European Review for Medical and Pharmacological Sciences 2019; 23: 5366-5373

LncRNA SNHG1 inhibits neuronal apoptosis in cerebral infarction rats through PI3K/Akt signaling pathway

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Abstract. – OBJECTIVE: To investigate the effects of long non-coding ribonucleic acid (lncRNA) small nucleolar RNA host gene 1 (SNHG1) on the neuronal apoptosis in rats with cerebral infarction through the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway.

MATERIALS AND METHODS: Male Sprague Dawley (SD) rats were divided into M group (model control group), N group (rat model of cerebral infarction) and R group (rat model of cerebral infarction plus lncRNA SNHG1) and then treated accordingly. 2,3,5-triphenyl tetrazolium chloride (TTC) staining was applied to detect the percentage of cerebral infarct volume and apoptosis of brain cells in the three groups of rats; hematoxylin and eosin (HE) staining was utilized to observe the pathological morphology of brain tissues, and Western blotting was performed to measure the protein levels of phosphorylated PI3K (*p*-PI3K) and p-Akt in the brain tissues.

RESULTS: The degree of neurological deficit in the N group was much higher than that in the M group ($p<0.05$), and it was decreased markedly in the R group compared with that in the N group, with statistically significant differences ($p<0.05$). In comparison with that in the M group, the cell apoptosis was aggravated notably in the N group and alleviated remarkably in the R group, and the differences were statistically significant ($p<0.05$). In the N group, the cerebral infarct volume accounted for 33.67% of the whole brain volume, and mild cerebral infarction was detected in the R group, with a percentage of cerebral infarct volume of 20.15%. N group had a more prominent increase in the cerebral infarct volume than the R group ($p<0.05$). Compared with those in the M group, the pyknotic nuclei and neuron staining of brain tissues were increased significantly, and the neuronal cell injury was aggravated in the N group, while markedly reduced pyknotic nuclei and neuron staining ($p<0.05$), as well as mild neuronal cell injury ($p<0.05$), were detected in the R group. The levels of p-PI3K and p-Akt proteins in the brain tissues declined remarkably in the N group compared with those in the R group ($p<0.05$).

CONCLUSIONS: The protective effect of lncRNA SNHG1 on the rats with cerebral infarction is correlated with the activation of the PI3K/Akt signaling pathway.

Key Words:
LncRNA SNHG1, PI3K/Akt signaling pathway, Cerebral infarction, Cell apoptosis.

Introduction

Cerebral infarction, also known as ischemic stroke, accounts for 80% of all the strokes and can easily cause disability and death, which is a common sudden brain disease[1]. Ischemia and hypoxia of the brain tissues can induce local cerebral necrosis or malacia, thus affecting various functions and activities of the patients[2]. The pathogenesis of cerebral infarction is complex. Friedrich and Lobs[3] have revealed that diabetes mellitus, heart disease, arteriosclerosis, cardiovascular disease, shock and other risk factors are the causes of cerebral infarction. Chen et al[4] have indicated that 71.8% of the patients with the ischemic cerebrovascular disease are accompanied by symptoms such as carotid atherosclerotic plaque and increased intima-media thickness at the same time. Improving blood circulation of the brain tissues, controlling hyperlipemia and diabetes mellitus and accelerating neurological function recovery are conventional therapies for cerebral infarction at present. Although these therapeutic methods can effectively improve the degree of ischemia and relieve neuronal cell injury in patients with cerebral infarction, they have little effect on reducing disability rate[5,6].

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Long non-coding ribonucleic acid (lncRNA), with a length of over 200 nucleotides, can be involved in the expression in multiple diseases and regulation of molecular mechanism. A large number of studies have manifested that lncRNAs are involved in cell development, which promote cell proliferation and inhibit apoptosis. It has been discovered through research that lncRNA small nucleolar RNA host gene 1 (SNHG1) is able to decrease the content of interleukin-1 beta (IL-1β), IL-6 and tumor necrosis factor-alpha (TNF-α), increase the content of IL-10 in the rat serum and exert neuroprotective effects. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signal is a transduction protein related to the phosphorylation process in cells[7,8]. According to the previous study[9], the PI3K/Akt signaling pathway is capable of regulating the physiological actions of cells inducing cell proliferation, metabolism, differentiation and apoptosis. The role of cell apoptosis in the nervous system injury has been gradually recognized in recent years, but the molecular mechanism of neuronal apoptosis after cerebral infarction has not been clarified yet.

Since the PI3K/Akt signaling pathway is an important signaling pathway for cell survival, research on the signal transduction pathways of neuronal apoptosis after cerebral infarction becomes one of the hotspots of research on ischemic cerebrovascular diseases. In this paper, a series of studies were conducted for the mechanism of the PI3K/Akt signaling pathway in inducing neuronal apoptosis after cerebral infarction, providing new directions for the early treatment of cerebral infarction.

Materials and Methods

Laboratory Animals and Grouping

The rats used in this experiment were purchased from the Hubei Provincial Center for Disease Control and Prevention. A total of 60 healthy male rats aged about 6 weeks and weighing 0.18-0.22 kg were selected and fed in cages (5 rats per cage) at 24°C, with humidity of 45%, a 12/12 h light/dark cycle and free access to food and water. After adaptation to the new environment for a week, all the 60 male rats were grouped with equal weight in each group. Moreover, they were utilized to establish the model of cerebral infarction, and divided into M group (model control group), N group (rat model of cerebral infarction) and R group (rat model of cerebral infarction plus lncRNA SNHG1). This study was approved by the Animal Ethics Committee of the Central South University Animal Center.

Main Reagents and Instruments

The main instruments and reagents applied in this research are as follows: kits for interleukins and tumor necrosis factors (eBioscience, San Diego, CA, USA), RNA extraction reagent RNAiso (TaKaRa, Otsu, Shiga, Japan), endothelial nitric oxide synthase and β-actin murine monoclonal antibody (Abcam, Cambridge, MA, USA), horseradish peroxidase (HRP)-labeled goat-anti-mouse IgG secondary antibody (Invitrogen, Carlsbad, CA, USA), normal saline (Changzhou Lanling Pharmaceutical Co., Ltd., Changzhou, China), TGL-16B high-speed centrifuge (Jintan Medical Instrument Factory, Jintan, China), BS110S electronic analytical balance and BCD-629WDEYU1 refrigerator (Sartorius Scientific Instruments, Beijing, China), hydroextractor (model: JJ-12J), embedding machine (model: JB-P5), pathological microtome (model: RM-P6) and ultrasonic atomizer (model: JB-L5; Scotsman Ice Systems, Shanghai, China), gel electrophoresis instrument and electroporator (Bio-Rad, Hercules, CA, USA) and BX50 biomicroscope and IX51 inverted microscope (Olympus Corporation, Tokyo, Japan).

Animal Model Establishment

After fasting for 12 h before the operation, the three groups of rats were fixed in the supine position and anesthetized through intraperitoneal injection of 1% pentobarbital sodium (dose: 0.1 g per kilogram of body weight). Then, the skin and fascia in the middle of the neck were cut open to find the common carotid artery, and a small incision on the right common carotid artery, which was located closest to the heart, was made using a pair of scissors. Later, an occluding suture was threaded into the common carotid artery from the incision until meeting apparent resistance in a depth of 17 mm. After that, the artery was ligated, the end of occluding suture was cut, and the incision was disinfected and sutured.

Dose and Route of Administration

At 3 h after successful modeling, the rats in each group were given corresponding drugs. M group received an injection of 20 mg/(kg·d) nimodipine, and N group was injected with 5 mL/kg normal saline. The RNA in the tissues in the R group and that in each group of cells were extracted using

extraction kits, which were utilized for subsequent experiments after injection for 14 consecutive days.

Neurological Function Score

After injection for 14 consecutive days, the limb status of the three groups of rats was observed, and the tail suspension test was performed. The rats without spasm and disturbance of consciousness were included for the present neurological scoring. The Zea Longa scoring standards were applied to evaluate the neurological function (Table I). 0 point represents for normal neurological function without disturbance, 1-2 points for mild neurological dysfunction, and 3-4 points for severe neurological dysfunction.

Observation of Percentage of Cerebral Infarct Volume in Rats Via 2,3,5-Triphenyl Tetrazolium Chloride (TTC) Staining

The three groups of rats were sacrificed after injection for 14 consecutive days, and the brain tissues were taken out under sterile conditions and preserved at -80°C for 5 min. The 2 mm-thick sections of the coronal plane starting from the frontal pole were stored in 1% TTC solution (Beyotime, Shanghai, China), followed by incubation at 37°C in the dark and flipping of the sections once every 5 min. When stained successfully, the normal brain tissues were red, and the tissues of cerebral infarction were white. The images were processed and analyzed using professional image analysis software, and the percentage of cerebral infarct volume was calculated.

Hematoxylin and Eosin (HE) Staining

After injection for 14 consecutive days, the three groups of rats are treated as follows: the brain tissues of the rats were taken out under sterile conditions after the rats were killed, fixed with 10% formaldehyde for 1 day and then prepared into paraffin-embedded sections. 4 μm-thick coronal sections were utilized for HE staining (Boster, Wuhan, China).

Table I. Neurological scoring standards.

Score	Scoring standard
0 point	No apparent neurological symptoms
1 point	Unable to fully extend the left forelimb
2 points	Circling to the left
3 points	Tumbling to the left when walking
4 points	Able to walk by itself, with disturbance

Brain Cell Apoptosis in Each Group of Rats Detected Via Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling (TUNEL) Assay

The prepared paraffin-embedded sections were subjected to TUNEL staining to detect the brain cell apoptosis in each group according to the instructions of the TUNEL assay kit (Roche, Basel, Switzerland). 5 non-overlapping fields of vision were randomly selected in every pathological section for observation under a light microscope, in which the yellowish-brown cells were apoptotic cells. Brain cell apoptosis rate = number of apoptotic cells/total number of cells × 100%.

Protein Levels of Phosphorylated PI3K (p-PI3K) and p-Akt Detected Via Western Blotting

A total of 100 mg brain tissues were taken from each group to extract the proteins using the radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China). Then, the bicinchoninic acid (BCA) method (Pierce, Waltham, MA, USA) was performed to determine the protein concentration as follows. The protein samples (30 μL per well) were separated by means of 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After that, the proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA), added with 5% skim milk powder and sealed at 22-25°C for 1 h, followed by washing with Tris-Buffered Saline and Tween 20 (TBST; Sigma-Aldrich, St. Louis, MO, USA). Subsequently, the corresponding primary antibodies were added for incubation at 4°C overnight, and secondary antibodies were added, followed by washing with TBST again and incubation at 22-25°C for 1 h. Finally, the membrane was washed, and the color was developed and exposed using enhanced chemiluminescence (ECL; Thermo Fisher Scientific, Waltham, MA, USA), followed by photography and preservation.

Statistical Analysis

In this paper, Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) was used for statistical analysis of data. *t*-test was performed for comparisons of neurological function score, cell apoptosis, cerebral infarct volume and p-PI3K and p-Akt proteins among M group, R group and N group. Univariate analysis was applied to analyze data in different

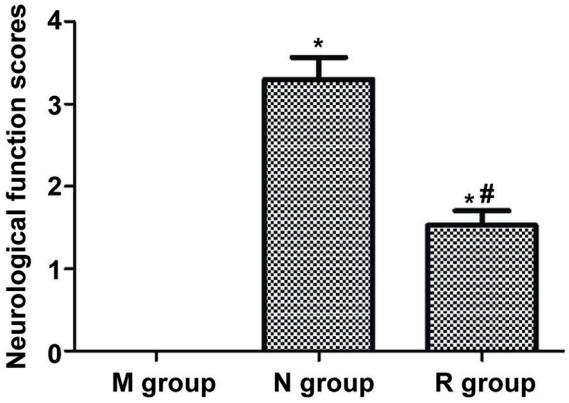

Figure 1. Neurological function scores in the M group, N group and R group. Note: *p<0.05 *vs.* M group, #p<0.05 *vs.* N group.

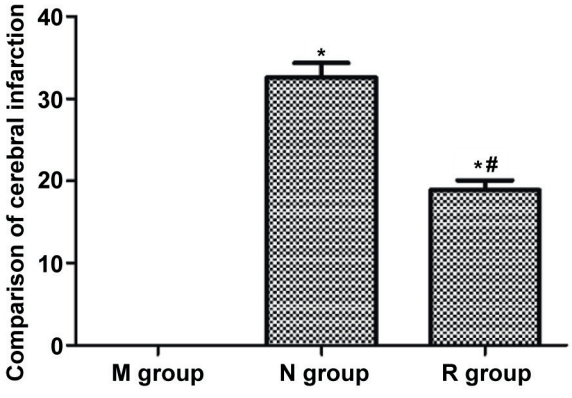

Figure 2. Comparison of cerebral infarction status among M group, N group and R group. Note: *p<0.05 *vs.* M group, #p<0.05 *vs.* N group.

groups, and calculation data were presented as mean ± standard deviation ($\bar{x}$±s). p<0.05 suggested that the difference was significant.

Results

Changes in Neurological Function in Each Group of Rats

The rats in the M group could move normally, those in the N group had an apparent limb movement disorder, and those in the R group had a mild limb movement disorder. The degree of neurological deficit in the N group was much higher than that in the M group (p<0.05), and it was decreased markedly in the R group compared with that in the N group. The differences between groups were statistically significant (p<0.05; Figure 1).

Cerebral Infarction Status in the Three Groups of Rats

The TTC staining results showed that white infarction foci existed in the brain tissues both in the N group and R group. The cerebral infarct volume was 33.67% in the N group and 20.15% in the R group, indicating a mild cerebral infarction. Compared with that in the R group, the increase in cerebral infarct volume was more significant in the N group (p<0.05), and there were statistically significant differences among the three groups (p<0.05; Figure 2).

HE Staining for Brain Tissues in the Three Groups of Rats

According to the HE staining results, in comparison with those in the M group, the pyknotic nuclei and neuron staining of brain tissue cel-

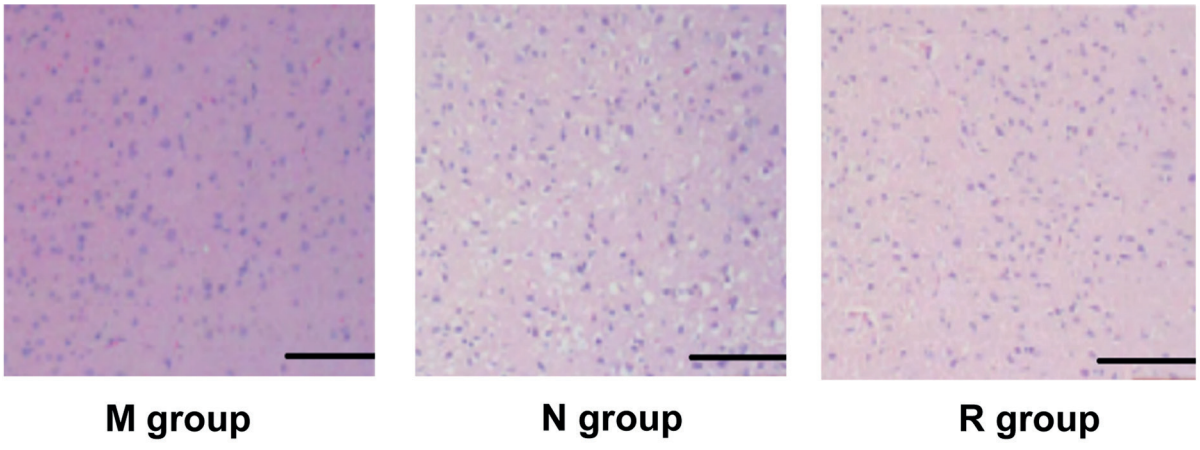

Figure 3. HE staining for brain tissues in M group, N group and R group (×200).

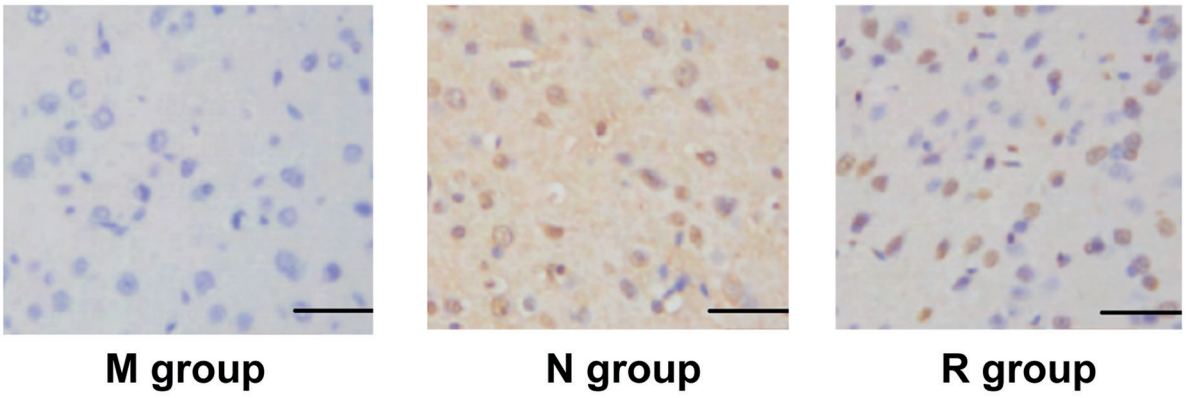

Figure 4. Comparison of TUNEL assay results of brain cell apoptosis in rats (×200).

ls were increased remarkably, and the neuronal cell injury was aggravated in the N group, while markedly reduced pyknotic nuclei and neuron staining ($p<0.05$), as well as alleviated neuronal cell injury ($p<0.05$), were detected in the R group (Figure 3).

Comparison of TUNEL Assay Results of Brain Cell Apoptosis in Rats

It was manifested in the TUNEL assay results that the N group had large quantities of yellowish-brown apoptotic cells compared with the M group, and the R group had remarkably fewer yellowish-brown apoptotic cells in the brain tissues than the N group (Figure 4). In comparison with that in the M group, the cell apoptosis was aggravated notably in the N group and relieved markedly in the R group, displaying statistically significant differences between groups ($p<0.05$; Figure 5).

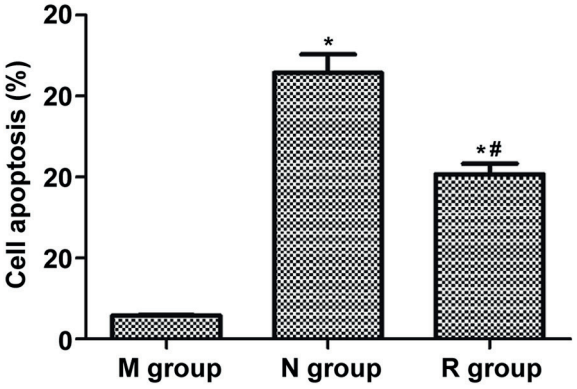

Figure 5. Comparison of brain cell apoptosis in rats. Note: *$p<0.05$ *vs.* M group, #$p<0.05$ *vs.* N group.

Effects of LncRNA SNHG1 on p-PI3K and p-Akt Protein Levels in Rat Brain Tissues Detected Via Western Blotting

The levels of p-PI3K and p-Akt proteins in the rat brain tissues were detected via Western blotting. It was shown that the levels of p-PI3K and p-Akt proteins in the brain tissues in the N group were prominently lower than those in the M group ($p<0.05$) and R group, and the differences between groups were statistically significant ($p<0.05$; Figure 6 and Figure 7).

Discussion

As the most common kind of sudden brain disease, cerebral infarction, also known as ischemic stroke, refers to the ischemic necrosis of brain cells caused by the inadequate blood supply to the brain tissues, which is a result of blood circulation disorder induced by embolic embolism in the cerebral vascular lesions or blood[10,11]. The clinical symptoms of the disease are mainly manifested as speech disorder and paralysis or

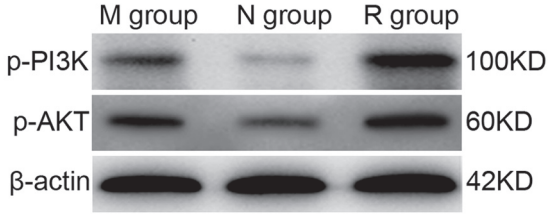

Figure 6. Expression of p-Akt protein in the brain tissues in M group, N group and R group.

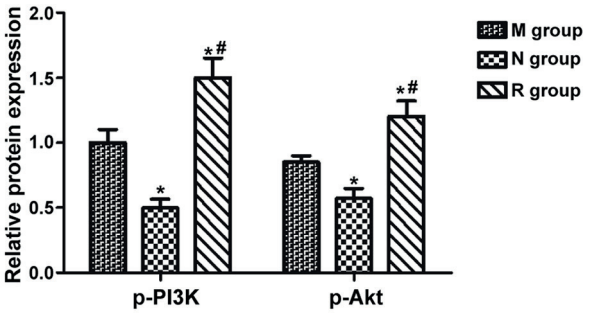

Figure 7. Differences in p-PI3K and p-Akt proteins in the brain tissues in M group, N group and R group. Note: *p<0.05 *vs.* M group, #p<0.05 *vs.* N group.

paraesthesia of the unilateral limb that occur suddenly in a resting state or sleep[12]. Currently, theories related to the pathogenesis of cerebral infarction include calcium overload in brain tissue cells, overexpression of inflammatory factors, the disequilibrium of energy metabolism *in vivo*, acidosis, brain tissue apoptosis and free radical injury *in vivo*. Corbyn et al[13] have shown that long-term cerebral ischemia will result in neuronal damage and apoptosis. Neurological deficit or cerebral infarction is the manifestation of brain tissue damage in rats with cerebral infarction[14]. In this research, it was discovered that the degree of neurological deficit after regaining consciousness was none, severe and mild in the M group, N group and R group, respectively. The rats in the M group could move normally, those in the N group had apparent limb movement disorder, and those in the R group had mild limb movement disorder. The neurological deficit in the N group was more significant than that in the M group (p<0.05), while it was alleviated markedly in the R group compared with that in the N group, with statistically significant differences between groups (p<0.05). In comparison with that in the M group, the cell apoptosis was aggravated notably in the N group and relieved markedly in the R group, and there were statistically significant differences between groups (p<0.05). The TTC staining results manifested that white infarction foci existed in the brain tissues in both N group and R group. The cerebral infarct volume was 33.67% in the N group and 20.15% in the R group, indicating mild cerebral infarction. Compared with that in the R group, the cerebral infarct volume was increased more markedly in the N group (p<0.05), displaying a statistically significant difference (p<0.05). Jacquin et al[16] verified that the changes in biological signals during development can regulate the level of lncRNA, and the dysregulation of lncRNA may affect the epigenetic information and be conducive to cell growth, resulting in uncontrollable tumor growth. It is also proven that lncRNA is capable of prominently ameliorating the cerebral infarction[15]. The intervention with lncRNA SNHG1 can remarkably ameliorate the neurological deficit and reduce the area of cerebral infarction in the rats. According to Martini et al[17], the leukocyte infiltration in the brain tissues after cerebral infarction can activate the pro-inflammatory and anti-inflammatory cytokines, further triggering inflammatory responses in the brain tissues. The pro-inflammatory cytokines can promote neural injury, while the anti-inflammatory cytokines (IL-10, TGF-β1, IL-3, etc.) can reduce such an injury. The HE staining results revealed that, in comparison with those in the M group, the pyknotic nuclei and neuron staining of brain tissue cells were increased significantly, and the neuronal cell injury was aggravated in the N group, while the R group had markedly decreased pyknotic nuclei and neuron staining (p<0.05) and alleviated neuronal cell injury compared with the N group (p<0.05). It was shown in Western blotting results that the levels of p-PI3K and p-Akt proteins in the brain tissues in the N group were prominently lower than those in the M group (p<0.05) and R group (p<0.05). Studies have demonstrated that apoptosis is an active "suicidal" process of cells. After cerebral infarction, the external and internal environments of the brain tissues are altered to some extent, and the apoptosis-related genes are activated by various factors, thus accelerating the cell apoptosis[18,19]. Zhang et al[20] have discovered the long non-coding RNA SNHG1 attenuates cell apoptosis by regulating miR-195 and BCL2-like protein 2 in human Cardiomyocytes. Koh and Lo [21] found that the levels of p-PI3K and p-Akt proteins in the brain tissues of rats with cerebral infarction are lower than those in the control group, and cerebral ischemia may lead to abnormality of the PI3K/Akt signaling pathway, which is a possible mechanism of exacerbated cerebral infarction. Therefore, the treatment of cerebral infarction can be realized through the lncRNA SNHG1-activated PI3K/Akt signaling pathway. The PI3K/Akt signaling pathway can be activated rapidly by injecting p-PI3K and p-Akt proteins, of which the PI3K is activated via autophosphorylation after binding to the corresponding sites[22]. Therefore, in this

paper, the levels of p-PI3K and p-Akt proteins in the brain tissues in the R group were higher than those in the N group, so it can be conjugated that the activated PI3K/Akt signaling pathway plays a vital role in cerebral infarction.

Conclusions

We detected that LncRNA SNHG1 can significantly improve the neurological function, reduce the infarction area and apoptosis rate and effectively accelerate the neural regeneration in rats with cerebral infarction, whose roles are closely correlated with the PI3K/Akt signaling pathway.

Funding Acknowledgments

This study was supported by the Hainan International Cooperation Project [KJHZ2013-08], Hainan Provincial Natural Science Foundation [817376] and Hainan Provincial Social Development Program [SF201303].

Conflict of interest

The authors declare no conflicts of interest.

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