Protective Mechanism of STAT3-siRNA on Cerebral Ischemia Injury

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Abstract. Nerve cells in ischemic brain injury will occur a series of complex signal transduction pathway changes and produce the corresponding biological function, thus affecting the central nervous system functionally different cells in the ischemic brain injury metabolism, division, Differentiation and death process, while changes in signal pathways also play an important role in the repair process of the post-ischemic nervous system. JAK/STAT pathway and vascular lesions have some relevance, but its exact mechanism after cerebral ischemia is not yet fully understood. This study is intended to further explore the JAK / STAT pathway in the functional site of STAT3 in neuronal ischemia Hypoxic injury and related molecular mechanisms, targeting these targets design intervention strategies to block the signal pathway, in order to provide a theoretical basis for the treatment of ischemic brain damage in this pathway.

1. Materials and Methods

1.1. Cell Line
PC12 cell line (adrenal pheochromocytoma) was purchased from Beijing Amethyst Biotech Corporation.

1.2. The Main Reagents and Equipment
DMEM medium: produced by the United States Gibco; fetal bovine serum: Beijing Dinguo company; penicillin, streptomycin: North China pharmaceutical production; thiazolyl blue, DMSO, mouse nerve growth factor (NGF) are produced in the United States Sigma; trypsin : The United States Sigma production; anti-STAT3 body: Wuhan Boster Biological Technology Company. Medical purification workbench: Suzhou purification equipment company; Model550 Enzyme Analyzer: the United States BIO-RAD; laser confocal microscope: Japan OLYMPUS.

1.3. Cell Grouping and Experimental Methods
PC12 cells were cultured with NCF medium for 6 days. PC12 cells were washed 3 times with oxygen-glucose deprived glucose-DMEM medium and incubated with cells in hypoxic-glucose deprivation-free DMEM medium. PC12 cells were placed in a homemade anoxic tank, Intake and outlet [3]; continue to incubate at 37 °C incubator.

PC12 cells were seeded on a six-well plate and stimulated with NGF (100 ng / ml) for 6 days. Cells were harvested for 3 h, 6 h, 9 h, 12 h and 24 h after oxygen deprivation. Remove the medium and wash once or twice with PBS. Add 0.1 ml per well on ice to the lysis buffer. Blow to complete contact
with the cells. Centrifuge at 12,000 rpm for 5 minutes and the supernatant is removed. Electrophoresis gels were prepared and subjected to SDS-PAGE. After the electrophoresis strip will be cut to the appropriate size, with transfer membrane buffer, 5min × 3 times. Cut the filter paper and NC membrane of the same size as the strips in advance and immerse them in transfer membrane buffer for 10 min. Turn on the power, constant current 1mA / cm², transfer 1.5hr. After the transfer, disconnect the power to remove the membrane, there will be protein standard staining, into the membrane staining solution after 50s, 50% methanol in several decolorization, to clear the background, and then double distilled water, air dry folder Save on two layers of filter paper. Wash the membrane with 0.01M PBS for 5 min × 3 times. Add coating solution, shake gently, room temperature 2hr. Discard the coating solution, wash the membrane with 0.01M PBS, 5min × 3 times. Add primary antibody, place it at 4 ℃ for more than 12 hours, discard primary antibody and 1% BSA, wash membrane with 0.01M PBS respectively, 5min × 4 times. Horseradish peroxidase-conjugated secondary antibody (diluted with 0.01 M PBS in the appropriate dilutions) was added and shaken gently for 2 hr at room temperature. Discard the secondary antibody, washed with 0.01M PBS, 5min × 4 times. Add color liquid, dark color to appear strip when placed in double distilled water to terminate the reaction [2-3].

1.4. Statistical Methods
Using SPSS 16.0 statistical software package, the mean of multiple samples was compared with one-way ANOVA. The two samples were compared by independent sample t-test. The measurement data were described by ± s. The test level was bilateral α = 0.05.

2. Results
2.1. PC12 cell Morphological Changes
After 3 days of culture, PC12 cells began to stop dividing. Gradually differentiated into cells with sympathetic neurons and started to grow neurites. After cultured for 5 days, most of PC12 cells turned into sympathetic neuron-like morphology, and the neurites gradually increased and extended to form a sparse network. With the extension of culture time, the sympathetic neuron-like cells gradually increase the body [4].

Cell survival rates at 3 h, 6 h, 9 h, 12 h, 16 h, and 24 h after OGD were measured by MTT assay. As shown in Fig. 1, the cell viability decreased significantly with OGD prolongation, while OGD12 h, the cell viability decreased more significantly. The survival rate of OGD cells at 24 h was 64.3%, which was significantly different from the control group (P <0.05).

![Figure 1. PC12 cell morphological changes](image-url)
2.2. STAT3-siRNA STAT3 Protein Expression Changes

STAT3 is an intrinsic marker of neuronal and synaptic functional status. Synaptophysin regulates the process of neuronal processes and contributes to synaptogenesis [5]. The expression of STAT3 protein in PC12 cells treated with oxygen-glucose deprivation model was detected by Western Blot. Compared with the control group, the expression of STAT3 protein was significantly increased as compared with the control group (P <0.05) Gradually lighter, protein expression of the band gradually become thicker.

![Figure 2. Cell viability at different times after PC12 glucose deprivation](image)

| Ratio of STAT3/β-actin | 0.5ug/ml | 1.0ug/ml | 2ug/ml |
|------------------------|---------|----------|--------|
| untransfected group    |         |          |        |

![Figure 3. STAT3-siRNA protein expression changes](image)

3. Discussion

Ischemic cerebrovascular disease is a complex, multi-factor, multi-level pathological process. A series of ischemic cascade and reperfusion injury triggered after stroke are the basic factors that cause the prognosis of stroke. Therefore, the study of the pathogenesis of ischemic cerebrovascular disease has important clinical significance. PC12 cells from (Rattus norvegicus) adrenal pheochromocytoma (a sympathetic nervous system tumors), the main secretion of catecholaminergic neurotransmitters including dopamine, norepinephrine and so on. Membrane NGF receptors, physiological levels induced by NGF, they stop dividing, grow neurites, differentiate into cells with sympathetic neurons, often used to analyze the role of neuronal differentiation and NGF molecular mechanism of research, but also Was used to study the mechanism by which growth factors regulate changes in the gene expression of nerve cells [6]. Oxygen glucose deprivation (OGD) is a model that simulates ischemia / hypoxia at the cellular level. Through the cell culture conditions change, including the cells into the
hypoxia tank or replace the sugar-free medium. Simulated cell damage in the case of ischemia / hypoxia, the cell will produce necrosis, apoptosis, autophagy and other phenomena [7]. In this study, PC12 cells were used to simulate cell hypoxia / hypoxia injury, the establishment of model of oxygen deprivation, OGD were observed 3 h, 6 h, 9 h, 12 h, 24 h, MTT assay cell viability, the results showed: As the time of oxygen deprivation increased, cell viability decreased from 99.7% to 64.3% (Figure 1).

To study the changes of important sites in JAK / STAT signal pathways after ischemic brain injury will provide a theoretical basis for finding key targets in neuroprotective substances and signal transduction pathways that can be applied in clinical practice, and will provide theoretical basis for developing ischemic brain Damage biological treatment and gene therapy to provide a theoretical basis, may have greater economic benefits.. The present study shows that STAT3 protein expression is positively correlated with PC12 cell viability after oxygen deprivation, STAT3 protein is involved in synaptic vesicle-mediated transport, neurotransmitter release, synapt vesicle cycling, and synaptogenesis [9] The study confirmed that STAT3 is a neuron and synaptic reflection of synaptic reflect neuronal synaptic function of the intrinsic markers of Western Blot detection of oxygen deprivation model of PC12 cells treated with STAT3 protein expression compared with the control group The longer the time of OGD, the lower the level of STAT3 protein expression and the lighter the protein expression band, suggesting that with the extension of OGD, the neuronal characteristics and synaptic function of neurons gradually decreased. STAT3 plays a crucial regulatory role in the release of neurotransmitter and participates in the early development of CNS. Therefore, the regulation of the expression of synaptophysin after cerebral ischemia may be used as a pathological and physiological change after diagnosis and treatment of cerebral ischemia As well as a way to study the neural regeneration and plasticity after cerebral ischemia.

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5. References and Notes
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