

# Model of Oxygen and Glucose Deprivation in PC12 Cells and Detection of HSP70 Protein

Jinting He<sup>1</sup>, Le Yang<sup>2</sup> and Yankun Shao<sup>1\*</sup>

<sup>1</sup>Department of Neurology, China-Japan Union Hospital, Jilin University, Changchun 130031, China

<sup>2</sup>China People's Hospital of Jilin Province, Changchun 130021, China;  
Email: \*yankunshao@163.com

**Abstract.** Objective: PC12 cell was used to set up a ischemia model by OGD and detected HSP70 protein. Methods: Use of PC12 cells induced by NGF stimulation into nerve cells, oxygen and glucose deprivation to build the nerve cells of oxygen and glucose deprivation model; using Western blot analysis of PC12 cells into neuron-like cells and oxygen-glucose deprivation model established. Results: The application of a final concentration of 50 ng / ml of NGF in DMEM complete medium PC12 cells showed a typical neuronal morphology with the increase in cell culture time. NGF culture time showed a positive correlation, the establishment of oxygen and glucose deprivation (OGD) training environment, the OGD after nerve element appears different degrees of damage, OGD can effectively induce the expression of HSP70. Conclusion: PC12 cell transformed into cells by NGF; the cell model of OGD was established.

## 1. Introduction

Ischemic brain damage related to signal transduction mechanisms have been widely attention of researchers. Notch signaling pathway members recently become a hot research ischemic cerebrovascular disease, ischemic injury, and other aspects of stem cell proliferation and regulation have been reported, therefore, the need to establish cerebral ischemic injury can be simulated in vitro model to study. This part of the experiment with the use of NGF in PC12 cells induced to differentiate into neuron-like cells were OGD (oxygen glucose deprivation, OGD) to establish a stable nerve cell ischemia and hypoxia model for further research on ischemic brain injury-related signal transduction foundation mechanism. Stress protein (stress protein, SP) is a heat shock response of the body cells in the environment of high temperature, hypoxia, oxidative stress induced, HP70 SP protein molecule the more important experimental study abroad often detect missing HP70 measure blood hypoxic injury

## 2. Materials and Methods

### 2.1. Material

*2.1.1. Cell Lines.* Rat adrenal pheochromocytoma (PC12) cells were purchased from Beijing Jin Zijing biomedical Technology Co.,

*2.1.2. Reagents DMEM medium: Gibco US company.* Trypsin: US Sigma production. FBS: Beijing Ding States company. MTT, DMSO, mouse nerve growth factor (NGF) are the United States Sigma production. anti-HSP70 body Wuhan Boster Biotechnology Limited. [1]



2.1.3. *The main instrument.* Model550 enzyme-linked analyzer: US BIO-RAD. Medical purification table; purification equipment company in Suzhou. Confocal microscopy: Japan OLYMPUS.

## 2.2. Methods

2.2.1. *PC12 cell culture and MAP2 staining confocal microscopy.* PC12 cells were cultured in DMEM 10% fetal calf serum (100 U / ml penicillin, 100 U / ml streptomycin), 37 °C 5% CO<sub>2</sub> in culture. 37 °C 5% CO<sub>2</sub> cells were cultured to 80% of the collection, use medium containing NGF were cultured 6 d, observe cell growth. Cells were removed from the 24 well plates climbing film, washed with precooled PBS 3 times, fixed in 4% formaldehyde 15 min, 0.1% Triton X - 100 transparent 10 min, washed with precooled PBS 3 times, 10% goat serum for 30 min, the supernatant; adding an anti-rabbit anti-mouse MAP2 200 $\mu$ l (1: 500), 4 °C overnight, washed with pre-cooled PBS 3 times, 5 min / times; FITC-labeled goat anti-rabbit IgG secondary antibody, using the anti-quenching Fengpian agents were mounted; using a laser scanning confocal microscope collection [2].

2.2.2. *PC12 cell model of oxygen glucose deprivation (OGD) establishmen.* The 300 mm diameter glass-stoppered vacuum dryer into a double-barreled rubber stopper, as out of the air intakes. Latex inner end of the *tracheal* tube (Bottom fluid level and above), the other end of the outer tube into the trachea (air-Linked). In anoxic tank inject approximately 50 ml of sterile double distilled water, 40g sodium hydrosulfite, anoxic tank is connected to the intake manifold and cylinder, open the cylinder valve, the gas flow rate of 300 ml / min, when the gas tank is full closure of the intake and outlet pipe

2.2.3. *HSP70 protein expression.* PC12 cells will NGF (100 ng / ml) stimulation after 6 d OGD3 h, 6 h, 9 h, 12 h, 16 h, 24 h later, the cells were collected and counted, prechilled cell lysates resuspended PC12 cells, ice for 30 min complete lysis of the cells. Lysates were collected; protein concentration was measured by *BCA* method [3]. Take the same content of protein cell lysate, was added a 5  $\times$  electrophoresis sample buffer, 100 °C boiled for 5 min, centrifuged, 12% SDS-PAGE electrophoresis (constant voltage 100 V, 3 h), after electrophoretic transfer membrane were completed (PVDF membrane, constant voltage 60 V, 4 °C ice bath electroporation 2 h). After transfection membrane with 5% skim milk blocking 2 h. According to an anti-instruction using 5% skim milk scaled appropriately diluted primary antibody, and then incubated with primary antibody overnight. PVDF membrane was washed with TBST, 15 min / time, 3 times, HRP secondary antibody was added, incubated at room temperature 2 h. TBST wash the membrane 10 min / times, three times, ECL color liquid exposure [4].

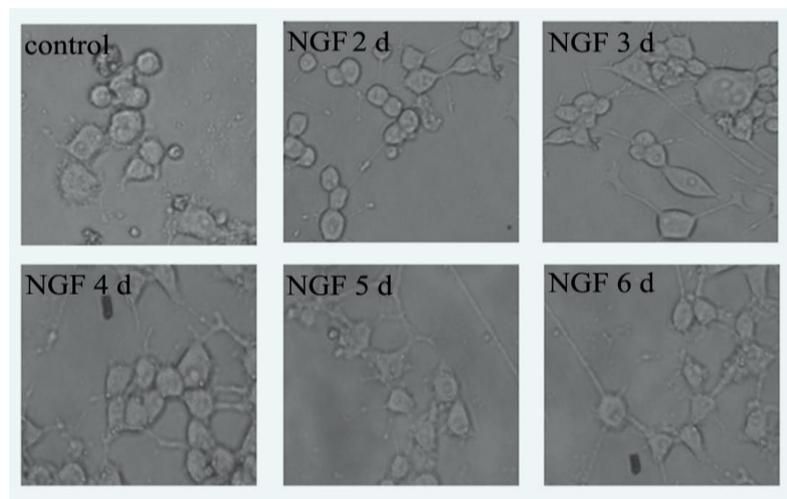
## 2.3. Stastitical Analysis

SPSS statistical software package (version 10.0) in the least significant difference method and analysis of variance procedures for pairwise comparisons between various sets of data mean to  $P < 0.05$  was considered statistically significant [5].

## 3. Results

### 3.1. NGF-Induced PC12 Cells into Neuron-Like Cells in Culture

With culture time, cell synaptic thickening growth, no significant increase in the number of Figure 1, training 1 d, cells grow shorter, smaller synapses; cultured 2 d, increasing the number of synapses and length; Training 3 -6d, synaptic length of up to 4 to 5 times the cell body [6], woven into a mesh, showing the typical neuronal morphology.

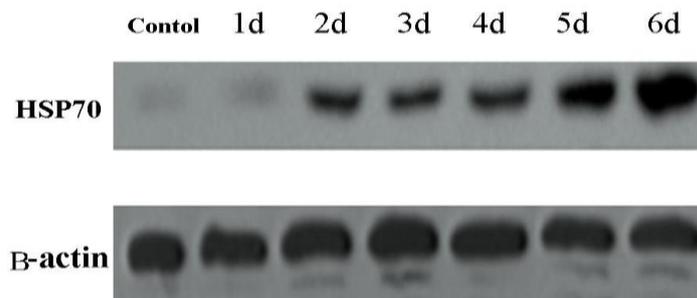


The morphological changes of PC12 cells after NGF activated ( $\times 200$ ). 100 ng/ml NGF was used to activate to PC12 cells for 1 d, 2 d, 3 d, 4 d, 5 d and 6 d.

**Figure 1.** NGF activated PC12 cells in culture.

### 3.2. OGD Upregulate the Expression of HSP70

Heat shock proteins (HSP) are a class of ischemic injury can be detected as late genes [7]. Figures 2, with the increase OGD time, HSP70 expression gradually increased, compared with control, OGD 6 h, the expression of HSP70 increased significantly, with a time-dependent OGD, OGD can effectively induce HSP70 expression.



**Figure 2.** The expressions of HSP70 after OGD for different time.

## 4. Discussion

PC12 cells are a rat adrenal pheochromocytoma cell line monoclonal, both nerve cell characteristics, but also has the characteristics of stably transfected generations can grow long processes in the effect of nerve growth factor, transforming into neuron-like cells, this Research on the use of experimental cell lines PC12 cells, grow postsynaptic NGF stimulation, with typical neuronal morphology. The results showed that, after PC12 cells induced by NGF, laser scanning confocal microscope synapse formation, showed NGF can induce PC12 cells MAP2, PC12 cells make synapses, neuron-like cell transformation. Ischemic brain injury refers to the local brain tissue (neurons, glial cells and Contact fibers). Because blood disorder nerve cell degeneration occurs, necrosis or transient loss of function, about 80% of cerebrovascular disease, clinical common disease, with high mortality and morbidity. Pathogenesis of ischemic brain injury in-depth study of great clinical significance [8]. Nerve cells OGD model (oxygen and glucose deprivation, OGD) was used at the cellular level more simulated clinical stroke process. In this study, the experimental cell lines PC12 cells, the cells continued by oxygen glucose deprivation OGD model to simulate the process of stroke, permanent partial necrosis of brain cells, lay the foundation for further experiments. On the basis of PC12 cells into neurons build on the use of continuous oxygen glucose deprivation OGD model, Stress protein (stress protein, SP) is

the body's cells in the environment of high temperature, hypoxia, oxidative stress [9], heat shock reactions induced by heavy metal poisoning, infection under the body against stress injury play an important role. HSPs can be divided HSP90, HSP70, HSP and small molecule ubiquitin four families. Which has the effect of HSP70 protect brain cells and enhance brain cells to hypoxia tolerance, further resistance lethal injury [10]. Transient non-fatal ischemic / hypoxic yet noticeable morphological changes before, HSP70 expression has quickly and effectively. Therefore experimental researches often detect HSP measure injury by hypoxia, the present results show that with the extension of OGD time, HSP70 expression was significantly increased, neuron-like cells in OGD when, through the expression of HSP70 protein effective to maintain cell function and survival.

## 5. Acknowledgments

The project supported by Jilin Provincial Department of Finance funds in China (No.Sczsy201512)and Jilin Provincial Department of Health funds(No.20152085).

## 6. References

- [1] Kumar MR, Bhat, Nityanand Maddodi. Transcriptional regulation of human MAP2 gene in melanoma: role of neuronal bHLH factors and Notch1 signaling. *Nucleic acids research*. 2006, 34(13): 3819–3832.
- [2] Koji Ikegami, Masahiro Mukai. TLL7 is a mammalian  $\alpha$ -tubulin polyglutamylase required for growth of MAP2-positive neurites. *The journal of biological chemistry*. 2006, 281(41): 30707–30716.
- [3] Larsena EC, Hatcher JF. Effect of D609 on phospholipid metabolism and cell death during oxygen-glucose deprivation in PC12 cells. *Neuroscience*. 2007, 46(3): 946–961.
- [4] Jae Young Lee, Joo-Woon Lee. Neuroactive conducting scaffolds: nerve growth factor conjugation on active ester-functionalized polypyrrole. *J. R. Soc. Interface*. 2009, 6: 801–810.
- [5] Damian C, Genetos, Whitney K. Oxygen tension modulates neurite outgrowth in PC12 cells through a mechanism involving HIF and VEGF. *J Mol Neurosci*. 2010, 40: 360–366.
- [6] Katarzyna Mnich, David P. Inhibition by anandamide of 6-hydroxydopamine induced cell death in PC12 cells. *International journal of cell biology volume*. 2010, 97: 10.
- [7] Ronchini C, Capobianco AJ. Induction of cyclin D1 transcription and CDK2 activity by Notch (ic): implication for cell cycle disruption in transformation by Notch(ic). *Mol Cell Biol*. 2001, 21: 5925–34.
- [8] Baonza A, Freeman M. Control of cell proliferation in the *Drosophila* eye by Notch signaling. *Dev Cell*. 2005, 8: 529–39.
- [9] Cheng P, Zlobin A, Volgina V, et al. Notch-1 regulates NF- $\kappa$ B activity in hemopoietic progenitor cells. *J Immunol*. 2001, 167: 4458–67.
- [10] Garces C, Ruiz-Hidalgo MJ, Font de Mora J, et al. Notch-1 controls the expression of fatty acid-activated transcription factors and is required for adipogenesis. *J Biol Chem*. 1997, 272: 29729–34.
- [11] Kumar MR, Bhat, Nityanand Maddodi. Transcriptional regulation of human MAP2 gene in melanoma: role of neuronal bHLH factors and Notch1 signaling. *Nucleic acids research*. 2006, 34(13): 3819–3832.