International Society for Cellular Therapy ISCT

Cytotherapy (2006) Vol. 8, No. 3, 215–227

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In vitro endothelial potential of human UC blood-derived mesenchymal stem cells

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Background

Human mesenchymal stem cells (MSC) possess powerful ex vivo *expansion and versatile differentiation potential, placing themselves at the forefront of the field of stem cell-based therapy and transplantation. Of high clinical relevance is the endothelial differentiation potential of MSC, which can be used to treat various forms of ischemic vascular disease.*

Methods

We investigated whether human umbilical cord blood (UCB)-derived MSC are able to differentiate in vitro *along an endothelial lineage, by using flow cytometry, RT-PCR and immunofluorescence analyzes, as well as an Ab array method.*

Results

When the cells were incubated for up to 3 weeks in the presence of VEGF, EGF and hydrocortisone, they began to express a variety of endothelial lineage surface markers, such as Flk-1, Flt-1, VE-Cadherin, vWF, VCAM-1, Tie-1 and Tie-2, and to secrete a specific set of cytokines. Differentiated cells were also found to be able to uptake low-density lipoprotein and form a tubular network structure.

Discussion

These observations have led us to conclude that UCB-derived MSC retain endothelial potential that is suitable for basic and clinical studies aimed at the development of vasculature-directed regenerative medicine.

Keywords

endothelial differentiation, human umbilical cord blood, mesenchymal stem cells.

Introduction

Mesenchymal stem cells (MSC) are derived from the embryonic mesoderm, distributed in many somatic tissues during fetal development and later confined to BM and a number of connective tissues in the adult [1]. They exhibit adherent, fibroblastic and clonal properties and express a unique cell-surface phenotype. Because the cells are highly expandable *ex vivo* and capable of differentiating along a variety of different cell lineages [2–5], they are regarded as one of the potential resources for stem cell-based therapy and transplantation. Basic and clinical studies are currently underway with the aim of curing connective tissue disease as well as neural and vascular disorders [6].

Endothelial progenitor cells (EPC) are also mesoderm-derived cells of high therapeutic potential. They give rise to endothelial cells (EC) and smooth muscle cells (SMC) in response to vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF)-BB, respectively [7]. There are two distinct forms of EPC: embryonic and adult [8]. Embryonic EPC, usually called angioblasts, form the vascular network of the embryo via both vasculogenesis (*in situ* formation and growth of blood vessels) and angiogenesis (remodeling and expansion of the pre-existing vascular network), whereas adult EPC have long been believed to mediate only angiogenesis in post-natal life. This traditional view, however, was challenged when a recent investigation revealed the neovasculogenetic activity of adult EPC [9], immediately raising the hope for therapeutic vasculogenesis [10]. Candidate cell populations of EPC were isolated from BM [11], peripheral blood (PB) [12,13] and newborn umbilical cord blood (UCB)

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DOI: 10.1080/14653240600735933

[13,14]. A number of animal studies have demonstrated that these cells are effective in treating peripheral [9,15–17], coronary [15,18,19] and cerebral [20,21] vascular diseases, which subsequently provided a basis for therapeutic [22,23] and diagnostic [24] application to human disorders.

However, adult EPC turned out not to be the only source for therapeutic vasculogenesis. Functional EC could be also derived from a variety of different cell sources, including embryonic stem cells (ESC) [7,25], BM-derived mononuclear cells (MNC) [26], hematopoietic stem cells (HSC) [27–29], MSC [5] and mesodermal adult progenitor cells (MAPC) [30]; as well as various PB- [31–34] and UCB-derived [35–37] cell populations. Recently, it has been shown that UCB contains a limited number of cells whose cellular properties, multilineage differentiation potential and even molecular content are highly similar to those of BM-derived MSC [38–44]. In this report, we demonstrate that these MSC-like cells from UCB can also be a potential source of functional EC. Because UCB appears to be more advantageous than other stem cell sources with respect to cell procurement, storage and transplantation [45], UCB-derived MSC are suitable for the development of cell-based therapeutics against various ischemic vascular diseases.

Methods

Isolation and culture of UCB-derived MSC

Human full-term UCB samples were collected with the mothers' consent in a blood collection bag containing citrate phosphate dextrose as anti-coagulant, and processed within 24 h. A fraction of MNC was separated by centrifugation in a Ficoll-Paque™ PLUS gradient (Amersham Biosciences, Uppsala, Sweden), washed with HBSS (Jeil Biotechservices, Daegu, Korea) and resuspended in low glucose (LG)-DMEM; Invitrogen Corporation, Grand Island, NY, USA), 20% FBS (JRH Biosciences, Lenexa, KS, USA), 2 mm L-glutamine, 1 mm sodium pyruvate and 1% antibiotics/antimycotics (Life Technologies, Gaithersburg, MD, USA) comprising 100 U/mL penicillin, 100 μg/mL streptomycin and 25 μg/mL amphotericin B. After 5 days, non-adherent cells were discarded and adherent cells were continued to culture with two medium changes/week. Prior to endothelial differentiation, the fifth-passage cells were plated at a density of 1×10^6 cells/cm^2 in culture medium and grown to cells with greater than 60% confluency.

Endothelial differentiation

UCB-derived MSC were incubated for up to 3 weeks in endothelial differentiation medium containing 100 ng/mL VEGF (Pangen Biotech, Seongnam, Korea), 50 ng/mL epidermal growth factor (EGF; Sigma-Aldrich, St Louis, MO, USA) and 1 μg/mL hydrocortisone, 5% FBS and 1% antibiotics/antimycotics. Endothelial differentiation was evaluated by flow cytometry, RT-PCR and immunofluorescence (IF). The functionality of differentiated cells was measured by capillary network formation and low-density lipoprotein (LDL) incorporation assays. In addition, the cytokine secretion profile of differentiated cells was determined with the use of cytokine Ab arrays.

Flow cytometric analysis

For detection of CD13, CD14, CD29, CD31, CD34, CD44 (β_1-integrin), CD45, CD49d, CD49e (α_5-integrin), CD54 (ICAM-1), CD90 (Thy-1), CD106, α-smooth muscle actin (ASMA), SH2 (CD105, endoglin), SH3 (CD73), HLA-ABC, HLA-DR and vascular cell adhesion molecule-1 (VCAM-1), the cells were harvested by treatment with 0.1% trypsin-EDTA, and detached cells were washed with cold PBS (Jeil Biotechservices) and incubated at 4°C for 20 min with the respective MAb (Becton Dickinson, San Jose, CA, USA), which were all conjugated with either FITC or PE. For detection of Willebrand factor (vWF), vascular endothelial (VE)-cadherin, Flt-1 (VEGF receptor 1) and Flk-1 (VEGF receptor 2), the cells were incubated with primary mouse Ab against vWF, VE-cadherin, Flk-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and Flt-1/VEGF-R1 (Sigma-Aldrich) at 4°C for 20 min, followed by staining with an FITC-conjugated secondary anti-mouse IgG (Becton Dickinson) for another 20 min. After washing with cold PBS, the cells were resuspended in 500 μL 2% FBS/PBS. PE- or FITC-labeled cells were then fixed with 2% formaldehyde/PBS and analyzed by flow cytometry (Epics XL; Beckman Coulter, Miami, FL, USA).

RT-PCR analysis

Total RNA was extracted using an RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufac-