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Original Article

Up-regulated phospho-STAT3 in ischemia reperfusion rat model and oxygen and glucose deprivation cell model

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Abstract: Signal transducer and activator of transcription factor 3 (STAT3), a member of the STAT protein family, plays a key role in neuroprotective when being activated through phosphorylation. However, the expression pattern of phospho-STAT3 (p-STAT3) in ischemic stroke remains poorly understood. Here we investigated the expression pattern and subcellular distribution of p-STAT3 in focal cerebral ischemia reperfusion rat model and oxygen and glucose deprivation (OGD) cell model using Western blot analysis and immunofluorescence staining. In Vitro, compared to control and sham-operated groups, Western blot analysis revealed that the p-STAT3 protein was significantly up-regulated in ischemic side hippocampus after ischemia reperfusion 24 hour. At the same time, immunofluorescence staining showed that the distribution of p-STAT3 was shifted from the cytoplasm to the nucleus at ischemia reperfusion 7 day. In vivo, the expression of p-STAT3 protein was increased in cultured neurons after OGD treated 1 hour, and reached its peak at OGD treated 3 hour. Also, OGD treatment promoted nuclear translocation of p-STAT3 in cultured neurons. Taken together, up-regulated expression of p-STAT3 protein in the ischemia reperfusion rat model and OGD cell model suggested that STAT3 was excessively activated by ischemia reperfusion and OGD, and p-STAT3 might be involved in the pathogenesis of ischemic stroke.

Keywords: Ischemic stroke, cerebral ischemia reperfusion, oxygen and glucose deprivation, p-STAT3

Introduction

Ischemic stroke is one of the most common and disability neurological disorders, which is a major public health concern in the global. It is reported that 15 million people suffer from a stroke worldwide each year, of these, nearly 5 million are left permanently disabled and another 6 million die. In addition, in developing countries, as the population aging and the increase of various kinds of cerebrovascular disease risk factors, stroke mortality will be increased by three times in the next 2 decades [1].

Energy failure and oxygen deprivation that occur in ischemic episodes can produce irreversible neuronal damage [2]. Signal transducer and activator of transcription factor 3 (STAT3), a member of the STAT family, is widely recognized as being a master regulator of the cellular functions and plays a key role in many physiological and pathological processes [3]. Currently, the research of STAT3 has been made significant progress, including the cellular localization, functional regulation, transcriptional control and posttranslational modification. Besides, as a latent cytosolic transcription factor, STAT3 can be translocated into the nucleus and bind to specific promoter sequences, exerting the transcriptional regulation function. At the same time, STAT3 also can be translocated into mitochondria, and participate in aerobic respiration and apoptosis [4]. In addition, STAT3 plays an important role in inflammation and tumorigenesis by regulating cell proliferation, differentiation and metabolism [5, 6]. A growing number of studies have reported that STAT3 has been linked to epilepsy and ischemia-induced retinopathy [7, 8]. What is more, the phosphorylated STAT3 (p-STAT3) protein expression is increased in ischemic brain tissue [9, 10] and has played an important neuroprotective role in ischemia neurons in the acute phase of ischemia [11-13]. Based on the physiological roles of p-STAT3, it is important to detect the

expression of p-STAT3 in focal cerebral ischemia reperfusion rat model and oxygen and glucose deprivation (OGD) cell model and investigate whether it is involved in the pathogenesis of ischemic stroke.

The present study was designed to determine the expression pattern of p-STAT3 in focal cerebral ischemia reperfusion rat model and OGD cell model using Western blot analysis and immunofluorescence staining, and to evaluate the role of p-STAT3 in the pathophysiology of ischemic stroke.

Materials and methods

Focal cerebral ischemia reperfusion rat model

All animal experimental procedures were performed according to the Institutional Animal Care Committee and the Ethical Commission of Zunyi Medical College, Zunyi, China. Adult male Wistar rats (6-8 weeks and weighing 280-320 g) were obtained from the Experimental Animal Center of Zunyi Medical College, and were randomly divided into five groups, including control group, sham-operated group and three focal cerebral ischemia reperfusion model subgroups stratified by the period of reperfusion time: 1, 3, and 7 days (n = 8 in the sham-operated and model 7 day groups; n = 5 in the remaining subgroups). Rats were intraperitoneally anesthetized with 3.5% Hydrated Chloral (3.5 mg/kg, Sigma), transient right middle cerebral artery occlusion (2 h) was established using a nylon monofilament suture, and focal cerebral ischemia reperfusion was induced as described previously [14]. According to the Longa's criteria [14], rats with neurologic deficits at scores 1 to 4 after anaesthesia resuscitation were included in the study. While the sham-operated rats only underwent vascular separation without filament insertion. Model rats were sacrificed at 1, 3, and 7 days after reperfusion, while the control and sham-operated rats were sacrificed at day 1, and the brain tissues were removed for analysis.

Tissue processing

Twenty five rats (n = 5 for each group) were randomly selected and sacrificed by decapitation after intraperitoneally anesthetized at the corresponding time points, the ischemia lateral hippocampal tissue of rats brain were dissected and stored in liquid nitrogen for subsequent Western blot analysis. The remaining six rats from the sham-operated and model 7-day groups (3 per group) were intraperitoneally anesthetized and perfused with 4% paraformaldehyde after saline irrigation, the brains were dissected and fixed in 4% paraformaldehyde for 24 h, and were dehydrated in 20% and 30% graded sucrose solution for each 48 h, then were sliced into 10-μm thick sections on a freezing microtome, followed mounted on polylysine-coated slides, and stored at -20°C for immunofluorescence.

Preparation of an OGD cell model

Neurons were cultured as described previously [15]. Less than 24 hours old, the neonatal Wistar rats were obtained from the Experimental Animal Center of Zunyi Medical College, Zunyi, China. After sterilizing the skin with 75% alcohol, the scalp was opened longitudinally to view the whole superior face of the brain. We carefully opened the skull along the midline and pulled open the two flaps to expose the brain, then carefully separated the hippocampus and removed the meninges and superficial blood vessels under a dissecting microscope (Nikon, Tokyo, Japan). We placed the hippocampus into a dish with 0°C D-Hanks medium (Sigma, St. Louis, MO, USA), sliced the tissue, and then added five volumes of 0.125% parenzyme (Sigma). We placed the dish into a 37°C, 5% CO_2 cell culture incubator to digest for 30 minutes; the digestion was stopped by adding an equal volume of growth medium composed of DMEM/F12 (Sigma), B-27 (2%; Gibco, Carlsbad, CA, USA), L-glutamine (0.5 mM; Gibco) and FBS (0.5%; Gibco). After centrifuging at 1000 rpm for 5 min, we discarded the supernatant and added growth medium. After blowing the tissue by pipette into suspension, we filtered the suspension through a 200-mesh cell-sieve. We took 5 ml cell suspension to count cells, and then diluted the cell suspension to 5×10^5 cells per ml and dispensed 1 ml into each well of a 24-well plate, into which had been put coverslips coated with polylysine (0.1%; Gibco). We took 2 ml of the cell suspension into each well of a six-well plate. At 24 hours after putting these neurons into a 37°C, 5% CO_2 cell culture incubator, we changed the medium to maintenance medium (DMEM/F12, B-27 (2%), and L-glutamine (0.5 mM)). We changed half the vol-

ume of maintenance medium every three days. Neurons were incubated for a minimum of 9 days before experimentation. On day 9 after beginning the incubation, neurons were exposed to OGD as described previously with little modification [16, 17]. Neurons were treated with OGD, 4 h in an environment containing 95% N_2, 5% CO_2 and D-Hanks medium. Neurons were then reverted to maintenance medium. At last, neurons were harvested for use in western blotting analysis or immunofluorescence.

Immunofluorescence analysis

Cultured neurons were selected randomly from the 24 well plates and divided into two groups (control and model group). Specimens were collected at 1 hour, 3 hours, 6 hours and 24 hours after treatment with OGD medium. In the control group, neurons were treated with normal cell culture fluid all the time. In the model group, after four hours of OGD medium exposure, the medium was changed to maintenance medium, and the cover slips were collected at the corresponding time. Subsequently, immunofluorescence was used to detect p-STAT3. Briefly, neurons were washed with PBS for 3-5 minutes, fixed in 4% paraformaldehyde for 30 minutes, washed with PBS 3-5 minutes, treated with 0.5% Triton X-100 (Gibco) for 20 minutes at room temperature, washed with PBS 3-5 minutes, and treated with 10% goat serum for 20 minutes at room temperature. Neurons were incubated with mouse polyclonal anti-p-STAT3 primary antibody (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C. Secondary antibody (goat anti-mouse FITC 1:50, Santa Cruz Biotechnology) was applied for two hours at room temperature, and washed with PBS for 3-5 minutes. Coverslips were mounted with 50% glycerin. For each sample, photos were collected using a laser scanning confocal microscope (Leica Microsystems, Wetzlar, Germany).

Frozen sections of rats were dried at 25°C for 8 min and fixed in acetone for 15 min, and washed with PBS three times (8 min per time), then were heated in a microwave oven at 98°C for 15 min in 10 mmol/L sodium citrate buffer (pH 6.0) for antigen retrieval. Followed, they were permeabilized with 0.2% Triton X-100 for 10 min and blocked in 10% normal goat serum for 60 min at 37°C, and were incubated in mouse polyclonal anti-p-STAT3 primary antibody (1:100, Santa Cruz) overnight at 4°C. On the second day, the sections were washed with PBS sufficiently (three times, 8 min per time) and incubated with secondary antibody (goat anti-mouse FITC 1:50, Santa Cruz) for 90 min at 37°C in the dark, then incubated in 4, 6-diamidino-2-phenylindole (DAPI Beyotime) at 37°C for 5 min. Finally, the sections were sufficiently washed with PBS (four times, 8 min per time) and mounted with antifade mounting medium (Beyotime). Fluorescence images were collected under a laser scanning confocal microscope (Leica, Wetzlar, Germany).

Western blotting analysis

The protein levels of p-STAT3 in both control and model groups were measured by western blotting. Cultural neurons were selected randomly from the six-well plate. Grouping and time points were the same as in the immunofluorescence experiment described above. At the corresponding time point, the neurons were washed with cold PBS and collected by centrifugation. The collected neurons were lysed in cell lysis buffer (100 μl) containing Tris-HCl (50 mM; pH 8.0), NaCl (150 mM), EDTA (1 mM), EGTA (1 mM), Triton X-100 (1%), phenylmethylsulfonyl fluoride (1 mM), with freshly added protease inhibitor cocktail (Calbiochem, San Diego, CA, USA) on ice for 30 minutes. Total protein was isolated from the ischemia lateral hippocampal tissue of rat's brain using a whole protein extraction kit (Beyotime). The protein concentration was measured by the Enhanced Bicinchoninic Acid Protein Assay Kit (Beyotime). For western blotting, 50 mg of protein was resolved on a 10% polyacrylamide gel and transferred to polyvinylidene difluoride (PVDF) membrane. PVDF membranes were blocked for 1 h at room temperature with 5% nonfat dried milk in PBS. The PVDF membrane was incubated with the appropriate primary antibody (mouse anti-p-STAT3 at 1:200 or rabbit anti-β-actin at 1:2500, Santa Cruz Biotechnology) in blocking buffer. The blots were washed 3-10 minutes each with PBS plus Tween-20 (0.1%). Next, the blots were incubated with the appropriate diluted HRP-tagged secondary antibody (1:1000, Santa Cruz Biotechnology) for 1 hour at room temperature. Finally, the blots were exposed according to the manufacturer's instructions to Super Signal West Pico Chemiluminescent HRP substrate (Pierce, Rockford, IL, USA), and bands were visualized

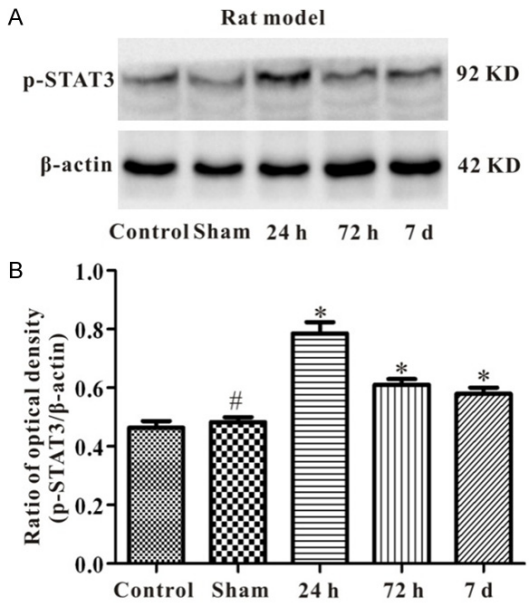

Figure 1. The p-STAT3 protein expression of right lateral hippocampus of rats was detected by western blot analysis, the relative optical density values of p-STAT3 protein bands were normalized to that of β-actin. A. p-STAT3 protein expression in right lateral hippocampus of ischemia reperfusion, control and sham-operated rats. B. The respective bar graph shows the p-STAT3 protein level was upregulated in the model rats compared with controls (n = 5 for each group, **P* < 0.05 versus control and sham-operated group, #*P* > 0.05 versus control group).

upon exposure to x-ray film. Band intensities were calculated with the Gelwork 4.1 image analysis system.

Statistical analysis

Data were expressed as mean ± SEM ($\bar{x}$±s). Statistical comparisons were made by one-way ANOVA with SPSS (version 18) software. *P* < 0.05 was considered significance.

Results

Ischemia reperfusion up-regulated the p-STST3 protein expression of ischemic lateral hippocampus

First, we detected the protein level of p-STAT3 in the ischemic lateral hippocampus of ischemia reperfusion rats using western blot analysis. Compared to the control and sham-operated groups, p-STAT3 protein expression was up-regulated at ischemia reperfusion 24 hour, 72 hour and 7 day (**Figure 1A**), and it was significantly higher than that of control and sham-operated rats (*P* < 0.05, **Figure 1B**), while there is no statistical significance between control group and the sham-operated group (*P* > 0.05, **Figure 1B**).

Ischemia reperfusion promoted p-STAT3 shifting from the cytoplasm to the nucleus

In order to determine whether there is influence in the subcellular distribution of p-STAT3 in ischemia neurons induced by ischemia reperfusion, immunofluorescence staining was performed on the hippocampus of ischemia reperfusion rats. In our results, p-STAT3 was mainly expressed in cytoplasm of hippocampal dentate gyrus neurons of sham-operated rats (**Figure 2A-C**). While in the 7 days of ischemia reperfusion rat's model, p-STAT3 was mainly expressed in the nucleus of the ischemia lateral hippocampal dentate gyrus neurons (**Figure 2D-F**).

OGD treatment increased the p-STST3 protein expression of cultured hippocampal neurons

To test whether the protein level of p-STAT3 was also altered in OGD cell model, we measured the dynamic changes of p-STAT3 protein expression in the OGD treated hippocampal neurons using western blot analysis. Compared to the control group, p-STAT3 protein expression was up-regulated at OGD treated 1 hour, and reached its peak at OGD treated 3 hour (**Figure 3A**). The p-STAT3 protein expression in the OGD treated hippocampal neurons was significantly higher than that in the control hippocampal neurons (*P* < 0.05, **Figure 3B**).

OGD treatment promoted nuclear translocation of p-STAT3 in cultured hippocampal neurons

To further verify whether there is nuclear translocation of p-STAT3 in OGD cell model, immunofluorescence staining was performed on the cultured hippocampal neurons. In the control group, p-STAT3 was detected in the cytoplasm predominantly (**Figure 4A**), while in the OGD treated neurons, p-STAT3 could be observed in both cytoplasm and nucleus (**Figure 4B-E**). Compared to the control group, the absolute fluorescence intensity of p-STAT3 was significantly increased at OGD treated 1 hour, reached to the peak at OGD treated 3 hour, and there

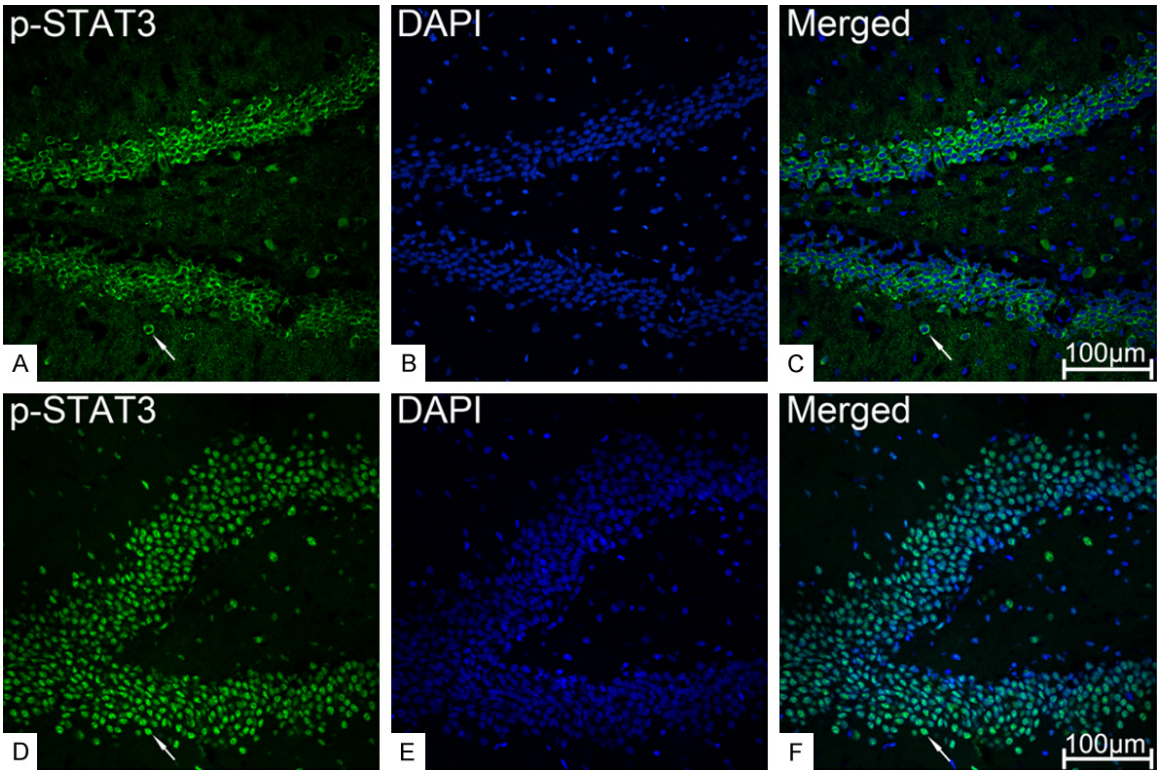

Figure 2. Immunofluorescence staining shows p-STAT3-positive cells in the right lateral hippocampal dentate gyrus of rats. In the sham-operated rats (A-C), p-STAT3 (green, A) was mainly expressed in the cytoplasm of neurons, but did not co-expressed with DAPI-positive nuclei (blue, B). In the 7 days of ischemia reperfusion rats model (D-F), p-STAT3 (green, D) was co-expressed with DAPI-positive nuclei (blue, E). White arrows: p-STAT3-positive cells. Scale bar = 100 μm.

were significant differences between the OGD treated and the control groups (**Figure 4F**, $*P < 0.05$).

Discussion

In present study, we described the expression pattern of p-STAT3 in focal cerebral ischemia reperfusion rat model and OGD cell model. The protein levels of p-STAT3 were up-regulated in the ischemic hippocampus and OGD treated neurons. Meanwhile, ischemia reperfusion injury and OGD intervention could promote nuclear translocation of p-STAT3 in hippocampal neurons and cultured neurons. Based on the findings, we believe that up-regulation the protein expression of p-STAT3 and the nuclear translocation of p-STAT3 following ischemia reperfusion injury and OGD intervention may contribute to the pathophysiology of ischemia stroke.

Ischemia stroke is caused by partial or complete occlusion of cerebral blood flow to the brain. Ischemic brain injury is a major cause of disability, dementia and death worldwide. In the ischemia stroke, ischemia-reperfusion injury of neurons is a complex pathophysiological process [18]. Previous study shows that energy metabolism disorder initiates a devastating cascade and leads to early dysfunction of neural network signal and ultimately to brain dysfunction [19, 20]. In this study, we applied a focal cerebral ischemia reperfusion rat model and an OGD cell model to mimic an ischemia stroke-like event. Ischemia reperfusion brain injury induced by transient middle cerebral artery occlusion (tMCAO) and hypoxic ischemia neuron injury induced by OGD are characteristic pathophysiological events of ischemic stroke [17, 21]. As we all known, the loss of aerobic metabolism and limitation of glucose following cerebral vascular occlusion has rapid and profound effects on neurons survival. During cerebral ischemia, accompanied by limited capacity for anaerobic respiration, few alternatives to glucose as fuel and the energy metabolism disorder, which has been shown to be associated with neuronal function damage [1].

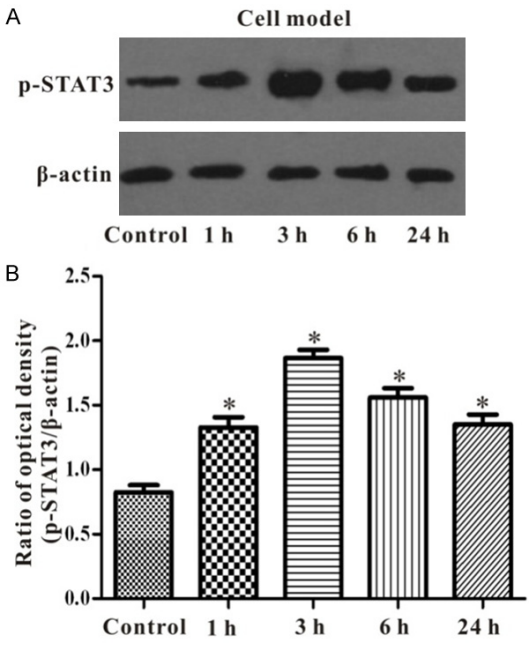

Figure 3. The protein expression of p-STAT3 in cultured hippocampal neurons was detected by western blot analysis, the relative optical density values of p-STAT3 protein bands were normalized to that of β-actin. A. p-STAT3 protein expression in control and OGD treated hippocampal neurons. B. The respective bar graph shows the p-STAT3 protein level was upregulated in the OGD treated hippocampal neurons compared with controls (n = 3 for each group, $*P < 0.05$ versus control group).

The degree of neurologic deficits induced by cerebral ischemia is associated with the reduction number of cortex neurons and hippocampal pyramidal neurons [22]. Although it is a common contributor to human morbidity, the pathological mechanisms involved in ischemia stroke are complex and not yet understood clearly. Previous study showed that cerebral ischemia could lead to serious brain deficits via various pathologic mechanisms, such as signal protein synthesis, neuronal apoptosis, and the release of neurotoxic substances [23]. In current study, the expression of p-STAT3 protein was significant up-regulated after ischemia reperfusion injury and OGD treatment compared with control group. At the same time, immunofluorescence staining suggested that p-STAT3 mainly expressed in the cytoplasm and axoplasm in the normal cultured hippocampal neurons and hippocampal dentate gyrus neurons. However, the p-STAT3 protein expression could be observed in the neurons nucleus after ischemia reperfusion injury and OGD treatment, so we speculate that up-regulation of p-STAT3 in the nucleus of OGD treated neurons and ischemic hippocampal neurons may be associated with the excessive activated STAT3.

STATs, a family of transcription factors, play an importment role in regulating cell growth, survival, differentiation, and motility. According to the structural features, STATs are divided into seven subclass members, including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6 [4, 24]. Previous studies identified that the STATs have already been clearly shown to play critical roles in the multiple systems and be correlated to many diseases [25, 26]. STAT5a/b was considered as biomarker and therapeutic target in prostate and breast cancer [27]. p-STAT6 was significantly decreased during the early stage of ischemic stroke [28]. STAT3, a latent cytosolic transcription factor, could mediate a variety of genes expression in response to cell stimuli, and play an important role in many cellular processes, such as cell growth and apoptosis. Recent studies demonstrate that STAT3 has played neuroprotective roles in ischemic injury neurons [11-13, 29], such as improving the recovery of neurological function through facilitating axon outgrowth and angiogenesis [11]. Besides, the excessive expression of p-STAT3 has been shown to contribute to maintain axon regeneration and neuronal survival by inhibiting apoptosis [30-35]. In this study, we found that up-regulated of p-STAT3 protein expression in focal cerebral ischemia reperfusion rat model and OGD cell model, which was in line with previous studies [9, 10]. At the same time, we also found that ischemia reperfusion injury and OGD intervention could promote nuclear translocation of p-STAT3 in vitro and in vivo experiments, which could exert the transcriptional regulation function through binding to specific promoter sequences. So, up-regulated the expression of p-STAT3 protein in ischemia reperfusion rat model and OGD cell model suggested that p-STAT3 might be involved in the pathogenesis of ischemic stroke.

In summary, the connection between ischemia stroke and the cell signal transduction pathway is complex and diverse, and the effect of STAT3 on the ischemia stroke is only one of numerous factors. The results of this research have provided a new experimental theory for further

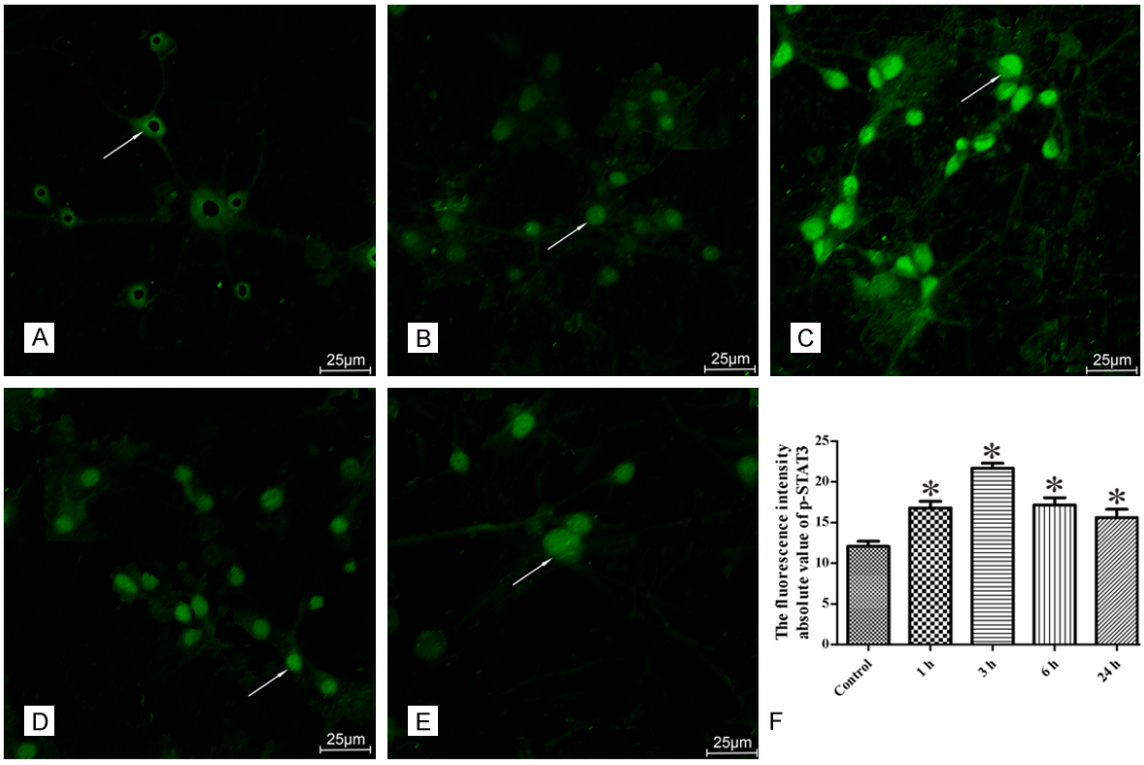

Figure 4. Immunofluorescence staining shows p-STAT3-positive cells in cultured hippocampal neurons. A. p-STAT3 (green) was detected in the cytoplasm predominantly in control neuros. B-E. p-STAT3 (green) could be observed in both cytoplasm and nucleus in OGD treated neurons. F. The respective bar graph shows the absolute fluorescence intensity of p-STAT3 was increased in the OGD treated neurons compared with controls (n = 3 for each group, $*P < 0.05$ versus control group). White arrows: p-STAT3-positive neurons. Scale bar = 25 μm.

study into the underlying mechanism of excessive activated STAT3 in neurons induced by ischemia reperfusion and OGD treatment. However, more research is needed to determine whether or not STAT3 can be used as biomarkers or therapeutic target for ischemic stroke.

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Disclosure of conflict of interest

None.

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References

[1] Baltan S, Morrison RS and Murphy SP. Novel protective effects of histone deacetylase inhibition on stroke and white matter ischemic injury. Neurotherapeutics 2013; 10: 798-807.

[2] Soria FN, Perez-Samartin A, Martin A, Gona KB, Llop J, Szczupak B, Chara JC, Matute C and Domercq M. Extrasynaptic glutamate release through cystine/glutamate antiporter contributes to ischemic damage. J Clin Invest 2014; 124: 3645-3655.

[3] Fletcher S, Drewry JA, Shahani VM, Page BD and Gunning PT. Molecular disruption of oncogenic signal transducer and activator of transcription 3 (STAT3) protein. Biochem Cell Biol 2009; 87: 825-833.

[4] Qi QR and Yang ZM. Regulation and function of signal transducer and activator of transcription 3. World J Biol Chem 2014; 5: 231-239.

[5] Levidou G, Sachanas S, Pangalis GA, Kalpadakis C, Yiakoumis X, Moschogiannis M, Sepsa A, Lakiotaki E, Milionis V, Kyrtsonis MC, Vassilakopoulos TP, Tsirkinidis P, Kontopidou F, Kokoris S, Siakantaris M, Angelopoulou M, Papadaki H, Kavantzas N, Panayiotidis P,

Patsouris E, Korkolopoulou P. Immunohistochemical analysis of IL-6, IL-8/CXCR2 axis, Tyr p-STAT-3, and SOCS-3 in lymph nodes from patients with chronic lymphocytic leukemia: correlation between microvascular characteristics and prognostic significance. Biomed Res Int 2014; 2014: 251479.

[6] Yuan S, Cao S, Jiang R, Liu R, Bai J and Hou Q. FLLL31, a derivative of curcumin, attenuates airway inflammation in a multi-allergen challenged mouse model. Int Immunopharmacol 2014; 21: 128-136.

[7] Mechoulam H and Pierce EA. Expression and activation of STAT3 in ischemia-induced retinopathy. Invest Ophthalmol Vis Sci 2005; 46: 4409-4416.

[8] Xu Z, Xue T, Zhang Z, Wang X, Xu P, Zhang J, Lei X, Li Y, Xie Y, Wang L, Fang M and Chen Y. Role of signal transducer and activator of transcription-3 in up-regulation of GFAP after epilepsy. Neurochem Res 2011; 36: 2208-2215.

[9] Planas AM, Soriano MA, Berruezo M, Justicia C, Estrada A, Pitarch S and Ferrer I. Induction of Stat3, a signal transducer and transcription factor, in reactive microglia following transient focal cerebral ischaemia. Eur J Neurosci 1996; 8: 2612-2618.

[10] Justicia C, Gabriel C and Planas AM. Activation of the JAK/STAT pathway following transient focal cerebral ischemia: signaling through Jak1 and Stat3 in astrocytes. Glia 2000; 30: 253-270.

[11] Hoffmann CJ, Harms U, Rex A, Szulzewsky F, Wolf SA, Grittner U, Lattig-Tunnemann G, Sendtner M, Kettenmann H, Dirnagl U, Endres M and Harms C. Vascular signal transducer and activator of transcription-3 promotes angiogenesis and neuroplasticity long-term after stroke. Circulation 2015; 131: 1772-1782.

[12] Jung JE, Kim GS and Chan PH. Neuroprotection by interleukin-6 is mediated by signal transducer and activator of transcription 3 and antioxidative signaling in ischemic stroke. Stroke 2011; 42: 3574-3579.

[13] Jung JE, Kim GS, Narasimhan P, Song YS and Chan PH. Regulation of Mn-superoxide dismutase activity and neuroprotection by STAT3 in mice after cerebral ischemia. J Neurosci 2009; 29: 7003-7014.

[14] Longa EZ, Weinstein PR, Carlson S and Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 1989; 20: 84-91.

[15] Koptyra M, Gupta S, Talati P and Nevalainen MT. Signal transducer and activator of transcription 5a/b: biomarker and therapeutic target in prostate and breast cancer. Int J Biochem Cell Biol 2011; 43: 1417-1421.

[16] Jang SS, Choi JH, Im DS, Park S, Park JS, Park SM, Joe EH, Jou I and Suh YH. The phosphorylation of STAT6 during ischemic reperfusion in rat cerebral cortex. Neuroreport 2014; 25: 18-22.

[17] Kaneko Y, Tajiri N, Shojo H and Borlongan CV. Oxygen-glucose-deprived rat primary neural cells exhibit DJ-1 translocation into healthy mitochondria: a potent stroke therapeutic target. CNS Neurosci Ther 2014; 20: 275-281.

[18] Xu ZC, Chen YM, Xu P, Liu H, Xie YL and Zeng KB. Epileptiform discharge upregulates p-ERK1/2, growth-associated protein 43 and synaptophysin in cultured rat hippocampal neurons. Seizure 2009; 18: 680-685.

[19] Fletcher L, Evans TM, Watts LT, Jimenez DF and Digicaylioglu M. Rapamycin treatment improves neuron viability in an in vitro model of stroke. PLoS One 2013; 8: e68281.

[20] Huang H, Chen YM, Zhu F, Tang ST, Xiao JD, Li LL and Lin XJ. Down-regulated Na(+)/K(+)-ATPase activity in ischemic penumbra after focal cerebral ischemia/reperfusion in rats. Int J Clin Exp Pathol 2015; 8: 12708-12717.

[21] Gong G, Yuan L, Cai L, Ran M, Zhang Y, Gong H, Dai X, Wu W and Dong H. Tetramethylpyrazine suppresses transient oxygen-glucose deprivation-induced connexin 32 expression and cell apoptosis via the ERK1/2 and p38 MAPK pathway in cultured hippocampal neurons. PLoS One 2014; 9: e105944.

[22] Zhang H, Xie M, Schools GP, Feustel PF, Wang W, Lei T, Kimelberg HK and Zhou M. Tamoxifen mediated estrogen receptor activation protects against early impairment of hippocampal neuron excitability in an oxygen/glucose deprivation brain slice ischemia model. Brain Res 2009; 1247: 196-211.

[23] Liu HB, Meng QH, Du DW, Sun JF, Wang JB and Han H. The effects of ABCG2 on the viability, proliferation and paracrine actions of kidney side population cells under oxygen-glucose deprivation. Int J Med Sci 2014; 11: 1001-1008.

[24] Chen H, Qu Y, Tang B, Xiong T and Mu D. Role of mammalian target of rapamycin in hypoxic or ischemic brain injury: potential neuroprotection and limitations. Rev Neurosci 2012; 23: 279-287.

[25] Hartman RE, Lee JM, Zipfel GJ and Wozniak DF. Characterizing learning deficits and hippocampal neuron loss following transient global cerebral ischemia in rats. Brain Res 2005; 1043: 48-56.

[26] Armanious H, Gelebart P, Mackey J, Ma Y and Lai R. STAT3 upregulates the protein expression and transcriptional activity of beta-catenin in breast cancer. Int J Clin Exp Pathol 2010; 3: 654-664.

[27] Wang Y, Qu A and Wang H. Signal transducer and activator of transcription 4 in liver diseases. Int J Biol Sci 2015; 11: 448-455.

[28] Bromberg J and Wang TC. Inflammation and cancer: IL-6 and STAT3 complete the link. Cancer Cell 2009; 15: 79-80.

[29] Chen H, Lin W, Zhang Y, Lin L, Chen J, Zeng Y, Zheng M, Zhuang Z, Du H, Chen R and Liu N. IL-10 Promotes Neurite Outgrowth and Synapse Formation in Cultured Cortical Neurons after the Oxygen-Glucose Deprivation via JAK1/STAT3 Pathway. Sci Rep 2016; 6: 30459.

[30] Battle TE and Frank DA. The role of STATs in apoptosis. Curr Mol Med 2002; 2: 381-392.

[31] Loddick SA, Turnbull AV and Rothwell NJ. Cerebral interleukin-6 is neuroprotective during permanent focal cerebral ischemia in the rat. J Cereb Blood Flow Metab 1998; 18: 176-179.

[32] Suzuki S, Tanaka K, Nogawa S, Dembo T, Kosakai A and Fukuuchi Y. Phosphorylation of signal transducer and activator of transcription-3 (Stat3) after focal cerebral ischemia in rats. Exp Neurol 2001; 170: 63-71.

[33] Wang T, Yuan W, Liu Y, Zhang Y, Wang Z, Zhou X, Ning G, Zhang L, Yao L, Feng S and Kong X. The role of the JAK-STAT pathway in neural stem cells, neural progenitor cells and reactive astrocytes after spinal cord injury. Biomed Rep 2015; 3: 141-146.

[34] Bareyre FM, Garzorz N, Lang C, Misgeld T, Buning H and Kerschensteiner M. In vivo imaging reveals a phase-specific role of STAT3 during central and peripheral nervous system axon regeneration. Proc Natl Acad Sci U S A 2011; 108: 6282-6287.

[35] Ben-Yaakov K, Dagan SY, Segal-Ruder Y, Shalem O, Vuppalanchi D, Willis DE, Yudin D, Rishal I, Rother F, Bader M, Blesch A, Pilpel Y, Twiss JL and Fainzilber M. Axonal transcription factors signal retrogradely in lesioned peripheral nerve. EMBO J 2012; 31: 1350-1363.