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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.

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)

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[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 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 $\alpha4\beta1$ 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 μg/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 600*g* for 10 min. The cell pellet was resuspended in the DMEM-LG supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 10 ng/ml fibroblast growth factor (FGF-2) (Royan Institute, Tehran, Iran) and 15% FBS. 10^3cells/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 5×10^3 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 1.6×10^7 cells/ml was prepared and micromass cultures were used by seeding 5 μL 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 3×10^4 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,κ (MOPC-21), FITC mouse IgG1,κ (MOPC-1) (BioLegend, CA, USA), and PE mouse IgG1,κ (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 μm pore size. The cells were initially trypsinzed at passages 3–4 and washed twice with serum-free DMEM-LG. 75×10^3 cells

were seeded to the upper side of the insert. FBS was used as chemoattractant. At the lower part of the insert, 600 μl 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 cells of the upper surface of the membrane were removed with a cotton swab and the insert was washed twice with PBS. The filters were fixed with formalin 10% and stained with hematoxylin. The membrane was cut out from the insert and mounted on the slide. The number of the migrated cells was counted in 10 random visual fields from each membrane at 400 × using a light microscope (Nikon, Tokyo, Japan). The migration index was determined as the average number of migrated cells in each sample of the test group divided by the average number of the migrated cells in the same sample of the positive control group.

2.5. Real time reverse transcriptase polymerase chain reaction (RT-PCR)

The expression of CXCR4, IGF1-R, PDGFRα, MMP2, MMP9, MTI-MMP, and TIMP2 was assessed in the term and the pre-term hUCV-MSCs using real-time PCR (RT-PCR). Total RNA extraction from the cells of passages 3–4 was done using phenol chloroform method according to the manufacturer's instructions (Pars-tous, Mashhad, Iran). 1 μg of the extracted RNA was used to produce the first strand cDNA. The amplification of the specified fragments was done using designed primers (Table 1) and SYBR premix EX taq II (Takara Bio INC, Shiga, Japan). The RT-PCR reaction profile was as follows: initial denaturation at 95 °C for 5 mins, 40 cycles (denaturation at 95 °C for 30 s, annealing temperature for 30 s for each gene (Table 1), extension at 72 °C for 45 s), and final extension at 72 °C for 5 mins. The quantitative evaluation of gene expression was done using Rotor-Gen™ 6000 sequence detection system (Corbett Life Science, Sydney, Australia). β-actin was used as reference gene to normalize data. The ratio of each gene's expression to the reference gene was calculated by $2^{-\Delta CT}$.

2.6. Statistical analyzes

The data regarding migration assay, surface expression of CXCR4 and expression of CXCR4, IGF1-R, PDGFRα, MMP2, MMP9, MTI-MMP, and TIMP2 genes in term and pre-term hUCV-MSCs were analyzed using SPSS software, version 20. Mann-Whitney *U* test was used. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. hUCV-MSCs characterization

47 term and pre-term human umbilical cords were transferred to the laboratory. Of the 33 term umbilical cords, hUCV-MSCs were isolated from 9 samples (27.27%). Of the 14 pre-term umbilical cords, hUCV-MSCs were isolated from 7 (50%) samples. The cells of both groups were expanded up to passages 3–4. The term and the pre-term hUCV-MSCs had spindle-shaped morphology and adhered to plastic (Fig. 1A). In both groups the cells were positive for CD73, CD90, and CD105 and negative for CD45 and CD34 (Fig. 1B). The term and pre-term hUCV-MSCs at passages 3–4 differentiated to the adipogenic, chondrogenic and osteogenic lineages (Fig. 2).

3.2. Migration assay

We assessed the migration rate of the hUCV-MSCs towards DMEM-LG containing 10% FBS. We found no migration towards serum-free media (negative control) in any group. Moreover, the number of cell migrating towards 30% FBS was considered as the positive control. The results of each sample in the test group were normalized with the results of the positive control of the same sample. The mean normalized percentage of migrated cells towards 10% FBS was 59.00% ± 10.12 and 45.4% ± 6.34 in the pre-term and the term hUCV-MSCs, respectively ($p = 0.03$) (Fig. 3). The migration rate of the pre-term hUCV-MSCs towards 10% FBS was significantly higher than the term hUCV-MSCs.

3.3. Cell surface expression of CXCR4

We evaluated the surface expression of the CXCR4 at passage 3–4 of the term and the pre-term hUCV-MSCs using flow cytometery. The mean percentage of cells expressing CXCR4 was 10.63% ± 2.06 and 16.15% ± 3.06 in the term and the pre-term hUCV-MSCs, respectively ($p = 0.02$) (Fig. 4). The mean percentage of the cells expressing CXCR4 was significantly higher in the pre-term than the term hUCV-MSCs.

3.4. Gene expression

The mRNA expression of CXCR4, IGF1-R, and PDGFRα in the term and the pre-term hUCV-MSCs were assessed using RT-PCR. The expression of the CXCR4 in the pre-term hUCV-MSCs was significantly higher than the term hUCV-MSCs (1.46 fold) ($p = 0.007$), while the expression of IGF1-R was significantly lower

Table 1
Sequences, product sizes and annealing temperatures of the primers, designed to assess the expression of βactin, CXCR4, IGF1-R, PDGFRα, MT1-MMP, MMP-2, MMP-9 and TIMP-2 in total mRNA extracted from the term and the pre-term hUCV-MSCs. Abbreviations: hUCV-MSCs: human umbilical cord vein mesenchymal stem cells.

Gene	Primer sequence	Product size (bp)	Annealing temperature (°C)
βactin	F: AGATCATTGCTCCTCCTGAG R: CTAAGTCATAGTCCGCCTAG	161	58
CXCR4	F: ACAGTCAACTTCTACAGCAG R: ATCCAGACGCCAACATAGAC	136	55
IGF1-R	F: AGCCTCCTGTGAAAGTGACG R: GTGCTTCCTTGTAGTAAACGG	137	56
PDGFRα	F: TAGTGCTTGGTCGGGTCTTG R: GATCTGGCCGTGGGTTTTAG	127	60
MT1-MMP	F: CAGCAACTTTATGGGGGTGAG R: TTGCCATCCTTCCTCTCGTAG	295	59
MMP-2	F: ATTCAGGAGCTCTATGGGGC R: ACAGTCCGCCAAATGAACCG	169	63
MMP-9	F: TGCCCGGACCAAGGATACAG R: GTTCAGGGCGAGGACCATAG	183	57
TIMP-2	F: GCAGATAAAGATGTTCAAAGGG R: TCGGCCTTTCCTGCAATGAG	129	60

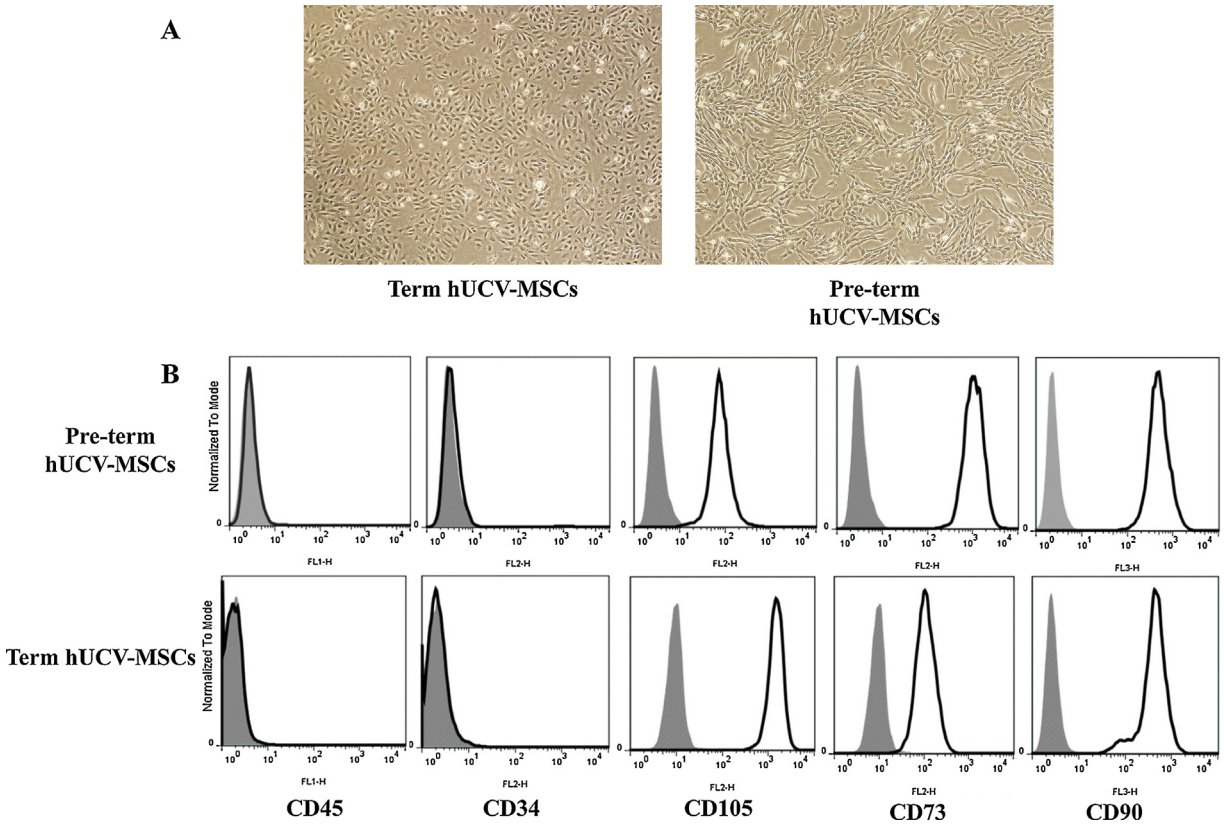

Fig. 1. Term and pre-term hUCV-MSCs characteristics. A: The light microscopic morphology of the term and the pre-term hUCV-MSCs at passage 4. B: the flow cytometery profile of the term and the pre-term hUCV-MSCs at passage 4. The hUCV-MSCs from both groups were positive for CD105, CD90 CD73 and negative for CD45 and CD34 cell surface markers. Magnification 60×. Abbreviations: hUCV-MSCs: human umbilical cord vein mesenchymal stem cells.

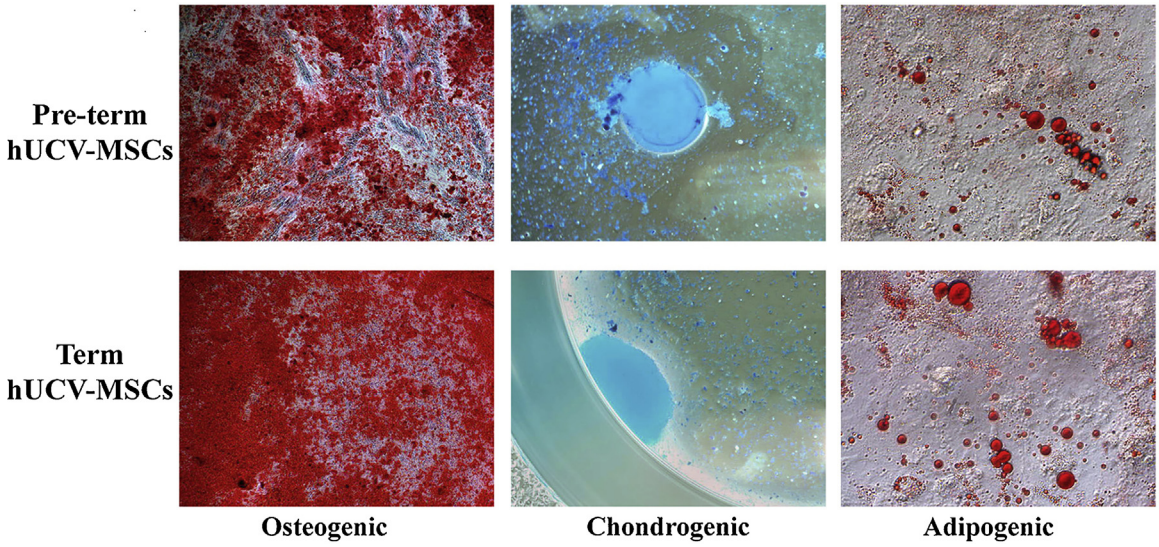

Fig. 2. Term and pre-term hUCV-MSCs differentiation. The light microscopic demonstration of osteogenic, chondrogenic and adipogenic differentiation of the term and the pre-term hUCV-MSCs at passage 3. Magnification 600×and 40×. Abbreviations: hUCV-MSCs: human umbilical cord vein mesenchymal stem cells.

in the pre-term cells (0.66 fold) ($p = 0.02$). PDGFRα had low expression in both groups but its expression was higher in the pre-term hUCV-MSCs (33.92 fold) ($p = 0.22$) (Fig. 5).

We also found that the expression of MMP2 was lower in the pre-term cells (0.78 fold) ($p = 0.08$). The expression of MMP9 was low in both groups but its expression was higher in the pre-term cells (1.34 fold) ($p = 0.56$). The expression of MT1-MMP gene was lower in pre-term cells (0.60 fold) ($p = 0.33$). TIMP-2 had a high expression in both groups but its expression was higher in the term hUCV-MSCs (0.76 fold) ($p = 0.31$) (Fig. 5).

4. Discussion

The ability of MSCs to regenerate and differentiate into various cell types, their immune evasion, and their Immunomodulatory properties have made them a suitable agent for cell therapy. The

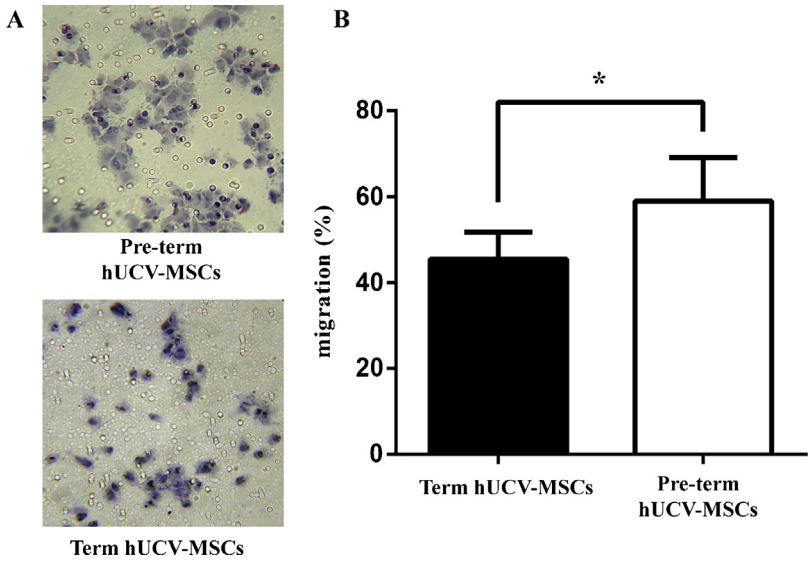

Fig. 3. Term and pre-term hUCV-MSCs migration assay. A: The light microscopic demonstration of the migrated term and pre-term hUCV-MSCs in response to the medium containing%10 FBS in the transwell migration assay (Magnification 400×). B: The pre-term hUCV-MSCs showed higher migration rate in response to the%10 FBS as compared to the term hUCV-MSCs. the columns represent mean ± SD of normalized percentage of the cells. ($p = 0.03$). The star shows the statistically significant difference ($p \leq 0.05$). Abbreviations: hUCV-MSCs: human umbilical cord vein mesenchymal stem cells.

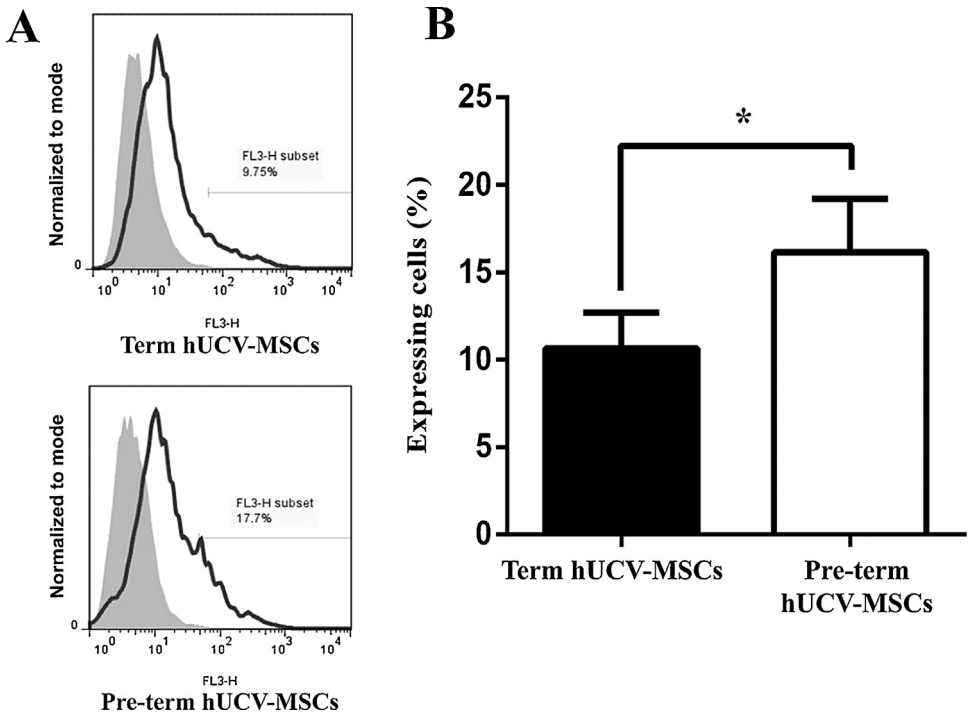

Fig. 4. Flow cytometery results for the CXCR4 expression on the term and the pre-term hUCV-MSCs at passages 3–4. A: histograms of one term and pre-term hUCV-MSCs shown as representatives of cell surface expression of the CXCR4 in both groups. B: Flow cytometery analyzes showed that pre-term hUCV-MSCs demonstrated significantly higher cell surface CXCR4 expression compared to the term hUCV-MSCs ($p = 0.02$). The star shows the statistically significant difference ($p \leq 0.05$). Abbreviations: hUCV-MSCs: human umbilical cord vein mesenchymal stem cells.

umbilical cord is one of the best sources of MSCs. Umbilical cord MSCs have phenotypic characteristics similar to MSCs derived from other sources such as bone marrow [38]. In our study, MSCs were isolated and cultured from the term and the pre-term human umbilical cords vein. We found no difference between the term and the pre-term hUCV-MSCs with respect to plastic adhesion, spindle-shaped morphology, expression of common MSCs surface markers, and lack of expression of hematopoietic markers. Moreover, both groups had the ability to differentiate into bone, cartilage, and fat tissues. The isolation rate of the pre-term cells was higher than the term hUCV-MSCs. This finding which is consistent with several previous studies showed that the number and the isolation rate of MSCs isolated from the preterm umbilical cord blood were higher than the term umbilical cord blood [33,39]. Gonzales et al. found that the proliferation rate of MSCs isolated from the pre-term was higher and the MSCs expressed Nanog, Sox-2 and SSEA-4 differentiation markers. The expression of these markers led to better differentiation flexibility [34].

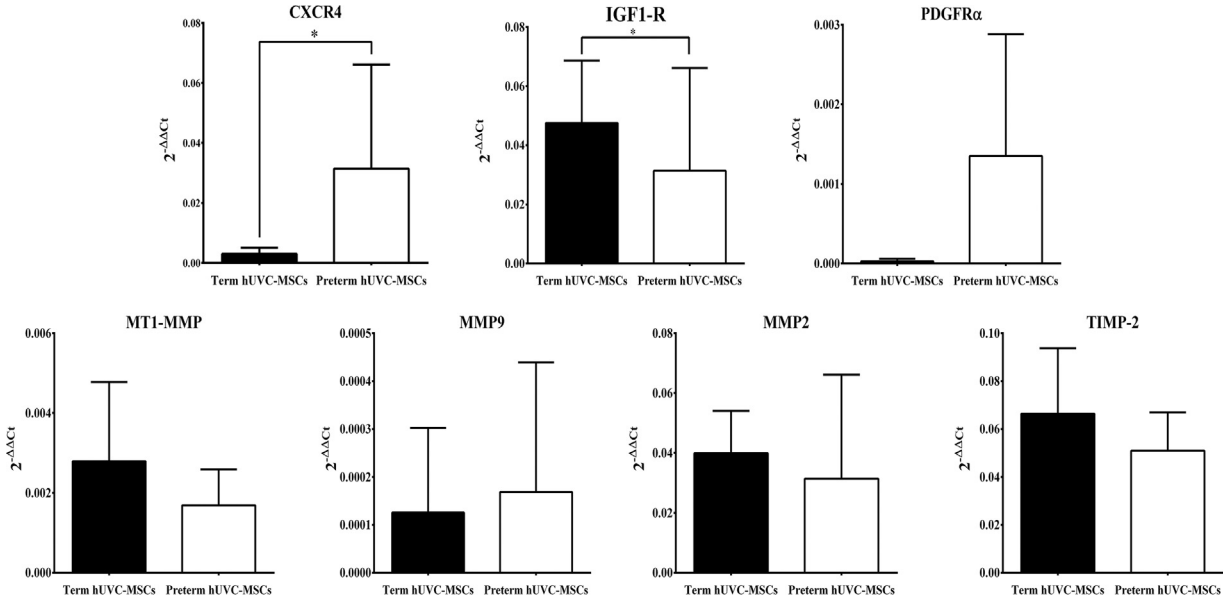

Fig. 5. Quantitative analysis of the term and pre-term hUCV-MSCs representing relative expression of the CXCR4, IGF1-R, PDGFRα, MMP2, MMP9, MT1-MMP and TIMP-2. The mRNA levels of CXCR4 were significantly higher in the pre-term hUCV-MSCs ($p = 0.007$), while the term hUCV-MSCs showed a higher expression of IGF1-R mRNA ($p = 0.02$). However, different mRNA levels of the PDGFRα, MMP-2, MMP-9, MT1-MMP and TIMP-2 in the term and the pre-term hUCV-MSCs were not statistically significant ($p > 0.05$). The star shows the statistically significant difference ($p \leq 0.05$). Abbreviations: hUCV-MSCs: human umbilical cord vein mesenchymal stem cells, IGF1-R: insulin-like growth factor 1 receptor, PDGFRα: platelet-derived growth factor receptor α, MMP: matrix metalloproteinase, MT1-MMP: membrane type 1-matrix metalloproteinase, TIMP-2: tissue inhibitor of metalloproteinase 2.

The migration ability of MSCs to injured tissues could be a determining factor in the success of cell therapy. The expression of chemokines and growth factors is increased in injured and inflamed tissues, leading to the recruitment of MSCs [8,9]. We compared the random migration rate of term and pre-term hUCV-MSCs towards FBS. In various studies, FBS was used as a suitable source of various chemoattractants to assess MSCs migration [40,41]. We found significantly higher migration rate in the pre-term hUCV-MSCs. Mainhenburg et al. compared the migration ability of the MSCs derived from adult bone marrow, fetal bone marrow, fetal lung, and adult adipose tissues. They found that the migration ability of the fetal lung MSCs was higher than the others [42]. Another study showed that the expression of proteins involved in migration such as cathepsin B, D, and prohibitin was lower in MSCs derived from the umbilical cord compared to the bone marrow and placenta. Moreover, migration inhibitors such as plasminogen activator inhibitor1 (PAI-1) and manganese superoxide dismutase were expressed at higher levels in the MSCs derived from the umbilical cord. Therefore, MSCs derived from the umbilical cord had lower migration ability [41]. It can be stated that the source of MSCs could be an effective factor in the migration ability of its cells. The age of the source is another determining factor in migration ability. In a rat model, as the age of the rat increased, the reaction ability of the cytoskeleton actin in the bone marrow MSCs decreased resulting in the reduced migration ability of these cells [43]. Comparison of the global gene expression in the bone marrow-derived MSCs of the old (24 months) and young (3 months) rats showed that the expression of the receptors important in MSCs migration (such as IL-1r2, IL-17rb, IL-18rap, and TGF-βr2) decrease with age. Moreover, the expression of TIMP-2 and MMP9 that are effective in cell invasion decreased in the cells isolated from the old rats. Therefore, the migration ability of the old rats' BM-MSCs towards the injured lung was low, leading to increased inflammation in the old rats' lung [44]. Fetal MSCs isolated at gestational weeks 8 and 9 expressed higher levels of integrin α4β1 and its ligand VCAM-1. This integrin and its receptor play an important role in MSCs migration and homing. Therefore, the migration ability of the MSCs is affected by gestational age [36]. We found that the migration rate of the pre-term hUCV-MSCs was significantly higher than the term cells. It seems that age of umbilical cord could be effective in the migration ability of MSCs.

Various chemokine axes could be influential in MSCs migration [8]. Several reports have confirmed the role of SDF-1/CXCR4 axis [9,22,45,46]. Although a small percentage of MSCs express CXCR4 [19], this axis is important because its suppression by inhibitor or neutralizing antibody can reduce MSCs migration to the bone and wound lesions [20,21]. In our study, the surface expression of CXCR4 was significantly higher in the pre-term hUCV-MSCs. Moreover, the CXCR4 mRNA level was also significantly higher in the pre-term hUCV-MSCs, which was consistent with its surface expression in the pre-term cells. Therefore, it is possible that the higher expression of CXCR4 is responsible for higher in vitro migration in the pre-term cells. The comparison of expression of different chemokine receptors genes in the human bone marrow and adipose-derived MSCs showed higher expression of CXCR4 in the adipose-derived MSCs [47]. It seems that the source of MSCs plays an important role in CXCR4 expression. Our study is the first report that compared the expression of CXCR4 in the hUCV-MSCs isolated from different gestational ages. We found that CXCR4 expression was significantly higher in the pre-term cells, which is consistent with the higher migration rate of these cells.

Growth factors are important for MSCs migration. In this regard, the role of IGF1, PDGF-BB and PDGF-AB has been confirmed [9,23,24,48]. We found that the expression of IGF1-R was significantly higher in the term hUCV-MSCs, while the expression of PDGF-Rα was higher in the pre-term cells. This difference was not statistically significant. The higher IGF1-R expression in the term cells did not correlate with our migration assay results

regarding the higher migration of the pre-term cells. This discrepancy could be attributed to the higher importance of other pathways in the hUCV-MSCs migration such as SDF-1/CXCR4 axis. The lower expression of PDGF and its receptor in the term and the pre-term cells could indicate its less important role in the hUCV-MSCs migration.

Studies have confirmed the important role of MMPs in the migration and invasion of MSCs [22,26,27,40]. MMP-2 interacts with MT1-MMP and TIMP-2 to control MSCs invasion capacity [27]. In our study, the expression of all these three genes were higher in the term hUCV-MSCs which were not statistically significant. MMP-9 had a low expression in both groups and was slightly higher in the pre-term cells. Previous studies have also reported low expression of MMP-9 in MSCs derived from other sources [22,27,40]. Ries et al. [27] found that the expression of MMP-2, MT1-MMP, and TIMP-2 was high in the BM-MSCs, while the expression of MMP9 was low and our results regarding the expression profile of MMP-2, TIMP-2 and MMP-9 were consistent with their findings. However, we found that the expression of MT1-MMP was low in the term and pre-term hUCV-MSCs compared to the mentioned study. We found no report comparing the expression of MMPs and their inhibitors in different gestational ages. It seems that the age of the umbilical cord does not affect these genes expression.

5. Conclusion

We found no difference between the term and the pre-term hUCV-MSCs with respect to phenotypic characterization and differentiation ability. The migration rate of the pre-term hUCV-MSCs was higher than the term cells. Considering the fact that the gene and cell surface expressions of CXCR4 was higher in the pre-term cells, it seems that CXCR4 is an important mediator of hUCV-MSCs migration. Pre-term hUCV-MSCs obtained from aborted fetuses or preterm births could be a suitable choice for cell therapy.

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Conflict of interest

The authors declare that they have no conflict of interest.

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