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Neural Stem Cell Transplantation Inhibits Apoptosis through Activation of MAPK/ERK Signaling Pathway in Cerebral Ischemia/Reperfusion Rats

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Abstract Apoptosis, a major pathogenesis of cerebral ischemia, is closely associated with the dysregulation of MAPK/ERK signaling pathway. The transplantation of neural stem cells (NSCs), as a therapeutic target of cerebral ischemia, could repair missing cells and the activation of endogenous cells to provide "self-repair". However, the neuroprotective mechanism of the transplantation of NSCs against cerebral ischemia injury is unclear. This study is to investigate the effect of the transplantation of NSCs on apoptosis and MAPK/ERK signaling in the cerebral ischemia/reperfusion rats to evaluate its neuroprotective role. These results suggest that the transplantation of NSCs provides a neuroprotective effect *via* decreasing neurological deficit and increasing the activity of MAPK/ERK signaling in MCAO-lesioned brains and restraining apoptosis. In view of the treatment and prevention of ischemic brain damage, the ability of NSCs to enhance the activity of MAPK/ERK signaling and inhibit apoptosis may be of great importance in the selection of neuroprotective agents.

Keywords: *neural stem cells, apoptosis, MAPK/ERK signaling, cerebral ischemia*

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1. Introduction

Stroke is the fourth killer and number one cause of adult disability in the United States. The estimated direct and indirect costs of stroke care in this country are $68.9 billion for 2009 [1]. In the Middle East and North Africa stroke is increasingly becoming a major health problem, with projections that deaths from it will nearly double by 2030 [2]. With a rapidly aging population in the developed world and increasing life expectancy in the developing world, stroke is becoming a growing problem around the globe [3,4,5,6]. Although current stroke treatments may prevent stroke from progressing, they can temporarily slow the worsening of stroke progress and improve the quality of life for those with stroke and their caregivers. Today, there is a worldwide effort under way to find better ways to treat stroke, delay its onset, and prevent it from developing.

Compelling research evidence has demonstrated that transplantation of neural stem cells (NSCs) on the brain after ischemic can play a critical role such as promoting subventricular zone cell proliferation and peri-infarct angiogenesis after focal cerebral ischemia [7], enhancing the recovery of damaged cells [8], secreting neurotrophic factors and neuropeptides [9], as well as enhancing structural plasticity and axonal transport and achieving recovery of brain function [10,11]. Previously we found that the transplantation of NSCs in the early stage could reduce focal infarct volume and improve the neurological functional recovery in the process of cerebral ischemia/reperfusion injury [12].

It is well-known that the MAPK/ERK signaling pathway acts a central mediator in signal transduction pathways involved in cell growth, proliferation, and survival under physiological and pathophysiologic conditions since the signal starts when a growth factor binds to the receptor on the cell surface and ends when the DNA in the nucleus expresses a protein and produces some change in the cell, such as cell division [13,14,15,16]. The neuroprotective role of MAPK/ERK signaling pathway in cerebral ischemia has been strongly evidenced including inhibiting inflammation and oxidative stress [17,18], improving the behavior impairment after cerebral ischemia [19,20], enhancing neurological rehabilitation and regeneration and limiting apoptosis procedure [21].

Apoptotic mechanism after cerebral ischemia is a major pathogenesis in stroke. Signaling cascades associated with apoptosis contribute to cell death after focal cerebral ischemia [22,23,24]. The activation of caspases 9 (the intrinsic apoptosis pathway), 8 (the extrinsic apoptosis pathway) and 3 (both extrinsic and intrinsic pathway) are critical steps [25]. Accordingly, we addressed the question whether the mechanism of neuroprotective effects by

transplantation of NSCs on cerebral ischemia contributes to activation of MAPK/ERK signaling pathway and inhibits expression of caspases 9, 8 and 3 after activation of MAPK/ERK pathway. This study analyzed that the transplantation of NSCs influenced on the expression of MAPK/ERK and caspases 9, 8 and 3 in the the hippocampus to investigate the neuroprotective mechanisms of the transplantation of NSCs against focal cerebral ischemia/reperfusion injury.

2. Materials and Methods

2.1. Animals

Seventy-two female Wistar rats (clean grade, weighing 200-250 g) and eighty E17 pregnant rats (about 250 g, grade Ⅱ of cleaning) were all provided by the Experimental Animal Center (License No. SCXK-Yue-2008-2010), Southern Medical University, Guangzhou, China. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) [26]. The animal experiments were performed according to internationally followed ethical standards and approved by the research ethics committee of Southern Medical University, Guangzhou, China.

2.2. Isolation and Culture of NSCs

Seventy-two pregnant rats about 250 g, grade Ⅱ of cleaning, were provided. At E17, the pregnant rat was anesthetized with 75% ethanol [27]. Remove the uterus along with the embryos and put it in a petridish containing icecold Hank's Buffered Salt Solution (HBSS). After dissecting the cortical tissues, mince it into small pieces with a scissors in a petridish containing ice-cold HBSS. Transfer the tissue in a 50 ml sterile tube. Spin it for 2 min. at room temperature (200~500g) and aspirate the supernatant. Add Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12, 20ng/mL EGF, 20ng/mL bFGF) (Sigma, USA), triturate 70x with a 10ml pipette. Filter the cell suspension through a cell strainer fitted with 70 μm mesh in to a 50ml sterile tube. Add another 3ml complete DMEM (CEMEM) to rinse the mesh. Incubate cells at 37°C with 5% CO_2 + 95% air. Change with fresh complete medium after 45 min. Treat cells with CEMEM containing 8μM Ara-c on day 4 for 3 days. Cells with drug-free CEMEM on day 7 grow cells for another 4 days and do experiments.

2.3. Identification of NSCs

Bromodeoxyuridine (BrdU) (mouse anti-BrdU, Santa Cruz, USA) uses nucleotide substitution to replace thymidine with uridine in the DNA structure of dividing cells both in vitro and in vivo. BrdU has been utilised in a number of in vitro and in vivo studies to label a variety of cellular sources from human olfactory epithelium and neural progenitors to NSCs [28]. NSCs were labelled 48hrs adding with BrdU (5μmol/L) and cultured 5 days in complete culture medium until the formation of a neurospheres [29]. Single cells dissociated from neurosphere were cultured in 96-well plates and formed single-cell cloning neurosphere 7 days later. The primary and single-cell cloning neurospheres were both positive for the immunofluorescent staining of nestin and were identified as NSC.

2.4. Model Establishment

A classical method for establishing a focal cerebral ischemia model is the middle cerebral artery occlusion (MCAO), which can be performed in a variety of species, including dogs, cats, rabbits, and rats. Because physiology, biochemistry, pathology, pathophysiology, and the cerebral vascular anatomy of humans are relatively similar to rats, the rat MCAO model was chosen for the present study. Ischemia was induced according to the method described by Longa et al. [30] by inserting a thread to block blood flow to the middle cerebral artery. Referring to the five-point scoring system of Longa et al. [30], rats with more than 2 scores were selected. The model rats were randomly divided into 2 groups: MCAO control and NSCs treatment group. Each group was divided into three subgroups: 3 day (n = 6), 7 day (n = 6) and14 day (n = 6).

2.5. Stereotactic Transplantation

Two days after middle cerebral artery occlusion, rats underwent stereotactic transplantation of 50000 E17 NSCs or 2μL PD98059 adjacent to ischemic hippocampus [31,32,33]. Rats were anesthetized with an intraperitoneal injection of 1% ketamine (30 mg/kg) and xylazine hydrochloride (4 mg/kg), and then positioned in a stereotaxic apparatus. The tip of the infusion cannula was implanted stereotaxically into the hippocampus in reference to the stereotaxic atlas (area postrema: -5 mm from interaural line; lateral: -1.3 mm from midline; dorsoventral: 1.8 mm above interaural line), and the cannula was cemented to three jeweler's screws attached to the skull [34]. MCAO animals were administered by intracerebral infusion of same volume saline. All rats received a stereotaxic unilateral lesion of the hippocampus.

2.6. Immunohistochemistry

Tissue samples were collected after rats were perfused transcardially with 4% paraformaldehyde in phosphate buffered saline (PBS) and were embedded in paraffin wax. Coronal slides (6μm thickness) were cut from various sections of the brain. After the coronal sections were rinsed in PBS 3 times, endogenous peroxidase activity was blocked by incubation of specimen with 3% H_2O_2 for 10 min. The sections were incubated with 10% normal goat serum. After the blocking serum was removed, the ABC immunohistochemical assay was carried out according to the protocols we described before Anti-Caspase 3, 8 and 9 proteins (Santa Cruz, USA) were prepared. Two hundred cells were counted and the intensity of staining for each of those cells was adjusted. Five grades were employed to express the degrees of staining, which represent 5 reaction coefficient respectively. The 5 products of every coefficient and the corresponding cell number were added up, which resulted in the value of a positive score. All slides were measured in duplicate. Those samples with a positive score over 10 or frequency over 5% were considered as positive.

2.7. Western Blotting

Western blotting assay was carried out previously [35]. Rat tissues were dissected and homogenized in T-PER buffer in the presence of protease inhibitors. Following homogenization, the lysates were centrifuged at 100,000×g, and the supernatants were saved for Western blot analysis. Equal amounts of lysates were subject to SDS-PAGE and Western blotting analysis using antibodies specific for the following antigens: p38MAPK (Anti-rabbit p38MAPK, Cell Signaling Technology, USA) (1:500) and *β*-tubulin (Bioss Inco., China) (1:200), pERK1/2 (Cell Signaling Technology, USA) (1:200) and *β*-tubulin (1:200), Caspase 3, 8 and 9 (1:200, 1:200 and 1:100, Santa Cruz, USA) and *β*-tubulin (1:2000). The optical densities of the specific bands were scanned and measured by image analysis software (HPIAS 2000, Tongji Qianping Company, Wuhan, China).

2.8. Statistical Analysis

Quantitative data were expressed as Mean ± SD. All statistical analyses were performed using SPSS software for Windows 13.0 (SPSS, Chicago, IL, USA). One-way analysis of variance was employed for multi-group comparison and *q* test for paired comparison. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Nscs Transplantation Improved Neurological Behavior Deficit

Neurological behavior scores was detected by Longa's 5 score method [30] on rats from each group at 3, 7, 14 d following NSCs transplantation. MACO rats exhibited behavioral changes including limb hemiparalysis, decreased activities, the opposite circling when walking, and tail chasing. The scores of neurological behavior in NSCs treatment groups was significantly decreased than MCAO model group ($P<0.01$). These results demonstrated that the transplantation of NSCs decreased the scores of neurological behavior, suggesting that NSCs transplantation improved neurological behavior deficit produced by cerebral ischemia/reperfusion injury (Figure 1).

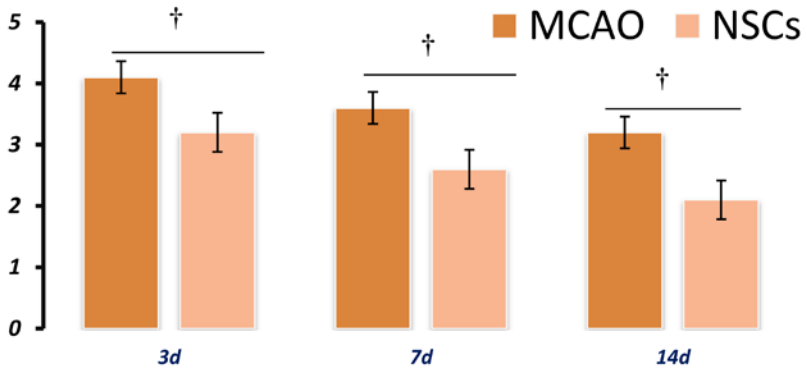

Figure 1. The scores of neurological behavior in the four groups. The scores of neurological behavior in NSCs treatment groups was significantly decreased than MCAO model group. † $P < 0.05$, *vs.* NSCs transplantation group. $n = 6$

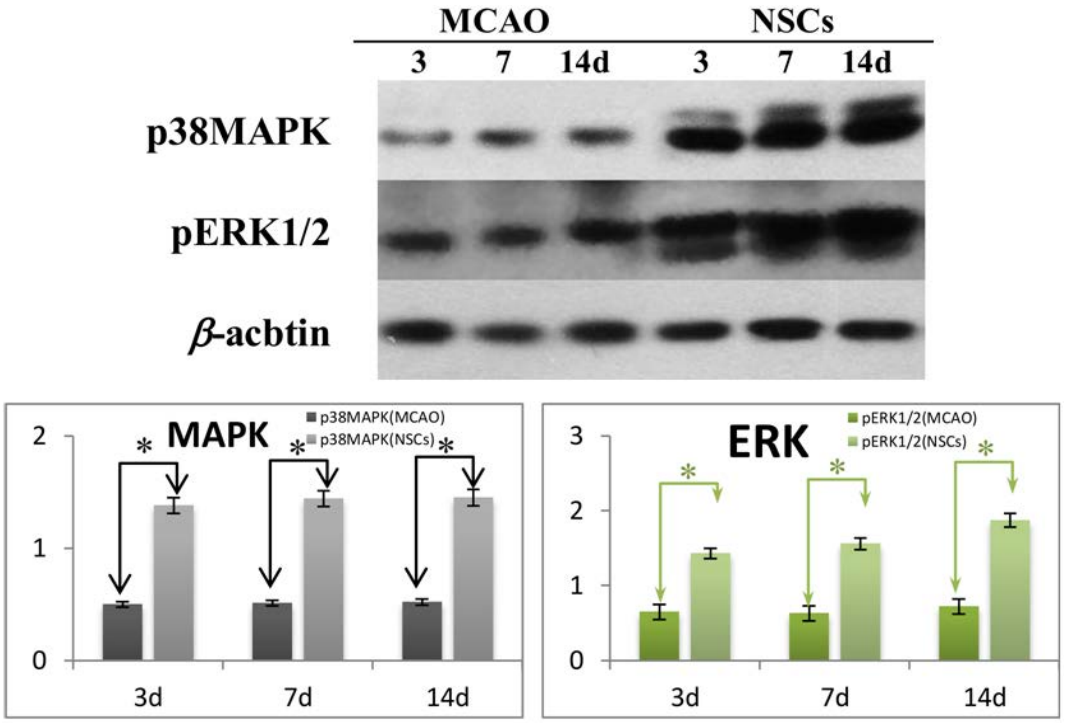

Figure 2. NSCs transplantation activated MAPK/ERK pathway. Columns depict relative densitometric values obtained by comparing with *β*-actin normalized densitometric values. At each corresponding time point following MCAO, the protein level of p38MAPK and pERK1/2 in NSCs treatment group was more significantly increased than that in MCAO animals (*$P<0.01$). $n = 6$

3.2. NSCs Transplantation Activated MAPK /ERK Pathway

This study detected the mechanism of the effect of NSCs transplantation on MAPK/ERK signaling pathway using Western blot, and try to clarify that NSCs transplantation on brain ischemia concerned with MAPK/ERK signaling pathway. At each time point following MCAO, the protein level of p38MAPK and pERK1/2 in NSCs treatment group was more significantly increased than that in MCAO model group ($P < 0.01$, Figure 2), indicating that NSCs transplantation may play a neuroprotective role *via* the activation of MAPK/ERK signaling.

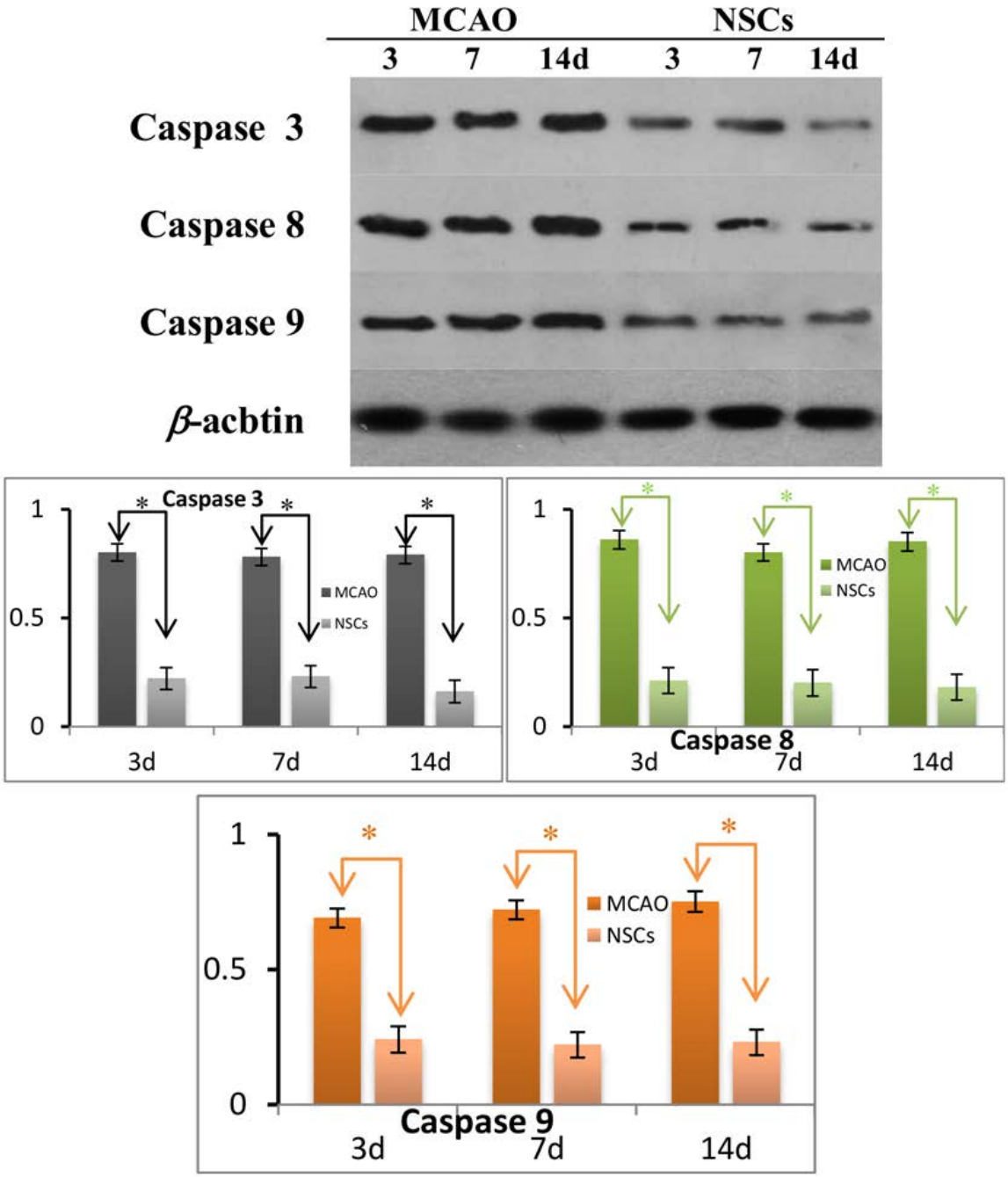

Figure 3. NSCs transplantation inhibited apoptosis pathway. Columns depict relative densitometric values obtained by comparing with *β*-actin normalized densitometric values. At 3, 7 and 14 days following MCAO, the protein level of Caspase 3, 8 and 9 in NSCs treatment group was more significantly decreased than that in MCAO animals (*$P < 0.01$). $n = 6$

3.3. NSCs transplantation Inhibited Apoptosis

The role of apoptosis in cerebral ischemia has been considered as a basic pathogenesis. To evaluate whether the neuroprotective effect on apoptosis in the pathogenesis of cerebral ischemia is due to NSCs transplantation, the effect of NSCs transplantation on the three apoptosis proteins including Caspase 3, 8 and 9 was examined. As Caspase 3, 8 and 9 are markers of apoptosis pathway, Caspase 3, 8 and 9 were measured by Western blot and immunohistochemistry to evaluate the activity of apoptosis. As shown in Figure 3 and Figure 4, Caspase 3, 8 and 9 were more downregulated through treatment of NSCs transplantation ($P < 0.01$, Figure 3 and Figure 4), suggesting that NSCs transplantation retarded the activity of apoptosis.

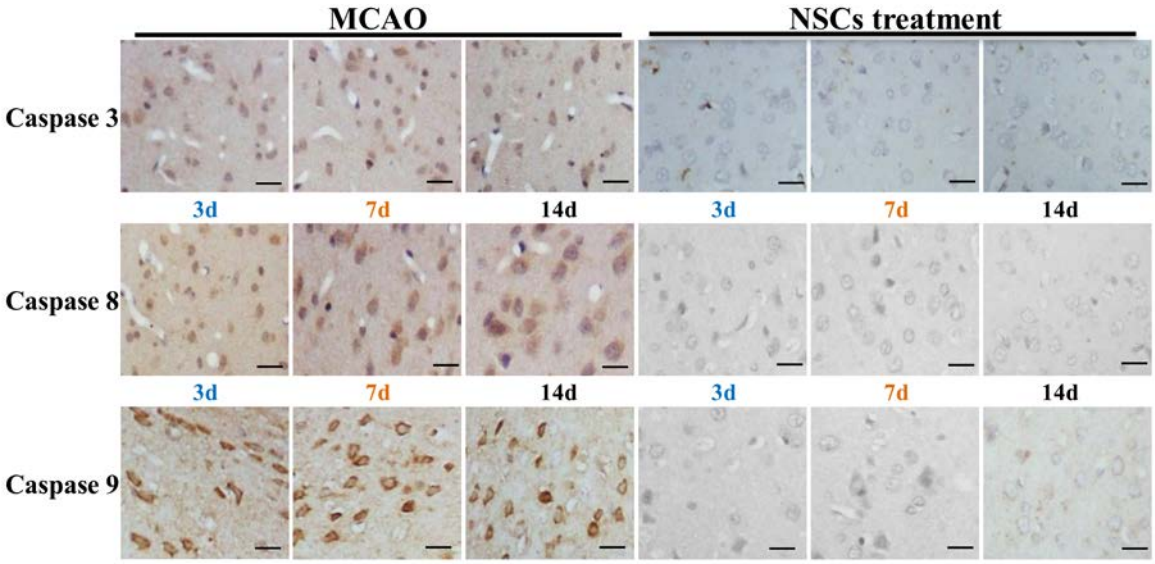

Figure 4. Immunohistochemistry for Caspase 3, 8 and 9. The number of positive cells of Caspases around the damaged cerebral cortex following ischemia/reperfusion injury was shown by the immune-staining images. At 3, 7 and 14 days after ischemia/reperfusion injury, there were many yellow-stained positive cells of Caspase 3, 8 and 9 in MCAO control rats, but in the NSCs treatment group, there were only a few or no positive cells of Caspase 3, 8 and 9. Results are shown as the mean ± SD. *P<0.001; n = 6. The scale bar is 25μm

4. Discussion

Cerebral ischemic injury can trigger a complex cascade of events such as initial tissue damage, neurological behavior deficit occur as early as several minutes after occlusion, neuroinflammation, as well as oxidative stress and apoptosis [36,37,38,39]. In the present study, research results demonstrated that the transplantation of NSCs in the early stage could improve the functional recovery in the process of cerebral ischemia/reperfusion injury and upregulate p38MAPK and pERK1/2 in the brain tissues following MCAO, implicating that the transplantation of NSCs activated MAPK/ERK signaling pathway. Furthermore, the transplantation of NSCs can inhibit the increased levels of Caspase 3, 8 and 9 in the process of cerebral ischemia/reperfusion injury. Therefore, the transplantation of neural stem cells may inhibit apoptosis through activation of MAPK/ERK signaling pathway in the pathogenic process of cerebral ischemia/reperfusion.

Emerging research evidence has noted that the MAPK/ERK pathway can play a neuropretective role in the process of cerebral ischemia injury which mechanisms include direct protection of neurons and oligodendrocytes [40,41], inhibition of inflammatory cytokines and mediators involved in the brains response to injury [42], oxidative stress and apoptosis triggered by cerebral ischemia insult [40,43,44,45]. In response to cerebral ischemia, neurons also activate survival/repair pathways including activation of MAPK/ERK which is linked to neuroprotection in experimental animal models of stroke [44]. NSCs possess high potencies of self-renewal and neuronal differentiation. Considering that several studies showed that the transplantation of NSCs contributes to neuro-protection *via* activation of the MAPK/ERK signaling pathway [46,47,48], this present study result that the transplantation of NSCs increased the expression of p38MAPK and pERK1/2 further confirmed the protective role of the transplantation of NSCs through MAPK/ERK activation in ischemic stroke.

Increasing number of research studies have clarified that apoptosis is a main pathogenesis in the process of cerebral ischemia injury and the transplantation of NSCs can inhibit apoptosis induced by cerebral ischemia damage [49,50,51,52,53]. This present study not only demonstrated that that the transplantation of NSCs limited apoptosis generating from MCAO administration, but found that the transplantation of NSCs can antagonize apoptosis by the inhibitor of MAPK/ERK signaling during cerebral ischemia as well. In view that the dysfunction of MAPK/ERK signaling pathway is an important contributor for apoptosis [54,55], the present research study discovered that the transplantation of NSCs can decrease apoptosis *via* activating MAPK/ERK signaling, and then play a neuropretective role in the process of cerebral ischemia injury.

Previously, our research findings from our group suggest that the transplantation of NSCs may provide a neuroprotective effect by reducing infarct size and decreases neurological deficit following cerebral ischemia/reperfusion injury [12]. Our recent research literature also noted that the neuroprotective effect of NSCs transplantation could correlated with the increased level of BDNF in MCAO-lesioned brains and PI3K/Akt pathway activation [56]. Here, whether the transplantation of NSCs is a feasible therapeutic option for cerebral ischemia was explored again. In conclusion, these findings suggest that the neuroprotective role for the transplantation of NSCs is correlated with many mechanisms. The present findings also further have evidenced that NSCs could become an effective therapeutic target for the treatment and prevention of ischemic brain damage.

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References

[1] Ovbiagele B, Nguyen-Huynh MN: Stroke epidemiology: advancing our understanding of disease mechanism and therapy. *Neurotherapeutics* 2011, 8(3):319-329.

[2] Tran J, Mirzaei M, Anderson L, Leeder SR: The epidemiology of stroke in the Middle East and North Africa. *J Neurol Sci* 2010, 295(1-2):38-40.

[3] Luker JA, Bernhardt J, Grimmer-Somers KA: Age and gender as predictors of allied health quality stroke care. *J Multidiscip Healthc* 2011, 4:239-245.

[4] Li N, Kong X, Ye R, Yang Q, Han J, Xiong L: Age-related differences in experimental stroke: possible involvement of mitochondrial dysfunction and oxidative damage. *Rejuvenation Res* 2011, 14(3):261-273.

[5] Hayashi T, Kawashima S, Nomura H, Itoh H, Watanabe H, Ohrui T, Yokote K, Sone H, Hattori Y, Yoshizumi M *et al*: Age, gender, insulin and blood glucose control status alter the risk of ischemic heart disease and stroke among elderly diabetic patients. *Cardiovasc Diabetol* 2011, 10(1):86.

[6] Giantin V, Semplicini A, Franchin A, Simonato M, Baccaglini K, Attanasio F, Toffanello ED, Manzato E: Outcome after acute ischemic stroke (AIS) in older patients: effects of age, neurological deficit severity and blood pressure (BP) variations. *Arch Gerontol Geriatr* 2011, 52(3):e185-191.

[7] Zhang P, Li J, Liu Y, Chen X, Lu H, Kang Q, Li W, Gao M: Human embryonic neural stem cell transplantation increases subventricular zone cell proliferation and promotes peri-infarct angiogenesis after focal cerebral ischemia. *Neuropathology* 2011, 31(4):384-391.

[8] Yu F, Morshead CM: Adult stem cells and bioengineering strategies for the treatment of cerebral ischemic stroke. *Curr Stem Cell Res Ther* 2011, 6(3):190-207.

[9] Lee HJ, Lim IJ, Lee MC, Kim SU: Human neural stem cells genetically modified to overexpress brain-derived neurotrophic factor promote functional recovery and neuroprotection in a mouse stroke model. *J Neurosci Res* 2010, 88(15):3282-3294.

[10] Yamashita T, Deguchi K, Nagotani S, Abe K: Vascular protection and restorative therapy in ischemic stroke. *Cell Transplant* 2011, 20(1):95-97.

[11] Andres RH, Horie N, Slikker W, Keren-Gill H, Zhan K, Sun G, Manley NC, Pereira MP, Sheikh LA, McMillan EL *et al*: Human neural stem cells enhance structural plasticity and axonal transport in the ischaemic brain. *Brain* 2011, 134(Pt 6):1777-1789.

[12] Zhao Y, Xie P, Zhu XF, Cai ZY: [Neural stem cell transplantation and nerve growth factor promote neurological recovery in rats with ischemic stroke]. *Nan Fang Yi Ke Da Xue Xue Bao* 2008, 28(7):1123-1126.

[13] Wabnitz GH, Nebl G, Klemke M, Schroder AJ, Samstag Y: Phosphatidylinositol 3-kinase functions as a Ras effector in the signaling cascade that regulates dephosphorylation of the actin-remodeling protein cofilin after costimulation of untransformed human T lymphocytes. *J Immunol* 2006, 176(3):1668-1674.

[14] Kwon DS, Kwon CH, Kim JH, Woo JS, Jung JS, Kim YK: Signal transduction of MEK/ERK and PI3K/Akt activation by hypoxia/reoxygenation in renal epithelial cells. *Eur J Cell Biol* 2006, 85(11):1189-1199.

[15] Madala SK, Schmidt S, Davidson C, Ikegami M, Wert S, Hardie WD: MEK-ERK pathway modulation ameliorates pulmonary fibrosis associated with epidermal growth factor receptor activation. *Am J Respir Cell Mol Biol* 2011.

[16] Kornasio R, Riederer I, Butler-Browne G, Mouly V, Uni Z, Halevy O: Beta-hydroxy-beta-methylbutyrate (HMB) stimulates myogenic cell proliferation, differentiation and survival via the MAPK/ERK and PI3K/Akt pathways. *Biochim Biophys Acta* 2009, 1793(5):755-763.

[17] Li Q, Han LP, Li ZH, Zhang JT, Tang MK: [Salvianolic acid B alleviate the disruption of blood-brain barrier in rats after cerebral ischemia-reperfusion by inhibiting MAPK pathway]. *Yao Xue Xue Bao* 2010, 45(12):1485-1490.

[18] Melani A, Gianfriddo M, Vannucchi MG, Cipriani S, Baraldi PG, Giovannini MG, Pedata F: The selective A2A receptor antagonist SCH 58261 protects from neurological deficit, brain damage and activation of p38 MAPK in rat focal cerebral ischemia. *Brain Res* 2006, 1073-1074:470-480.

[19] He QP, Ding C, Li PA: Effects of hyperglycemic and normoglycemic cerebral ischemia on phosphorylation of c-jun NH2-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK). *Cell Mol Biol (Noisy-le-grand)* 2003, 49(8):1241-1247.

[20] Ferrer I, Friguls B, Dalfo E, Planas AM: Early modifications in the expression of mitogen-activated protein kinase (MAPK/ERK), stress-activated kinases SAPK/JNK and p38, and their phosphorylated substrates following focal cerebral ischemia. *Acta Neuropathol* 2003, 105(5):425-437.

[21] Maddahi A, Edvinsson L: Enhanced expressions of microvascular smooth muscle receptors after focal cerebral ischemia occur via the MAPK MEK/ERK pathway. *BMC Neurosci* 2008, 9:85.

[22] Zhang Y, Yao W, Qiu J, Qian W, Zhu C, Zhang C: The involvement of down-regulation of Cdh1-APC in hippocampal neuronal apoptosis after global cerebral ischemia in rat. *Neurosci Lett* 2011.

[23] Siegel C, Li J, Liu F, Benashski SE, McCullough LD: miR-23a regulation of X-linked inhibitor of apoptosis (XIAP) contributes to sex differences in the response to cerebral ischemia. *Proc Natl Acad Sci U S A* 2011, 108(28):11662-11667.

[24] Connell BJ, Saleh M, Khan BV, Saleh TM: Apocynin may limit total cell death following cerebral ischemia and reperfusion by enhancing apoptosis. *Food Chem Toxicol* 2011.

[25] Putcha GV, Harris CA, Moulder KL, Easton RM, Thompson CB, Johnson EM, Jr.: Intrinsic and extrinsic pathway signaling during neuronal apoptosis: lessons from the analysis of mutant mice. *J Cell Biol* 2002, 157(3):441-453.

[26] NIH committee recommends against revising the Guide. *Physiologist* 2007, 50(1):23.

[27] Yu H, Cao B, Feng M, Zhou Q, Sun X, Wu S, Jin S, Liu H, Lianhong J: Combinated transplantation of neural stem cells and collagen type I promote functional recovery after cerebral ischemia in rats. *Anat Rec (Hoboken)* 2010, 293(5):911-917.

[28] Brannvall K, Bogdanovic N, Korhonen L, Lindholm D: 19-Nortestosterone influences neural stem cell proliferation and neurogenesis in the rat brain. *Eur J Neurosci* 2005, 21(4):871-878.

[29] Lehner B, Sandner B, Marschallinger J, Lehner C, Furtner T, Couillard-Despres S, Rivera FJ, Brockhoff G, Bauer HC, Weidner N *et al*: The dark side of BrdU in neural stem cell biology: detrimental effects on cell cycle, differentiation and survival. *Cell Tissue Res* 2011, 345(3):313-328.

[30] Longa EZ, Weinstein PR, Carlson S, Cummins R: Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 1989, 20(1):84-91.

[31] Zhang F, Wang S, Signore AP, Chen J: Neuroprotective effects of leptin against ischemic injury induced by oxygen-glucose deprivation and transient cerebral ischemia. *Stroke* 2007, 38(8):2329-2336.

[32] Harting MT, Sloan LE, Jimenez F, Baumgartner J, Cox CS, Jr.: Subacute neural stem cell therapy for traumatic brain injury. *J Surg Res* 2009, 153(2):188-194.

[33] Luan Z, Yin GC, Hu XH, Qu SQ, Wu NH, Yan FQ, Qian YM, Jin HY, Gong XJ: [Treatment of an infant with severe neonatal hypoxic-ischemic encephalopathy sequelae with transplantation of human neural stem cells into cerebral ventricle]. *Zhonghua Er Ke Za Zhi* 2005, 43(8):580-583; discussion 580.

[34] Yoshida H, Date I, Shingo T, Fujiwara K, Kobayashi K, Miyoshi Y, Ohmoto T: Stereotactic transplantation of a dopamine-producing capsule into the striatum for treatment of Parkinson disease: a preclinical primate study. *J Neurosurg* 2003, 98(4):874-881.

[35] Cai Z, Zhao Y, Yao S, Bin Zhao B: Increases in beta-amyloid protein in the hippocampus caused by diabetic metabolic disorder are blocked by minocycline through inhibition of NF-kappaB pathway activation. *Pharmacol Rep* 2011, 63(2):381-391.

[36] Onteniente B, Rasika S, Benchoua A, Guegan C: Molecular pathways in cerebral ischemia: cues to novel therapeutic strategies. *Mol Neurobiol* 2003, 27(1):33-72.

[37] Noh SJ, Lee SH, Shin KY, Lee CK, Cho IH, Kim HS, Suh YH: SP-8203 reduces oxidative stress via SOD activity and behavioral deficit in cerebral ischemia. *Pharmacol Biochem Behav* 2011, 98(1):150-154.

[38] Friefeld SJ, Westmacott R, Macgregor D, Deveber GA: Predictors of quality of life in pediatric survivors of arterial ischemic stroke and cerebral sinovenous thrombosis. *J Child Neurol* 2011, 26(9):1186-1192.

[39] Codaccioni JL, Velly LJ, Moubarik C, Bruder NJ, Pisano PS, Guillet BA: Sevoflurane preconditioning against focal cerebral ischemia: inhibition of apoptosis in the face of transient

improvement of neurological outcome. *Anesthesiology* 2009, 110(6):1271-1278.

[40] Irving EA, Barone FC, Reith AD, Hadingham SJ, Parsons AA: Differential activation of MAPK/ERK and p38/SAPK in neurones and glia following focal cerebral ischaemia in the rat. *Brain Res Mol Brain Res* 2000, 77(1):65-75.

[41] Franceschini D, Giusti P, Skaper SD: MEK inhibition exacerbates ischemic calcium imbalance and neuronal cell death in rat cortical cultures. *Eur J Pharmacol* 2006, 553(1-3):18-27.

[42] Chen ZB, Huang DQ, Niu FN, Zhang X, Li EG, Xu Y: Human urinary kallidinogenase suppresses cerebral inflammation in experimental stroke and downregulates nuclear factor-kappaB. *J Cereb Blood Flow Metab* 2010, 30(7):1356-1365.

[43] Jia J, Chen X, Zhu W, Luo Y, Hua Z, Xu Y: CART protects brain from damage through ERK activation in ischemic stroke. *Neuropeptides* 2008, 42(5-6):653-661.

[44] Park EM, Joh TH, Volpe BT, Chu CK, Song G, Cho S: A neuroprotective role of extracellular signal-regulated kinase in N-acetyl-O-methyldopamine-treated hippocampal neurons after exposure to in vitro and in vivo ischemia. *Neuroscience* 2004, 123(1):147-154.

[45] Chen XQ, Lau LT, Fung YW, Yu AC: Inactivation of bad by site-specific phosphorylation: the checkpoint for ischemic astrocytes to initiate or resist apoptosis. *J Neurosci Res* 2005, 79(6):798-808.

[46] Gakhar-Koppole N, Hundeshagen P, Mandl C, Weyer SW, Allinquant B, Muller U, Ciccolini F: Activity requires soluble amyloid precursor protein alpha to promote neurite outgrowth in neural stem cell-derived neurons via activation of the MAPK pathway. *Eur J Neurosci* 2008, 28(5):871-882.

[47] Gan HT, Tham M, Hariharan S, Ramasamy S, Yu YH, Ahmed S: Identification of ApoE as an autocrine/paracrine factor that stimulates neural stem cell survival via MAPK/ERK signaling pathway. *J Neurochem* 2011, 117(3):565-578.

[48] Peng CH, Chiou SH, Chen SJ, Chou YC, Ku HH, Cheng CK, Yen CJ, Tsai TH, Chang YL, Kao CL: Neuroprotection by Imipramine against lipopolysaccharide-induced apoptosis in hippocampus-derived neural stem cells mediated by activation of BDNF and the MAPK pathway. *Eur Neuropsychopharmacol* 2008, 18(2):128-140.

[49] Zhang P, Li J, Liu Y, Chen X, Kang Q, Zhao J, Li W: Human neural stem cell transplantation attenuates apoptosis and improves neurological functions after cerebral ischemia in rats. *Acta Anaesthesiol Scand* 2009, 53(9):1184-1191.

[50] Abe K: [Gene-stem Cell therapy for ischemic stroke]. *Brain Nerve* 2009, 61(9):1043-1049.

[51] Kameda M, Shingo T, Takahashi K, Muraoka K, Kurozumi K, Yasuhara T, Maruo T, Tsuboi T, Uozumi T, Matsui T *et al*: Adult neural stem and progenitor cells modified to secrete GDNF can protect, migrate and integrate after intracerebral transplantation in rats with transient forebrain ischemia. *Eur J Neurosci* 2007, 26(6):1462-1478.

[52] Hsieh WY, Hsieh YL, Liu DD, Yang SN, Wu JN: Neural progenitor cells resist excitatory amino acid-induced neurotoxicity. *J Neurosci Res* 2003, 71(2):272-278.

[53] Levison SW, Rothstein RP, Romanko MJ, Snyder MJ, Meyers RL, Vannucci SJ: Hypoxia/ischemia depletes the rat perinatal subventricular zone of oligodendrocyte progenitors and neural stem cells. *Dev Neurosci* 2001, 23(3):234-247.

[54] Qu J, Zhao M, Teng Y, Zhang Y, Hou K, Jiang Y, Yang X, Shang H, Qu X, Liu Y: Interferon-alpha sensitizes human gastric cancer cells to TRAIL-induced apoptosis via activation of the c-CBL-dependent MAPK/ERK pathway. *Cancer Biol Ther* 2011, 12(6):494-502.

[55] Sarkar S, Mazumdar A, Dash R, Sarkar D, Fisher PB, Mandal M: ZD6474, a dual tyrosine kinase inhibitor of EGFR and VEGFR-2, inhibits MAPK/ERK and AKT/PI3-K and induces apoptosis in breast cancer cells. *Cancer Biol Ther* 2010, 9(8):592-603.

[56] Yu Zhao, Shengtao Yao, Shijun Wang. 2010. Neural stem cell transplantation in the hippocampus of rats with cerebral ischemia/reperfusion injury activated the phosphatidylinositol-3 kinase/Akt pathway and increased brain-derived neurotrophic factor expression. *Neural Regeneration Research*. 21(01):1605-1610.