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Human Wharton's jelly cells can be induced to differentiate into growth factor-secreting oligodendrocyte progenitor-like cells

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ABSTRACT

Human Wharton's jelly-derived mesenchymal stromal cells (hWJ-MSCs) are capable of differentiating into neural and astroglia-like cell types. However, a reliable means of inducing the selective differentiation of hWJ-MSCs into oligodendrocyte progenitor cells (OPCs) *in vitro* has not yet been established. In this study, the OPC-like differentiation of hWJ-MSCs was characterized using and immunoblotting. The hWJ-MSC-derived OPC-like cells were able to secrete nerve growth factors and promote neurite outgrowth *in vitro*. These results show that hWJ-MSCs can be induced to differentiate into cells with the morphologic, phenotypic and functional characteristics of OPC-like cells.

1. Introduction

Difficulties in regenerating lost cells, replacing damaged myelin, and re-establishing functional neural connections hinder the regenerative ability of the injured mammalian central nervous system (CNS) (Liu et al., 2000). Demyelination of intact axons and disruption of action potential propagation resulting from the death of oligodendrocytes are important factors contributing to the loss of function (Liu et al., 2000; Keirstead et al., 2005). Hence, remyelination of otherwise intact axons through transplantation of myelin-producing cells could enhance action potential conduction and restore functional deficits. Embryonic stem cells (ESCs), which are able to expand and differentiate into oligodendrocyte precursor cells (OPCs) or oligodendrocytes *in vitro* and *in vivo*, provide a potentially unlimited source of cells suitable for such transplantation therapy (Louro and Pearse, 2008). However, ethical considerations limit the practical use of ESCs and many researchers have therefore begun to explore the beneficial effects of adult stem cells. As one kind of adult stem cells, human bone-marrow-derived mesenchymal stem cells (hBM-MSCs) have been intensively studied, but harvesting these cells involves a highly invasive procedure (Kingham et al., 2007). Furthermore, the number, differentiation potential and frequency of MSCs derived from bone marrow decline with increasing age (Kern et al., 2006). Recently, human MSCs isolated from Wharton's jelly from the umbilical cord have been identified as an alternative source of pluripotent stromal cells. They can be easily obtained and proliferate rapidly in culture. Like other MSCs, Wharton's jelly-derived MSCs (hWJ-MSCs) are immunologically compatible and amenable to stable transfection (Wang et al., 2004). More importantly, hWJ-MSCs are isolated from fetal structures during the perinatal period and, like umbilical cord blood, could be better tolerated following transplantation, resulting in a lower incidence of graft versus host disease (Troyer and Weiss, 2008; Wang et al., 2004).

hWJ-MSCs can be induced to differentiate into osteogenic, chondrogenic, adipogenic, and myogenic cells in culture (Wang et al., 2004). Moreover, recent studies have shown that hWJ-MSCs are able to break germ layer commitment and differentiate into cells expressing neural and astroglia-like properties (Fu et al., 2004). However, it is difficult to induce them to differentiate into oligodendrocytes, and the majority of transdifferentiation studies using MSCs have focused on neurons, rather than oligodendrocytes (Kennea et al., 2009; Fujimura et al., 2005; Sanchez-Ramos et al., 2000). No reliable means of selectively inducing the differentiation of hWJ-MSCs into OPCs or oligodendrocytes currently exist, under either *in vitro* or *in vivo* conditions.

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Here, we present the first report showing that hWJ-MSCs can be induced to selectively differentiate into cells with the morphologic and immunophenotypic characteristics of OPC-like cells under culture conditions. We also demonstrated that the induced OPC-like cells were able to secrete neurotrophic factors and promote neurite outgrowth *in vitro*.

2. Materials and methods

2.1. Preparation of hWJ-MSCs

hWJ-MSCs for this study were a gift from Health & Biotech Company (Guangdong, Guangzhou, China). Umbilical cords (n=24; gestational age, 39–40 weeks) were obtained from local maternity hospitals after normal deliveries. Fresh human umbilical cords were obtained after birth with the written consent of the parents, and were collected in Hanks' balanced salt solution (HBSS) (Gibco, USA) at 4 °C. Following disinfection in 75% ethanol for 30 s, the umbilical cord vessels were removed while still in HBSS. The mesenchymal tissue (in Wharton's jelly) was then diced into cubes of about 0.5–1 cm^3 and centrifuged at 1200 rpm for 5 min. Following removal of the supernatant fraction, the precipitate (mesenchymal tissue) was washed with serum-free DMEM/F12 (Gibco) and centrifuged at 1000 rpm for 5 min. The precipitate was then enzymatically dissociated for 30 min at 37 °C using 0.075% collagenase type II (Sigma, St. Louis, MO, USA), and further digested with 0.125% trypsin/EDTA (Gibco) at 37 °C for 30 min. The suspension was neutralized with 10% (v/v) fetal bovine serum (FBS, Gibco) and cells were counted under a microscope with the aid of a hemocytometer. Cells were washed three times with phosphate-buffered saline (PBS) and then plated into uncoated plastic 25-cm^2 flasks (Corning, Glendale, Arizona, USA) in DMEM/F12 (1:1) (Gibco) supplemented with 10% FBS (HyClone, Logan, UT, USA), 100 U/ml penicillin–streptomycin (Sigma), and 1% glutamine (Sigma). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and passaged at 60–80% confluency. Cells of passage four to six were used in this study.

2.2. Conversion of hWJ-MSCs into nestin-positive hWJ-MSCs

To obtained nestin-positive cells, hWJ-MSCs were dissociated in 0.125% trypsin/EDTA, and 1×10^6 cells/ml were then plated in DMEM/F12 supplemented with 10 ng/ml epidermal growth factor (EGF, R&D, Minneapolis, MN, USA) and N2 (1:100, Invitrogen, USA) for 3 days. Immunocytochemical staining was used to detect the percentage of nestin-positive cells.

2.3. Conversion of nestin-positive hWJ-MSCs into neurospheres

Nestin-positive hWJ-MSCs were initially dissociated in 0.125% trypsin/EDTA. Then 1×10^5 cells/ml dissociated nestin-positive hWJ-MSCs were plated into low-attachment plastic 25-cm^2 flasks in neural stem cell medium (NSC medium), composed of neurobasal medium (NB medium, Invitrogen), 20 ng/ml EGF (Peprotech, UK), 20 ng/ml basic fibroblast growth factor (bFGF, Peprotech) and B27 (1:50, Gibco) at 37 °C in 5% CO_2. Fresh bFGF and EGF were added every 3–4 days and the NSC medium was renewed once a week. Four to five days post-differentiation in NSC medium, sphere formation could be observed. The neurospheres were passaged by mechanical trituration when their average diameters reached 100 μm. The neurospheres were expanded for an additional 4–6 weeks (3–4 passages) before the next step in the differentiation protocol was started.

2.4. Conversion of neurospheres into OPC-like cells

The procedures in this step were performed according to the previously described protocol (Fu et al., 2007). Firstly, hWJ-MSC-derived neurospheres were seeded at a concentration of 1×10^5 cells/ml in NSC medium supplemented with a reduced dose (10 ng/ml) of bFGF/EGF to keep the neurospheres small. The cultures were fed every 3 days by replacing half of the NSC medium with the same volume of fresh OPC medium, composed of NB medium, 10 ng/ml platelet-derived growth factor (PDGF)-AA (Sigma), 10 ng/ml bFGF (Sigma) and sonic hedgehog (shh, 100 ng/ml, Sigma). Six days later, the medium was all replaced with fresh OPC medium. After 4 days in OPC medium, many cells with bipolar or tripolar processes showing the morphological characteristics of OPCs were seen migrating out from the neurospheres. The cultures were passaged when the cells reached 90% confluence.

2.5. Differentiation of OPC-like cells

To induce OPC differentiation, we triturated the OPCs and replated them on poly-L-lysine- and laminin (Sigma) double-coated coverslips at 2×10^4 cells/coverslip. For oligodendrocyte differentiation, the OPC medium was replaced by DMEM/F12 supplemented with 3% FBS, and 15 nM F3. The OPCs were allowed to differentiate for 3 days.

As a positive control, we investigated the differentiation ability of hESCs (H1 line of hESC) towards oligodendroglia lineage using the same protocol.

2.6. Immunocytochemistry

The immunocytochemistry procedure has been described previously (Zhang et al., 2009). The primary antibodies used were mouse anti-O4 antibody (1:400), and rabbit anti-PDGF receptor (PDGFR) antibody (1:400) (both from R&D), mouse anti-fibronectin antibody (1:800), mouse anti-nestin antibody (1:800) (both from Chemicon, Billerica, MA, USA), and rabbit anti-MBP antibody (Sigma, 1:600). The secondary antibodies used were Alexa Fluor 594 goat anti-rabbit (1:200) IgG and Alexa Fluor 488 goat anti-mouse IgG (1:200) (both from Molecular Probes, Eugene, Oregon, USA). The nuclei were counterstained with Hoechst 33342 (Invitrogen). For negative controls, the primary antibodies were replaced with normal goat serum or cells were stained without secondary antibody. No specific positive staining was detected in either case. Samples were examined using a fluorescence microscope and an IM50 imaging system (Vertrieb, Deutschland, Leica, Germany). The positively stained cells were counted in 30 random fields (× 200). All immunocytochemical experiments were repeated twice in eight independent experiments.

2.7. Western blot

For Western blot analysis, cells were washed with PBS and then lysed with RIPA buffer. Protein concentration was determined using the Bradford method. Equal amounts (50 μg) of total cellular protein were denatured at 100 °C for 5 min. After gel electrophoresis and electrophoretic transfer onto PVDF membranes (Bio-Rad, 1000 Alfred Nobel Drive Hercules, CA, USA), membranes were blocked with skimmed milk for 1 h, and incubated with primary antibodies including anti-nestin, anti-fibronectin, anti-PDGFR, anti-O4 antibodies (all from Chemicon) and anti-MBP (Sigma) overnight at 4 °C. Antibody binding was visualized using peroxidase-conjugated secondary antibodies, and the blots were developed using an enhanced chemiluminescence method. β-actin served as an internal protein control. All Western blot experiments were repeated at least three times.