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Original article

Determination and comparison of the expression levels of various germ cell-specific genes in human bone marrow- and peripheral blood-derived mesenchymal stem cells

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Abstract

Background: A number of reports have shown that mesenchymal stem cells (MSCs) from various sources expressed a number of germ cell (GC)-specific genes innately, but almost none of these studies were quantitative in nature. This study, however, tried to determine and compare the expression levels of some famous GC-specific genes in human male bone marrow (BM)- and peripheral blood (PB)-MSCs.

Methods: Human BM- and PB-MSCs were isolated using a density gradient factor and centrifuging. Then, Passage 3 of both cell types were characterized through flow cytometric analysis and the differentiation test. They were then evaluated by real time RT-PCR for the expression levels of GC-specific genes.

Results: Both cell types obviously expressed c-kit, Dazl, Fragilis, Itgb1, Nanos3, Oct4 and Blimp1 at high levels. The only exception was Itgb1, which was expressed at a higher level in PB-MSCs than in BM-MSCs ($p<0.05$). The levels of expression for other markers were almost similar in both cell types. Different levels of Itgb1 expression could be due to their different niches. Furthermore, both cell types were negative for Fkbp6, Stra8, Scp3, Tex13 and Vasa, and they both expressed Stella weakly. Moreover, it should be stated that BM-MSCs had low expression levels of Piwil2, and that this marker was not expressed in PB-MSCs.

Conclusion: Overall, it could be concluded that BM- and PB-MSCs have a little bit different GC-specific gene expression patterns. Although these results are very revealing, many detailed and creative research studies need to be conducted to find appropriate ways of using MSCs in cell-based therapies of infertility.

Keywords: Bone marrow, Peripheral blood, Mesenchymal stem cells, germ cell-specific genes, expression levels

Introduction

Mesenchymal stem cells (MSCs) are of pluripotent adult stem cells which have the ability to be differentiatiated into several cell types. They exist in almost all tissues of the body and can produce various cell types in vitro like hepatocytes, neurons, adipocytes, osteocytes, chondrocytes, myocytes, germ cells (GCs), etc (1-5). Because of this high

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differentiation capability, researchers have concentrated on MSCs as a suitable source for use in cell therapy and regenerative medicine. Several studies were conducted in order to clarify the biology of MSCs and the characterization of their sources as well. Simultaneously, several research studies have been conducted on derivation of GCs from MSCs and infertility treatment. Generally, almost all scientists have designed their studies based on their knowledge on GC biology to make the in vitro culture system similar to GC natural niche. Several factors have been tested in order to produce GCs in the laboratory from MSCs , and researchers ultimately found that under stimulation of some factors, MSCs can be differentiated into GCs in vitro. Some of these inductive factors were retinoic acid (RA) (5-9), transforming growth factor beta (TGFβ) 1 (10), bone morphogenetic protein (BMP) 4 (11, 12), and BMP8b (13), which caused the induction or elevation of the expression of some GC-specific markers in MSCs and the formation of germ-like cells in the culture. Of interest, the utilized cells in these studies were from different sources. MSCs from bone marrow (BM) (5, 7, 10, 14), adipose tissue (15), amniotic membrane (16), fetus lung (8), and umbilical cord Wharton's jelly (17, 18) have been used in these investigations, and scientists have shown that the cells acquired GC characteristics after treatment with the aforementioned factors. One of the most interesting findings reported in some of the studies was the expression of a number of GC-specific markers in untreated MSCs. For example, a number of research groups indicated that untreated MSCs expressed Vasa (6, 7, 14, 19), Stra8 (7, 20, 21), Dazl (7, 19), Stella (7, 14, 19, 20), Oct4 (6-9, 14, 19, 20), c-Kit (7, 8, 14, 19, 20), Fragilis (14, 19, 20), etc. Nevertheless, the reported results were quite different, and some studies reported contradictory results. Moreover, the number of genes which were evaluated in previous studies were really few. Additionally, no comparative study was performed in order to determine the gene expression profile of MSCs from different sources. Thus, in this study, we isolated MSCs from human BM and peripheral blood (PB) samples and after characterization, tested the expression levels of 14 GC-specific genes, c-kit, Dazl, Fkbp6, Fragilis, Itgb1, Nanos3, Oct4, Piwil2, Blimp1, Stella, Stra8, Scp3, Tex13, and Vasa, in passage-3 (P3) of both cell types. Some of these genes are mostly expressed in primordial germ cells (PGCs) like Stella, Fragilis, Nanos3, Blimp1, Oct4, and Vasa and the others are expressed in adult GCs (5, 22). Results of such studies can certainly help us have a better understanding of MSCs biology and select a suitable type of MSCs for use in GC production and cell-based therapy of infertility.

Materials and Methods

Isolation and culture of human bone marrow (BM) and peripheral blood (PB) mesenchymal stem cells (MSCs)

After gaining approval from the ethical committee of Babol university of Medical Sciences (MUBABOL.REC.1393.8), BM samples were collected from three healthy fertile men, who were candidate of bone surgery (bone fracture or knee joint replacement) with their consent, and PB samples were also collected from the same patients or the volunteers again with their consent. First of all, all donors were informed about the purpose of the sampling, and they were then asked to sign the consent forms. BM and PB-MSCs were isolated based on the methods described in previous studies (14, 23) with a little alteration. Briefly, an equal volume of culture medium was added to BM and PB samples. The mixture was slowly layered on a density gradient (Inno-Train, Germany) cushion and centrifuged at 1800 rpm for 20 min. at room temperature. Mononuclear cells layer was carefully collected, and after being washed twice with phosphate buffer saline (PBS), the resulted cells were cultured in complete culture medium comprising high glucose Dulbecco's modified eagle medium [DMEM (Gibco, Paisley, UK)], 15 % fetal bovine serum [FBS, (Gibco, Paisley, UK)], 100 U/ml penicillin G, and 100 U/ml streptomycin (Gibco, Paisley, UK). The cells were then transferred to humidified cell culture incubator (37˚C and 5% CO2). The culture medium was changed two times a week and the cells were sub-cultured prior to complete cunfluency (70-80%) using trypsin/EDTA (Gibco, Paisley, UK). Cultures were evaluated on a daily basis, and changes were noted. Photographs were taken if necessary.

Characterization of isolated bone marrow (BM) and peripheral blood (PB)-derived MSCs

In order to confirm the mesenchymal stemness of isolated cells, BM and PB isolated cells were checked morphologically. Then, their differentiation capacity into mesenchymal tissues (bone and adipose) and their profile of expression of surface CD markers were also evaluated with flow cytometry.

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Morphological evaluation of Bone marrow (BM) and peripheral blood (PB)-derived MSCs

From the primary culture, the cultures were evaluated with regard to the cells morphology and the growth on a daily basis. Many photographs were taken in this regard.

Differentiation test

Osteogenic differentiation

Osteogenesis was induced in the P3 BM and PB-isolated cells. The cells were treated with osteogenic medium containing high glucose DMEM (Gibco) supplemented with 0.1 lM dexamethasone (Sigma, St. Louis, MO, USA), 10 mM beta glycerol phosphate (Sigma), 0.2 mM ascorbic acid (AsA; Sigma), and 10% FBS (Gibco, Paisley, UK) for 21 days. At the end of the treatment period, the cells were stained with Alizarin red stain in order to detect the mineralized cell aggregates. The induction of the expression of two bone-specific genes- Osteo calcin and Runx2- was also checked by reverse transcription polymerase chain reaction (RT-PCR).

Adipogenic differentiation

Adipogenic medium comprised of high-glucose DMEM (Gibco) supplemented with 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma), 1 µM hydrocortisone (Sigma), 0.1 mM indomethacin (Sigma) and 10% FBS (Gibco). P3 BM and PB-derived cells were treated with adipo-inductive medium for 21 days while the medium was changed every 3-4 days. To confirm the differentiation of treated cells into adipocytes, the cells were subjected to Oil red O staining and gene expression analysis for LPL and PPARγ.

Flow cytometry

P3 of BM- and PB-MSCs were detached from the bottom of culture dish by trypsinization and single cell suspensions were prepared by pipetting of the cellular suspensions. The cell suspensions were centrifuged and the supernatant were discharged. The cells were incubated with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated antibodies against CD44, CD73, CD90, CD105, CD31, CD45 and CD34 (Pharmingen BD, San Diego, CA, USA) for 30 min at 4˚C. Next, the cells were fixed and analyzed with FACS Calibur cytometer (Becton–Dickinson, San Jose, CA, USA) with CellQuest software.

Growth curve

The growth characteristics of BM and PB-derived MSCs were evaluated by the generation of their growth curves. Therefore, 5000 P3 cells/well were seeded in 24-well plates, and the cells in each three wells were counted on a daily basis up to 9 days using a hemocytometer. The growth curves of BM- and PB-derived MSCs were plotted after the calculation of the average number of the cells each day.

RNA extraction, RT-PCR and real time RT-PCR

RT-PCR was performed to detect the expression of bone and fat tissue specific genes in MSCs treated with osteogenic and adipogenic media, respectively. On the other hand, to determine and compare the expression

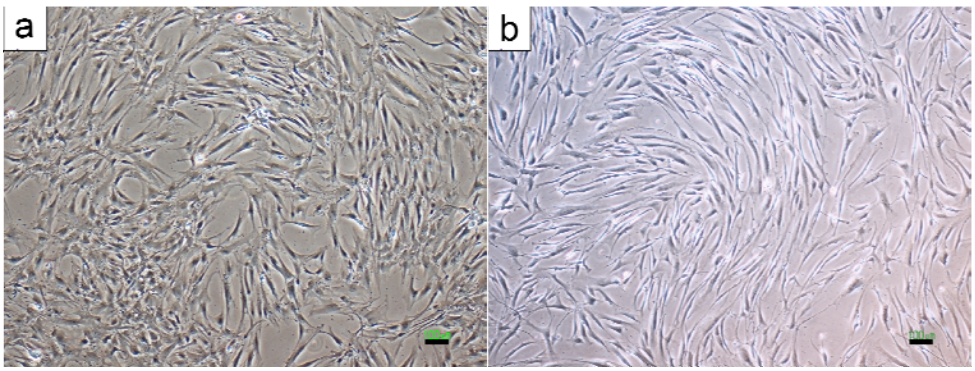

Fig. 1: Passage 3 human bone marrow mesenchymal stem cells (BM-MSCs) (*a*) and peripheral blood (PB)-MSCs (*b*). Both of the cell types were elongated spindle-shaped cells that tightly attached to the bottom of cell culture flasks (bar= 100 µm).

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levels of the GC-specific genes, c-kit, Dazl, Fkbp6, Fragilis, Itgb1, Nanos3, Oct4, Piwil2, Blimp1, Stella, Stra8, Scp3, Tex13, and Vasa, real time RT-PCR was performed on BM- and PB-derived MSCs. Testicular germline cells were also tested as a positive control.

RNA was extracted using Nucleospin® RNA extraction kit (Macherey-Nagel GmbH & Co., Düren,Germany) and DNase I (Macherey-Nagel GmbH & Co., Düren,Germany) treatment was performed in order to eliminate any DNA contamination. Then, cDNA was constructed from 2 µg of total RNA using cDNA synthesis kit (Takara, Shiga, Japan), which was done based on the manufacturer's instructions.

To perform PCR reaction for the detection of the expression of bone and fat genes, TEMPase Hot start PCR master mix (Ampliqon, Skovlunde, Denmark) was utilized.

The levels of expression of GC-specific genes in BM and PB-MSCs were assessed by real time RT-PCR using an Applied Biosystems Real-Time PCR (ABI step1-plus, USA) and Master SYBR Green Kit (ABI, USA). The obtained data were analyzed with REST© software (24). Real time RT-PCR reactions for each sample were duplicated. All of the primers used in this study are shown in Table 1.

Statistical analysis

Version 18 SPSS software and the simple t-test were used for data analysis. $P<0.05$ was considered to be statistically significant.

Results

A few days after culturing the BM and PB mononuclear cells, a number of cells started to grow in the culture flasks. The culture medium was changed every 3-4 days and the cells were subcultured by trypsinization prior to complete confluency. P3 cell were used to confirm that the isolated cells were MSCs. For confirmation, the morphology of the cells, the differentiation capacity and the expression of surface CD markers were evaluated.

Morphology of bone marrow (BM) and peripheral blood (PB)-derived MSCs

The cells grown in both BM and PB cultures were elongated and fibroblastic cells. These cells were highly proliferative and colonogenic. Since the number of MSCs in PB is very low, it took about 20 days for primary culture to reach almost 80% confluency in 25 cm3 flasks, although this time was about 7 days for

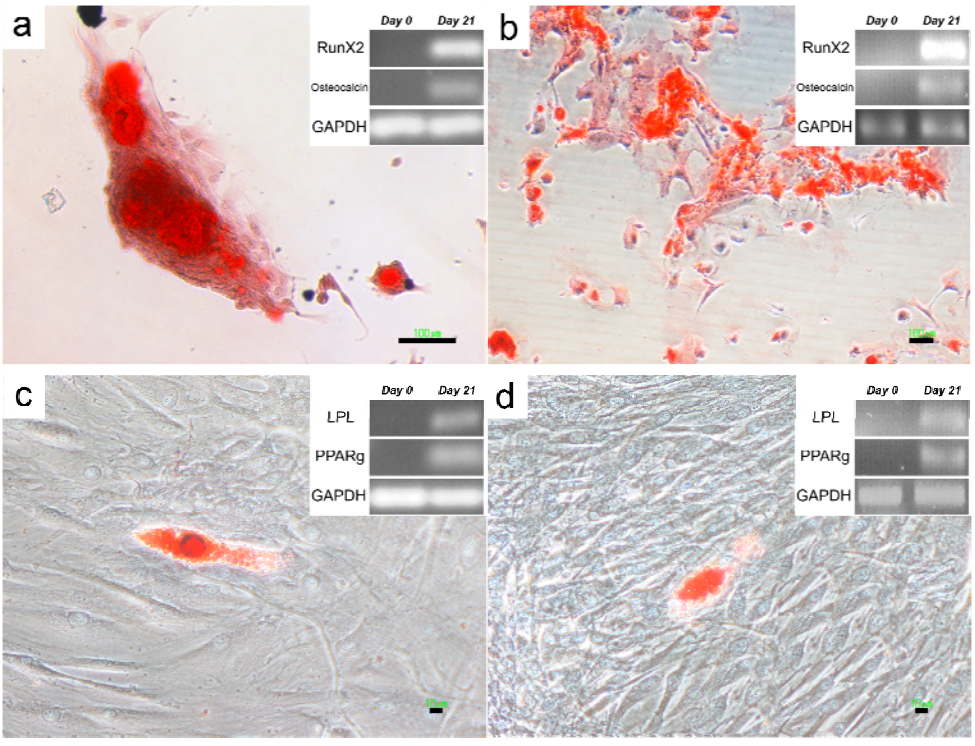

Fig. 2: Differentiation test. Three weeks treatment with osteogenic medium induced differentiation into osteocytes in both human BM-MSCs (*a*) and PB-MSCs (*b*) which confirmed by Alizarin Red staining and RT-PCR and detection of the expression of bone-specific genes as well (bar= 100 µm). Furthermore, Oil red O staining showed that some of the cells in both of the cell types differentiated into adipocytes and expressed fat tissue-specific markers (bar= 10 µm). *c*: BM-MSCs, *d*: PB-MSCs.

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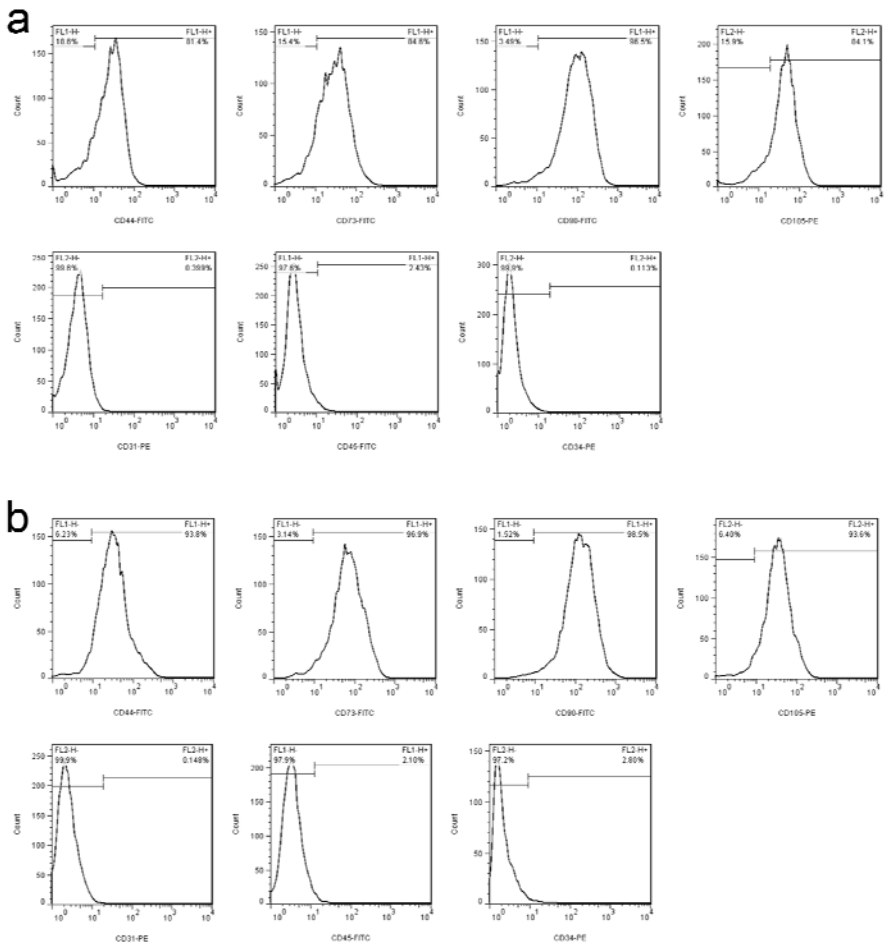

Fig. 3: Flow cytometric analysis of passage 3 human BM-MSCs (*a*) and PB-MSCs (*b*) revealed that both of the cell types highly expressed mesenchymal markers, CD44, CD73, CD90 and CD105, while they were negative for hematopoietic markers, CD31 and CD45 and endothelial marker, CD34.

BM-MSCs. P3 BM and PB-MSCs are shown in the Fig. 1a,b.

Differentiation test

Osteogenic differentiation

Some days after the start of the treatment, the morphology of BM- and PB-derived cells gradually changed and some cell aggregates were formed in the culture. Staining with Alizarin red indicated that these cell aggregates of both BM and PB groups were positively stained red, confirming the existence of mineralized compartments and therefore differentiation of MSCs into bone tissue. Moreover, RT-PCR analysis revealed that, the expression of the two bone-specific markers, RunX2 and Osteocalcin, were induced in BM and PB-derived cells through the treatment with osteogenic medium for 21 days (Fig. 2a,b).

Adipogenic differentiation

After 21 days treatment with adipogenic medium, a number of cells in both BM and PB groups showed accumulation of lipid droplets in their cytoplasm. These droplets stained red with Oil red O staining. Furthermore, molecular analysis showed that adipogenic treatment induced the expression of two fat tissue-specific markers, LPL and Pparγ, in the cells of both groups (Fig. 2c,d).

Flow cytometric analysis

The results of flow cytometry revealed that both BM- and PB-MSCs were highly positive for CD44, CD73, CD90 and CD105, while they had almost no expression of CD31, CD45 and CD34 on their surface (Fig. 3a,b).

Growth curve

Plotting growth curves for BM and PB-MSCs indicated that there were not any significant differences between the growth rates of the two cell types. The curves are shown in Fig. 4a,b.

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Table 1 Primers used for RT-PCR and real time RT-PCR.

Gene name	Sequence A. N.*	Forward primer	Reverse primer	Size (bp)
Gapdh	NM_002046.5	5'- GGAAGGTGAAGGTCGGAGTC	5'-CCTGGAAGATGGTGATGGG	231
Runx2	NM_001015051.3	5'-GTCCATCCACTCTACCACCC	5'-TGAAATGCTTGGGAACTGCC	136
Osteocalcine	NM_199173.5	5'-TCACACTCCTCGCCCTATTG	5'-TCTCTTCACTACCTCGCTGC	134
LPL	NM_000237.2	5'-CGAGATGGAGAGCAAAGCC	5'-GAATGAGGTGGCAAGTGTCC	176
PPARγ	NM_005037.5	5'-ATGGTTGACACAGAGATGCC	5'-GCAGGCTCCACTTTGATTG	242
c-Kit	NM_001093772.1	5'-CCTACCATCGGCTCTGTCTG	5'-TGGACACAGACACAACAGG	120
Dazl	NM_001190811.1	5'-TCGTTTCCAGCCTTTGTCAC	5'-TCCCATTGCTACCGTTCCAG	125
Fkbp6	NM_001281304.1	5'-TACGCCTATGGAACGCTGG	5'-ACTCCCGTTCCGTAGCTG	184
Fragilis	NM_021034.2	5'-ATGTCGTCTGGTCCCTGTTC	5'-GGGATGACGATGAGCAGAAT	205
Itgb1	NM_002211.3	5'-CGGGTTTCACTTTGCTGGAG	5'-TCTGGACAAGGTGAGCAATAG	134
Nanos3	NM_001098622.2	5'-CTACACCTCCGTCTACAGCC	5'-ACCTCTGAAACCTGCTCCTC	127
Oct4	NM_002701.5	5'-CCCGAAAGAGAAAGCGAACC	5'-TACAGAACCACACTCGGACC	153
Piwil2	NM_001135721.1	5'-CAGCAGAGAAGTGGACAAGC	5'-CCCAAAGACTGAGGTGTTCC	192
Blimp1	NM_001198.3	5'-GGGAGAATGTGGACTGGGTAG	5'-CTCTGCCAATCCCTGAAACC	136
Stella	NM_199286.2	5'-AAGACCAACAAACAAGGAGCC	5'-AAGATTTATGGCTGAAGTGGC	150
Stra8	NM_182489.1	5'-GCTCCAGTTCAGTGCTTGC	5'-TCATCGTCAAAGGTCTCTGTG	184
Scp3	NM_001177949.1	5'-GAAATCTGGGAAGCCGTCTG	5'-CAACTCCAACTCCTTCCAGC	209
Tex13	NM_031274.3	5'-GAAAGAGAGAGACAAGGAGC	5'-TCCCTCTGCTCTTCTTCTGC	177
Vasa	NM_024415.2	5'-AGGTAGTTTCCGAGGTTGCC	5'-CGTTCACTTCCACTGCCAC	174

Molecular analysis

The evaluation of the expression levels of GC-specific genes in human BM- and PB-MSCs with real time RT-PCR revealed that both cell types expressed c-kit, Dazl, Fragilis, Itgb1, Nanos3, Oct4 and Blimp1, vigorously. Except for the Itgb1 with had a higher expression in PB-MSCs than in BM-MSCs ($p<0.05$), the other genes had almost similar expression levels in the two cell types. Moreover, both cells did not express Fkbp6, Stra8, Scp3, Tex13 and Vasa. Besides, Piwil2 was not expressed in PB-MSCs. Although it had a weak expression in BM-MSCs, although the difference between the expression levels was not statistically significant ($p>0.05$). In addition, both BM- and PB-MSCs weakly expressed Stella. Although the expression level was higher in PB-MSCs, the difference again was not statistically significant ($p>0.05$), either. All results are shown in Fig 5a,b.

Discussion

As previously stated, a number of studies reported that MSCs expressed GC-specific markers innately and also possessed the ability to differentiate into germ-like cells both in vitro and in vivo (5, 7, 9, 13, 14, 25-27). Various cell sources such as MSCs from human, rat,

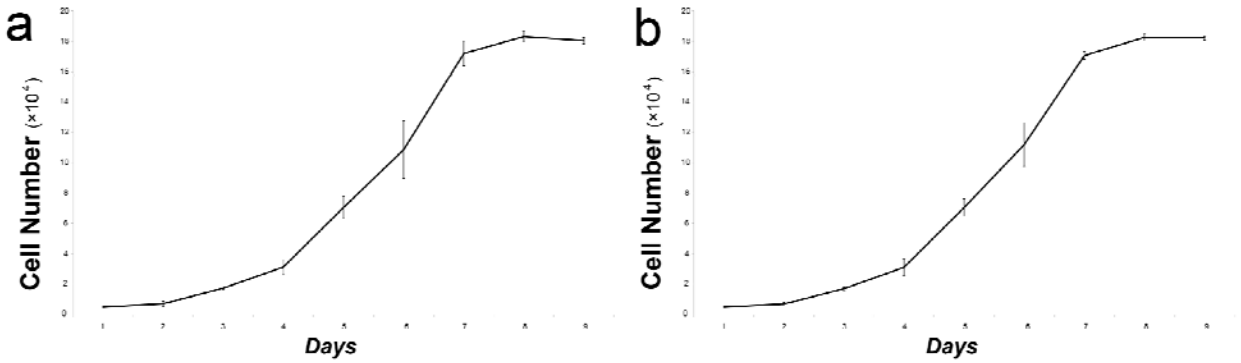

Fig. 4: Growth cure plotted using passage 3 of the two tested cell types and revealed that both of the cell types, human BM-MSCs (*a*) and PB-MSCs (*b*), had roughly similar growth rates.

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mouse and ram BM (5, 10, 20, 28), human umbilical cord (9, 18), human lung tissue (8) and mouse amniotic membrane (16) have been used in previous studies, which led to various contradictory results. Hence, in the present study we decided to determine the profile of the expression of 14 GC-specific genes in BM- and PB-MSCs and compare the expression levels of the genes in these two cell types. Furthermore, this is the first study of this kind which evaluates the expression pattern of GC genes in human PB-MSCs.

In this study, we isolated MSCs from human BM and PB samples. The cells were characterized based on the criteria previously explained by Dominici et al. which included the attachment of the cells to the bottom of culture dish, the capacity of differentiation into other cell types of mesenchymal lineage and the expression of MSCs known CD markers (29). We observed that both BM- and PB-MSCs were adhesive, elongated and spindle-shaped cells, which were successfully differentiated into osteocytes and adipocytes after 21 days treatment with osteogenic and adipogenic media, respectively. Moreover, flow cytometric analysis showed that the both cell types were strongly positive for some famous MSC CD markers including CD44, CD73, CD90 and CD105, and were negative for hematopoietic, CD31 and CD45, and endothelial ,CD34, surface markers.

After characterization, we used the P3 of both BM- and PB-MSCs for the evaluation of the expression of GC-specific genes with real time RT-PCR. As can be seen in the Fig. 5, our findings revealed that both cell types obviously expressed c-kit, Dazl, Fragilis, Itgb1, Nanos3, Oct4 and Blimp1, while they did not express Fkbp6, Stra8, Scp3, Tex13 and Vasa. Moreover, the expression level of Itgb1 in PB-MSCs was significantly ($p<0.05$) higher than its expression level in BM-MSCs. The two cell types only showed very low expression levels of Stella. Also, a very weak expression of Piwil2 was only observed in BM-MSCs. PB-MSCs did not express this marker, however.

C-kit is a GC marker which plays an important role in the differentiation of both male and female GCs and gametogenesis (30, 31). Reports on the expression of this gene in MSCs are controversial. A couple of studies have reported that MSCs from human, mouse and rat BM (7, 14, 19, 20, 27), mouse amniotic membrane (16) and also human fetal lung (8) expressed c-kit. While others indicated that mouse BM-MSCs (5) and human umbilical cord MSCs (9) did not express c-kit. In this study, we found that both BM- and PB-MSCs showed high levels of c-kit expression, which were comparable to the expression level of testicular cells. Although the expression level of c-kit in PB-MSCs was higher than that in BM-MSCs, it was not statistically significant ($p>0.05$).

Dazl has been shown to be an important marker of GCs, which is involved in GC development and can regulate the differentiation of GCs (32, 33). Like the case of c-kit, there are conflicting reports on the expression of Dazl in MSCs. Some studies have reported that human, ram, mouse and rat BM-MSCs (5, 13, 14, 20, 21), human fetal lung MSCs (8), human Wharton's jelly MSCs (34) and also mouse amniotic membrane MSCs (16) did not express Dazl, whereas others claimed that Dazl expression was observed in human and mouse BM-MSCs (7, 19). Similar to the results of the latter reports, we observed that BM- and PB-MSCs expressed Dazl at high and almost the same levels.

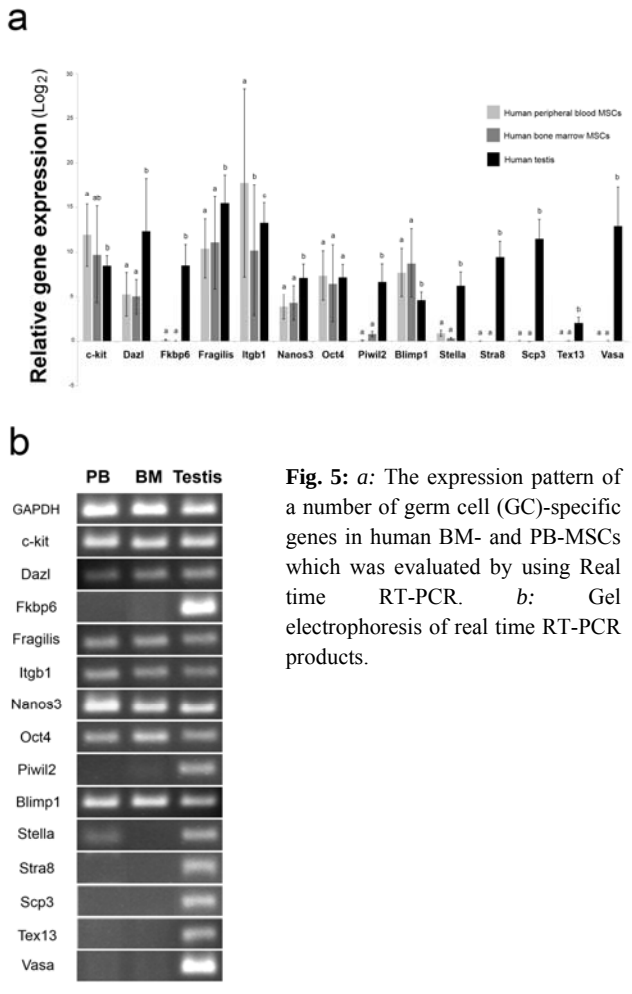

Fig. 5: *a:* The expression pattern of a number of germ cell (GC)-specific genes in human BM- and PB-MSCs which was evaluated by using Real time RT-PCR. *b:* Gel electrophoresis of real time RT-PCR products.

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Fkbp6 is a GC specific gene which is essential for male fertility (35, 36). Our results showed that both BM- and PB-MSCs were negative for Fkbp6 expression. There is no similar study for comparison, however.

One of the most important genes, involved in formation of PGCs and also the development and competence of GCs is Fragilis (37, 38). Two studies indicated that male human BM-MSCs (14) and female mouse BM-MSCs (19) expressed Fragilis. Moreover, our previous study revealed that male and female rat BM-MSCs showed high expression of Fragilis. In the current study, we found the same results. Both BM- and PB-MSCs had high and almost equal expression levels of Fragilis.

Itgb1 is a known marker of mouse spermatogonia, which is necessary for the migration and development of PGCs and has a role in human spermatogenesis (39-41). A number of research groups have shown that the MSCs of human BM (7), lung (8) and umbilical cord (18), and also ram BM-MSCs (10) expressed Itgb1 innately. In contrast, the others have reported that Itgb1 was not expressed in mouse and rat BM-MSCs (5, 27). We found that both BM- and PB-MSCs expressed Itgb1 at high levels, and the level of expression in PB-MSCs was higher than those in BM-MSCs and testicular cells ($p<0.05$).

Nanos3 is a marker of male and female PGCs, which is necessary for GC development, and any mutation in this marker can result in the loss of whole GCs and infertility (42, 43). Our results showed that both BM- and PB-MSCs expressed Nanos3 at high and almost the same levels. To the best of our knowledge, there is no other study which has evaluated the expression of Nanos3 in MSCs.

Oct4 is a famous pluripotency marker which is highly expressed in PGCs and has a vital role in the specification, establishment and survivance of germ cells (44, 45). All previous studies which evaluated the expression of Oct4 in MSCs have reported that MSCs from various sources expressed Oct4 (5-7, 9, 14, 16, 19), but the only quantitative study which estimated the expression levels of this marker in MSCs was our previous study on rat BM-MSCs. We found that both male and female rat BM-MSCs expressed Oct4 at high and roughly equal levels (20). In the current study, our result was similar to those of the previous studies. Oct4 was expressed in BM- and PB-MSCs at high levels, although there were not any significant differences between the expression levels.

Piwil2 is one of the most important GC markers which is expressed in premeiotic male GCs and can play an important role in the self-renewal of testicular SSCs and spermatogenesis. This marker also helps to repair DNA damage in GCs (20, 46). A number of researchers have reported that mouse BM-MSCs did not express Piwil2 (5), whereas the other researchers teams have shown that human and ram BM-MSCs (13, 14) and also mouse amniotic membrane MSCs (16) expressed Piwil2. Furthermore, in one of our previous studies, we found that both male and female rat BM-MSCs highly expressed Piwil2 and that male BM-MSCs expressed Piwil2 at significantly higher levels than female cells (20). In the current study, we observed that BM-MSCs showed a low expression level of Piwil2, whereas the marker was not expressed in PB-MSCs.

Blimp1 is activated early in GC developmental process and expressed in PGCs. Mutation in this marker can result in failure of PGCs for performing proliferation and migration (22, 47). There is no report on the expression of Blimp1 in MSCs. We observed that both of the tested cell types, BM- and PB-MSCs, expressed Blimp1 at high levels and the levels of expression were almost the same.

Stella is a GC marker, which is expressed early in the developmental process of GC and has an important role in the specification of PGCs (44, 47). A number of research studies have shown the expression of Stella in BM-MSCs of human (7, 14), mouse (19) and rat (20), whereas others have reported the contradictory results (5, 9, 21, 27, 34). In the current research, we observed a weak expression of Stella in both cell types. Although there were not any significant differences ($p>0.05$) between the expression levels, the expression level in PB-MSCs was higher than that in BM-MSCs.

Stra8 is expressed in the premeiotic stage of both male and female GCs and is vital for triggering meiosis in gametogenesis process (20, 48). Reports about the expression of this marker in MSCs are controversial. A couple of reports have stated that human and mouse BM-MSCs , human Wharton's jelly and lung MSCs ans also mouse amniotic membrane MSCs did not express Stra8 (5, 8, 14, 16, 18), whereas others have shown that human (7) , mouse (21) and rat BM-MSCs (20) expressed this marker. Our results were similar to the results of the first group. Both BM- and PB-MSCs were negative for Stra8.

Scp3 is the marker of both male and female GCs, which is expressed during meiosis (49). Like other markers, reports on the expression of this gene in

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MSCs is controversial. A study has reported that human fetal BM-MSCs expressed Scp3 innately (7), whereas the same research team has reported that human fetal lung-derived MSCs did not express the marker (8). Our evaluations showed that neither BM- nor PB-MSCs expressed Scp3.

Tex13 is a male GC specific marker, which is involved in the final stages of GC development (50). We found that, despite the testicular GCs, both BM- and PB-MSCs were negative for the expression of Tex13.

Vasa is one of the most known GC markers, which expressed form gonocyte stage until the end of the GC differentiation procedure (45). Most of the previous studies have shown that MSCs from different sources expressed Vasa (7, 14, 16, 19, 20, 23), but in this study, we observed that Vasa was expressed neither in BM-MSCs nor in PB-MSCs. This was similar to the findings of some other research studies (5, 8, 9, 27, 34).

As can be seen, BM- and PB-MSCs showed different expression levels of Itgb1. Integrins are adhesion molecules, which are involved in the cell migration and play critical roles in the normal formation of various tissues. Moreover, it has been shown that PGCs lacking Itgb1 fail to migrate and colonize the gonads (51). Nevertheless, perhaps higher expression level of Itgb1 in PB-MSCs is related to the fact that these cells are constantly moving and circulating with the blood. In addition, BM-MSCs, which are residing cells in the bone marrow and migrate only if it is needed, show lower expression levels of Itgb1.

Overall, this is the first report which evaluated and compared the expression levels of GC-specific markers in two types of human MSCs. Certainly, these data will help to clarify the uncovered zones of MSCs biology. Moreover, the results can help us to select the suitable cell source for using in stem cell-based therapies of infertility.

Conclusion

As stated above, BM- and PB-MSCs vigorously expressed a couple of GC-specific genes (c-kit, Dazl, Fragilis, Itgb1, Nanos3, Oct4 and Blimp1) and showed a weak expression of Stella.

Piwil2 was expressed only in BM-MSCs at very low levels, and the other tested markers, including Fkbp6, Stra8, Scp3, Tex13 and Vasa, were not expressed in both cell types. PB-MSCs expressed higher levels of Itgb1 than BM-MSCs, which probably occurred as a result of their different niches. This difference in the expression levels of the genes can perhaps affect their functions, which needs to be investigated in the future studies. All in all, both BM- and PB-MSCs expressed a number of genes, which are specific to both PGCs and/or adult GCs with some differences in the expression levels of Itgb1, Piwil2 and Stella.

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Conflict of interest

None declared.

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