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The bone marrow mononuclear cells reduce the oxidative stress of cerebral infarction through PI3K/AKT/NRF2 signaling pathway

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Abstract. – OBJECTIVE: To evaluate the mechanism of bone marrow mononuclear cells (BMMNCs) in reducing the oxidative stress after cerebral infarction through PI3K/AKT/NRF2 signaling pathway.

MATERIALS AND METHODS: 96 healthy SD rats, which were 6-8-week old, weighting about 250-280 g, were selected for the study. The middle cerebral artery occlusion model (MCAO) was established in SD rats using the suture method. The rats were randomly divided into sham operation group, model group, BMMNCs group and PI3K inhibitor group. 24 rats in each group were selected. 200 μl phosphate-buffered saline (PBS) solution was injected into the caudal vein of the rats in the model group, 200 μl PBS solution containing 5×10^6 BMMNCs that obtained by gradient centrifugation was injected into the rats in the BMMNCs group, meanwhile, in the PI3K inhibitor group, LY294002 (10 mmol/L/kg) was injected into the lateral ventricle of the brain. After the 3d, 7d and 14d, the modified neurological severity scores (mNSS) were used to evaluate the neurological function. The volume of cerebral infarction was assessed by TTC staining, the VEGF, BDNF, TNF-α, IL-1β, malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels were detected by ELISA.

RESULTS: The mNSS and the volume of cerebral infarction of the model group were significantly higher than those of the sham operation group ($p<0.05$), while the mNSS and the volume of cerebral infarction of the BMMNCs group were lower than those of the model group, higher than those of the sham operation group ($p<0.05$). The VEGF, BDNF, TNF-α, IL-1β, MDA, SOD and GSH-Px levels of the model group were significantly higher than those of the sham operation group ($p<0.05$).

CONCLUSIONS: BMMNCs can reduce the oxidative stress, apoptosis, and inflammatory reaction through PI3K/AKT/NRF2 signaling pathway, thus promoting the secretion of nerve and vascular cytokines, improving the neurological function and reducing the infarct scope.

Key Words

Bone marrow mononuclear cells (BMMNCs), PI3K/AKT/NRF2 signaling pathway, Cerebral infarction, Oxidative stress.

Introduction

The acute cerebral infarction which is a cerebrovascular disease that causes serious disability and increases in the mortality rate. It has multiple complications which further involves the nerve injury in the early and late stages. These injuries are such as apoptosis, oxidative stress, inflammatory response, calcium overload, neurotoxicity, autophagy, energy metabolism disorder and so forth, thereby affecting the nerve rehabilitation process[1,2]. Stem cell is a kind of cells with the potential of multi-directional differentiation and self-renewal, which in vitro can be differentiated into cellular components with neural function and also can express in multiple neurotrophic factors[3]. The animal model also confirms that bone marrow mononuclear cells (BMMNCs) can be homing to sites of cerebral infarction, so as to promote the nerve remodeling[4]. Due to the various advantages such as convenient material, simple manufacture, no immunogenicity, safety and economy, bone marrow stem cells are widely used in research and clinical practice. Nowadays, these cells mainly used in the treatment of serious diseases such as diabetic foot, myocardial ischemia, renal injury, cerebral infarction and so forth[5,6]. The research has proved that PI3K/AKT/NRF2 signaling pathway is involved in cell proliferation, differentiation, apoptosis, inflammation and other reactions[7], which further play an important role in the occurrence and development of cerebral infarction. This study aims to analyze

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the mechanism of bone marrow mononuclear cells (BMMNCs) in reducing oxidative stress after cerebral infarction through PI3K/AKT/NRF2 signaling pathway, thereby providing reference basis for stem cell targeted therapy.

Materials and Methods

Animal source: A total of 96 healthy Sprague-Dawley (SD) rats, which were 6-8 weeks old, weighting 250-280 g, were selected for the study group. No limit for the male or female has been taken. The rats were purchased from Shanghai Silaike Experimental Animal Co., Ltd. with the certificate number was 2015000405526, and the license number was SC-SCXK (Shanghai, China) 2015-0004. The experiment was performed after the normal feeding and adapting to the environment for one week. The middle cerebral artery occlusion model (MCAO) was established in SD rats using the suture-occluded method, and the thread was purchased from Beijing Shadong Biology Co., Ltd (Beijing, China)

Research method: The rats were randomly divided into sham operation group, model group, BMMNCs group and PI3K inhibitor group. An equal number of rats, i.e. 24 rats, were taken in each and every group. The sham operation group was considered to be same as the model group operation, except that insertion of nylon thread was not considered in the sham operation group. After the 24h of successful modeling, 200 µl phosphate-buffered saline (PBS) solution was injected into the caudal vein of the rats in the model group, 200 µl PBS solution containing 5×10^6 BMMNCs that obtained by gradient centrifugation was injected into the rats in the BMMNCs group, meanwhile, in the PI3K inhibitor group, LY294002 (10 mmol/L/kg) was injected into the lateral ventricle of the brain.

Extraction of BMMNCs: The rats have received the continuous intraperitoneal injection with 50 mg/kg 5-bromodeoxyuridine (BrdU, Sigma-Aldrich Co, St. Louis, MO, USA). After 14 days, intraperitoneal anesthesia with 4% chloral hydrate (300 mg/kg) and soaking in 75% alcohol for 10 min were carried out. The femur and tibia bones were then separated out. For rinsing purposes, the sterile D-Hank's solution was used further. The cavum medullare was repeatedly washed with the heparin saline. After that, the single cell suspension was prepared and added into the centrifugal tube with the equal volume of lymphocyte separation solution (Jiangsu Biyuntian Technology Co., Ltd. Jiangsu, China). The gradient centrifugation (1500 g, 30 min) was adopted. After the middle buffy coat had been extracted, L-DMEM was added, according to the volume of the ratio 1:2. Subsequently, the centrifugation (1500 g, 20 min) was performed. After then, the cell precipitation was taken and washed by PBS solution. Finally, continued centrifugation with the force of 1500 g for 5min was accomplished.

Injecting LY294002 into the lateral ventricle: Under the local anesthesia, LY294002 (10 mmol/L/kg) was injected into the local left lateral ventricle by the guidance of brain stereotactic instrument. The coordinates were -0.8 mm of bregma, 1.5 mm of the lateral of the center line and below 3.5 mm of the dura.

Observation index: After the follow-up period of 3 d, 7 d and 14 d, the modified neurological severity scores (mNSS) was used to evaluate the neurological function. The assessment includes a total of four aspects such as movement, feeling, reflection and the balance beam. The higher the scores, the weaker the function would be. The volume of cerebral infarction was assessed by triphenyl tetrazolium chloride (TTC) staining. The vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), TNF-α, IL-1β, malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels were detected by ELISA method. PI3K, AKT, NRF2, bax, bcl-2 and caspase-3 levels were detected by RT-PCR and Western blot analysis. The survival, migration, distribution and differentiation of BMMNCs in the brain were detected by the immunofluorescence technique.

TTC staining method: After the dissection off the head of rats, the brain tissues were cut into 3 mm thick coronal slices from anterior to posterior into a total of five groups. The slices were placed into 2% TTC solution (Beijing Zhongshan Jinqiao Biology Co., Ltd. Beijing, China), for staining at 37 c for 30 min away from the source of light. The normal tissues were stained in red color, and infarct tissues were stained in white color. After fixing with 4% paraformaldehyde, the photography was taken and observed. The images were collected and analyzed by Image J1.46 software. The volume of cerebral infarction (%) = ((cerebral infarction volume – (right hemisphere volume – left hemisphere volume)) / left hemisphere volume ×100%.

ELISA method: After the tissues had been homogenated, the centrifugation with the force of 3000 g was performed for 30 min, and then the supernatant was taken out. The reagents were purchased from Invitrogen (Carlsbad, CA, USA). The microplate reader was purchased from Bio-Rad Co (Hercules, CA, USA), which was strictly operated in accordance with the instructions.

RT-PCR method: The total RNA in cells were extracted by the conventional trizol reagent. The concentration and purity were determined by UV spectrophotometer. Reverse transcription kit was adopted to synthesize the cDNA. According to the GeneBank sequence, the primer sequences were synthesized by Shanghai Shenggong Co., Ltd., which were shown as follows:
PI3K: (F)5'-CAAAGCCGAGAACCTATTGC-GAG -3', (R)5'-GTTTGACTTCGCCATCTAC-CAC-3', 323bp; AKT: (F)5'-GTGGAGGAC-CAGATGATGC-3', (R)5'-TGCCCCTGCTAT-GTGTAAG-3', 453bp; NRF2: (F)5'-TCAG-CGACGGAAAGAGTA-3', (R)5'-TGG-GCAACCTGGGAGTAG-3', 201bp; bax: (F)5'-GGTTTCATCCAGGATCGAGCAGG-3', (R)5'-ACAAAGATGGTCACGGTCTGCC-3', 445bp; bcl-2: (F)5'-ACTACTTCTCCCGC-CGCTAC-3', (R)5'-GAAATCAAACAGAGG-CCGCATG-3', 332bp; caspase-3: (F)5'-TAC-CAGTGGAGGCCGACTTC-3', (R)5'-GCA-CAAAGCGACTGGATGAAC-3', 103bp; GAP-DH(F): 5'-CGCGAGAAGATGACCCAGAT-3', R:5'-GCACTGTGTTGGCGTACAGG-3', 225 bp.

The reaction system was cDNA 2 μl + each of upstream and downstream primers 3 μl + Taq polymerase 0.5 μl + dNTPs 1 μl + MgCl2 3 μl + 10×Buffer 5 μl + ddH2O2 2.5 μl. The reaction conditions were 95 c for 5 min, 95 c for 30 s, 58 c for 30 s and 72 c for 60 s, a total of 30 circulations, and 72 c for 10 min was the end. PCR products were identified by 2% agarose gel electrophoresis. The ultraviolet spectral imaging was analyzed by the gel imaging analysis system. The digital photography was used for gray value analysis. The results were expressed by the $2^{-\Delta\Delta Ct}$ method.

Western blot method: After the tissues had been homogenated, RIPA lysis buffer was added and the total protein of the cells was extracted. The Coomassie brilliant blue method was further used for rough quantifying. The amount of protein in each sample was performed by the dose standardization detection using β-actin antibody before detecting the protein. 30 μg of the total protein was taken and separated by 8% SDS-PAGE. The separation zone was transferred to the Polyvinylidene difluoride (PVDF) membrane. Then, rabbit anti-mouse PI3K, AKT, NRF2, bax, bcl-2 and caspase-3 monoclonal antibody (1:2000, R&D Co, Minneapolis, MN, USA) were added, which was placed static overnight. Then goat anti-rabbit polyclonal antibody secondary antibody (1:500, Santa Cruz Biotechnology, Santa Cruz, CA, US) was further added and incubated at room temperature for 4h. Subsequently, PBS was used for washing, and ECL was adopted for the color development. The results were scanned and preserved further. The Lab works 4.5 gel imaging software (Invitrogen, Carlsbad, CA, USA) was used for the semi-quantitative analysis, which was expressed by integrate optical density (IOD).

Immunofluorescence staining: 4% chloral hydrate was used for local anesthesia and, then, the rapid cardiac perfusion fixation was performed. After cutting off the head of rats, the brain tissues were placed in 4% paraformaldehyde at 4c overnight. Then it was transferred into the 30% sucrose solution until the brain tissues sank to the bottom of the solution. The continuous coronal section was performed using frozen section method and with the thickness about 10 μm. Then the desmolysis, neutralization, rinsing and sealing by goat serum for 1 h was performed. Then the incubation of primary antibody rabbit anti-mouse BrdU, GFAP, Iba1 monoclonal antibody (1:1000, Applied Biosystems Co, City Foster, CA, USA) was performed at 4°C overnight successively. The sample was washed with PBS. Then secondary antibody of goat anti-rabbit monoclonal antibody (1:200, Santa Cruz Company, CA, USA) was incubated for 6h away from the source of light. Then the photography under the fluorescence microscope (Olympus, Tokyo, Japan) was carried out for analysis. The percentage of positive cells was calculated by the semi-quantitative method. Therein, GFAP + cells were stained red, BrdU + cells were stained green, Iba1 + cells were stained red, GFAP + BrdU + cells were stained yellow, and Iba1+ BrdU + cells were stained yellow.

Statistical Analysis

SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The measurement data were expressed as mean±standard deviation. The single factor ANOVA analysis was used for the comparison among the groups. LSD t-test was used for the pairwise comparison. The variance analysis of repeated measurement data was used for the comparison in each group. $p<0.05$ suggested that the difference had statistical significance.

Table I. Comparison of mNSS score between the groups.

Groups	3d	7d	14d
Sham operation group	4.5±1.2	4.2±1.1	4.3±1.2
Model group	17.2±1.9	16.6±2.2	16.2±2.3
BMMNCs group	13.5±2.6	10.8±2.8	8.6±2.4
PI3K inhibitor group	16.9±2.3	16.5±2.1	16.2±2.2

Table II. Comparison of the volume of cerebral infarction between the groups.

Groups	3d	7d	14d
Sham operation group	3.3±1.1	3.5±1.2	3.4±1.2
Model group	25.5±5.6	32.8±8.9	38.5±12.8
BMMNCs group	21.4±5.3	17.6±4.5	13.5±4.2
PI3K inhibitor group	22.2±5.5	28.9±6.7	35.6±9.5

Results

Comparison of mNSS: The mNSS of the model group was statistically significant higher values than that of the sham operation group with the significant p-value of <0.05. The mNSS of the BMMNCs group was lower than that of the model group and higher than that of the sham operation group, and it was gradually decreased with the treatment time extension with significant p-value <0.05. There was no difference in comparison between the PI3K inhibitor group and the model group with a non-significant p-value of more than 0.05 (Table I).

Comparison of the volume of cerebral infarction: The volume of cerebral infarction of the model group was significantly higher than that of the sham operation group, showing an increase with the time extension (p<0.05). The volume of cerebral infarction of the BMMNCs group was lower than that of the model group and higher than that of the sham operation group, and it was gradually decreased with the treatment time extension with significant p-value <0.05. There was no difference in comparison between the PI3K inhibitor group and the model group with a non-significant p-value of more than 0.05 (Table II)

Comparison of the results of ELISA method: The index level of the model group was significantly higher than that of the sham operation group (p<0.05). The VEGF and BDNF levels of the BMMNCs group were higher than those of the model group, while other indexes were lower than those of the model group (p<0.05). There was no difference in comparison between the PI3K inhibitor group and the model group with a non-significant p-value of more than 0.05 (Table III).

Comparison of the results of the RT-PCR method: The index level of the model group was significantly higher than that of the sham operation group (p<0.05). The PI3K, AKT, NRF2 and bcl-2 mRNA levels of the BMMNCs group were higher than those of the model group, while bax

Table III. Comparison of the results of ELISA method (μmol/L).

Groups		Sham operation group	Model group	BMMNCs group	PI3K inhibitor group
VEGF	3d	12.3±4.2	26.9±12.2	55.8±16.9	31.2±13.2
	7d	13.5±4.3	27.2±13.2	75.6±22.2	34.2±11.5
	14d	13.3±4.5	28.8±14.4	92.3±34.5	35.5±12.7
BDNF	3d	7.5±2.6	25.6±12.6	48.5±16.5	33.6±12.6
	7d	7.3±2.5	24.9±15.2	66.3±24.4	35.2±11.9
	14d	7.4±2.3	26.8±13.4	85.7±32.6	34.9±12.5
TNF-α	3d	12.6±5.5	34.5±12.5	21.2±6.6	32.2±13.2
	7d	13.2±5.4	42.2±20.6	16.9±5.9	40.6±18.7
	14d	11.9±5.2	36.9±16.7	13.5±5.2	36.7±15.6
IL-1β	3d	15.8±6.3	45.6±23.3	26.7±8.7	41.1±23.2
	7d	16.6±6.7	61.5±32.6	20.5±8.2	57.8±24.5
	14d	17.2±6.5	58.7±24.5	18.9±6.7	55.6±25.6
MDA	3d	23.6±10.5	89.6±24.5	56.7±23.2	85.5±25.8
	7d	24.5±9.6	124.5±42.3	52.3±16.8	112.6±32.5
	14d	25.2±8.9	113.7±46.5	45.9±15.7	103.4±42.1
SOD	3d	31.6±11.2	76.9±23.3	62.3±20.5	72.3±23.5
	7d	32.5±12.6	91.5±31.2	54.8±16.4	86.9±22.6
	14d	33.8±13.3	102.3±45.5	50.2±15.9	94.5±34.7
GSH-Px	3d	42.5±15.8	215.4±89.6	125.4±45.5	198.7±45.5
	7d	44.6±19.6	236.6±142.2	113.6±42.3	212.3±86.5
	14d	46.7±18.7	198.7±65.7	98.5±35.6	221.5±92.2

Table IV. Comparison of the results of RT-PCR method.

Groups		Sham operation group	Model group	BMMNCs group	PI3K inhibitor group
PI3K	3d	0.0635±0.0059	0.1325±0.0325	0.3152±0.1324	0.1026±0.0253
	7d	0.0578±0.0067	0.1426±0.0426	0.3065±0.1258	0.1247±0.0163
	14d	0.0659±0.0058	0.1258±0.0515	0.3359±0.1623	0.1132±0.0538
AKT	3d	0.0548±0.0043	0.1635±0.0923	0.2589±0.1124	0.1524±0.0624
	7d	0.0724±0.0062	0.1452±0.0824	0.2764±0.1321	0.1362±0.0732
	14d	0.0665±0.0078	0.1521±0.0527	0.2854±0.1027	0.1458±0.0829
NRF2	3d	0.0534±0.0092	0.1569±0.0626	0.3126±0.1526	0.1326±0.0832
	7d	0.0438±0.0034	0.1457±0.0548	0.3259±0.1424	0.1248±0.0921
	14d	0.0584±0.0062	0.1323±0.0732	0.3348±0.1529	0.1407±0.0767
bax	3d	0.1026±0.0532	0.3569±0.1023	0.1649±0.0832	0.3269±0.1324
	7d	0.0968±0.0072	0.4214±0.1524	0.1524±0.0765	0.3564±0.1125
	14d	0.0768±0.0063	0.4658±0.1326	0.1426±0.0825	0.3958±0.1632
bcl-2	3d	0.1124±0.0659	0.2535±0.1028	0.4213±0.1234	0.2652±0.0827
	7d	0.1063±0.0324	0.2324±0.1123	0.4654±0.1526	0.2528±0.1023
	14d	0.0965±0.0082	0.2158±0.1324	0.4527±0.1324	0.2467±0.0968
caspase-3	3d	0.0765±0.0072	0.5214±0.2136	0.3526±0.1127	0.5023±0.2354
	7d	0.0724±0.0092	0.5656±0.2215	0.3359±0.1025	0.5124±0.2657
	14d	0.0639±0.0053	0.6124±0.2659	0.3247±0.1063	0.5327±0.1985

and caspase-3 mRNA levels were lower with significant p-value <0.05. There was no difference in comparison between the PI3K inhibitor group and the model group with a non-significant p-value of more than 0.05 (Table IV).

Comparison of the results of Western blot method: The index level of the model group was significantly higher than that of the sham operation group (p<0.05). The PI3K, AKT, NRF2 and bcl-2 protein levels of the BMMNCs group were higher than those of the model group, while bax and caspase-3 protein levels were lower with significant p-value <0.05. There was no difference in comparison between the PI3K inhibitor group and the model group with a non-significant p-value of more than 0.05 (Table V).

Comparison of the results of immunofluorescence: The astrocyte (GFAP positive) and microglia (Iba1 positive) in ischemic penumbra of the BMMNCs group were significantly more than those of the model group and the PI3K inhibitor group, and it was increased with the time extension (p<0.05). There was no difference in comparison between the PI3K inhibitor group and the model group with a non-significant p-value of more than 0.05 (Table VI).

Table V. Comparison of the results of Western blot method.

Groups		Sham operation group	Model group	BMMNCs group	PI3K inhibitor group
PI3K	3d	0.07±0.02	0.12±0.03	0.32±0.13	0.10±0.03
	7d	0.06±0.02	0.13±0.03	0.35±0.14	0.11±0.03
	14d	0.08±0.02	0.13±0.03	0.34±0.12	0.12±0.03
AKT	3d	0.05±0.01	0.14±0.03	0.29±0.15	0.12±0.03
	7d	0.04±0.01	0.14±0.03	0.30±0.16	0.11±0.03
	14d	0.06±0.01	0.15±0.04	0.32±0.17	0.13±0.03
NRF2	3d	0.07±0.01	0.15±0.04	0.34±0.15	0.14±0.03
	7d	0.08±0.01	0.13±0.04	0.36±0.14	0.15±0.05
	14d	0.09±0.02	0.12±0.03	0.37±0.19	0.13±0.03
bax	3d	0.05±0.01	0.36±0.12	0.21±0.12	0.33±0.16
	7d	0.06±0.01	0.38±0.13	0.18±0.06	0.34±0.13
	14d	0.04±0.01	0.41±0.22	0.19±0.07	0.37±0.15
bcl-2	3d	0.08±0.01	0.22±0.12	0.38±0.14	0.25±0.11
	7d	0.10±0.03	0.23±0.11	0.39±0.15	0.24±0.08
	14d	0.11±0.03	0.25±0.13	0.37±0.13	0.26±0.12
caspase-3	3d	0.02±0.01	0.48±0.24	0.32±0.12	0.45±0.21
	7d	0.03±0.01	0.51±0.25	0.31±0.15	0.46±0.22
	14d	0.04±0.01	0.53±0.26	0.27±0.09	0.48±0.23

Table VI. Comparison of the results of immunofluorescence (%).

Groups		Sham operation group	Model group	BMMNCs group	PI3K inhibitor group
GFAP positive	3d	5.2±2.3	5.6±2.5	12.2±5.3	5.9±2.4
	7d	5.3±2.4	5.5±2.6	15.4±5.5	6.2±2.6
	14d	4.7±2.2	5.7±2.8	18.2±5.6	6.3±2.5
Iba1 positive	3d	4.6±2.1	5.4±2.4	15.6±5.3	5.8±2.5
	7d	4.8±2.3	5.3±2.5	16.7±5.6	5.6±2.4
	14d	4.7±2.6	5.2±2.3	17.8±5.7	5.7±2.6

Discussion

BMMNCs considered being the mixed cell group which includes mesenchymal stem cells, endothelial progenitor cells, hematopoietic stem cells, etc. The experiments *in vivo* and *in vitro* have confirmed that stem cells can directionally differentiate neural cells and glial cells in reducing the cerebral ischemia reperfusion injury, promote angiogenesis, decrease the permeability of blood-brain barrier, inhibit oxidative stress, inflammatory and apoptosis reaction, stabilize cell membrane, improve the energy metabolism and ion permeability[8,9]. Through this study, it is concluded that BMMNCs may exert the above functions through the PI3K/AKT/NRF2 signaling pathway, thereby improving the neurological function and reducing the infarct scope.

PI3K/AKT/NRF2 is nowadays considered to be the most important anti-oxidative stress pathway[10], while NRF2 is a key factor in cellular defense stress injury. NRF2 can induce II phase enzyme gene expression and produce HO-1 with a protective effect. HO-1 can catalyze heme generating biliverdin that would form to be bilirubin, which is a strong free radical scavengers. MDA can indirectly reflect the content of oxygen free radical. SOD and GSH-Px are the important antioxidant enzymes, which can scavenge free radical and protect the cell membrane. Furthermore, PI3K/AKT signaling transduction pathway has multiple biological functions, such as regulating cell proliferation, migration, survival, differentiation, apoptosis and so forth[11]. BMMNCs in vivo and in vitro can secrete many active cytokines, such as VEGF, BDNF, stromal cell derived factor-1 (SDF-1), etc. Secreting cytokines further maintain the characteristic of stem cells, promote the nerve regeneration, and provide an indispensable microenvironment which further improves the stem cell function such as self-renewal, activation, proliferation and differentiation. Kinnaird et al[12] proposed that the action of transplanted cells is the key to exert the therapeutic effect. Paracrine is the most important factor that can promote the angiogenesis. The receptors of VEGF are widely distributed in endothelial cells, neural stem cells, neuron, glial cells and so forth, which can promote tissue regeneration after brain injury. BDNF can prevent neuronal apoptosis through the tyrosine receptor signal transduction pathway. The inflammatory reaction is accompanied by the whole process of cerebral infarction. So, we can consider that the inflammatory reaction is activated by cerebral infarction, while the process of cerebral infarction is promoted by an inflammatory reaction. BMMNCs can effectively regulate the inflammatory reactions, such as TNF-α and IL-1β expressions, thus improving the degree of brain injury[13]. Bax/bcl-2 and caspase-3 are the key molecules in regulating cell apoptosis in vivo[14]. The ratio of pro-apoptotic molecule Bax and anti-apoptotic molecule bcl-2 determines the direction of apoptosis. Caspase-3 is the terminal molecule of many apoptotic pathways. BMMNCs, on the one hand, can inhibit the cell apoptosis and on the other hand, it also re-differentiated into mature nerve cells, thus promoting the recovery of neural function[15-17].

GFAP is a cytoskeletal protein of astrocytes, while the amount of astrocytes is multiple, which can support and contact with neurons, regulate and control the microenvironment of neurons and so forth[18]. Microglia is the main immune cells in the brain, which can play an important role in promoting the cell growth, repairing and removal after the activation[19]. To precisely regulate the stem cell homing, the differentiation and the maturation ability are significant factors in determining the therapeutic value. This study has indicated the functional characteristics of PI3K/AKT/NRF2 signaling pathway in acute cerebral infarction that intervened by BMMNCs, which provides an important reference basis for the further study.

Conclusions

BMMNCs can reduce the oxidative stress, apoptosis and inflammatory reaction through PI3K/AKT/NRF2 signaling pathway, thus promoting the secretion of nerve and vascular cytokines, improving the neurological function and reducing the infarct scope.

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Conflict of Interest
The Authors declare that they have no conflict of interests.

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