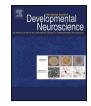
Contents lists available at ScienceDirect



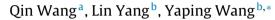
International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu



CrossMark

Enhanced differentiation of neural stem cells to neurons and promotion of neurite outgrowth by oxygen-glucose deprivation



^a Department of Otorhinolarynology, The Second Xiangya Hospital, Central South University, Changsha 410011, Hunan, China
^b Department of Anesthesiology, The Second Xiangya Hospital, Central South University, Changsha 410011, Hunan, China

ARTICLE INFO

Article history: Received 28 February 2015 Received in revised form 7 April 2015 Accepted 20 April 2015 Available online 22 April 2015

Keywords: Neural stem cells Oxygen–glucose deprivation Proliferation Differentiation

ABSTRACT

Stroke has become the leading cause of mortality worldwide. Hypoxic or ischemic insults are crucial factors mediating the neural damage in the brain tissue of stroke patients. Neural stem cells (NSCs) have been recognized as a promising tool for the treatment of ischemic stroke and other neurodegenerative diseases due to their inducible pluripotency. In this study, we aim to mimick the cerebral hypoxic-ischemic injury in vitro using oxygen-glucose deprivation (OGD) strategy, and evaluate the effects of OGD on the NSC's neural differentiation, as well as the differentiated neurite outgrowth. Our data showed that NSCs under the short-term 2 h OGD treatment are able to maintain cell viability and the capability to form neurospheres. Importantly, this moderate OGD treatment promotes NSC differentiation to neurons and enhances the performance of the mature neuronal networks, accompanying increased neurite outgrowth of differentiated neurons. However, long-term 6 h and 8 h OGD exposures in NSCs lead to decreased cell survival, reduced differentiation and diminished NSC-derived neurite outgrowth. The expressions of neuron-specific microtubule-associated protein 2 (MAP-2) and growth associated protein 43 (GAP-43) are increased by short-term OGD treatments but suppressed by long-term OGD. Overall, our results demonstrate that short-term OGD exposure in vitro induces differentiation of NSCs while maintaining their proliferation and survival, providing valuable insights of adopting NSC-based therapy for ischemic stroke and other neurodegenerative disorders.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Cerebral ischemia limits the supply of glucose and oxygen to the brain tissue, and is proposed as a causal factor for neuron degeneration and the onset of stroke and Alzheimer type of changes in aged brain (Kalluri et al., 2007; Lipton, 1999). Hypoxic environment imposes detrimental impact on cell survival. Activation of apoptotic pathway in brain neurons has been characterized as a fundamental process involved in neurodegenerative diseases (Friedlander, 2003). In animal models of stroke, DNA damage and structural features of apoptosis are frequently observed, whereas the inhibition of caspase, an important family of proteins mediating apoptosis, and the enhanced expression of anti-apoptotic proteins, Bcl-2 family can ameliorate the brain injury (Lawrence et al., 1996; MacManus et al., 1993). In an analysis of human cerebellum following global ischemia, a significant number of TUNEL (terminal dUTP nick end-labeling)-positive granule cells were observed, indi-

http://dx.doi.org/10.1016/j.ijdevneu.2015.04.009 0736-5748/© 2015 Elsevier Ltd. All rights reserved. cating elevated DNA fragmentation in ischemic stroke patient's brain (Huttner et al., 2014). Moreover, the level of caspase-3 that does not express in normal human cerebral neurons increases under the condition of ischemic degeneration (Krajewska et al., 1997).

Despite numerous investigations into the pathological mechanism of stroke, there are limited effective therapies available for stroke patients. Neural stem cells (NSCs) are self-renewing cell population residing in the central nervous system, possessing the capability to differentiate into multiple cell lineages in brain such as astrocytes, neurons and oligodendrocytes (Bachoo et al., 2002; Gage, 2000; Johansson et al., 1999). NSCs play important roles in neurogenesis after neurological injuries so that the endogenous and transplanted NSCs have recently been recognized as promising tools for the treatments of neurodegenerative disorders (Hicks et al., 2009). Cerebral ischemia is shown to activate NSCs, which participate in the subsequent neural regeneration (Macas et al., 2006). However, the low survival percentage and limited neural differentiation of NSCs hinder the extensive application of NSC-based therapy. Therefore, understanding of determinants for NSC's differentiation under the ischemic condition can lead to

^{*} Corresponding author. Tel.: +86 731 85295978; fax: +86 731 85295978. *E-mail address:* ypwang1234@163.com (Y. Wang).

possibilities for development of cell transplantation therapy after stroke (Hicks et al., 2008).

The current study investigates the synergistic impact of time-course oxygen-glucose withdrawal on NSCs' proliferation, differentiation and NSC-derived neurite outgrowth. We establish the culture of adult neural stem cells under the time-course OGD treatments as an in vitro ischemic model. How OGD treatments regulate NSC's cell viability, survival and differentiation, as well as differentiated neurite outgrowth were further examined. We found that mild OGD treatment retained NSCs' viability and proliferation, facilitated neural differentiation, and improved the performance and outgrowth of NSC-derived mature neurons. These findings might provide novel implications to the application of NSC in the regenerative medicine.

2. Materials and methods

2.1. NSCs culturing

NSCs were derived from both hemispheres hippocampus of postnatal day 1 ICR rat (Animal Center in Central South University). Hippocampus was removed from blood vessels and meninges to be collected in falcon tubes in Hank's balanced salt solution (HBSS) at 4°C, then rinsed with HBSS solutions for two times. After centrifugation (1000 r/min for 5 min), tissues were digested in TryplE (Life Technologies, USA) for 15 min at 37 °C, then gently triturated mechanically by using pipette tips to NSC suspension in DMEM-F12 medium containing 2% B-27. NSCs were cultured at 37 °C in a humidified incubator with 5% O₂. For proliferation and neuroshpere assays, EGF (20 ng/mL), FGF-2 (20 ng/mL) and heparin (5 ug/mL) were added into the neural stem cell basal media. For differentiation assays, the serum-free and growth factors-free basal medium was used. Media was changed half-weekly and NSCs were passed weekly. The NSCs were used for the experiments during passages 5-10 after isolation. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Central South. The IACUC committee members at Central South University approved this study. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. OGD treatment

To initiate OGD treatment, NSCs were cultured in 60 mm dish. Cells were then washed twice with PBS and immersed in glucose-free NBM-B27 media (Neurobasal glucose free media, Invitrogen, USA) before transferred into the sterile hypoxic humidified chamber, which maintains the atmosphere of 5% CO_2 in 95% N_2 . The chamber was incubated at 37 °C for different durations (2 h, 4 h, 6 h and 8 h) before the cells being used for experimental assays.

2.3. Immunostaining

Cells were washed with PBS, fixed in 4% paraformaldehyde for 45 min and then blocked and permeabilized for 90 min. Primary antibodies against β -tubulin (Sigma, USA) and MAP-2 (Abcam, USA) were incubated for 90 min followed by incubation of secondary antibody for 60 min. DAPI staining was then performed.

2.4. Calcium imaging

Standard solutions (contains 150 mM NaCl, 5 mM KCl, 2 mM CaCl, 1 mM MgCl₂, 10 mM HEPES, 10 mM D-glucose, pH 7.3) were used to wash cells. Cells were washed twice and then loaded with

2.5 μ mole Fluo-4-AM (Dojindo Laboratories, Japan) and pluronic F-127 (Sigma, USA). Cells were then incubated at 37 °C for 45 min. Fluo-4-AM was removed followed by extended incubation in standard solution for 25 min. Cells were then imaged with Carl Zeiss scanning confocal microscope. The excitation and emission wavelengths for Fluo-4-AM were 488 nm and 510 nm, respectively. The frequency of the oscillations was calculated as number of spikes per min. The relative fluorescence amplitudes of the calcium spikes Δ F/F was measured by normalizing the fluorescence for each cell to the average fluorescence intensity.

2.5. Western blot

Cell were washed with PBS and lysed in RIPA lysis buffer (20 mM Tris–HCl, pH 7.6, 150 mM NaCl, 1% Triton X-100 and 2 mM PMSF) with protease and phosphatase inhibitors, which were added upon use. Protein concentration was quantified by BCA assay system (Biorad, USA). For electrophoresis, 25 μ g of total protein extracts were loaded onto the gel. The following primary antibodies were used and incubated at 4 °C overnight, rabbit polyclonal MAP-2 antibody and mouse monoclonal GAP-43 antibody was from Santa Cruz Biotechnology. Caspase-9 and Bcl-2 primary antibodies were purchased from Sigma. Rabbit monoclonal GAPDH antibody was obtained from Cell Signaling. Image J was used to quantify the optical density of western blot results.

2.6. MTT assay

For the measurement of metabolic activity, 3-(4, 5-dimethyl-2-thiazoyl)-2, 5-diphenyltetrazolium bromide (MTT) was used according to manufacturer's instructions (Roche). The cells were incubated with 0.5 mg/mL MTT at the incubator with 5% O_2 at 37 °C, and incubated with lysis buffer overnight in the incubator. The optical density of solubilized formazan was measured at 570 nm on a plate reader (BioRad).

2.7. LDH assay

Neural stem cells were stored on ice in 0.5 mL of cold Assay Buffer. They were centrifuged at $10,000 \times g$ for 15 min at 4 °C and the supernatant was collected. Positive controls were diluted 1 in 9 in Assay buffer. 2–50 μ L of sample was added into a 96-well plate, which was topped up to 50 μ L with Assay buffer later. 0, 2, 4, 6, 8 and 10 μ L of the 1.25 mM NADH standard were added into 96-well plate, respectively, for generating NADH standard. 50 μ L of reaction mix (including 48 μ L of Assay Buffer and 2 μ L of Substrate Mix Solution) was added to each sample. OD_{450nm} at T1 and T2 were measured for further data analysis.

2.8. Statistical analysis

Results were collected as the average of at least five independent experiments. The significance between the experimental groups was analyzed by Kolmoorov–Smirnov (K–S) test. All the data were presented by the mean \pm SEM, *p < 0.05 and #p < 0.01.

3. Results

3.1. Effects of OGD treatment on NSC viability

After purified in vitro for at least 30 days after isolation, to confirm the neural stemness of the established NSC population, immunostaining was conducted with the antibody specifically targeted to nestin and SOX2, neural progenitor markers (Supplementary Fig. S1) (Rietze et al., 2001). The nuclei were counterstained by DAPI. We observed that the majority of the cell

Download English Version:

https://daneshyari.com/en/article/2785713

Download Persian Version:

https://daneshyari.com/article/2785713

Daneshyari.com