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PTPN21 protects PC12 cell against oxygen-glucose deprivation by activating cdk5 through ERK1/2 signaling pathway

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ABSTRACT

PTPN21, a cytosolic non-receptor tyrosine phosphatase isolated from human skeletal muscle, was reported to promote neuronal survival. Nevertheless, it is not clear whether PTPN21 plays a role in hypoxia ischemia-induced neuronal injury. A proper understanding of the PTPN21 mechanism in neuron growth regulation is limited. In this study, we investigated the neuroprotective effects and potential mechanism of PTPN21 on oxygen glucose deprivation (OGD)-injured PC12 cells. The ischemic stroke model of PC12 cells was made by OGD for 2 h, after transfection of the PTPN21 siRNA and pcDNA 3.1 PTPN21(+). Cell viability was tested using the MTT and CCK-8 assay. Apoptotic cells were estimated by Annexin V-FITC/PI staining and caspase-3 activity using the Caspase-3 Assay Kit; the PTPN21, cdk5, ERK1/2 and p-ERK1/2 levels were estimated by qRT-PCR and Western blot. We found that the PTPN21 markedly increased cell viability, inhibited apoptosis. We also found that PTPN21 inhibited caspase-3 activity and down-regulating the Bax/Bcl-2 ratio. Furthermore, the expression of cdk5 protein was up-regulated by PTPN21 by activating ERK1/2 signaling pathway. Finally, our results showed that cdk5 siRNA or ERK1/2 signaling inhibitor PD98059 attenuated the accelerative effect of pcDNA3.1 PTPN21(+) on cell proliferation and apoptosis in PC12 cells. In short, it appears that PTPN21 may protect the PC12 from ischemia injury by upregulating cdk5 via ERK1/2 signaling pathway.

1. Introduction

Hypoxic-ischemic is one of the leading causes of irreversible cerebral damage (Yoshizuka et al., 1996). Although the mechanism of cerebral injury resulting from hypoxic-ischemia is complex, neurons have been suggested as being one of the main targets (Manzanero et al., 2013; Baron et al., 2014). Increasing evidence suggests that the cell death noted during cerebral ischemia is mainly due to neuronal apoptosis, considered to result from a complex pathophysiological cascade. In fact, it is the predominant form of cerebellar damage caused by ischemia (Wang et al., 2014). At present, it has been well established that two general pathways of apoptosis, the intrinsic and extrinsic pathways are involved in ischemia-induced neuronal apoptosis (Franklin, 2011; Jordan et al., 2011). However, the precise mechanisms for inducing neuronal apoptosis and survival by ischemia have not been investigated thoroughly.

PTPN21 (protein tyrosine phosphatase, non-receptor type 21), also known as PTPD1, is a member of the protein tyrosine phosphatases (PTPs) super-family which catalyze the de-phosphorylation of phosphotyrosine. PTPs play an essential role in normal development and physiology and a higher expression level of PTPs by neural cells and have been implicated in various types of CNS disease (Hendriks et al., 2013). PTPN21 is a cytosolic non-receptor tyrosine phosphatase, which was first isolated from the human skeletal muscle in 1994 (Moller et al., 1994). While its function is still not fully understood, recent advances suggest that PTPN21 exerts its major effect on cell adhesion, growth, scattering, and migration (Carlucci et al., 2008, 2010; Roda-Navarro and Bastiaens, 2014). Lam et al. (Lam and Lin, 2001) reported that overexpression of PTPN21 reduced cell viability and induced cell apoptosis in carcinoma cells, whereas Plani-Lam et al. (2015) advocated that PTPN21 promoted neuronal survival. Thus, the role PTPN21 in cell growth remains controversial.

PTPN21 is critical in a variety of signaling cascades from many surface receptors in various cell types. Although the function of PTPN21 has not been fully investigated, this molecular entity has been shown to associate with or activate Src tyrosine kinase and Elk-1, stimulate the extracellular regulated protein kinases (ERK), ErbB4/NRG3 pathway (Cardone et al., 2004; Barr et al., 2009; Plani-Lam et al., 2015, 2016). ERK1/2 is one members of the MAPK family which is involved in cerebral protection. Activation of ERK1/2 plays a crucial role in neuronal death evoked by various stimuli such as nitric oxide, hypoxia/ischemia and hydrogen etc. (Cheng et al., 2010; Wang et al., 2013; Zakharova

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et al., 2014). Cyclin dependent kinase 5 (cdk5), which is actived primarily in differentiated cells like neurons, is a pivotal regulators in several important biological process like cell apoptosis and survival. Researchers have made it known that cdk5 is hyperactivated in several models of neuronal apoptosis and critical for neuronal survival.

In this study, our purpose is to investigate the role of PTPN21 in PC12 cells after oxygen-glucose deprivation (OGD). The protective effect of PTPN12 on cell proliferation and apoptosis after OGD injury was investigated. Subsequently, the possible mechanisms of PTPN21 protecting PC12 cells from OGD were explored.

2. Materials and methods

2.1. Cell culture

The PC12 cell line was purchased from the Cell Bank of Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Cells were plated in 96-well plate pre-coated with Poly-L-Lysine at a density of 5×10^4 cells/well and grown in high-glucose Dulbecco's Modified Eagle Medium (DMEM, 4.5 g/l glucose) supplemented with 10% fetal bovine serum (Gibco, Rockville, MD, USA), and penicillin/streptomycin (Sigma, St. Louis, MO, USA) at 37 °C under 5% CO2. After plating cells for 24 h, 50 ng/ml nerve growth factor (NGF; Promega, Madison, WI, USA) was added to the above culture medium and cultured for 7 days. The medium was replaced every second day. In all experiments, only NGF-stimulated PC12 cells were used.

2.2. Oxygen-glucose deprivation

Cells were exposed to oxygen glucose deprivation (OGD) as previously mentioned (Mo et al., 2012). Briefly, cells were washed twice with balanced salt solution and then exposed to OGD medium without glucose (in mM: NaCl 116, $CaCl_2$ 0.9, $MgSO_4$ 0.8, KCl 5.4, NaH_2PO_4 1, phenol red 110, pH 7.4). And then placed in an anaerobic chamber containing 94% N2 and 5% CO_2 for 4 h at 37 °C. OGD was terminated by adding 5 mM glucose to medium and transferred back to full culture medium with oxygen for 24 h. Control cells were cultured in the same buffer containing 5 mM glucose at 37 °C in a regular 5% CO_2 incubator.

2.3. Plasmids and siRNA transfection

PTPN21 siRNA was purchased from the Santa Cruz Biotechnology (Santa Cruz, CA, USA). For knocking down of PTPN21 and cdk5, PTPN21 siRNA and cdk5 siRNA were synthesized and annealed (Ambion Research, Shanghai, China). Control and PTPN21 siRNA or scramble were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturers recommended protocol. Then 48 h post transfection, cells were harvested and the degree of PTPN21 level was measured. For the over-expression of PTPN21, pcDNA 3.1(–) and PTPN21(+) (Invitrogen) were transfected by Lipofectamine 2000 (Invitrogen) as indicated by the manufacturer. At 48 h after transfection, cells were harvested and measured the expression level of PTPN21.

2.4. Quantitative real-time PCR (RT-qPCR)

RNA was isolated using Trizol Reagent (Invitrogen, USA)) according to the manufacturer's protocol. 2 μg RNA was reverse transcribed following the manufacturer's protocol of Omniscript RT kit (Qiagen, Valencia, CA, USA). RT-PCR was performed with SYBR® Premix Ex TaqTM (Takara, Japan). β-actin was used as endogenous control. The primers for PTPN21 was designed as follows: PTPN21 forward primer, 5′- CGGTGGGTAGATTTGGAAAA -3′, reverse primer, 5′-TGCTTCCATCTGTTTGTTGC-3′. Results were normalized to β-actin expression using the ΔΔC(t) method.

2.5. Western Blot assay

Cells were harvested and lysed with lysis buffer for 30 min on ice to extract the total protein from PC12 cells. The protein concentration thus obtained was measured using Bio-Rad Protein Assay reagent (Bio-Rad, Hercules, CA, USA). About 30 μg of protein were separated on 10% SDS-PAGE, and then transferred onto PVDF membrane (Millipore, Boston, MA, USA). The PVDF membrane was saturated in 5% skim milk for 4 h. Rabbit anti-PTPN21 antibody, rabbit anti-cdk5 antibody, rabbit anti-p-ERK1/2 and ERK1/2 antibody (Abcam, Cambridge, MA, UK) were incubated with membrane overnight at 4 °C. This was followed by 1 h incubation with the HRP-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology). The protein bands were visualized using the chemiluminescence-emanating ChemiDocTM MPImaging System (Bio-Rad, USA). The intensity of relative protein expression was measured by densitometry (Image J) and corrected for variable protein recovery using beta-actin protein measurement as a surrogate for total protein.

2.6. Annexin V-FITC/PI staining

Cell apoptosis after OGD was assessed using Annexin V fluorescein isothiocyanate (FITC)/apoptosis detection kit (Sigma, San Francisco, CA, USA). The cells were harvested after 24 h of OGD by trypsinization and re-suspended in a binding buffer. Cell suspension incubated with 5 μl annexin V-FITC and 10 μl propidium iodide for 15 min at 15–25 °C. The cells thus stained were analyzed via a BD FACS Calibur™ flow cytometer (BD Biosciences, Piscataway, NJ, USA).

2.7. CCK-8 assay

PC12 cells were planted in a 96-well cell culture plates with OGD injury for 24 h. Then CCK-8 regent come from Dojin Chemical Laboratory (Kumamoto, Japan) was added to each well and the culture cluster was settled at 37 °C incubation chamber for 2 h. Using a microplate reader (Bio-Rad) to detected the optical density (OD). Cell viability was expressed as a percentage of untreated controls. The experiment was repeated 3 times in triplicate.

2.8. MTT assay

PC12 cells were planted in a 96-well cell culture plates at a concentration of 1×10^4 cells/well in the OGD medium for 24 h. Then using a medium containing 0.5 mg/ml MTT to replaced the cell culture supernatants and cultured cells for another 4 h. The supernatants were removed and added 100 ml dimethyl sulfoxide. Absorbance reading was taken at 450 nm.

2.9. Caspase-3 activity assay

Caspase-3 activity was determined by a caspase-3 colorimetric protease assay kit (Abcam, Cambridge, MA, USA) according to the manufacturers recommended protocol. In brief, cells were suspended in lysis buffer and incubated on ice for 10 min. The reaction buffer and DEVD-AFC substrate were then added before being read with the following filters: excitation filter 400 nm and emission filter 505 nm.

2.10. Statistical analysis

Continuous variables were expressed as mean ± standard deviation (mean ± S.D.). Measurement data were analyzed using Student's *t*-test. P-values < 0.05 were considered to indicate statistically significant differences. All statistical analyses were performed using SPSS 18.0 statistical software (SPSS; Chicago, IL, USA).

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