Neurobiology of Disease 43 (2011) 86–98

Contents lists available at ScienceDirect

Neurobiology of Disease

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

Adult neural precursors isolated from post mortem brain yield mostly neurons: An erythropoietin-dependent process

Giovanni Marfia [a], Laura Madaschi [a], Francesca Marra [a], Mauro Menarini [b], Daniele Bottai [a], Alessandro Formenti [c], Carmelo Bellardita [c], Anna Maria Di Giulio [a], Stephana Carelli [a,d], Alfredo Gorio [a,d,*]

[a] Laboratory of Pharmacology, Department of Medicine, Surgery and Dentistry, University of Milan, Polo H. San Paolo, via A. di Rudinì 8, Milan, Italy
[b] Ospedale Montecatone, via Montecatone 37, Imola, Bologna, Italy
[c] Department of Human Physiology, School of Medicine, University of Milan, via Mangiagalli 32, Italy
[d] Clinical Pharmacology, IRCCS Humanitas, via Manzoni 56, Rozzano, Milan, Italy

ARTICLE INFO

Article history:
Received 23 December 2010
Revised 2 February 2011
Accepted 7 February 2011
Available online 13 February 2011

Keywords:
Adult neural progenitors
Erythropoietin
Gene regulation
Hypoxia- inducible factor-1 (HIF-1)
mTOR
Neural plasticity

ABSTRACT

This study was aimed at the isolation of neural precursor cells (NPCs) capable of resisting to a prolonged ischemic insult as this may occur at the site of traumatic and ischemic CNS injuries. Adult mice were anesthetized and then killed by cervical dislocation. The cadavers were maintained at room temperature or at 4 °C for different time periods. Post mortem neural precursors (PM-NPCs) were isolated, grown *in vitro* and their differentiation capability was investigated by evaluating the expression of different neuronal markers. PM-NPCs differentiate mostly in neurons, show activation of hypoxia-inducible factor-1 and MAPK, and express both erythropoietin (EPO) and its receptor (EPO-R). The exposure of PM-NPCs to neutralizing antibodies to EPO or EPO-R dramatically reduced the extent of neuronal differentiation to about 11% of total PM-NPCs. The functionality of mTOR and MAPK is also required for the expression of the neuronal phenotype by PM-NPCs. These results suggest that PM-NPCs can be isolated from animal cadaver even several hours after death and their self-renewable capability is comparable to normal neural precursors. Differently, their ability to achieve a neural phenotype is superior to that of NPCs, and this is mediated by the activation of hypoxia-induced factor 1 and EPO signaling. PM-NPCs may represent good candidates for transplantation studies in animal models of neurodegenerative diseases.

Introduction

Traumatic lesions of the cord are characterized by a secondary process of degeneration, that is a complex condition of ischemia-like syndrome and neuroinflammation leading to a large expansion of the early mechanical damage (Bottai et al., 2008, 2010; Moreno-Manzano et al., 2009, 2010; Popovich et al., 2003; Ronaghi et al., 2010). A successful transplantation of neural precursors requires their survival in such an unfavorable environment. We have previously reported that neural precursor cells (NPCs), accumulate at spinal cord injury (SCI) site and improve the rate of hind limb functional recovery. Their viability in the injured spinal cord, however, lasts 12–20 days, then NPCs are phagocytated by macrophages (Bottai et al., 2008). A similar outcome was observed with embryonic stem cells (Bottai et al., 2010) In view of such results we aimed at isolating adult neural stem cells from the subventricular zone (SVZ) after a prolonged global ischemia. The isolation of ischemia-resistant neural precursors may supply cells with properties different from NPCs, and able to survive in the unfavorable environment of the traumatically injured central nervous system (Molcanyi et al., 2007; Popovich et al., 2003).

Oxygen is an important energy source for cell metabolism (Bruick, 2003), and its utilization is tightly regulated in the CNS (Hoge and Pike, 2001). Lower oxygen levels determine hypoxia, that affects cellular proliferation and differentiation, and may have salutary effects on neural precursors (Chen et al., 2007; Studer et al., 2000) and neurons (Moreno-Marzano et al., 2010; Zhu et al., 2005). For instance dopamine expression is induced by hypoxia in bone marrow-derived mesenchymal stem cells and P19 cells (Semenza, 2000; Wu et al., 2008). Hypoxia may also positively influence stem cell differentiation (Santilli et al., 2010). The cellular responses to hypoxia are manifested by activation of the hypoxia-inducible factor-1 (HIF-1), a transcriptional factor consisting of two subunits, the O_2-regulated HIF-1 α subunit and the O_2-independent HIF-1 β subunit. Under hypoxic conditions, HIF-1 α and β dimerize and migrate to the nucleus; this enhances the expression of several key target genes such as erythropoietin (EPO), vascular endothelial growth factor, tyrosine hydroxylase and OCT4, which acts as regulators of cellular proliferation and differentiation (Adelman and Simon, 2002; Ramírez-Bergeron and Simon, 2001; Richard et al., 1999).

Abbreviations: PM-NPCs, Post-Mortem Neural Precursor Cells; EPO, Erythropoietin; EPO-R, Erythropoietin Receptor; NPCs, Neural Precursor Cells; SCI, Spinal Cord Injury; SVZ, Sub Ventricular Zone.

* Corresponding author at: Laboratory of Pharmacology, Department of Medicine, Surgery and Dentistry, University of Milan, Polo H. San Paolo, via A di Rudinì 8, Milan, Italy. Fax: +39 0250323033.
E-mail address: alfredo.gorio@unimi.it (A. Gorio).

Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2011.02.004

Hypoxia also activates ERK1/2, which phosphorylates HIF-1α directly, thereby enhancing HIF-1α transcriptional activity (Berra et al., 2000; Minet et al., 2000). In this study we report that we were able to isolate from the SVZ adult neural stem cells resistant to the ischemic condition present at several hours after mouse death (6 and 16 h). These cells were called Post-Mortem Neural Precursor Cells (PM-NPCs). Their proliferation is similar to that of NPCs obtained at killing time (T0) (Gritti et al., 1996), while their differentiation yields about 33% β tubulin III- and 36% of MAP2-positive cells compared to 10–12% of NPCs. Differentiated PM-NPCs show higher HIF-1α activation, express both EPO and EPO-R, and active voltage-dependent Ca^{++} channels. Such a higher differentiation requires the functionality of mTOR and MAPK systems and is prevented by exposure to anti-EPO and anti-EPO-R antibodies. This study shows the existence of novel type EPO-dependent adult neural stem cells, that can be extracted from the brain of cadavers, and may represent a more successful approach to the treatment of traumatic CNS injuries.

Materials and methods

Ethics statement

All animals were handled in strict accordance with good animal practice as defined by the Italian Guidelines for Laboratory Animals which conform to the European Communities Directive of November 1986 (86/609/EEC), and all animal work was approved by the Review Committee of the University of Milan.

The animals were kept for at least 3 days before the experiments in standard conditions (22 ± 2 °C, 65% humidity, and artificial light between 08:00 a.m. to 08:00 p.m.).

Post-mortem neural precursor cells derivation

Adult CD-1 albino mice weighing 25–30 g (Charles River) were anesthetized by intraperitoneal injection of 4% chloral hydrate (0.1 ml/10 g body weight) and then killed by cervical dislocation. The cadavers were maintained at room temperature (25 °C) for 0, 6 and 16 h (T0, T6 and T16) or at 4 °C for 6 h (T6 4 °C) (Fig. 1A). Their brains were removed after the indicated periods and the area encompassing the SVZ surrounding the lateral wall of the forebrain ventricle was dissected using fine scissors. Primary cultures and cultivation of NPCs, their differentiation and immunostaining were performed as previously described (Bottai et al., 2008; Gritti et al., 1999).

The cells were plated at 3500 cells/cm^2 in the appropriate volume of the aforementioned medium in a 25 cm^2 flask at 37 °C in a humidified 5% CO_2 atmosphere.

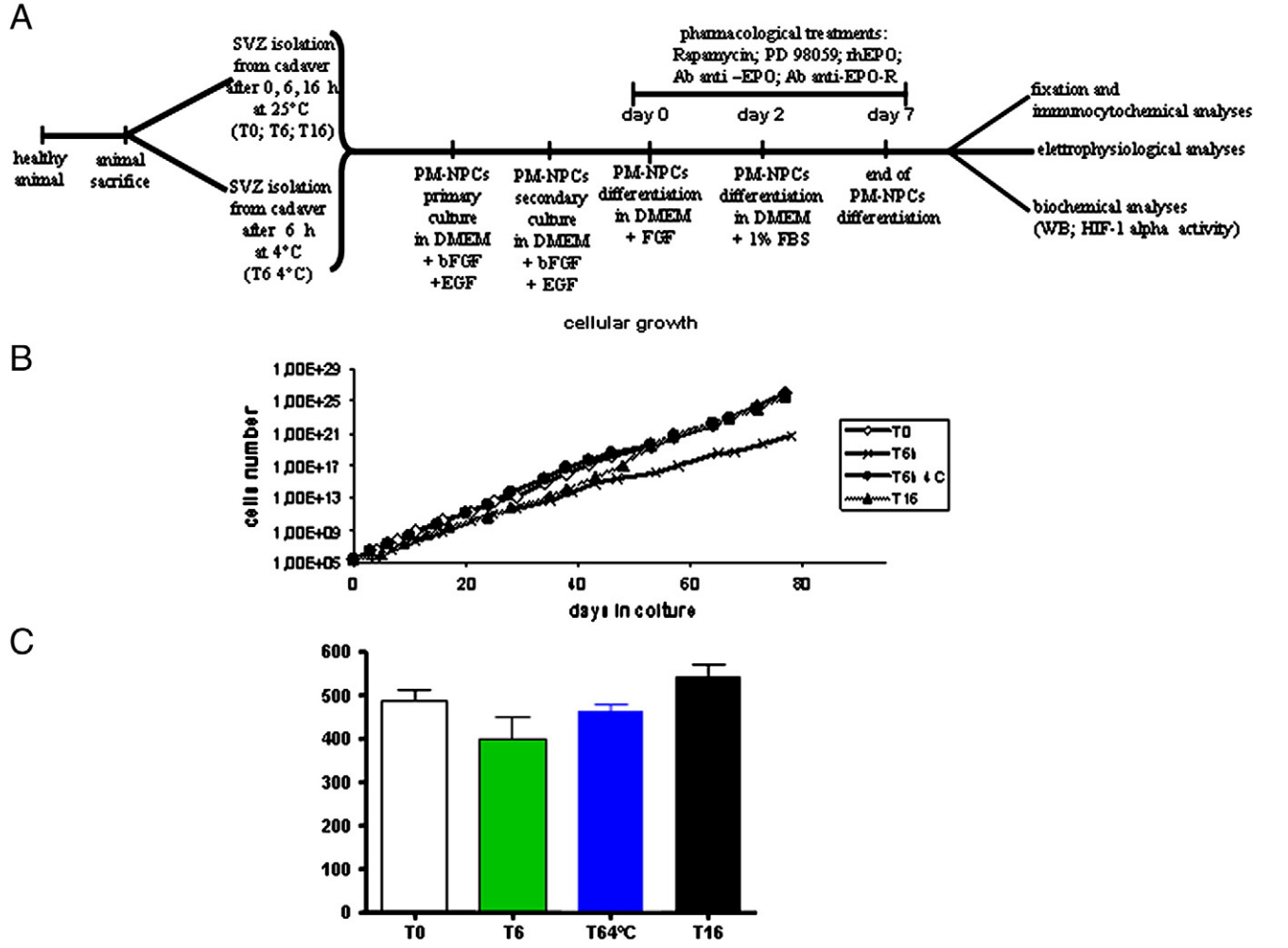

Fig. 1. Experimental scheme and PM-NPC perpetuation and population analysis. (A) Temporal scheme of experimental design illustrating the sequence of procedures. (B) The perpetuation and continuous growth properties of PM-NPCs are comparable of T0 NPCs. Cells were plated in growth medium at the density of 10,000 cells/cm^2 (Gritti et al., 1996, 1999). (C) PM-NPCs population analysis was performed by counting the number of neurospheres generated after 10 days of culture. It is represented the number of neurospheres generated by 3000 cells plated in a single well. The experiment was performed three times in triplicate for each cell sources and was repeated at passage 7, 15 and 30 without observing significant differences. Data are expressed as the mean of three independent experiments with similar results ± standard error.