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Characterization of p75 neurotrophin receptor expression in human dental pulp stem cells

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ABSTRACT

Human adult dental pulp stem cells (DPSC) are a heterogeneous stem cell population, which are able to differentiate down neural, chondrocyte, osteocyte and adipocyte lineages. We studied the expression pattern of p75 neurotrophin receptors (p75NTR), a marker of neural stem cells, within human DPSC populations from eight donors. p75NTR are expressed at low levels (<10%) in DPSC. Importantly, $p75^+$ DPSC represent higher expression levels of SOX1 (neural precursor cell marker), SOX2 (cell pluripotency marker) and nestin (neural stem cell marker) in comparison to $p75^-$ DPSC. Our results suggest that $p75^+$ hDPSC may denote a subpopulation with greater neurogenic potential.
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1. Introduction

p75NTR is a well-conserved transmembrane proneurotrophin/neurotrophin receptor which plays critical roles in the maintenance of nerve cell viability. For instance, the interaction between p75NTR and sortilin leads to the formation of a complex capable of inducing neuronal death (Nykjaer et al., 2004, 2005). In contrast, the association of p75NTR to the tyrosine receptor kinases (Trk) promotes neuronal cell survival (Verdi et al., 1994; Culmsee et al., 2002). In the presence of sortilin, p75NTR binds to all pro-neurotrophins with high affinity, it also binds to all mature neurotrophins with low affinity. Neurotrophins that have been found to be associated with p75NTR include brain derived neurotrophin factor (BDNF), nerve growth factor (NGF), neurotrophin 3 (NT3) and neurotrophin 4/5 (NT4/5) (Hempstead, 2006). It should be noted that mature neurotrophin binding, or the absence of sortilin, may result in the activation of signaling pathways supporting cell survival via p75NTR in the absence of Trks (Dubanet et al., 2015; De la Cruz-Morcillo et al., 2016).

Besides its trophic function, p75NTR has been proposed to be a neural stem cell marker which defines a cell population with neurogenic potential in the adult brain subventricular zone (SVZ) (Young et al., 2007) and brain subgranular zone (SGZ) (Catts et al., 2008). SVZ cells with high p75NTR expression levels isolated using fluorescence activated cell sorting (FACS) have greater neurogenicity than SVZ cells moderately expressing p75NTR, whereas low p75NTR-expressing SVZ cells fail to generate cells of neural lineages (Young et al., 2007). Moreover, p75NTR knockout mice have demonstrated decreased neuroblast cell numbers in the SVZ as well as a reduction in the size of the hippocampus and dentate gyrus layer compared to their wild-type littermates (Young et al., 2007; Catts et al., 2008).

Nevertheless, prior to this study, there was no conclusive report indicating p75NTR as a reliable neural stem cell marker in other nervous tissues, some evidence indirectly suggests that p75NTR is associated with neuroblasts in the peripheral nervous system, such as the gut (Joseph et al., 2011; Becker et al., 2012) and dorsal root ganglia (DRG) (Rifkin et al., 2000; Li et al., 2007). p75NTR is also highly expressed in neural crest (NC) cells that give rise to peripheral nervous systems during neural development in the embryo,

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yet its expression is down-regulated in postnatal mature cells (Yiu and He, 2006; Wilson et al., 2004). Therefore, some of the p75NTR positive NC cells may remain present in mature nervous tissues and may be responsible for tissue regeneration at these sites.

Adult human dental pulp stem cells (DPSC), taken from the pulp of the third molar tooth, are multipotent (osteogenic, dentinogenic, adipogenic, chondrogenic and neurogenic) cells derived from NC cells (Abe et al., 2012) and possess neurogenic potential (Arthur et al., 2008; Kiraly et al., 2009). The use of DPSCs in transplantation has given many positive therapeutic outcomes when applied in animal disease models, especially in stroke. For instance, in the peri-infarct striatum, transplanted DPSCs are able to differentiate into astrocytes and neurons, though the preference depends on the proximity to the infarcted site (Leong et al., 2012). The neurogenic property of DPSC also promotes cerebral functional repair when used to treat the post-stroke brains in rats (Leong et al., 2012; Sugiyama et al., 2011), although the functional improvement may also have been resulted from DPSC-dependent paracrine effects (Leong et al., 2012). A previous study has found that only a small percentage (~2.3%) of engrafted DPSC survived in the infarcted brain, and the majority of them are differentiated into astrocytes and neurons (Leong et al., 2012). This evidence suggests that a small subpopulation of cells may exist within human DPSC populations, which represent greater neurogenic potential and ability to survive within the damaged brain. However, we cannot exclude other possibilities that may result in this survival characteristic, e.g. the intracerebral route of DPSC cell administration and/or anatomical site of injection. Identifying this population may aid in refining human DPSC therapy against neurological diseases, such as stroke.

Here in this study we characterized the expression of the neural stem cell marker p75NTP in human DPSC, with the aim of identifying a subpopulation with a greater potential to differentiate into cells of the neural lineage. We examined the expression levels of p75NTR, as well as other neural cell molecules such as the mesenchymal stem cell (MSC) marker CD146. We also assessed the co-expression of p75NTR with other neural stem cell markers, including nestin, SOX1, SOX2 and SOX9. We have found that $p75NTR^{+}$ DPSC coincide with high percentage of cells expressing neural stem cell markers mentioned above, which suggests that p75NTR can serve as a reliable marker of a small cell sub-population with high neurogenic potential within the total DPSC population.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents were purchased from Sigma-Aldrich unless otherwise specified.

2.2. Cell culture

BSRc is a control BSR (a clone of baby hamster kidney fibroblasts) cell line without p75NTR expression, as described in (Rogers et al., 2006). SH-SY5Y is a human neuroblastoma cell line. BSRc and SH-SY5Y cells were cultured in high glucose (4.5 g/L) Dulbecco's modified Eagle medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS, Bovogen Biologicals) and 1% penicillin-streptomycin-glutamine (Life Technologies), under standard conditions (37 °C, 100% humidity, 5% CO_2). Cells were passaged twice per week into new flasks to avoid intimate cell contact.

2.3. Human DPSC culture

Human DPSC were isolated from adult impacted molars as described previously (Arthur et al., 2008). In order to obtain a comprehensive p75NTR expression profile of human DPSC, several cell populations from eight different individuals were used in this study, and these are noted as: DPSC1, DPSC2, DPSC3, DPSC4, DPSC5, DPSC6, DPSC7 and DPSC8. Cells were cryo-preserved in dimethyl sulfoxide (10% v/v) and FBS (Bovogen Biologicals), (90% v/v) and thawed for subsequent experiments in a-modified Eagle's medium supplemented with 20% (v/v) (FBS), 100 μM L-ascorbic acid 2-phosphate (Novachem), 2 mM L-glutamine (GibcoBRL), 100U/ml penicillin (GibcoBRL) and 100 μg/ml streptomycin (GibcoBRL), under standard incubation conditions (37 °C, 100% humidity, 5% CO_2). Sub-confluent colonies were trypsinized (0.025% trypsin, 1 × EDTA in phosphate buffered saline (PBS), GibcoBRL), centrifuged (200 × g, 2 min) and passaged.

2.4. Immunofluorescence

Human DPSC were sub-cultured onto poly-L-lysine (10 μg/ml) and laminin (10 μg/ml, GibcoBRL) coated round cover slips (13 mm in diameter). Cultured cells were fixed with 4% (w/v) paraformaldehyde in PBS for 15 minutes, blocked and permeablized in blocking buffer (0.2% (v/v) Triton X-100, 0.5% (v/v) donkey serum, 2% (w/v) bovine serum albumin in PBS) for 30 minutes, incubated overnight with mouse anti-p75 mlr2 IgG primary antibody (1:750, Rogers et al., 2006) in blocking buffer, washed with PBS and blocking buffer, followed by the incubation with Alexa Fluor 647 anti-mouse IgG (1:200, Molecular Probes) as the secondary antibody for two hours. For double immunofluorescence staining, rabbit anti-nestin (1: 250, Abcam), rabbit anti-SOX1 (1:300, Millipore), rabbit anti-SOX2 (1:1000, Chemicon) and rabbit anti-SOX9 (1:200, Sigma Aldrich) were also applied after p75 staining, anti-GFAP (glial fibrillary acidic protein, 1:250) was purchased from Dako (Cat. No. Z0334). Cy3 anti-rabbit IgG (1:400, Jackson ImmunoResearch) were used as the secondary antibody to identify bound primary antibodies. All coverslips were mounted with Prolong Gold antifade reagent (Invitrogen).

2.5. Microscopy and image analysis

Images were taken in five representative fields using a TCS SP5 spectral scanning confocal microscope (Leica microsystems, Wetzlar, Germany) or an Olympus BX51 microscope epifluorescence microscope (Olympus innovation, USA). Images were processed and analysed, ensuring at least 500 cells in total were counted using imageJ (http://imagej.nih.gov/ij/).

2.6. Flow cytometry

Flow cytometry analysis was performed as previously described (Rogers et al., 2010). Antibody concentrations: anti-p75NTR Alexa Fluor 647 (50 μg/ml, BD pharmingen); anti-CD146 phycoerythrin (PE) (1:5, BD pharmingen); anti-nestin PE (1:100, R&D system); anti-MHCI (major histocompatibility complex class I) PE (1:5, BD pharmingenn) and anti-MHCII PE (1:5, BD pharmingen). For single colour staining, cells were incubated with anti-p75NTR, anti-MHCI, anti-MHCII for 30 min in the dark at 4 °C. For double colour anti-p75NTR and anti-CD146 staining, cells were washed twice with PBS and then incubated with anti-CD146 for 30 min in the dark at 4 °C, washed twice with PBS followed by further incubation with anti-p75NTR for 30 min in the dark at 4 °C. For double colour anti-p75NTR and anti-nestin staining, cells were first incubated with anti-p75NTR, cells were washed twice with PBS and then incubated with anti-CD146 for 30 min in the dark at 4 °C. Cell were washed again twice with PBS followed by further incubation with anti-nestin for 30 min in the dark at 4 °C. At the end of the incubation period, cells were fixed upon the addition of 4% paraformaldehyde for 15 min and then permeabilised with SAP buffer (0.1% (w/v) saponin, 0.05% (w/v) sodium azide in Hank's balanced salt solu-

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