression of miRNA-144, and inhibition of miRNA-144 alleviates brain injury in ICH mice [13]. Yuan B et al. reported that miR-367 expression was down-regulated and it could reduce inflammation response and brain damage in ICH [14]. In addition, miR-590-5p has been reported to be down-regulated in peripheral blood from patients with ICH [7]. MiR-590-5p was also reported with inflammation response [15,16]. However, whether miR-590-5p alleviates ICH-induced brain injury

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and neuroinflammation in ICH is still unknown and need to be investigated.

In this study, we first examined the mRNA expression level of miR-590-5p in perihematomal tissues of ICH mice, and found that it was down-regulated. In LPS-induced microglia cells, miR-590-5p level was also down-regulated at 24 h post-LPS compared with that of control group. In addition, our results indicated that miR-590-5p overexpression reduced brain damage through targeting Peli1 gene expression. Altogether, our findings suggest that miR-590-5p is a promising molecular target for ICH treatment.

2. Materials and methods

2.1. ICH model

C57BL/6 male mices (8–10 weeks, 22 ± 2 g) were obtained from the animal center of the Fourth Military Medical University (Xi'an, China). Mice was housed in individual cages with free access to standard laboratory chow diet and water. The ICH model was performed as previously described. Briefly, mice was intraperitoneally anesthetized with 400 mg/kg chloral hydrate, and then placed in a stereotaxic frame (Stoelting, Kiel, WI, USA). Under stereotactic guidance, 50 μL of autologous blood (ICH group) or 0.9% saline (Sham group) was injected straight at 25 μL/min into the caudate nucleus (0.8 mm posterior, 1.5 mm right lateral and 3.5 mm deep relative to bregma) over a period of 5 min, and the microsyringe was indwelt for another 5 min before being slowly withdrawn. Next, the pinhole was sealed using the bone wax, and the scalp was closed. In the course of the study, mice was housed in separate cages at room temperature. Experiments comply with animal care guidelines approved by the ethics committee of the Animal Ethics Committee of Xi'an Xidian Group Hospital.

2.2. Intracerebroventricular (ICV) injection

The ICV injection was performed as previously described. Briefly, miR-590-5p mimic and its scramble control, the recombinant plasmid pcDNA3.1-Peli1 (pcPeli1) and pcDNA3.1 negative control (pc-control) (Genepharma, Shanghai, China) were added to Lipofectamine™ 3000, and then the solution was lowly injected into the ipsilateral ventricle 15 min after ICH under the guidance of the stereotaxic instrument. In sham and ICH group, the equal of normal saline was administered. Mice was sacrificed for further analysis at 24 h after ICH.

2.3. Cell culture and transfection

Primary microglia cells were isolated as previously described [17,18]. In brief, cerebral hemispheres from C57BL/6mice (1-day old) were minced, digested by 0.1% trypsin, resuspended with Dulbecco's Modified Eagle Media (DMEM) containing 10% fetal bovine serum, and then plated into a six-well plate coated with poly-L-lysine. After approximately 14 days, the cell purity identified with Iba1 and CD11b+ staining. The microglia cultures used were >95% pure. For transfection, cells were seeded into plates, incubated for 15 h, and then transfected with miRNAs or recombinant plasmid using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions.

2.4. Neurological deficit scores

The neurological deficit tests were performed using the modified Neurological Severity Score (mNSS) at 24 h after ICH. The tests included motor, sensory, reflex, and balance tests. The range of scores for mNSS is from 0 to 18 (normal score: 0–3; maximal deficit score: 18). The investigator was blinded to the experimental groups. The experiment was repeated three times.

2.5. Cerebral water content

The analysis of cerebral water content was evaluated as previously reported [19]. Briefly, after the test of Neurological deficit scores, mice was euthanized and the brain tissues were removed. Next, the wet weight of brain samples was immediately weighed on an electric analytic balance, and then samples dried at 100 °C for 24 h to obtain the dry weight. Brain water content was calculated as follows: brain water content (%) = (wet weight-dry weight)/wet weight × 100%.

2.6. ELISA

The perihematomal brain tissues were collected at 24 h after ICH, and then were homogenized by lysis buffer. Next, samples were centrifuged at 12,000 g for 30 min, and the supernatants were collected. The concentration of TNF-α, IL-1β, and IL-6 was determined with an ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

2.7. Dual-luciferase reporter assay

For vector construction, the Peli1-3′-untranslated region (UTR) sequence containing the miR-590-5p binding side (named WT) and the Peli1-mutated-3′-UTR fragment containing putative miR-590-5p binding sites (named Mutant) were amplified by PCR. Then, these sequences were cloned into the pGL3 miReport vector (Promega, Madison, WI, USA) to generate the Luc-pGL3-Peli1-3′-UTR plasmid (Peli1-WT) or Luc-pGL3-Peli1-mut-3′-UTR plasmid (Peli1-MUT). For the luciferase assay, 293T cells were cultured in 24-well plates, and then co-transfected with miR-590-5p mimic or negative control and the mutant or wild type Peli1 plasmid using Lipofectamine 3000. After transfection for 48 h, firefly and Renilla luciferase activities were measured using a Dual-Luciferase Reporter assay system (Promega, Madison, WI, USA). The relative luciferase activity was reported as luciferase activity/Renilla luciferase activity. Renilla luciferase activities were evaluated as an internal control. All experiments were performed at least three times.

2.8. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from perihematomal brain tissues using Trizol reagent (Invitrogen, Life Technologies, CA, USA) according to the instructions of the manufacturers, and its concentration and purity were detected at 260/280 nm by a NanoDrop ND-1000 instrument (Thermo Scientific, Waltham, MA, USA). cDNA synthesis was performed using a PrimeScript RT reagent Kit (TaKaRa, China) according to the manufacturer's protocol. Next, to examine the mRNA expression level, the qRT-PCR analysis was performed using the miScriptSYBR®green PCR Kit (Qiagen, Redwood City, CA, USA) according to the manufacturer's protocol. U6 and β-actin were used as internal controls. Relative mRNA expression level was calculated by using the $2^{-\Delta\Delta Ct}$ method, and normalized to β-actin/U6 mRNA. All qRT-PCRs were performed in triplicates.

2.9. Western blotting

Total protein was extracted from perihematomal brain tissues using RIPA lysis buffer. The protein concentration was determined using a Bichinonic Acid Assay kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Equal amounts of protein from each sample were separated by sodium dodecyl sulphate-polyacrylamide gel

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