Comparison of human mesenchymal stem cells derived from dental pulp, bone marrow, adipose tissue, and umbilical cord tissue by gene expression

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Aims. Our aims were to characterize human mesenchymal stem cells isolated from various tissues by pluripotent stem cells gene expression profile.
Methods. Four strains of dental pulp stem cells (DP-MSCs) were isolated from dental pulp tissue fragments adhered to plastic tissue culture dishes. Mesenchymal stem cells derived from umbilical cord tissue (UBC-MSCs) were isolated with the same technique. Bone marrow derived mesenchymal stem cells (BM-MSCs) were isolated from nucleated cells of bone marrow obtained by density gradient centrifugation. Human mesenchymal stem cells from adipose tissue (AT-MSCs) were isolated by collagenase digestion. All kinds of MSCs used in this study were cultivated in low glucose DMEM containing 5% or human platelet extract. All stem cell manipulation was performed in GMP conditions. Expression of 15 pluripotent stem cells genes on the level of proteins was measured by Proteome Profiler Human Pluripotent Stem Cell Array. Induction of MSCs to *in vitro* differentiation to adipocytes, osteoblasts, chondroblasts was achieved by cultivation of cells in appropriate differentiation medium.
Results. All MSCs tested were phenotypically similar and of fibroblastoid morphology. DP-MSCs and UBC-MSCs were more proliferative than bone marrow BM-MSCs and AT-MSCs. Protein expression of 15 genes typical for pluripotent stem cells distinguished them into two groups. While the gene expression profiles of BM-MSC, AT-MSCs and UBC-MSCs were similar, DP-MSCS differed in relative gene expression on the level of their products in several genes.
Conclusions. Dental pulp mesenchymal stem cells cultivated *in vitro* under the same conditions as MSCs from bone marrow, adipose tissue and umbilical cord tissue can be distinguished by pluripotent stem cell gene expression profile.

Key words: dental pulp, bone marrow, adipose tissue, umbilical cord tissue, mesenchymal stem cells, gene expression

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INTRODUCTION

Adult stem cells are present in a variety of tissues in the human body. The most studied adult stem cell population is the mesenchymal stem cells (MSCs). MSCs possess multi-lineage differentiation potential; they produce a variety of cytokines, chemokines, growth factors that mostly in paracrine fashion are involved in the regeneration of used and damaged tissues. MSCs can be readily isolated from several tissues like bone marrow, adipose tissue, umbilical cord tissue and from post-natal dental pulp tissues and other tissues. Multipotent mesenchymal progenitor cells known as dental pulp stromal/stem cells (DP-MSCs), with high proliferative potential for self-renewal are intensively studied because of their neural characteristics[1,2]. DP-MSCs can play a potential role in peripheral neural regeneration[3]. DP-MSCs as cells of ectomesenchymal origin can serve as an excellent source for generation induced pluripotent cells[4]. Recently it was shown that DP-MSC are a promising source for cell-based therapy for immune diseases such as systemic lupus erythematosus[5].

Generally, mesenchymal (stromal) stem cells have several interesting properties responsible for the induction of endogenous reparatory processes in the body. For instance human bone marrow mononuclear concentrates containing MSCs were shown to be an effective therapeutic strategy for "no option" patients with critical limb ischemia preventing limb amputations[6]. The quality of the BM-MSCs as gene expression and cell surface markers, determined the success in the ischemic ulcers treatment[7]. Discoveries of the immunomodulatory functions of MSCs have suggested that they might have therapeutic use in treating immune diseases[8,9]. MSCs also possess tumor tropic property; they can be recruited by tumors and metastases[10]. Genetically modified MSCs are the basis for cancer gene therapy using suicide genes (reviewed in[11,12]). MSCs represent novel promising therapeutic tools in emerging regenerative medicine. MSCs of different origin are being tested in a large number of clinical trials for at present untreatable diseases. As of August 2013, the public clinical trials database http://clinicaltrials.gov showed 344 clinical trials using MSCs for a very wide range of therapeutic applications.

MATERIALS AND METHODS

Cell cultures

All donors of adipose tissue, bone marrow, teeth, umbilical cord tissue and blood platelets provided informed written consent.

Human BM-MSCs were isolated from bone marrow aspirates taken from the iliac crest of normal adult donors by procedures described previously[7]. Briefly, BM-MSCs were isolated from nucleated bone marrow cells obtained by density gradient centrifugation by adherence to plastic dishes. The separated fraction was resuspended in a complete culture medium DMEM low glucose (1 g/L) supplemented with 5% human platelet extract (PE) and incubated at 37 °C in humidified atmosphere with 5% CO_2.

Human AT-MSCs were isolated from lipoaspirates using a collagenase type VII digestion and plastic adherence technique as described previously[9]. The material was obtained from healthy individuals undergoing elective lipoaspiration.

Dental pulp stem cells (DP-MSCs) were isolated from dental pulp tissue fragments adhered to plastic tissue culture dishes. The same technique was used to obtain umbilical cord tissue mesenchymal stem cells (UBC-MSCs).

For expansion of all kinds of MSCs, the cells were seeded at 4000 cells/cm^2 to plastic flasks and grown with medium exchange every 2-3 days. The cells were then harvested with trypsin/EDTA, resuspended at 1×10^6 cells/ml in 10% dimethylsulfoxide and 30% human serum albumin and frozen in 1 ml aliquots in liquid nitrogen.

Platelet extract preparations

Platelet extract was prepared from human platelets of healthy blood donors by procedures described previously[10]. Briefly, platelets in bags contained $5x10^{11}$/L platelets in blood plasma were twice frozen at -80 °C and subsequently thawed at 37 °C. Lysed platelets and platelets bodies were eliminated by centrifugation and the supernatant was filtered through a 0.22 µm GP Millipore Express Plus Membrane Stericup and designated as platelet extract (PE). PE isolates were negative for bacterial, fungal and mycoplasma contaminations.

Differentiation of MSCs in vitro

The ability of MSCs to differentiate to adipocytes, osteoblasts and chondrocytes *in vitro* was evaluated using Human Mesenchymal Stem Cell Functional Identification Kit (R&D SYSTEMS Minneapolis, MN 55413).

Protein concentration was measured by Pierce™ BCA Protein Assay Kit (Thermo SCIENTIFIC) according to the manufacturer's instructions.

Expression of MSCs genes

Expression of 15 pluripotent stem cell genes on the level of proteins was measured by Proteome Profiler Human Pluripotent Stem Cell Array (R&D SYSTEMS Minneapolis, MN 55413). Relative expression levels of individual genes were detected according to the manufacturer's instructions. Briefly, cultured MSCs were rinsed with PBS, disintegrated by lysis buffer and the cell extract was prepared by centrifugation according to the recommendations of the provider. Total protein concentration was determined. The same amount of protein was used for incubation of all arrays and procedure was performed as directed by the manufacturer. Membranes were exposed to Western HRP Substrate (Luminata™ Forte), covered with plastic wrap and exposed to X-ray film. Pluripotent stem cell array data on developed X-ray film was quantified by scanning the film by a transmission mode scanner. ImageJ software (NIH) was used for the quantitative evaluation.

Statistics

SigmaPlot 11.0 software for Windows (Systat Software, Germany) was used for statistical analysis. Student's t-tests were used for the data that passed the Shapiro-Wilk normality test and the Mann-Whitney Rank Sum test for non parametric data.

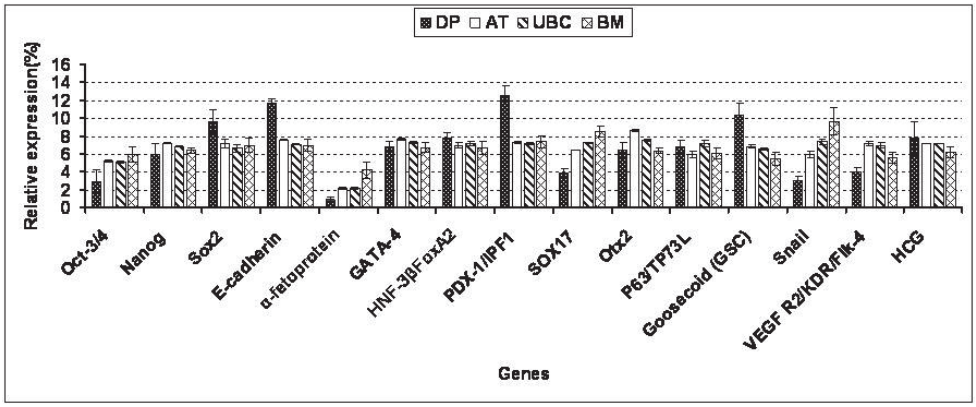

Fig. 1. Protein expression of 15 pluripotent stem cell genes. Cell extracts of BM-MSCs (n=10), AT-MSCs (n=4), UBC-MSCs (n=4) and DP-MSCs (n=4) were examined by Proteome Profiler Human Pluripotent Stem Cell Array and quantified by ImageJ program.

RESULTS

Relative expression of 15 pluripotent stem cell genes on the level of proteins

Tissue cultures of BM-MSCs derived from early passage of ten bone marrow donors, four AT-MSCs, four umbilical tissue derived MSCs and four MSCs prepared from dental pulp tissue were examined for relative expression of 15 genes by Proteome Profiler Human Pluripotent Stem Cell Array. The data quantified by ImageJ software are presented in Fig. 1.

Relative expression of all tested genes in MSCs derived from bone marrow, adipose tissue and umbilical tissues did not differ significantly. In DP-MSCs higher relative expression was found for the following genes: E-cadherin, Goosecold (GSC), PDX-1/IPF1 (statistical significance $P \leq 0.001$) and, Sox2 ($P=0.013$) in comparison with expressions in BM-MSCs. Lower relative expression of genes Snail ($P \leq 0.001$) Sox17 ($P=0.006$) and gene Oct-3/4 ($P=0.006$) was detected in the same comparison. Thus DP-MSCs differed substantionally from MSCs derived from bone marrow, adipose tissue and umbilical cord tissue.

Differentiation of MSCs *in vitro*

DP-MSCs can be induced to all differentiation lineages required for MSCs characterization *in vitro*. Interestingly, while BM-MSCs and AT-MSCs showed a gradual loss of osteogenic differentiation potential with increasing number of passages *in vitro*, DP-MSCs on the other hand had an increasing potential (Fig. 2.).

Presence of cell surface markers

All tested MSCs were examined for expression of cell surface markers by flow cytometry. All MSCs tested expressed CD44, CD90, CD105 markers and were negative

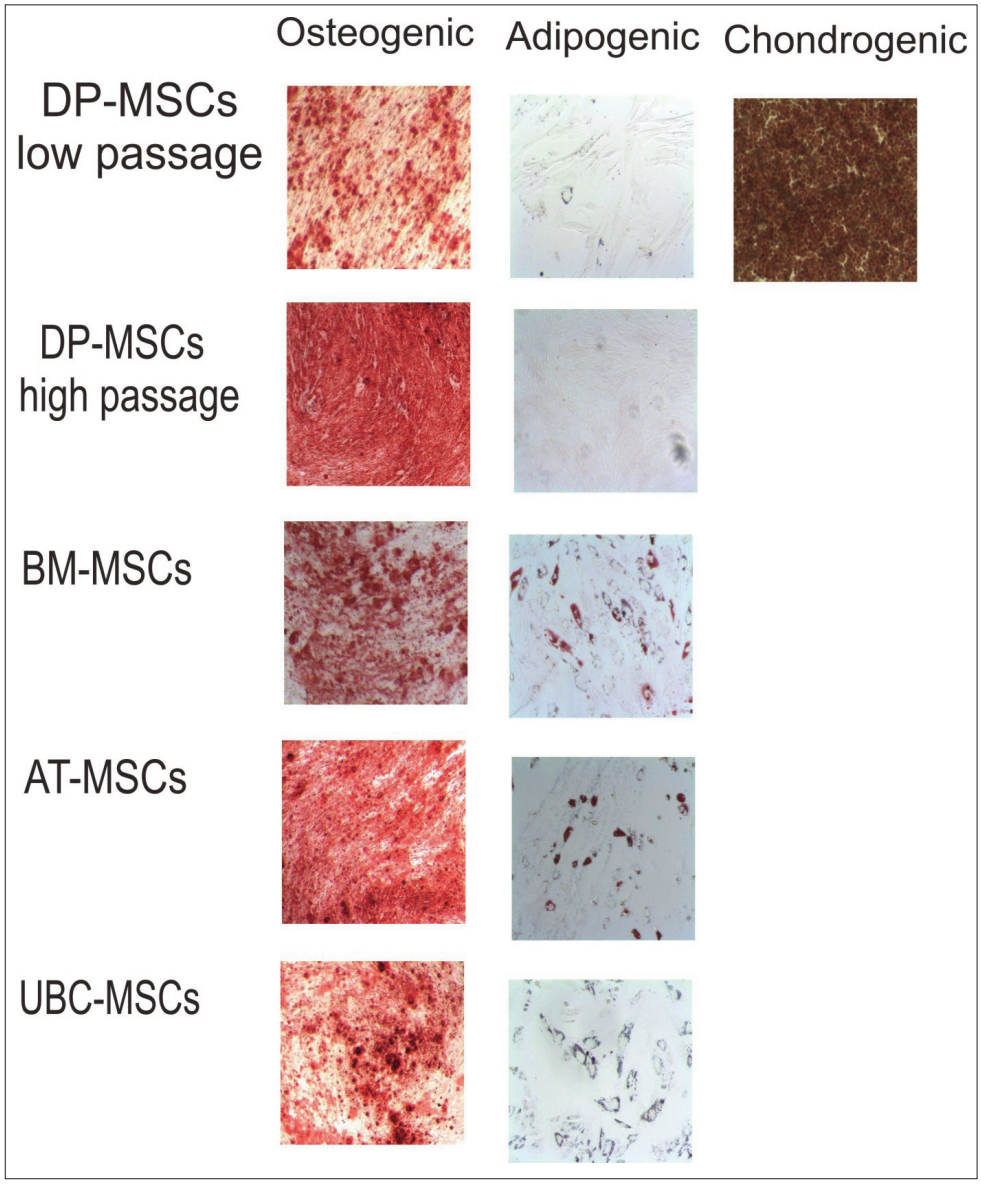

Fig. 2. Differentiation of BM-MSCs, AT-MSCs, UBC-MSCs and DP-MSCs *in vitro*. Human Mesenchymal Stem Cell Functional Identification Kit was used for *in vitro* induction of differentiation of all MSCs into osteogenic, adipogenic and chondrogenic lineages.

for expression of CD34, CD45 surface antigens (data not shown).

DISCUSSION

Adult human MSCs are factories that produce a large number of bioactive factors that induce in the body, the molecular processes of regenerative paths. Bone marrow-derived MSCs (BM-MSCs) secrete factors that act in a paracrine manner to promote complex endogenous reparatory processes. MSCs act through interactions with the endogenous cells and tissues. They are also responsive to their environment and can modify their activities and functions depending on the biomolecular context. MSCs can accelerate wound closure by modulating the inflammatory environment, promoting the formation of a well-vascularized granulation matrix, encouraging the migration of keratinocytes, and inhibiting apoptosis of wound healing cells. Numerous *in vivo* studies provide overwhelming evidence that the biologically active compounds secreted by BM-MSCs play the main role in their therapeutic potential. An important part of the healing process is suppression of the immune response and/or immunomodulation induced by MSCs. There is no evidence that applied cells are transformed into target tissue. Therefore, understanding the gene expression profile that indicates their lineage-specific proclivity is fundamental to the development of successful cell-based therapies.

Our comparison of human mesenchymal stem cells derived from different tissues revealed no differences in cell morphology or in the expression of surface markers typical for mesenchymal stem cells. However, a comparison of the protein levels of several genes typical for pluripotent stem cells led to classifying these MSCs into two groups. There were no significant differences in the level of 15 pluripotent stem cell genes of BM-MSCs, AT-MSCs and UBC-MSCs. We found highly significant differences in the amount of protein products in DP-MSCs. All cells were cultivated in the same culture fluid with human growth factors from platelets.

Observed differences in gene expression of pluripotent stem cell genes in DP-MSCs might reflect their embryonic stem cell origin. Dental pulp is made of ecto-mesenchymal elements, containing neural crest-derived cells, which display plasticity and multipotential capabilities[13]. Mixed embryonic origin of DP-MSCs might be connected with our observation of the lower level of transcription factor characteristic for embryonic stem cells Oct-3/4 in comparison with BM-MSCs. Determination of Oct3/4 on protein level rules out the role of several Oct-4 pseudogenes present in normal genome. Higher expression of gene Goosecoid, which acts as a transcription factor is obviously connected with its involvement in embryogenesis, where plays a role in craniofacial and rib cage development[14].

We found in DP-MSCs higher expression of product of gene E-cadherin - calcium dependent adhesive molecule and lower expression of gene Snail. Snail acts as the E-cadherin repressor. Snail is a zinc finger transcriptional repressor that downregulates the expression of ectodermal genes within the mesoderm. Cadherins and transcription factor Snail are specific markers of Epithelial-Mesenchymal Transition (EMT). EMT plays a role in maintenance of embryonic mesoderm, growth arrest, survival and cell migration[15,16]. Higher expression of E-cadherin and lower expression of Snail favor DP-MSCs for reparative functions where EMT plays an important role. This might also reflect our observation of the ability of DP-MSCs to undergo osteogenic differentiation despite the large number of passages *in vitro* that differ from the other MSCs. This is in agreement with observation of Hara and coworkers[17,18] that expression of bone morphogenic protein 4 in DP-MSCs is much higher than in BM-MSCs.

The transcription factor PDX1 plays a critical role in glucose-induced insulin gene transcription in adult β-cells. High expression of Pdx1 in DP-MSCs suggests that those cells might be useful for treatment of diabetes and/or could be a good source of cells for transdifferentiation to beta cells[19].

The product of Sox2 gene and Sox 17 both important transcriptional regulators are required for stem-cell maintenance in the central nervous system[20]. Sox2 may function as a switch in neuronal development. Higher expression of Sox2 reflects probably the ability of DP-MSCs to be induced *in vitro* to undergo neuronal differentiation – a property that differs from the other MSCs. This supports our observation that DP-MSCs cultivated in neurobasal medium form spheroids.

MSCs isolated from different tissues have heterologous populations of clones with various growth and/or regenerative potential. Gene expression profiles might be one of characterizing for use in future clinical applications. Microarray analysis was used to compare the global gene expression profiles of high growth/multipotential clones with low growth potential cell clones derived from 3 stromal tissues[21].

Whether the difference in gene expressions in DP-MSCs compared with BM-MSCs, AT-MSCs and UBC-MSCs is reflected in their secretome, remains to be analysed.

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