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Research paper

Effects of mesenchymal stem cell-derived cytokines on the functional properties of endothelial progenitor cells

Witchayaporn Kamprom[a,b], Pakpoom Kheolamai[b,c,d], Yaowalak U-Pratya[b,e], Aungkura Supokawej[f], Methichit Wattanapanitch[g], Chuti Laowtammathron[b], Surapol Issaragrisil[b,e,*]

[a] Department of Immunology, Faculty of Medicine Siriraj hospital, Mahidol University, Bangkok, Thailand
[b] Siriraj Center of Excellence for Stem Cell Research, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[c] Division of Cell Biology, Faculty of Medicine, Thammasat University, Pathumthani, Thailand
[d] Center of Excellence in Stem Cell Research, Faculty of Medicine, Thammasat University, Pathumthani, Thailand
[e] Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[f] Faculty of Medical Technology, Mahidol University, Bangkok, Thailand
[g] Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

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ABSTRACT

Human mesenchymal stem cell (hMSC) is a potential source for cell therapy due to its property to promote tissue repair. Although, it has been known that hMSCs promote tissue repair via angiogenic cytokines, the interaction between hMSC-derived cytokines and the endothelial progenitor cells (EPCs), which play an important role in tissue neovascularization, is poorly characterized. We investigate the effect of cytokine released from different sources of hMSCs including bone marrow and gestational tissues on the EPC functions in vitro. The migration, extracellular matrix invasion and vessel formation of EPCs were studied in the presence or absence of cytokines released from various sources of hMSCs using transwell culture system. The migration of EPCs was highest when co-culture with secretory factors from placenta-derived hMSCs (PL-hMSCs) compared to those co-culture with other sources of hMSCs. For invasion and vessel formation, secretory factors from bone marrow-derived hMSCs (BM-hMSCs) could produce the maximal enhancement compared to other sources. We further identified the secreted cytokines and found that the migratory-enhancing cytokine from PL-hMSCs was PDGF-BB while the enhancing cytokine from BM-hMSCs on invasion was IGF-1. For vessel formation, the cytokines released from BM-hMSCs were IGF1 and SDF-1. In conclusion, hMSCs can release angiogenic cytokines which increase the migration, invasion and vessel forming capacity of EPCs. We can then use hMSCs as a source of angiogenic cytokines to induce neovascularization in injured/ischemic tissues.

1. Introduction

Human mesenchymal stem cells (hMSCs) are multipotent stem/progenitor cells which are present in bone marrow, adipose tissue, and postnatal sources including umbilical cord, placenta, the Wharton's jelly, and amnion (Bunnell et al., 2008; In't Anker et al., 2004; Lee et al., 2004; Wang et al., 2004). hMSCs are considered to be a potential source for cellular therapy in several disorders such as acute graft-versus-host disease (acute GvHD), myocardial infarction, and neurological diseases (Uccelli et al., 2008; Williams et al., 2013) due to their immunomodulatory property and multilineage differentiation capacity (Jiang et al., 2002; Nauta and Fibbe, 2007; Pittenger et al., 1999). The therapeutic effects of hMSCs are believed due to soluble factors or cytokines released from hMSCs rather

Abbreviations: AM-hMSCs, amnion-derived human mesenchymal stem cells; ANGPT1, angiopoietin 1; ANGPT2, angiopoietin 2; BM-hMSCs, bone marrow-derived human mesenchymal stem cells; CFSE, 5-(6)-carboxyfluorescein diacetate succinimidyl ester; EPCs, endothelial progenitor cells; FGF2, fibroblast growth factor 2; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; HUVECs, human umbilical vein endothelial cells; IGF1, insulin-like growth factor 1; IGF2, insulin-like growth factor 2; IL6, interleukin 6; IL8, interleukin 8; PDGFβ, platelet-derived growth factor beta; PL-hMSCs, placenta-derived human mesenchymal stem cells; PlGF, placenta growth factor; SDF1, stromal cell-derived factor 1; TGFβ, transforming growth factor beta; UC-hMSCs, umbilical cord-derived human mesenchymal stem cells; VEGFA, vascular endothelial growth factor A; VEGFR2, vascular endothelial growth factor receptor 2; vWF, Von willebrand factor; WJ-hMSCs, Wharton's jelly-derived human mesenchymal stem cells.

* Corresponding author at: Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. Tel.: +66 2 419 4448 50; fax: +66 2 411 2012.
E-mail address: surapolsi@gmail.com (S. Issaragrisil).

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than their direct effect (Meirelles Lda et al., 2009). hMSC-derived soluble factors can exert immunomodulatory effect, reduce tissue inflammation and prevent apoptosis of several cell types in injured tissues which might be the underlying mechanism in the improvement of tissue repair and inflammatory disease (Gnecchi et al., 2008; Wang et al., 2014). hMSCs can stimulate neovascularization in the injured ischemic tissue via pro-angiogenic cytokines which enhance endothelial cell proliferation and migration leading to neovascularization and tissue restoration (Hung et al., 2007; Kamihata et al., 2001; Li et al., 2010). It was the belief that mature endothelial cells in the local vasculature are responsible for postnatal neovascularization. However, recent studies have shown the important role of endothelial progenitor cells (EPCs) in vascular homeostasis and postnatal neovascularization in both physiological and pathological conditions (Asahara et al., 1999; Melero-Martin et al., 2007; Tepper et al., 2005). There are no reported information on the effects of cytokines released from hMSCs on the endothelial progenitor cells. In order to better understand the therapeutic effect of hMSCs on neovascularization, we investigated the effects of soluble factors released from hMSCs on the EPC functions including proliferation, migration, invasion and vessel formation capacity in vitro, and also identified those factors that play roles on various EPC functions. Soluble factors released from gestational tissues-derived hMSCs were chosen to study due to easily and non-invasive collection compared to bone marrow-derived hMSCs (BM-hMSCs).

2. Materials and methods

2.1. Subjects

This study was approved by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University which was in accordance with the Declaration of Helsinki, the Belmont Report, CIOMS Guidelines, and ICH-GCP. Human bone marrow samples were obtained from healthy volunteers after giving written informed consent. The gestational tissues (umbilical cord, Wharton's jelly, placenta, and amnion) were obtained from healthy newborns after receiving written informed consent from their mothers.

2.2. Isolation and culture of hMSCs

Ten milliliter of heparinized bone marrow was collected for hMSC isolation. Bone marrow-derived mononuclear cells were then isolated using IsoPrep® (Robbins Scientific Corporation, USA) density gradient centrifugation, washed twice with PBS (GIBCO™, Invitrogen Corporation, USA), and re-suspended in complete medium which is Dulbecco's Modified Eagle Medium (DMEM) (GIBCO™, Invitrogen Corporation, USA) supplemented with 10% (v/v) Fetal Bovine Serum (FBS) (Lonza, USA), 100 U/ml penicillin (General Drug House CO., Ltd, Thailand), and 100 μg/ml streptomycin (General Drug House CO., Ltd, Thailand). Cell suspensions were then plated in 25 cm^2 culture flask (Corning, USA) at a density of 2×10^5 cells/cm^2. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and the medium was replaced every 3 days throughout the entire culture period.

For hMSC isolation from gestational tissues, umbilical cord, Wharton's jelly, placenta, and amnion were cut into small pieces and digested by incubation with 0.25% (w/v) trypsin-EDTA (GIBCO™, Invitrogen Corporation, USA) for 30 min at 37 °C. Cell suspensions were washed twice with PBS, re-suspended in complete medium and plated in 25 cm^2 culture flask (Corning, USA). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and the medium was replaced every 3 days throughout the entire culture period.

2.3. Characterization of cultured hMSCs by flow cytometry

The 3rd–5th passage of hMSCs were characterized for hMSC surface markers by incubation with the following mouse anti-human antibodies: anti-CD45-PerCP (BD Pharmingen, USA), anti-CD34-PE (Biolegend, USA), anti-CD90-FITC (AbD Serotec, USA), anti-CD73-PE (BD Pharmingen, USA), and anti-CD105-PE (Miltenyi Biotec, Germany) for 30 min at 4 °C in the dark. After incubation with the antibodies, cell pellets were washed twice with PBS and fixed with 1% (w/v) paraformaldehyde in PBS. Flow cytometry was performed by FACS calibur™ Flow cytometer using CellQuest™ software (Becton Dickinson, USA).

2.4. Osteogenic and adipogenic differentiation of cultured hMSCs

The 3rd–5th passage hMSCs were used to assess their adipogenic and osteogenic differentiation potentials. For adipogenic differentiation, 5×10^4 cells were cultured in NH AdipoDiff® Medium (Miltenyi Biotec, Germany). Medium was replaced every 3 days according to the manufacturer's instruction. After culture for 3 weeks, cells were stained with 0.5% (w/v) Oil Red O (Sigma Aldrich, USA), in isopropanol for 20 min at room temperature, to determine the number of hMSC-derived adipocytes in culture.

For osteogenic differentiation, 5×10^4 cells were cultured in NH OsteoDiff® Medium (Miltenyi Biotec, Germany). Medium was replaced every 3 days according to the manufacturer's instruction. After culture for 3 weeks, cells were stained with 40 mM Alizarin Red S (Sigma Aldrich, USA) for 20 min at room temperature to determine the number of hMSC-derived osteocytes in culture.

2.5. Cultures and characterization of EPCs from umbilical cord blood

Twenty milliliter of heparinized umbilical cord blood was collected for EPC isolation. Umbilical cord blood-derived mononuclear cells were then isolated using IsoPrep® (Robbins Scientific Corporation, USA) density gradient centrifugation, washed twice with PBS (GIBCO™, Invitrogen Corporation, USA), re-suspended in endothelial cell growth medium [endothelial basal medium-2 (LONZA, Germany), supplemented with EGM-2 single aliquots (LONZA, Germany) containing 2% (v/v) fetal bovine serum (FBS), 5 μg/ml epidermal growth factor, 200 μg/ml hydrocortisone, 0.5 μg/ml vascular endothelial growth factor, 10 μg/ml basic fibroblast growth factor, 20 μg/ml long R3 Insulin-like growth factor 1 and 1 mg/ml ascorbic acid], and plated in an individual well of 6-well plate coated with 10 μg/ml human fibronectin (Amersham Biosciences, USA) at a density of 1×10^6 cells/well. After culture for 3 days, the non-adherent cells were removed and fresh medium was added. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and medium was replaced every 3 days throughout the entire culture period.

The cultured cells were characterized for EPC surface markers by incubation with the following mouse anti-human antibodies: anti-CD34-PE (R&D Systems, USA), anti-VEGFR2-PE (R&D Systems, USA), anti-CD146-FITC (R&D Systems, USA), and anti-vWF-FITC (R&D Systems, USA) for 15 min at 4 °C in the dark. Cell pellets were then washed twice with PBS and fixed with 1% (v/v) paraformaldehyde in PBS. Flow cytometry was performed by FACScalibur™ flow cytometer (Becton Dickinson, USA) using CellQuest® software.

2.6. Study of hMSC-derived cytokines on the functional properties of EPCs

2.6.1. Effect on proliferation

2×10^5 hMSCs derived from five distinct tissues were cultured in 2 ml DMEM supplemented with 2% (v/v) FBS for 48 h to generate