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Original research article

# Effect of gestational age on migration ability of the human umbilical cord vein mesenchymal stem cells

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ABSTRACT

*Purpose:* Migration ability of mesenchymal stem cells (MSCs) towards chemotactic mediators is a determinant factor in cell therapy. MSCs derived from different sources show different properties. Here we compared the migration ability of the term and the pre-term human umbilical cord vein MSCs (hUCV-MSCs).

*Materials/Methods:* MSCs were isolated from term and pre-term umbilical cord vein, and cultured to passage 3–4. Migration rate of both groups was assessed in the presence of 10% FBS using chemotaxis assay. Surface expression of CXCR4 was measured by flow cytometery. The relative gene expression of CXCR4, IGF1-R, PDGFRα, MMP-2, MMP-9, MT1-MMP and TIMP-2 were evaluated using real time PCR.

*Results:* The isolation rate of the pre-term hUCV-MSCs was higher than the term hUCV-MSCs. Phenotype characteristics and differentiation ability of the term and pre-term hUCV-MSCs were not different. The migration rate of the pre-term hUCV-MSCs was more than the term hUCV-MSCs. Gene and surface expressions of the CXCR4 were both significantly higher in the pre-term hUCV-MSCs ($P \leq 0.05$). The mRNA levels of PDGFRα, MMP-2, MMP-9, MT1-MMP and TIMP-2 showed no significant difference between the two groups.

*Conclusion:* Our results showed that the gestational age can affect the migration ability of the hUCV-MSCs.

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## 1. Introduction

Mesenchymal stem cells (MSCs) are heterogeneous cells which can be isolated from fetal and adult human tissues [1]. These cells are identified by adhesion to plastic, simultaneous expression of CD105, CD73 and CD90, no expression of CD14, CD34, CD45, and HLA-DR, along with differentiation to osteogenic, chondrogenic, and adipogenic lineages [2,3].

MSCs can regenerate and differentiate into various cell lines [4]. The low immunogenicity of MSCs [5] and their ability to modulate immune responses [6] has made them suitable candidates for cell therapy in regenerative medicine and immune therapies.

Similar to leukocytes, the migration of MSCs is a multistage cascade of activation, adhesion, and transmigration [7]. These cells migrate to tissues in response to chemokines and growth factors [7–11]. Endogenic MSCs migrate to injured and inflamed tissues and participate in tissue repair and modulate immune responses [10,11]. The migration of transplanted MSCs to ischemic brain [12], infarcted myocardium [13], demyelinated lesions [14], and ischemic kidney [15] as well as healthy tissues such as bone marrow [16] has been previously reported. As a regenerative and therapeutic agent, the rate of MSCs migration to the target tissue is of crucial importance [17].

The SDF-1/CXCR4 axis is one of the important pathways in the recruitment and homing of MSCs into different tissues [18]. Small populations of MSCs express the active C-X-C receptor 4 (CXCR4) [19]. However, the inhibition of the SDF-1/CXCR4 with SDF-1 neutralizing antibody or synthetic inhibitor prevents the migration of MSCs to bone and lesions [20,21]. Moreover, in vitro migration

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assays have shown MSCs that strongly express CXCR4 migrate towards SDF-1, and the inhibition of CXCR4 would suppress migration [22]. Insulin-like growth factor (IGF) and platelet-derived growth factor (PDGF) are important stimulators of MSCs migration [9]. PDGF-AB is a strong stimulator of MSCs migration in vitro [9,23,24]. IGF-1 and IGF-2 bond with IGF1-R and stimulate MSCs migration [9,24]. The increased expression of IGF-1 in the bone marrow MSCs of the rat, increased the migration of MSCs to the injured myocardium through paracrine activation of the SDF-1/CXCR4 axis, which would enhance cardiac function [25].

Matrix metalloproteinases (MMPs) have an important role in the transmigration of MSCs through the extracellular matrix [26]. MSCs produce various membrane-type and secretory MMPs. MMP-2 is a secretory gelatinase which is highly expressed in MSCs. This enzyme interacts with the tissue inhibitor of matrix metalloproteinase 2 (TIMP-2) and has a key role in MSCs invasion [27]. The membrane type 1 MMP (MT1-MMP) which activates MMP-2 and MMP-9 is a main mediator of MSCs invasion too [22,26]. In hypoxic conditions, increase in the expression of MT1-MMP increases the migration of MSCs, and its inhibition by the antibody reduces migration [28].

Abundance, accessibility, no ethical limitations, high isolation and proliferation rate, and similar phenotype characteristics along with other tissues' MSCs, have made human umbilical cord a suitable source for MSCs [29,30]. MSCs can be obtained from fetal and prenatal tissues and can also be isolated at different gestational ages [31,32]. MSCs isolated from preterm sources have some differences from those isolated from term and adult sources. The number of these cells, proliferation rate, expression of multi-lineage differentiation markers and plasticity are higher in preterm MSCs compared to term and adult ones [33–35]. Moreover, MSCs isolated from different fetal tissues in the first trimester have longer telomeres with more telomerase activity than MSCs derived from adult bone marrow [35]. Fetal MSCs isolated at gestational weeks 8 and 9 express higher levels of integrin $\alpha 4\beta 1$ and its ligand VCAM-1, and their expression decrease with gestational age [36]. Mitogen-induced lymphocyte proliferation is more inhibited in the one co-cultured with fetal MSCs compared to adult MSCs [37]. Considering the importance of MSCs migration rate in the outcome of cell therapy, in this study, we aimed to compare the migration ability of the term and pre-term hUCV-MSCs.

## 2. Materials and methods

### 2.1. MSCs isolation and culture

After obtaining written informed consent from pregnant women, umbilical cords were collected from term Caesarean sections (n = 7, 38–40 weeks) and pre-term Caesarean sections or legal abortions (n = 7, 20–28 weeks). The umbilical cords were transferred to the laboratory in Hanks' Balanced Salt Solution (HBSS) containing 300 U/ml Penicillin, 300 $\mu$g/ml streptomycin and 2ug/ml amphotericin B. All samples were processed within 2 h. The umbilical cord vein was catheterized and its inside was washed twice with HBSS. Then, the vein was filled with collagenase IV (Gibco, Life Technologies, NY, USA) 0.1% (w/v) in antibiotic-containing DMEM-LG (Gibco, Life Technologies, Paisley, UK). The two proximal ends of the umbilical cord were clamped and incubated for 20 min at 37 °C. Endothelial and sub-endothelial cells were collected by intravenous washing with DMEM containing 15% FBS (Gibco, Life Technologies, NY, USA) (v/v) and antibiotics. The cell suspension was centrifuged at 600g for 10 min. The cell pellet was resuspended in the DMEM-LG supplemented with 100 U/ml penicillin, 100 $\mu$g/ml streptomycin, 10 ng/ml fibroblast growth factor (FGF-2) (Royan Institute, Tehran, Iran) and 15% FBS. $10^3$cells/$cm^2$ were plated in 25 $cm^2$ flasks and incubated at 37 °C with 95% humidity and 5% $CO_2$. After 48 h, the non-adherent cells were removed and the media were replaced, the media replacement was repeated every 72 h. Cells were subcultured up to passages 3–4 at cell confluency of 70–80% with trypsin-EDTA (0.25%).

### 2.2. Differentiation of term and pre-term hUCV-MSCs in adipogenic, chondrogenic and osteogenic lineages

To confirm the identity of hUCV-MSCs, the cells of passages 3–4 were cultured in adipogenic, chondrogenic and osteogenic differentiation media. For osteogenic differentiation, the cells were seeded in $5 \times 10^3$ cells/$cm^2$ in 24-well plates and were incubated with osteogenesis differentiation medium consisting of 90% STEMPRO Osteocyte/Chondrocyte Differentiation Basal Medium and 10% STEMPRO Osteogenesis supplement (Gibco, Life Technologies, NY, USA). The media were changed every three days. After 21 days, the cells were stained with Alizarin Red S and characterized for calcium deposition.

For chondrogenic differentiation, a cell suspension containing $1.6 \times 10^7$ cells/ml was prepared and micromass cultures were used by seeding 5 $\mu$L droplets of this cell suspension in the center of the wells of 96-well plates. Chondrogenic differentiation medium consisting of 90% STEMPRO Osteocyte/Chondrocyte Differentiation Basal Medium and 10% Chondrogenesis supplement (Gibco, Life Technologies, NY, USA) was added to the wells. The media were changed every two days. After 14 days, the cells were stained with Alcian blue solution 1% and characterized for proteoglycan formation.

For adipogenic differentiation, cells were seeded in $3 \times 10^4$ cells/$cm^2$ in 24-well culture plate and were incubated with adipogenic differentiation medium (Stem Cell Technology Research Center, Tehran, Iran). The media were replaced every three days. After 21 days, the cells were stained with Oil Red O and characterized for presence of fat globules.

### 2.3. Flow cytometry

The expressions of CD45, CD34, CD90, CD73, CD105, and CXCR4 in term and pre-term hUCV-MSCs were assessed at passages 3–4 using flow cytometry. To assess the mentioned antigens, monoclonal antibodies for PE anti-human CD34 (581), FITC anti-human CD45 (HI30), PerCP/Cy5.5 anti-human CD90 (5E10), PE anti-human CD73 (A2D), PE anti-human CD105 (43A3), and PE/Cy5 anti-human CD184 (12G5) (BioLegend, CA, USA) were used. Initially, the cells were trypsinized and washed twice using staining buffer (PBS containing 5% FBS). About $10^5$ cells along with monoclonal antibodies were incubated for 45 min at 4 °C in the dark. Then, they were washed twice with staining buffer and resuspended in the same buffer. PerCP/Cy5.5 mouse IgG1,$\kappa$ (MOPC-21), FITC mouse IgG1,$\kappa$ (MOPC-1) (BioLegend, CA, USA), and PE mouse IgG1,$\kappa$ (P3.6.2.8.1) (eBioscience, CA, USA) were used as control isotypes. Cells data were collected by FACS caliber (Becton Dickinson, NJ, USA) and analyzed using FlowJo version 7.6 (FLOWJO, LLC, OR, USA).

### 2.4. In vitro migration assay

The random migration rate of term and pre-term hUCV-MSCs was assessed using transwell inserts (SPL Life Sciences Co., Ltd, Gyeonggi-do, Korea) with a membrane filter of 6.5 mm diameter and 8 $\mu$m pore size. The cells were initially trypsinzed at passages 3–4 and washed twice with serum-free DMEM-LG. $75 \times 10^3$ cells were seeded to the upper side of the insert. FBS was used as chemoattractant. At the lower part of the insert, 600 $\mu$l DMEM-LG without FBS was used as the negative control, medium containing FBS 30% as the positive control, and FBS 10% as the test. The plates were incubated at 37 °C, $CO_2$ 5%, and humidity 95% for 6 h. Then the

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