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Research Report

Preconditioning in lowered oxygen enhances the therapeutic potential of human umbilical mesenchymal stem cells in a rat model of spinal cord injury

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ABSTRACT

Human umbilical cord mesenchymal stem cells (UCMSCs) have recently been shown to hold great therapeutic potential for the treatment of spinal cord injury (SCI). However, the number of engrafted cells has been shown to decrease dramatically post-transplantation. Physioxia is known to enhance the paracrine properties and immune modulation of stem cells, a notion that has been applied in many clinical settings. We therefore hypothesized that preconditioning of UCMSCs in physioxic environment would enhance the regenerative properties of these cells in the treatment of rat SCI. UCMSCs were pretreated with either atmospheric normoxia (21% O_2, N-UCMSC) or physioxia (5% O_2, P-UCMSC). The MSCs were characterized using flow cytometry, immunocytochemistry, and real-time polymerase chain reaction. Furthermore, 10^5 N-UCMSC or P-UCMSC were injected into the injured spinal cord immediately after SCI, and locomotor function as well as cellular, molecular and pathological changes were compared between the groups. We found that N-UCMSC and P-UCMSC displayed similar surface protein expression. P-UCMSC grew faster, while physioxia up-regulated the expression of trophic and growth factors, including hepatocyte growth factor (HGF), brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor(VEGF), in UCMSCs. Compared to N-UCMSC, treatment with P-UCMSC was associated with marked changes in the SCI environment, with a significant increase in axonal preservation and a decrease in the number of caspase-3+ cells and ED-1+ macrophages. These changes were accompanied by improved functional recovery. Thus, the present study indicated that preculturing UCMSCs under 5% lowered oxygen physioxic conditions prior to transplantation improves their therapeutic potential for the treatment of SCI in rats.

1. Introduction

Spinal cord injury (SCI) is one of the most devastating forms of trauma incurred by humans. SCI induces a cascade of secondary tissue damage that limits spontaneous neural tissue regeneration and the effectiveness of regenerative therapies (Rowland et al., 2008). SCI repair remains a major therapeutic challenge. Cell-based transplantation therapy has emerged as a promising strategy to improve the hostile environment of the lesion site, as well as functional recovery in cases of neurological disorder, including ischemic stroke, hemorrhagic stroke, traumatic brain injury (TBI), and spinal cord injury (Ahn et al., 2013; Sahni and Kessler, 2010; Zacharek et al., 2010). Among candidate stem cell populations for SCI transplantation, marrow mesenchymal stem cells (MSCs) are preferred because they are multi-potent and have the capacity to differentiate into various cell types, including chondrocytes, osteoblasts, neurons, and cardiomyocytes. MSCs are currently one of the most useful tools for application in tissue engineering and regenerative medicine (Lindenmair et al., 2012; Parekkadan and Milwid, 2010). Among MSCs used for transplantation, human umbilical cord mesenchymal stem cells (UCMSCs) are easier to isolate and expand, and exhibit greater proliferative activity (Gong et al., 2012). Furthermore, UCMSCs do not express class II human leukocyte antigen (HLA-DR), what could allow their penetration across full allogeneic MHC barriers; UCMSCs are not only able to differentiate into cells of mesodermal origin, but can also become neuroderm (Bicknese et al., 2002). These properties make UCMSCs less immunogenic and result in higher immunosuppression activity than bone marrow derived mesenchymal stromal cells

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(BMSCs) (Barcia et al., 2015). Our previous study demonstrated that UCMSC transplantation could significantly reduce the extent of astrocytic activation, thereby increasing axonal preservation and decreasing the number of caspase-3 + and ED-1 + cells after SCI (Zhilai et al., 2012). More recently, UCMSC-mediated spinal cord regeneration has been introduced into clinical medicine, and promising initial results have been achieved (Cheng et al., 2014).

However, as shown in previous studies, the vast majority of grafted cells are diminished 24 h after grafting into hypoxic sites (Amsalem et al., 2007; Nakamura et al., 2007; van der Bogt et al., 2009). Recently, various strategies have emerged to enhance the therapeutic potentials of MSCs. Several studies have shown that embryonic stem (ES) cells and BMSCs pretreated with physioxia are much more resistant to necrotic and apoptotic insult, have increased odds of survival in vitro and in ischemic tissue, and exhibit additional functional benefits after transplantation into the ischemic brain and heart (Theus et al., 2008; Wei et al., 2012; Yu et al., 2013). Drela demonstrated that a low-oxygen atmosphere promoted the proliferation of UCMSCs and maintained these cells in an undifferentiated state (Drela et al., 2014). However, whether physioxic preconditioning enhances UCMSC-based treatment of SCI has not been demonstrated.

In the present study, we sought to determine whether physioxic preconditioning of UCMSCs could increase trophic factor support, promote cell survival, decrease apoptosis, and inhibit the inflammatory cell infiltration necessary for tissue repair. In combination, these effects result in increased neurogenesis and improved functional recovery after SCI.

2. Results

2.1. Characterization of human UCMSCs in culture under 21% and 5% oxygen

Isolated UCMSCs exhibited fibroblast-like morphology after two passages in a standard 21% O_2 atmosphere. To evaluate the influence of a low-oxygen atmosphere on the morphology, immunophenotype and proliferation of UCMSCs in vitro, UCMSCs were exposed to a low oxygen concentration (5% O_2) for 24 h. These cells remained fully viable, similar to control UCMSCs passaged under air atmosphere conditions. Flow cytometric analysis revealed that growth in 5% O_2 did not change the phenotype of UCMSCs; both populations were negative for CD45 and HLA-DR ($\leq$2% positive) expression, and produced high levels of CD90 and CD44 ($\geq$98% positive) (Fig. 1B). Consistent with previous reports,

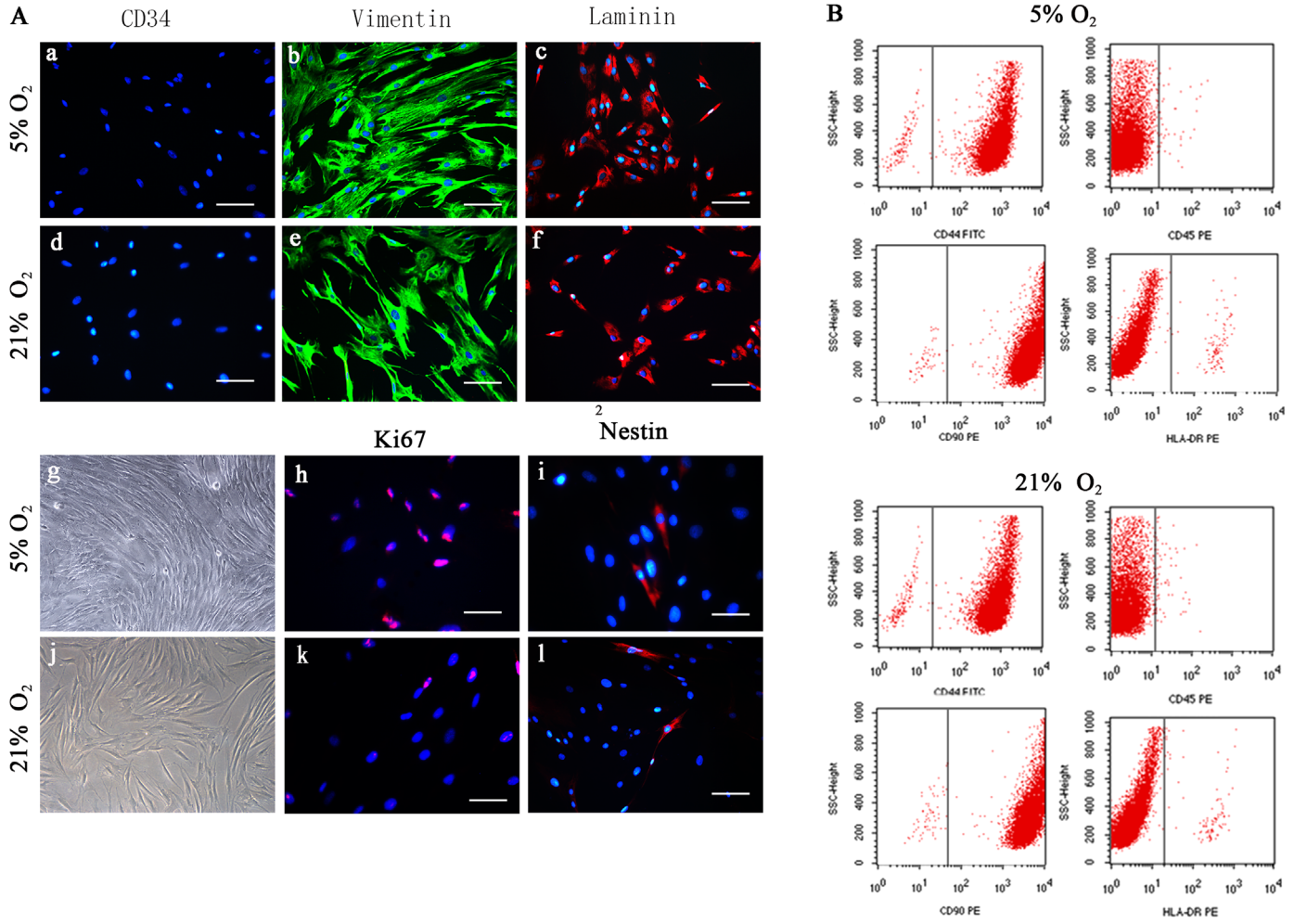

Fig. 1. (A) Characteristics of human UCMSCs cultured under standard (21% O_2) and lowered physiological (5% O_2) conditions. UCMSCs growing at different oxygen concentrations exhibited similar profiles of typical surface marker expression: negative for CD34 (a, d), while positive for Vimentin (green; b, e) and Laminin (green; c, f). The percentages of P-UCMSC and N-UCMSC expressing Ki67 were 78.45 ± 8.56% and 56.36 ± 6.28%, respectively ($p < 0.05$), while nestin was expressed in 12.32 ± 5.6% of P-UCMSC and 8.85 ± 4.2% of N-UCMSC ($p > 0.05$). (scale bars: 100 μm in a-f and l; scale bars: 50 μm in h, i and k). (B)Flow cytometry analysis of the expression of different markers in UCMSCs cultured under 5% and 21% O2 conditions. UCMSCs in both culture conditions were $CD44^+CD90^+CD45^-HLA\text{-}DR^-$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)