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Mesenchymal stromal cells from umbilical cord Wharton's jelly trigger oligodendroglial differentiation in neural progenitor cells through cell-to-cell contact

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Abstract

Background aims. Wharton's jelly mesenchymal stromal cells (WJ-MSCs) might be ideal candidates to treat perinatal brain damage. Their secretome has been shown to have beneficial effects on neuroregeneration, in part through interaction with neural progenitor cells (NPCs). However, it remains unclear whether cell-to-cell contact decisively contributes to this positive effect. The objective of this study was to elucidate the mechanism through which differentiation in NPCs is triggered after exposure to WJ-MSCs. Furthermore, given that WJ-MSCs can be derived from term (tWJ-MSCs) or preterm (ptWJ-MSCs) deliveries and that WJ-MSCs might be used for transplantations independent of gestational age, the influence of tWJ-MSCs versus ptWJ-MSCs on the differentiation capacities of NPCs was studied. *Methods.* The effect of tWJ-MSCs and ptWJ-MSCs on the expression of neuroglial markers in NPCs was assessed in co-culture (CC), conditioned medium (CM) or transwell CC experiments by immunocytochemistry, real-time polymerase chain reaction and Western blot. Additionally, mass spectrometry was used to study their secretomes. *Results.* NPCs showed an increased expression of glial markers after CC with WJ-MSCs or exposure to WJ-MSC-CMs. CC had a more prominent effect on the expression of glial markers compared with CM or transwell CCs. tWJ-MSCs more strongly induced the expression of mature oligodendroglial markers compared with ptWJ-MSCs. A possible role in enhancing this maturation could be attributed to the laminin α2-subunit. *Conclusions.* Cell-to-cell contact between WJ-MSCs and NPCs induces oligodendrogenesis on NPCs, whereas trophic factor secretion is sufficient to promote astrogenesis. Thus, transplanting WJ-MSCs may promote endogenous neuroregeneration in perinatal brain damage.

Key Words: *mesenchymal stromal cells, neural progenitor cells, neuroregeneration, oligodendrogenesis, Wharton's jelly*

Introduction

Worldwide, 11% of all neonates are born preterm (<37 weeks' gestational age). Complications from preterm birth are the second most common cause of death in children under 5 years of age and the most important cause of long-term disability. Many survivors of early preterm birth suffer from necrotizing enterocolitis, bronchopulmonary dysplasia, hearing loss, partial loss of vision or brain damage. Brain damage includes major motor deficits such as cerebral palsy but also cognitive and behavioral abnormalities [1,2]. The underlying cause of perinatal brain damage is white matter brain injury (WMI), which is characterized by a loss of oligodendrocytes as well as oligodendrocyte progenitor cells leading to an overall hypomyelination of the brain [3].

To date, there is no cure for WMI. Stem cell therapy might be a promising therapeutic approach that could be effective by either directly replacing lost cells of the oligodendrocyte lineage or by stimulating endogenous neural progenitor cells (NPCs) to adopt a neuroprotective or neuroregenerative function [4,5]. NPCs are multipotent cells of the brain that are able to differentiate both *in vitro* and *in vivo* into all cell lineages of the brain. Moreover, in the case of brain

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injury, NPCs migrate toward the site of injury and support the brain's regenerative capacities by secreting trophic factors or replenishing the lost cells [6,7].

Mesenchymal stromal cells (MSCs) derived from the Wharton's jelly (WJ) of the umbilical cord represent an appropriate stem cell graft for the future treatment of WMI. WJ-MSCs are easily collectable, immunomodulatory, non-tumorigenic and known to secrete proangiogenic as well as neuroprotective factors [8–10]. Importantly, it has been shown that MSCs are neuroprotective by stimulating the proliferation and differentiation of NPCs [11,12]. However, little is known about the mechanisms through which MSCs and NPCs interact and how MSCs can induce differentiation in NPCs. It has been suggested, however, that many of the beneficial effects of MSCs are mediated by factors they secrete [12].

Cord tissue and placenta are in a close relationship with maternal metabolic conditions. Diseases during pregnancy might therefore affect the properties of these tissues and subsequently the ones of the cells they harbor [13,14]. It is barely known how pregnancy complications affect the properties of WJ-MSCs [15]. However, from MSCs derived from other tissues, it is known that the age of the donor highly affects their self-renewal capacities [16]. Moreover, MSCs from different tissues secrete different factors and respond differently when transplanted into the sites of injury [8]. Thus, WJ-MSCs derived from preterm deliveries might be substantially different from those derived from healthy term deliveries. Given that WJ-MSCs would be used for transplantation independent of gestational age, it is crucial to understand the properties of WJ-MSCs from term and preterm birth.

In this work, we investigated the effects of WJ-MSCs derived from term and preterm deliveries on the differentiation of NPCs *in vitro*. In addition, the importance of trophic factors versus direct cell-to-cell contact for the induction of changes in NPCs was studied.

Methods

Isolation and primary culture of human WJ-MSCs

After informed consent, umbilical cords from healthy term (>37 weeks' gestational age) and preterm deliveries (27–36 weeks) were collected. Preterm birth was either idiopathic or due to twin pregnancy. The study was approved by the institutional review board of the Bern University Hospital and the Canton of Bern.

The WJ-MSCs were isolated from the umbilical cords as described previously [17]. Briefly, the umbilical cords were disinfected in 70% ethanol, vessels and amnion were removed and the cord chopped into small fragments and digested for 3 h in 270 U/mL collagenase II (Worthington Biochemical Corporation) at 37°C/5% CO_2. The cells were expanded in Dulbecco's Modified Eagle Medium (DMEM)/F12 supplemented with 10% fetal calf serum (FCS), 2 mmol/L GlutaMAX, 100 units/mL penicillin and 100 mg/mL streptomycin (Thermo Fisher Scientific). WJ-MSCs were positive for CD105, CD73 and CD90 and negative for myeloid and hematopoietic cell lineage-specific antigens (CD19, CD45, CD34, and HLA-DR) and were able to differentiate into osteocytes, chondrocytes and adipocytes as described previously [17].

Direct co-culture of human WJ-MSCs and rat NPCs

WJ-MSCs derived from either term (tWJ-MSCs; n = 6; passage 5 p5.) or preterm (ptWJ-MSCs; n = 6; p5) deliveries were seeded at a density of 1000 cells/cm^2 in DMEM/F12 containing 10% FCS on poly-L-ornithine-coated (10 µg/mL, Sigma-Aldrich) and laminin-coated (6 µg/mL, Millipore) dishes. After 24 h, cells were washed with phosphate-buffered saline (PBS) before rat NPCs (p3, Millipore) were added at a density of 5000 cells/cm^2 and left for 96 h in rat neural stem cell basal medium (NB, Millipore) supplemented with basic fibroblast growth factor (FGF-b, 10 ng/mL, Millipore). After 2 days, fresh FGF-b was added to all cultures. As a control, NPCs alone were plated in identical conditions. For subsequent immunocytochemistry experiments, WJ-MSCs were stained with 2 µg/mL chloromethyl-DiI (CM-DiI), a red fluorescent dye that stains cell membranes and lipids (Thermo Fisher Scientific) before co-culture (CC) with NPCs. For Western blot analysis, NPCs were isolated after 4 days of CC by negative selection magnetic cell separation using human anti-CD90 MicroBeads (Miltenyi Biotec) against WJ-MSCs according to the manufacturer's instructions.

Generation of human WJ-MSC conditioned medium

tWJ-MSCs and ptWJ-MSCs were left to grow overnight in DMEM/F12 containing 10% FCS at a density of 10.6×10^3 cells/cm^2. The next day, cells were washed with PBS, and the medium was replaced by DMEM supplemented with 4.5 g/L D-Glucose (Thermo Fisher Scientific), 2 mmol/L GlutaMAX, and 110 mg/L pyruvate (Thermo Fisher Scientific). Conditioned medium (CM) was collected after 24 h and frozen at –80°C until use.

Culture of rat NPCs with human WJ-MSC CM

To assess the effect of the CM on NPCs, the latter were seeded at a density of 5000 cells/cm^2 on poly-L-ornithine and laminin coated dishes in NB medium supplemented with 10 ng/mL FGF-b. After 24 h, NPCs were washed with 1 × PBS, and cultured in 30%

CM and 70% NB medium supplemented with FGF-b for 96 h. After 2 days, fresh FGF-b was added to all cultures. As a control, NPCs were cultured in 30% DMEM supplemented with 2 mmol/L GlutaMAX and 110 mg/L pyruvate, and 70% NB medium containing 10 ng/ml FGF-b for 96 h.

Indirect transwell co-culture of human WJ-MSCs and rat NPCs

To avoid direct contact between WJ-MSCs and NPCs but having a continuous exchange of trophic factors during CC (96 h), a transwell (TW) CC system was established. One day before starting the TW CC, WJ-MSCs were seeded at a density of 1000 cells/cm^2 in DMEM/F12 containing 10% FCS on poly-L-ornithine- and laminin-coated membrane inserts (Thermo Fisher Scientific, 0.4-μm pores). In parallel, NPCs were seeded on poly-L-ornithine and laminin coated six-well plates at a density of 5000 cells/cm^2 in NB medium supplemented with 10 ng/mL FGF-b. The next day, both cell types were washed with 1 × PBS. Fresh NB medium supplemented with FGF-b was added to the six-well plates. The inserts containing WJ-MSCs were placed above the NPC cultures, and NB medium supplemented with FGF-b was also added to the inserts. TW CCs were incubated for 96 h. After 2 days, fresh FGF-b was added to all cultures. As a control, empty poly-L-ornithine and laminin coated inserts were placed above NPC cultures for 4 days.

Immunocytochemistry

After 4 days of direct CC, the cells in chamber slides were fixed with ice-cold methanol (Sigma), and blocked with 1 × PBS containing 1% bovine serum albumin (Sigma) and 0.25% Triton X 100 (Sigma). Fixed cells were stained with a rabbit polyclonal antibody against rat myelin basic protein (MBP, 1:200; Millipore), a mouse monoclonal antibody against rat glial fibrillary acidic protein (GFAP, 1:1000; Millipore), and a rabbit polyclonal antibody against microtubule-associated protein 2 (MAP2, 1:200; Abcam), followed by the detection with an anti-rabbit or anti-mouse immunoglobulin G Alexa Fluor 488 antibody (1:200, Thermo Fisher Scientific). Nuclei were counterstained with 4-6-diamidino-2-phenylindole-dihydrochloride (Sigma). Images were examined with a DM6000 B microscope (Leica Microsystems).

RNA and protein isolation

RNA and protein were isolated using the QIAshredder and the AllPrep DNA/RNA/Protein Mini Kit (Qiagen). The concentration of RNA was measured by Nanodrop spectrometry (Thermo Fisher Scientific). RNA quality was determined with the optical density 260/230 nm ratio, which was between 1.8 and 2.1. Total protein concentration was determined with the bicinchoninic acid protein assay kit (Sigma).

Reverse transcription and real-time polymerase chain reaction

Up to 5 μg of RNA were reverse transcribed using the SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific).

The gene expression of *Map-2* (TaqMan gene expression GE. assay ID: Rn00565046_m1), *Gfap* (TaqMan GE assay ID: Rn01460869_g1), *Mbp* (TaqMan GE assay ID: Rn00690431_m1), galactocerebroside (*GalC*, TaqMan GE assay ID: Rn01517759_m1), chondroitin sulfate proteoglycan 4 (*Cspg4*, TaqMan GE assay ID: Rn00578849_m1) and platelet-derived growth factor receptor alpha (*Pdgfra*, TaqMan GE assay ID: Rn01417534_g1; all Thermo Fisher Scientific) was assessed by real-time polymerase chain reaction (PCR) in NPCs alone as well as in NPCs in CM, after direct CC and TW CC. The PCR was run with the following program on a 7300 Real Time PCR System (Thermo Fisher Scientific): 2 min at 50°C, 10 min at 95°C, followed by 45 cycles of 15 seconds at 95°C and 1 min at 60°C. The transcripts were normalized to the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data were analyzed using the 7300 System Software (Thermo Fisher Scientific) and expressed as fold-change relative to NPCs cultured alone.

Western blot analysis

Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 15% gel for MBP detection and on a 4% to 20% gradient gel (BioRad) for GFAP detection, transferred onto a polyvinylidene fluoride membrane, blocked with 5% milk and analyzed with a rabbit polyclonal antibody against MBP (1:400, Millipore) or a mouse monoclonal antibody against rat GFAP (1:1000, Millipore). Horseradish peroxidase–coupled donkey anti-rabbit or sheep anti-mouse (1:1000, GE Healthcare Life Sciences) secondary antibodies were used. Binding was detected by chemiluminescence using the Amersham ECL Prime Western blotting reagent (GE Healthcare Life Sciences). ImageJ software (National Institutes of Health) was used for pixel summation of individual bands, and pixel intensities were corrected for background. MBP and GFAP intensities were standardized to the corresponding β-actin bands. The MBP and GFAP expression of direct CC, TW CC and CM were compared to NPCs alone.