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Relationship between the MAPK/ERK pathway and neurocyte apoptosis after cerebral infarction in rats

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Abstract. – OBJECTIVE: The aim of this study was to explore the relationship between the mitogen-activated protein kinase (MAPK)/extracellular regulated protein kinase (ERK) pathway and neurocyte apoptosis after cerebral infarction in rats.

MATERIALS AND METHODS: Neural stem cells were isolated from rats by establishing the cerebral infarction model and sham model. Isolated cells were cultured in complete culture medium in vitro. Real-time quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) was used to detect the messenger ribonucleic acid (mRNA) expression of ERK1 and ERK2 in the MARK pathway. Western blotting was applied to examine the activation of the MAPK/ERK pathway and neuron-specific markers. The expression of neuron-specific enolase (NSE) was detected via immunofluorescence. Cell activity and apoptosis were measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and flow cytometry, respectively.

RESULTS: The mRNA expressions of ERK1 and ERK2 in neural stem cells increased in a time-dependent manner after cerebral infarction in rats. The expressions of ERK1, ERK2, cyclin D1, Nestin, NSE and glial fibrillary acidic-protein (GFAP) in neural stem cells were significantly decreased after being treated with SCH772984. Cell activity, proliferation and differentiation were markedly inhibited. However, cleaved-caspase 3 protein and apoptosis rate were remarkably increased.

CONCLUSIONS: The MAPK/ERK pathway seriously affects neurocyte apoptosis after cerebral infarction in rats. When the MAPK/ERK pathway is inhibited, neurocyte apoptosis is remarkably increased after cerebral infarction in rats.

Key Words:
MAPK/ERK pathway, Cerebral infarction, Neurocyte, Apoptosis

Introduction

Cerebral infarction is an important cerebrovascular disease, which is the second leading cause of death after ischemic heart disease[1]. Due to high morbidity and mortality rates, it seriously threatens human health[2]. When blood supply to the brain is blocked, ischemic cerebral infarction occurs[3]. Cerebral infarction caused by cerebral ischemia can easily lead to irreversible neuronal damage[4]. Its main mechanism of action involves excitatory toxic and side effects, depolarization in acute infarction, as well as delayed inflammation and apoptosis[5]. Most cerebral infarction is ischemic, and is mainly correlated with related influencing factors, including gender, age, hypertension, hyperlipidemia and diabetes history. It may lead to vascular embolism or thrombosis of the cerebral aorta, thus resulting in subsequent tissue death and reduced blood flow in the affected area[6,7]. At present, the transduction mechanism of the signal pathway in cerebral infarction remains unclear.

Mitogen-activated protein kinases (MAPKs) play a crucial role in signal transduction. It is reported that the MAPK/extracellular regulated protein kinase (ERK) pathway is affected by different stressors interacting between cells and receptor ligands[8]. Studies have shown that the MAPK/ERK signal transduction affects the destruction of the blood-brain barrier and enhances the activation of inflammatory factors in neurons after ischemic stroke[9,10]. Meanwhile, it also exerts certain effects on the expression of cerebrovascular receptors in human arteries[11]. In addition, it plays an important role in regulating the proliferation and differentiation of neurons. Zhen et al[12] have demonstrated that the MAPK/ERK signal transduction is crucial to the regulation

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of inflammation and apoptosis during cerebral infarction. Multiple studies have explored the role of the MAPK/ERK pathway in cerebrovascular diseases or cerebral infarction. However, the relationship between the MAPK/ERK signaling pathway and neurocyte apoptosis after cerebral infarction in rats has not been elucidated.

Therefore, the aim of this work was to explore the role of the MAPK/ERK pathway in neurocyte apoptosis in rats with cerebral infarction *via* targeting the pathway.

Materials and Methods

Materials

Main Reagents and Instruments

Chloral hydrate was purchased from Qingdao Yulong Algae Co., Ltd. (Qingdao, China); ND-2000 nucleic acid-protein quantitative analyzer from NanoDrop (Madison, WI, USA); SuperScript IV reverse transcription (RT) kit from Thermo Fisher Scientific (Waltham, MA, USA); Real Time fluorescence-quantitative Polymerase Chain Reaction (qRT-PCR) instrument from Xi'an Tianlong Science and Technology Co., Ltd. (Xi'an, China); SCH772984 from MEC, radioimmunoprecipitation assay (RIPA) lysis buffer from MultiSciences (Hangzhou, China); ERK1 and ERK2 antibodies from Cell Signaling Technology (CST; Danvers, MA, USA); Cyclin D1 antibody from Shanghai Anyan Trade Co., Ltd. (Shanghai, China); cleaved-caspase 3 primary antibody from CST (Danvers, MA, USA); Nestin, neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP) and β-actin antibodies from Thermo Fisher Scientific (Waltham, MA, USA); and enhanced chemiluminescence (ECL) apparatus from Shanghai Tellgen Life Science and Technology Co., Ltd. (Shanghai, China).

Methods

Establishment of the Cerebral Infarction Rat Model

A rat model of cerebral infarction was established by sewing. Intraperitoneal injection of 2% pentobarbital sodium was used for intraoperative anesthesia. Then 2% isoflurane was inhaled through the anesthesia machine to maintain anesthesia. At the middle cervical incision, the left common carotid artery and the external carotid artery were separated and ligated. Meanwhile, an incision was made at the bifurcation of the left common carotid artery. Then, a single strand of nylon thread was inserted into the internal carotid artery about 18 mm deep along the incision. Subsequently, the internal carotid artery was ligated and sutured with nylon thread. After blocking for 2 h, the thread was pulled out and reperfusion was conducted. After anesthesia, the rats were put back into cages and fed freely. This study was approved by the Animal Ethics Committee of the Chongqing Medical University Animal Center.

Real Time quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total ribonucleic acid (RNA) was extracted from cells according to the instructions of TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The absorbance values at 260 nm and 280 nm were measured using an ND-2000 nucleic acid protein quantitative analyzer, respectively. The concentration of extracted total RNA was determined. RT was carried out according to the instructions of SuperScript IV RT kit. Finally, RT products were subjected to qRT-PCR detection. Primer sequences used in this study were as follows: ERK1, F: 5'- -GGCTCTATGGATTACCCAATC-3', R: 5'-CCAGTGTTCGTTCCTCGGA-3'; ERK2, F: 5'-G CAGGACCTTTGAAGATTTTGTGAG-3', R: 5'-GACTTTATTCTGCTGGGTGAACTCTCCG-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Neurocyte Culture

MAPK/ERK inhibitor treatment: SCH772984 was dissolved in a certain amount of dimethyl sulfoxide (DMSO), and the concentration was adjusted to 0.5 μM. Then, cell concentration was adjusted to 1×10^6/mL, and the cells were paved on plates. After incubation with SCH772984 for 1 h, the cells were washed and cultured with complete medium for subsequent experiments.

Western Blotting

Cells were first lysed with RIPA, followed by centrifugation to obtain supernatant for Western blotting. After membrane transfer, the membrane was blocked with 0.5% bovine serum albumin-phosphate-buffered saline (BSA-PBS) for 1 h. Then the membrane was incubated with primary antibodies of ERK1, ERK2, cyclin D1, cleaved-caspase 3, Nestin, NSE, GFAP, and β-ac-

tin at 4°C overnight. After membrane washing, the horseradish peroxidase-immunoglobulin G (HRP-IgG) secondary antibody was added for reaction at room temperature for 1 h. ECL was used for color development, and the photos were taken. Finally, E-Gel Imagergel software was used to analyze the absorbance of color strips.

Cell Activity

3-(4,5)-dimethylthiahiazo (-z-yl)-3,5-diphenytetrazoliumromide (MTT) assay was adopted to determine cell proliferation activity. Exponentially growing cells were first seeded into 24-well plates at an initial density of 1×10^5/well. Then, the cells were treated with the corresponding methods in the MAPK/ERK inhibitor group and the control group, followed by culture for a specified time. After that, MTT (0.2 mg/mL) was added in each well for 2 h of incubation. The medium was replaced with acidified isopropanol (isopropanol solution of 0.04N HCl), and the plate was incubated at room temperature for 1 h. The colorimetric absorbance of samples was determined *via* the Spectramax M5 enzyme reader.

Cell Apoptosis

Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) method was used to detect cell apoptosis. Cultured cells treated with SCH772984 were washed with phosphate-buffered saline (PBS), centrifuged and re-suspended in 200 μL binding buffer. Then, Annexin V-FITC/PI was added and mixed for 15 min, followed by the addition of 300 μL binding buffer. Finally, cell apoptosis was detected *via* flow cytometry.

Indirect Immunofluorescence Detection

Rat neural stem cells were inoculated onto the coverslip, washed with PBS and fixed with 4% paraformaldehyde for 30 min. Then, the rabbit anti-mouse NSE antibody was added for incubation with the coverslip at 4°C overnight. After PBS washing, the FITC fluorescent antibody was added for incubation at room temperature for 1 h. After washing with PBS, the coverslip was observed and photographed under a fluorescent microscope.

Statistical Analysis

GraphPad Prism 5.0 (La Jolla, CA, USA) was used for statistical analysis. The experimental data were represented by mean ± standard error of the mean. $p<0.05$ represented a significant difference, and $p<0.01$ represented an extremely significant difference.

Results

Expression of ERK in Neural Stem Cells of Rats With Cerebral Infarction

First, qRT-PCR was carried out to detect the sequential expression of the MAPK/ERK pathway in neural stem cells of rats with cerebral infarction. The mRNA expressions of ERK1 and ERK2 in neural stem cells of rats with cerebral infarction at different periods (0 d, 1 d, 3 d, 5 d, 7 d, and 9 d) were detected, respectively. The results revealed that the mRNA expressions of ERK1 and ERK2 increased significantly with the prolongation of culture time (Figure 1A, 1B). Preliminary results demonstrated that the MAPK/

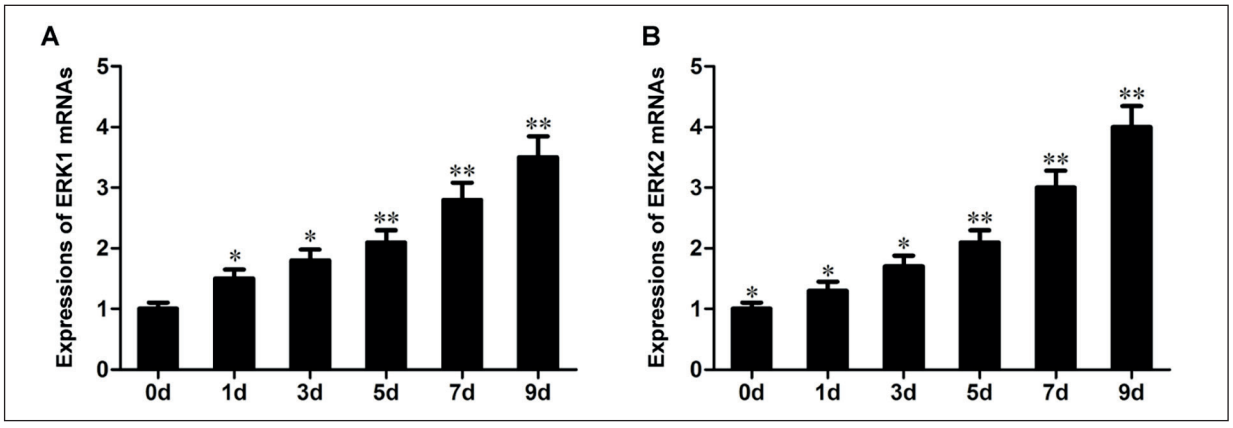

Figure 1. Expressions of ERK1 and ERK2 in MAPK/ERK pathway in neural stem cells at different time periods. ***A,*** Expressions of ERK1 mRNA at different periods detected *via* qRT-PCR. ***B,*** Expressions of ERK2 mRNA at different periods detected *via* qRT-PCR. *$p<0.05$.

ERK pathway might be related to the growth of neural stem cells in rats with cerebral infarction.

Influences of MAPK/ERK Pathway Inhibitor on Proliferation and Apoptosis of Neural Stem Cells, as well as the Expression of Neural Marker Proteins

To explore the influences of the MAPK/ERK pathway in rats with cerebral infarction, the effect of SCH772984 was detected. SCH772984 is a MAPK/ERK pathway inhibitor. Subsequently, we investigated the effect of SCH772984 on the proliferation and apoptosis of neural stem cells as well as the expression of neural marker proteins (including ERK1, ERK2, Cyclin D1, cleaved-caspase 3, Nestin, NSE, and GFAP). The results manifested that the expressions of ERK1, ERK2, cyclin D1, cleaved-caspase 3, Nestin, NSE, and GFAP in the SCH772984 group were significantly reduced compared with those of the blank and control groups. Specifically, ERK1, ERK2, cyclin D1, Nestin, NSE, and GFAP decreased to about 1/4, 1/3, 1/2, 1/2, 1/3, and 1/3 of that in the control group, respectively. However, the expression of cleaved-caspase 3 remarkably increased to about 3 times of the control group ($p<0.01$) (Figure 2A-2G).

Influence of MAPK/ERK Pathway Inhibitor SCH772984 on the Activity of Neural Stem Cells in Rats

Additionally, the influence of the MAPK/ERK pathway inhibitor SCH772984 on the activity of neural stem cells in rats was further investigated in this study. Neural stem cells were first treated with the MAPK/ERK pathway inhibitor SCH772984. Subsequently, the proliferation activity of neural stem cells in rats with cerebral infarction was evaluated by MTT assay. As shown in Figure 3, the proliferation activity of rat neural stem cells increased with the prolongation of culture time. However, the SCH772984 group exhibited significantly lower cell activity than the blank group. Meanwhile, the SCH772984 group exhibited significant differences when compared with the blank group and the control group at 24 h ($p<0.05$) (Figure 3).

Influence of MAPK/ERK Pathway Inhibitor SCH772984 on the Apoptosis of Neural Stem Cells

The influence of the MAPK/ERK pathway on the apoptosis of rat neural stem cells was further analyzed. Rat neural stem cells were treated with SCH72984. Apoptosis was detected by flow cytometry using the Annexin V-FITC/PI method. According to the results, compared with the blank group, the apoptosis rate in the SCH772984 group was markedly increased [(3.2%±0.3%) in the blank group, (3.5%±0.4%) in the control group and (12.5%±1.1%) in the SCH772984 group] ($p<0.05$)] (Figure 4A, 4B). SCH772984 significantly promoted the apoptosis of neural stem cells. The inhibition of the MAPK/ERK pathway remarkably increased the apoptosis of neural stem cells.

Influence of MAPK/ERK Pathway Inhibitor SCH772984 on the Differentiation of Rat Neural Stem Cells into Neurons

Ultimately, the influence of the MAPK/ERK pathway on the differentiation of rat neural stem cells into neurons was studied. The expression of NSE in neurons after SCH772984 treatment was analyzed by immunofluorescence, and its differentiation was determined. The results indicated that, compared with the blank group and the control group, the number of positive neurons in the SCH772984 group was significantly decreased ($p<0.01$) (Figure 5). SCH772984 markedly inhibited the differentiation of neural stem cells in rats. However, the MAPK/ERK pathway remarkably promoted the differentiation of neural stem cells.

Discussion

Cerebral infarction is one of the most important causes of death in human diseases. It has caused great harm to the whole world, which has also become a severe burden to the society[13]. In recent years, great progress has been made in medical science worldwide. However, the disability and mortality rates caused by cerebral infarction remain high[14]. Therefore, it is particularly important to study its internal mechanism of action. In this work, the influences of the MAPK/ERK pathway on neurocyte apoptosis in rats with cerebral infarction from the perspective of the MAPK/ERK signaling pathway were analyzed.

The results revealed that the mRNA expressions of ERK1 and ERK1 in neural stem cells of rats with cerebral infarction increased in a time-dependent manner. Previous studies have shown that the ERK expression is positively correlated with infarct volume after acute cerebral infarction[15,16]. In neurons affected by ischemic

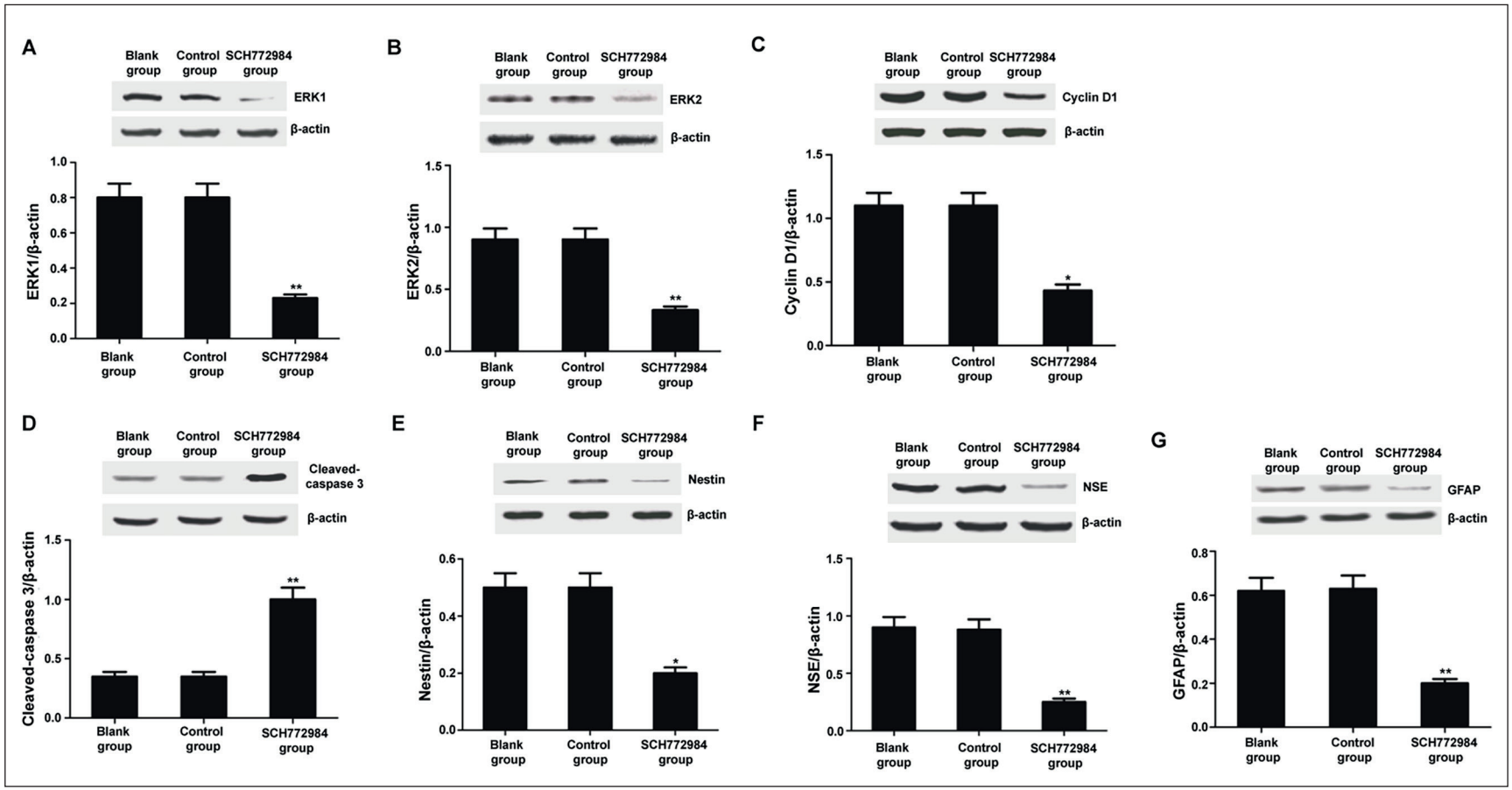

Figure 2. Cell proliferation and apoptosis of neural stem cells, as well as the expression of neural marker proteins. ***A-G***, The protein expressions of ERK1, ERK2, cyclin D1, cleaved-caspase 3, Nestin, NSE, and GFAP detected by Western blotting. *$p<0.05$ *vs.* blank group and control group, and **$p<0.05$ *vs.* blank group and control group.

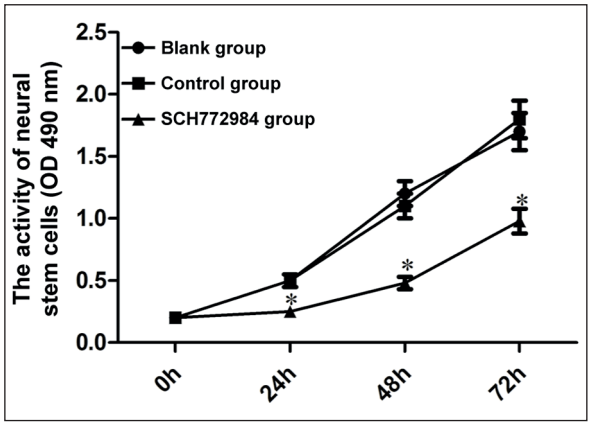

Figure 3. Influence of SCH772984 on the activity of neural stem cells in rats determined *via* MTT assay. **p*<0.05 represented a significant difference.

cerebral infarction, various mechanisms can lead to excessive activation of ERK[17]. Furthermore, the activation of MAPK, including ERK, JNK and P38, has been found in the ischemic region immediately after occlusion of the middle cerebral artery[18].

Besides, our study demonstrated that the MAPK/ERK pathway inhibitor SCH772984 significantly decreased the expression of ERK1, ERK2, cyclin D1, Nestin, NSE, and GFAP, whereas markedly promoted the expression of cleaved-caspase 3. SCH772984 is a MAPK/ERK pathway inhibitor that completely blocks the expression of some vascular smooth muscle contraction genes[19]. Cyclin D1, Nestin, GFAP, and cleaved-caspase 3 are related to cell proliferation and apoptosis, respectively[20]. The activation of the ERK pathway (a subfamily of the MAPK family) affects the expression of various genes regulating cell cycle, including cyclin D1[21]. Neonatal rat ventricular myocytes treated with SCH772984 resulted in a significant dose-dependent inhibition of cell cycle reentry and Nestin expression. In cerebral artery occlusion, the expression of GFAP is increased[22].

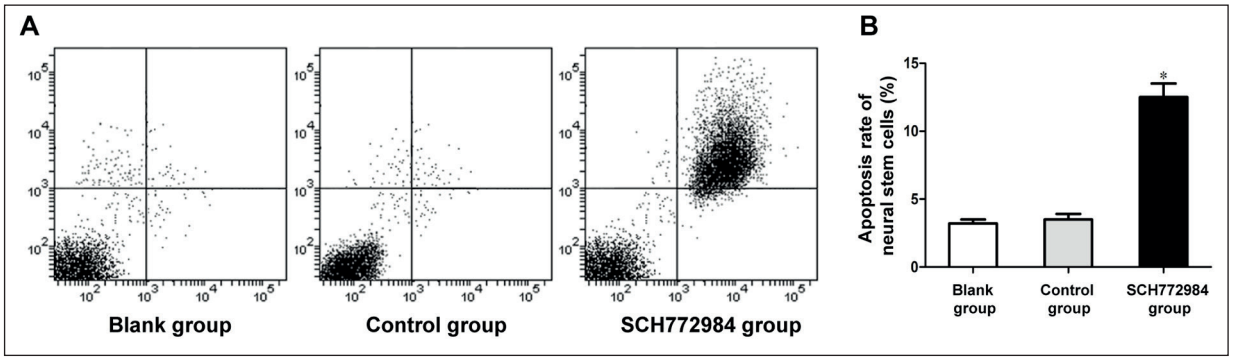

Figure 4. Apoptosis of rat neural stem cells. ***A,*** Flow cytometry graph of apoptosis. ***B,*** Apoptosis rate of neural stem cells in cerebral infarction rats. **p*<0.05 *vs.* blank group and control group.

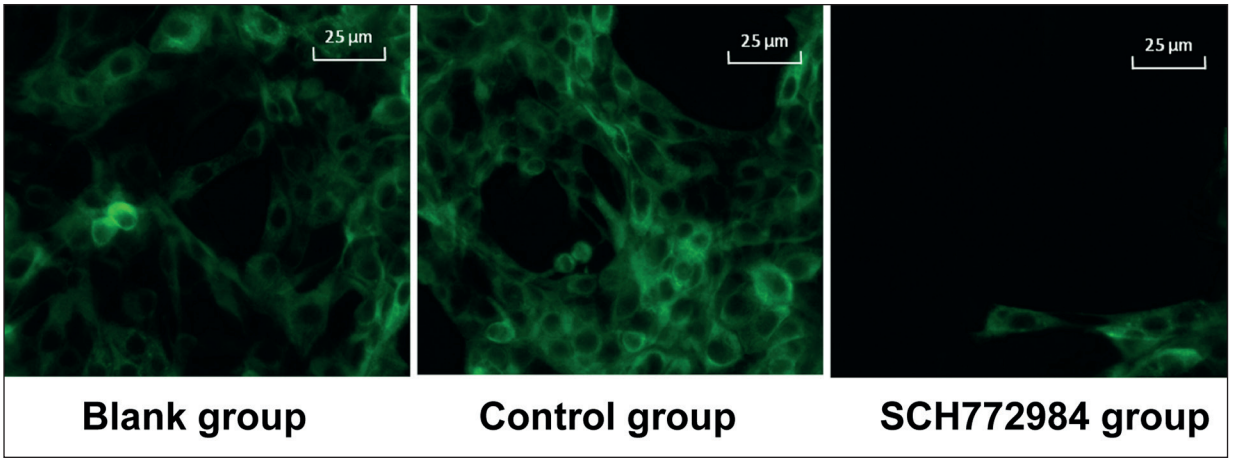

Figure 5. NSE in rat neural stem cells detected by immunofluorescence (Magnification: 100×).

Zhen et al[23] have found that the inhibitory effect of SB203580 on p38 MAPK significantly up-regulates the expression of cleaved-caspase 3 and inhibits the neuroprotective effect of hypoxic post-conditioning on cerebral ischemia/reperfusion injury.

To further demonstrate the influence of the MAPK/ERK pathway on neurocyte apoptosis in cerebral infarction rats, the effect of SCH772984 on the proliferation activity of neurons in cerebral infarction rats was detected. The results showed that SCH772984 evidently weakened the proliferation activity of neurons. Flow cytometry revealed that SCH772984 could significantly increase neurocyte apoptosis. Gupta et al[24] have confirmed that the MAPK/ERK pathway is involved in the apoptosis of microvascular endothelial cells. Tran et al[25] have reported that MAPK/ERK is superior to apoptosis signaling from Fas, tumor necrosis factor (TNF) and TNF-related apoptosis-inducing ligand receptors. Roy et al[22] have demonstrated that the inhibition of the MAPK/ERK pathway leads to the activation of Forkhead box O transcription factors. Meanwhile, this may eventually lead to cell cycle arrest and apoptosis of pancreatic cancer. Satoh et al[26] reported that the MAPK/ERK kinase inhibitor U0126 has neuroprotective effects on oxidative stress in mouse nerve cell lines and rat primary cultured cortical neurons. In addition, the expression of NSE in neuron cells was detected by indirect immunofluorescence to determine its differentiation. The results showed that SCH772984 significantly inhibited the differentiation of neural stem cells in rats. This indicated that the MAPK/ERK pathway was closely related to the differentiation of neurocytes in cerebral infarction rats. Studies of Xu et al[27] have manifested that the MAPK/ERK signal transduction mediates vascular endothelial growth factor-induced bone marrow stem cells to differentiate into endothelial cells.

Conclusions

We showed that the MAPK/ERK pathway inhibits cell proliferation, apoptosis and expression of neural marker proteins. Moreover, it reduces the proliferation activity and promotes the apoptosis of neurocytes in cerebral infarction rats.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Rodrigo R, Fernandez-Gajardo R, Gutierrez R, Matamala JM, Carrasco R, Miranda-Merchak A, Feuerhake W. Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities. CNS Neurol Disord Drug Targets 2013; 12: 698-714.
2) Jia L, Hao F, Wang W, Qu Y. Circulating miR-145 is associated with plasma high-sensitivity C-reactive protein in acute ischemic stroke patients. Cell Biochem Funct 2015; 33: 314-319.
3) Lee DH, Lee YJ, Kwon KH. Neuroprotective effects of astaxanthin in oxygen-glucose deprivation in SH-SY5Y cells and global cerebral ischemia in rat. J Clin Biochem Nutr 2010; 47: 121-129.
4) Siniscalchi A, Gallelli L, Malferrari G, Pirritano D, Serra R, Santangelo E, De Sarro G. Cerebral stroke injury: the role of cytokines and brain inflammation. J Basic Clin Physiol Pharmacol 2014; 25: 131-137.
5) Melani A, Pugliese AM, Pedata F. Adenosine receptors in cerebral ischemia. Int Rev Neurobiol 2014; 119: 309-348.
6) Gibson CL. Cerebral ischemic stroke: is gender important? J Cereb Blood Flow Metab 2013; 33: 1355-1361.
7) Liu C, Zhong L, Tian XL, Han YC. Protective effects of 8-MOP on blood-brain barrier via the Nrf-2/HO-1 pathway in mice model of cerebral infarction. Eur Rev Med Pharmacol Sci 2018; 22: 4278-4287.
8) Son Y, Cheong YK, Kim NH, Chung HT, Kang DG, Pae HO. Mitogen-activated protein kinases and reactive oxygen species: how can ROS activate MAPK pathways? J Signal Transduct 2011; 2011: 792639.
9) Cao G, Jiang N, Hu Y, Zhang Y, Wang G, Yin M, Ma X, Zhou K, Qi J, Yu B, Kou J. Ruscogenin attenuates cerebral ischemia-induced blood-brain barrier dysfunction by suppressing TXNIP/NLRP3 inflammasome activation and the MAPK pathway. Int J Mol Sci 2016; 17: 1418.
10) Fann DY, Lim YA, Cheng YL, Lok KZ, Chunduri P, Baik SH, Drummond GR, Dheen ST, Sobey CG, Jo DG, Chen CL, Arumugam TV. Evidence that NF-kappaB and MAPK signaling promotes NLRP inflammasome activation in neurons following ischemic stroke. Mol Neurobiol 2018; 55: 1082-1096.
11) Ansar S, Eftekhari S, Waldsee R, Nilsson E, Nilsson O, Saveland H, Edvinsson L. MAPK signaling pathway regulates cerebrovascular receptor expression in human cerebral arteries. BMC Neurosci 2013; 14: 12.
12) Zhen Y, Ding C, Sun J, Wang Y, Li S, Dong L. Activation of the calcium-sensing receptor promotes apoptosis by modulating the JNK/p38 MAPK pathway in focal cerebral ischemia-reperfusion in mice. Am J Transl Res 2016; 8: 911-921.
13) Pang X, Li T, Feng L, Zhao J, Zhang X, Liu J. Ellagic acid-induced thrombotic focal cerebral ischemic model in rats. J Pharmacol Toxicol Methods 2014; 69: 217-222.

14) Godinez-Rubi M, Rojas-Mayorquin AE, Ortuno-Sahagun D. Nitric oxide donors as neuroprotective agents after an ischemic stroke-related inflammatory reaction. Oxid Med Cell Longev 2013; 2013: 297357.

15) Liu C, Du Q, Zhang X, Tang Z, Ji H, Li Y. Clematichinenoside serves as a neuroprotective agent against ischemic stroke: the synergistic action of ERK1/2 and cPKC pathways. Front Cell Neurosci 2016; 9: 517.

16) Ou Z, Tao MX, Gao Q, Zhang XL, Yang Y, Zhou JS, Zhang YD. Up-regulation of angiotensin-converting enzyme in response to acute ischemic stroke via ERK/NF-kappaB pathway in spontaneously hypertensive rats. Oncotarget 2017; 8: 97041-97051.

17) Huang J, Ye X, You Y, Liu W, Gao Y, Yang S, Peng J, Hong Z, Tao J, Chen L. Electroacupuncture promotes neural cell proliferation in vivo through activation of the ERK1/2 signaling pathway. Int J Mol Med 2014; 33: 1547-1553.

18) Li W, Huang R, Shetty RA, Thangthaeng N, Liu R, Chen Z, Sumien N, Rutledge M, Dillon GH, Yuan F, Forster MJ, Simpkins JW, Yang SH. Transient focal cerebral ischemia induces long-term cognitive function deficit in an experimental ischemic stroke model. Neurobiol Dis 2013; 59: 18-25.

19) Long X, Cowan SL, Miano JM. Mitogen-activated protein kinase 14 is a novel negative regulatory switch for the vascular smooth muscle cell contractile gene program. Arterioscler Thromb Vasc Biol 2013; 33: 378-386.

20) D'Amico AG, Scuderi S, Saccone S, Castorina A, Drago F, D'Agata V. Antiproliferative effects of PACAP and VIP in serum-starved glioma cells. J Mol Neurosci 2013; 51: 503-513.

21) Xie G, Yang S, Chen A, Lan L, Lin Z, Gao Y, Huang J, Lin J, Peng J, Tao J, Chen L. Electroacupuncture at Quchi and Zusanli treats cerebral ischemia-reperfusion injury through activation of ERK signaling. Exp Ther Med 2013; 5: 1593-1597.

22) Roy Choudhury G, Ryou MG, Poteet E, Wen Y, He R, Sun F, Yuan F, Jin K, Yang SH. Involvement of p38 MAPK in reactive astrogliosis induced by ischemic stroke. Brain Res 2014; 1551: 45-58.

23) Zhu P, Zhan L, Zhu T, Liang D, Hu J, Sun W, Hou Q, Zhou H, Wu B, Wang Y, Xu E. The roles of p38 MAPK/MSK1 signaling pathway in the neuroprotection of hypoxic postconditioning against transient global cerebral ischemia in adult rats. Mol Neurobiol 2014; 49: 1338-1349.

24) Gupta K, Kshirsagar S, Li W, Gui L, Ramakrishnan S, Gupta P, Law PY, Hebbel RP. VEGF prevents apoptosis of human microvascular endothelial cells via opposing effects on MAPK/ERK and SAPK/JNK signaling. Exp Cell Res 1999; 247: 495-504.

25) Tran SE, Holmstrom TH, Ahonen M, Kahari VM, Eriksson JE. MAPK/ERK overrides the apoptotic signaling from Fas, TNF, and TRAIL receptors. J Biol Chem 2001; 276: 16484-16490.

26) Satoh T, Nakatsuka D, Watanabe Y, Nagata I, Kikuchi H, Namura S. Neuroprotection by MAPK/ERK kinase inhibition with U0126 against oxidative stress in a mouse neuronal cell line and rat primary cultured cortical neurons. Neurosci Lett 2000; 288: 163-166.

27) Xu J, Liu X, Jiang Y, Chu L, Hao H, Liua Z, Verfaillie C, Zweier J, Gupta K, Liu Z. MAPK/ERK signalling mediates VEGF-induced bone marrow stem cell differentiation into endothelial cell. J Cell Mol Med 2008; 12: 2395-2406.