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Comparison of Mesenchymal Stem Cells Obtained from Different Human Tissues

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We studied mesenchymal stem cells from human bone marrow, adipose tissue, skin, placenta, and thymus. Morphological study and cytofluorometrical analysis by the main marker genes (CD10, CD13, CD31, CD44, CD90, CD105) were carried out. Mesemchymal stem cells of the studied tissues during isolation and culturing were morphologically similar and did not differ by the expression of the main marker genes.

Key Words: *mesenchymal stem cells; expression of marker genes*

Mesenchymal stem cells (MSC) were first described by Fridenstein in the bone marrow are now intensively studied. Great interest to this object can be explained by the possibility of using these cells for various medical purposes. The bone marrow, which was considered to the main source of MSC in the organism, was thoroughly studied for many years. Ample data were accumulated on clinical application of hemopoietic and mesenchymal bone marrow stem cells [2,5-7]. The main characteristics of bone marrow MSC are fibroblast-like morphology, adhesion to plastic, morphological heterogeneity of population, differentiation under certain conditions into adipocytes, chondrocytes, osteocytes, myocytes, cardiomyocytes, neural cells and other cell types, expression of marker genes (CD13, CD44, CD90, CD105, Stro1, and some other markers) and the absence of expression of genes typical of hemopoietic cells (CD34, CD45) [1,3,8,9]. There are data on the presence of MSC-like cells in other organs and tissues. P.Zuk *et al.* described MSC in the adipose tissue [9,10]. Similar data were presented by Japanese scientists [4]. Stem cells were detected in practically all organs and tissues, but their origin is still poorly studied.

Mesenchymal and parenchymal components are present in all organs. It is obvious, that stem cells in different organs are committed. The identity of MSC from different sources is still not proven. Only few attempts to clarify this question were undertaken. For instance, comparative characteristics of MSC from the bone marrow and adipose tissue were presented by P. Zak *et al.* [10]. According to these data MSC from these sources are similar by their morphology, expression of marker genes, and differentiation potential.

The aim of the present study was to compare MSC-like cells from different sources: human bone marrow, adipose tissue, placenta, thymus, and skin.

MATERIALS AND METHODS

We studied human bone marrow, adipose tissue, placenta, skin, and thymus. The bone marrow, fat, and skin were obtained from healthy adult donors aging 13-58 years (mean age 25 years). Placenta specimens were obtained during normal labor, thymus samples were obtained from stillborn infants. All donors and/or their parents gave informed consent for the use of their tissues for scientific purposes.

Bone marrow samples (10-15 ml) were obtained from the iliac bone by aspiration biopsy under local anesthesia. Fat specimens (3-5 ml) were obtained from a small cut in the umbilical area under local anesthesia. Skin specimens (1.0-1.5 cm^2) were obtained during surgery. The cells from different sources (fat, skin, placenta, and thymus) were isolated and cultured under identical conditions. The only exception was the use of different types of collagenase for the treatment

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of different tissues. Bone marrow was treated routinely and separated on a Ficoll gradient for obtaining mononuclear cell fraction. Bone marrow MSC were cultured under the same conditions. Collagenases 1, 2, and 4 (Invitrogene) were used for tissues.

The bone marrow was diluted 1:1 with PBS, layered onto a Ficoll-Paque gradient (Pharmacia), and centrifuged at 400*g* and 10°C for 30 min. Medium cell layer was collected, washed with PBS, and centrifuged at 200*g* and room temperature for 10 min, and after erythrocyte lysis with 160 mM NH_4Cl washed again and seeded at a density of 1×10^6 cells per cm^2. Tissue fragments were washed in PBS, minced, and treated with 0.075% collagenase for 30 min at 37°C. The enzyme was inactivated with DMEM supplemented with 10% FBS (Gibco), repeatedly centrifuged at 200*g* and room temperature for 10 min, and repeatedly washed in PBS (erythrocytes were lyzed when needed as for bone marrow cells). Tissue fragments were removed by filtering through 10-μ Nylon filters (Millipore). The cells were seeded onto uncoated plastic at a density of 1×10^6 cells per cm^2 according to the same scheme. On the next day the medium was replaced. The cells were cultured in DMEM (Gibco) with low glucose content (1 g/liter) and containing 10% FBS, 2 mM glutamine, 1% penicillin and streptomycin under standard conditions (37°C and 5% CO_2). The medium was replaced every 3rd-4th day. After attaining 80% confluence, the cells were detached with 0.25% trypsin/EDTA and seeded at a density of 6-10 cells per cm^2. Cytofluorimetrical analysis was performed after 2 and 5 passages and after 3-month culturing. The samples were analyzed on an EPICS-V Elite flow cytofluorimeter (Coulter) using a logarithmic scale of fluorescence. Fluorescence was excited with an Innova-90-6 argon laser (Coherent). The cells with fluorescence intensity below the main peak were considered to die through apoptosis. FITC-labeled monoclonal antibodies CD44, CD90, CD105/Endoglin, CD45, CD34, CD10, CD13, and CD31 (Beckman Coulter and Coltak) were used. Before the analysis on the flow cytofluorimeter, the cells were detached with 0.25% trypsin/EDTA, fixed in 2% cold formaldehyde for 30 min, and washed with PBS buffer containing 2% FBS, 0.2% Tween 20. Cell aliquots (1×10^6) were incubated in this buffer containing the above listed monoclonal antibodies. The data were processed using EXPO32 software (Applied Cytometry Systems).

RESULTS

For all cultures the lag period was 24-48 h. Log-phase slightly differed depending on the culture. The first cell monolayer (80% confluence) was attained at different terms: 2-3 weeks in culture for bone marrow cells and 7-9 days for adipose cells. Thymic cells grew intensively and attained the first monolayer on days 8-10; the cells from the placenta grew slowly and attained 80% confluence after 3-4 weeks. Proliferative activity of all tested cells did not decrease after 3 months. The doubling rate for the cell populations in the log-phase was calculated by the formula $\log N1/\log N_2$, where N_1 is the number of cells in the confluent monolayer and N_2 is the number of seeded cells. The rate of proliferation depended on donor age. The cells obtained from young donors grew more rapidly.

MORPHOLOGICAL CHARACTERISTICS OF CELLS

Figure 1 shows bone marrow MSC: individual flattened cells and cell monolayer. MSC population is very heterogeneous by cell size (from 10 to 300 μ for attached cells) and shape. Small cells predominated in early passages, but in later passages they became less numerous. There were several cell types: thin spindle-shaped, typical fibroblast-like, and mantle cells. The intensity of cell grown under these conditions did not change with time. Bone marrow MSC grew similarly over 3-month culturing. The rate of their proliferation depended on the donor, rather than on culturing time. Spontaneous differentiation, mainly into astrocytes and adipocytes, was sometimes observed in cell population.

Adipose cells did not differ morphologically from bone marrow cells. Heterogeneity of the population was also observed. The same picture was noted for cell shape (Fig. 1, *b*). The same directionality of cells was seen in the monolayer with the formation of "fish schools". However, the initial number of stem cells in the fat far surpassed that in the bone marrow. In 3 cases we compared bone marrow and adipose MSC from the same donor. The yield of MSC from 1 cm^3 fat is higher by one order of magnitude than from the same volume of the bone marrow. The fraction of small cells in the fat predominates and persists for longer time than in the bone marrow. In the same patients proliferative activity of cells form the fat was not lower, but was even higher than that of bone marrow MSC.

After isolation and culturing under specified conditions, typical MSC-like cells similar to the cells from the bone marrow and fat, grew from the skin. They were characterized by the same shape and size, and the same monolayer appearance (Fig. 1, *c*). Proliferative activity of MSC from the fat and skin of the same donor was similar.

In cell population from the placenta mantle cells predominated, but in the monolayer the cells looked and behave similarly to other cell populations. The

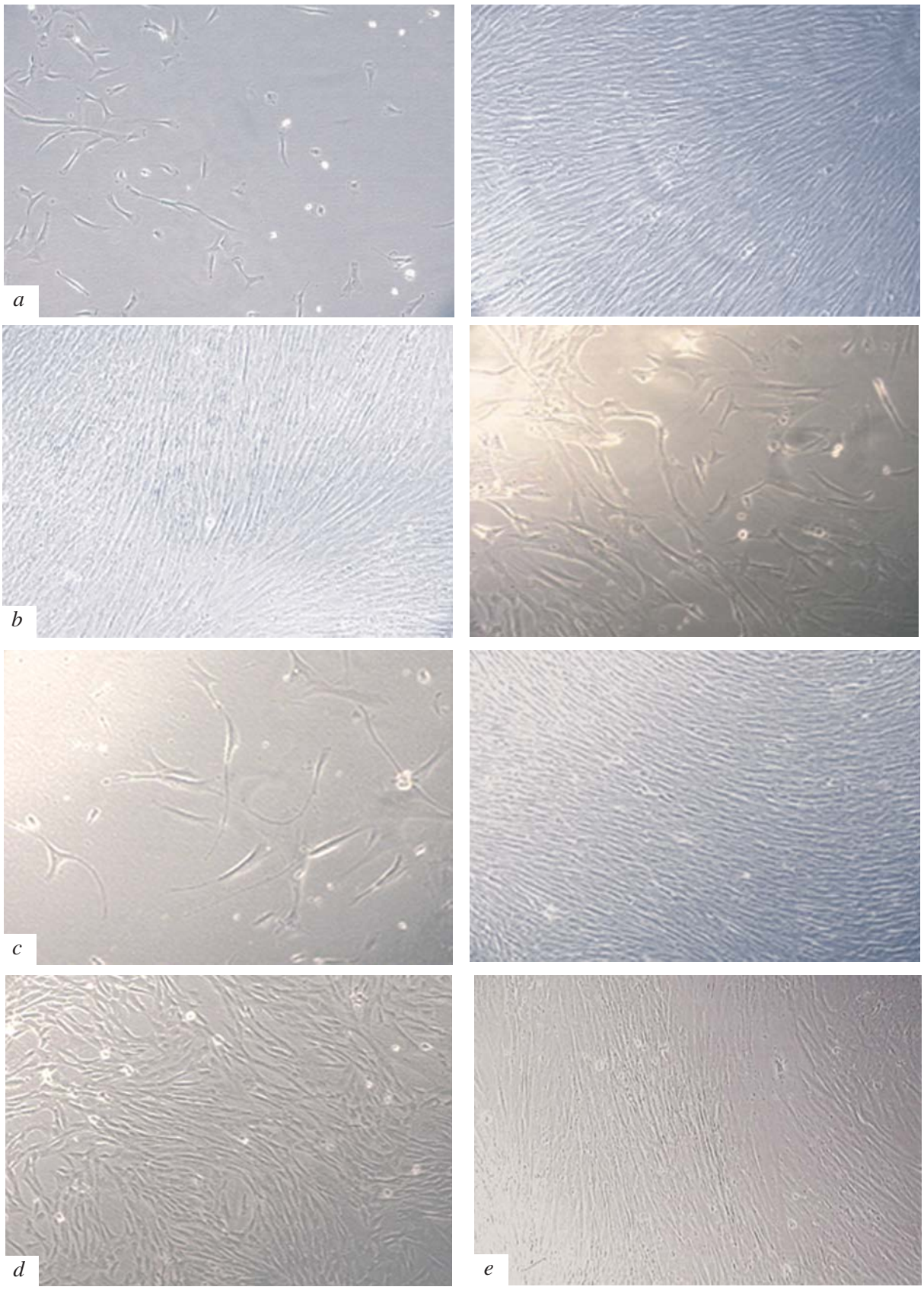

Fig. 1. Mesenchymal stem cells (MSC) obtained from different sources. *a*) bone marrow, primary cells and monolayer; *b*) cells from the adipose tissue, monolayer and solitary cells before the first passage; *c*) cells from the skin; ×100; *d*) placenta cells; *e*) thymic cells; ×100.

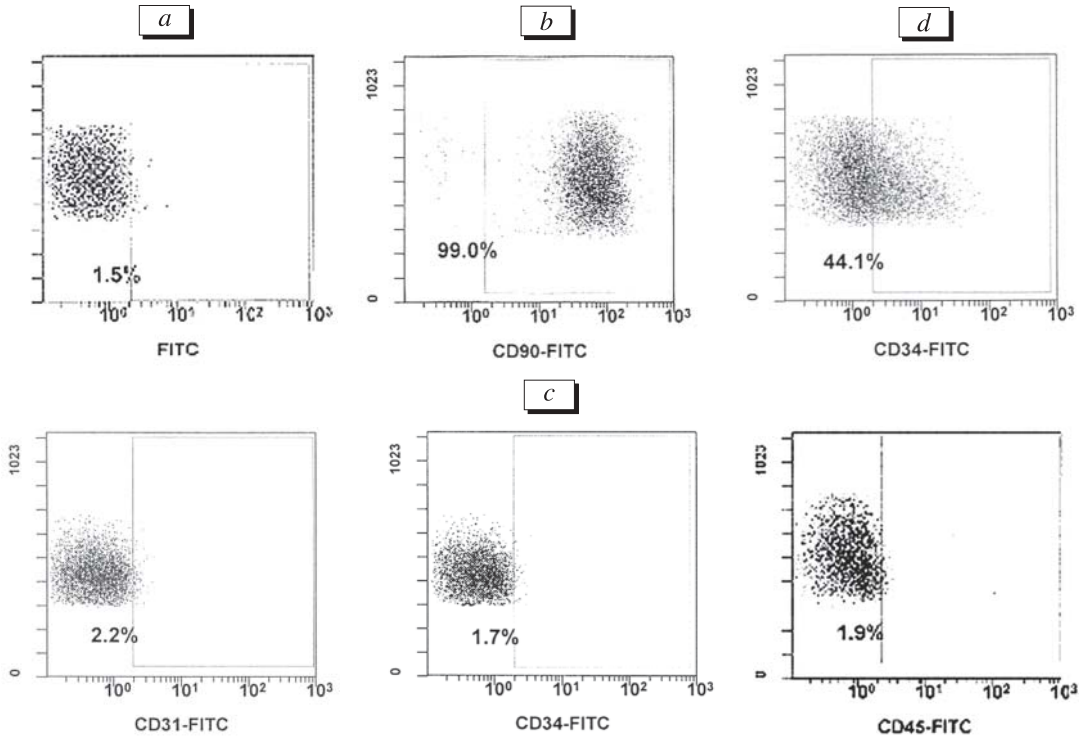

Fig. 2. Cytofluorimetric analysis of the expression of marker cells in MSC from different sources. *a*) background values; *b*) expression of CD90; *c*) expression of negative markers CD31, 34, 45; *d*) expression of CD34 in thymic cells.

fraction of small cells was less numerous at the start of the experiment and throughout culturing. The same cell types and heterogeneity by the cell size were noted. Proliferative activity was lower than in other cell populations (Fig. 1, *d*).

Thymic cells morphologically differed from other cells, despite their heterogeneity and similar behavior in the monolayer (Fig. 1, *e*). Predominance of mantle cells was noted. The fraction of small cells was not numerous. Proliferative activity at the initial stages of culturing was high and comparable to that of fat and skin cells. A peculiar feature was rapid spontaneous differentiation into nerve cells (often after 3-4 passages) with the corresponding decrease in proliferative activity.

GENE EXPRESSION

All cells were characterized cytofluorimetrically by the expression of the following genes: CD44, CD90, CD105, CD13, CD10, CD31, CD34, and CD45. The analysis was repeated trice over 3-month culturing

TABLE 1. Expression of Marker Genes in MSC Isolated from Different Sources

Gene	Bone marrow	Fat	Skin	Thymus	Placenta
Background	1.3	1.5	1.5	2.2	1.8
CD44	87.8	98.9	88.9	98.6	99.0
CD90	99.0	99.7	99.7	99.0	95.7
CD105	97.9	99.9	99.3	98.5	99.3
CD13	90.2	95.6	96.8	96.8	90.1
CD31	1.4	2.2	1.7	5.0	4.0
CD34	0.9	1.7	1.4	44.1	3.1
CD45	0.9	1.9	1.1	1.9	2.1
CD10	41.7	90.4	89.9	6.2	51.1
Nestin	—	—	—	10.1	—

Note. dash: not measured.

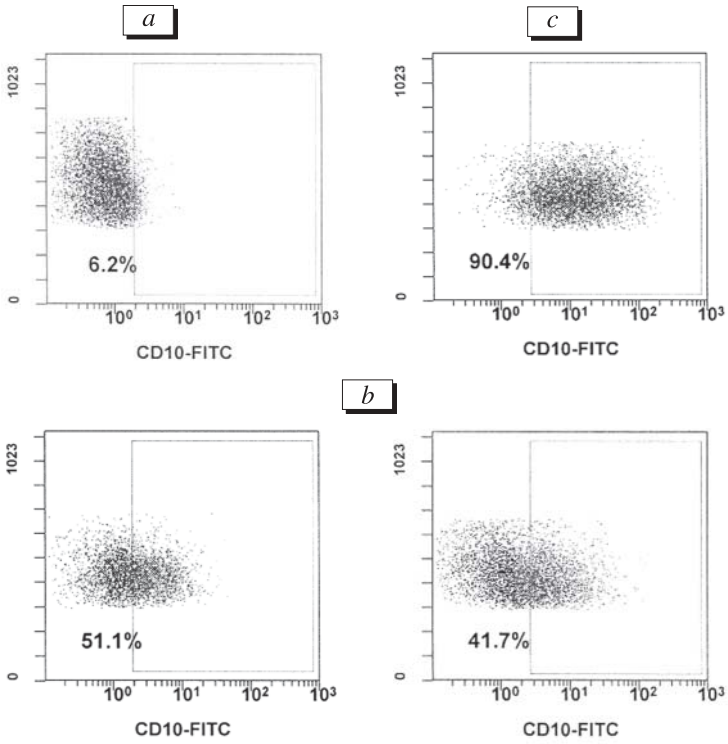

Fig. 3. Expression of CD10 in thymic cells (*a*), cells from the bone marrow and placenta (*b*), cells from the fat (*c*).

(after 2nd, 5th, and 10th passages). The background did not exceed 2% (Fig. 2, *a*). Expression of the main marker genes of MSC (CD44, CD90, CD105, and CD13) was similar in all studied cells. Expression of these genes was very high: 87.0-99.7% (Fig. 2, *b*) and did not change throughout culturing. Expression of CD34, CD45, and CD31 genes did not exceed the background values throughout the experiment (Fig. 2, *c*). The only exception was thymic cells: expression of CD34 gene attained 41.1% (mean 35%, Fig. 2, *d*) and remained unchanged throughout the experiment.

Expression of CD10 gene in thymic cells was ~6% (Fig. 3, *a*) and in bone marrow and placenta cells about 40% (Fig. 3, *b*). In fat and skin cells this parameter attained 90.4% (mean 85%, Fig. 3, *c*) and remained unchanged throughout culturing. Thymic cells were additionally characterized by nestin, expression of this marker was 10.1% (Fig. 3, *d*).

The identity of mesenchymal cells from different sources is still not proven. Descriptive reports devoted to cells of a certain tissue without comparative characteristics predominate among scientific publications in this field. These data cannot be correctly compared because of differences in isolation and culturing of these cells. Measuring of gene expression was usually performed only once and gave contradicting results, probably due to different culturing conditions. The main conclusion from our data is unique character of MSC-like cells obtained from different sources. It can be concluded that MSC first described by Fridenstein in the bone marrow and usually named stromal can be found in different tissues and organs. We present comparative data on MSC from human bone marrow, fat, skin, placenta, and thymus. These cells have similar morphological characteristics and do not differ by the expression of the main marker genes CD13, CD44, CD45(-), CD90, and CD105 (Table 1). However, we noted some specific features in the morphology and gene expression in cells obtained from different sources. These peculiarities were probably determined by the presence of different progenitor cells in the populations. A fraction of small cells (<10 μ) predominating at the early stages of culturing should be noted. Of particular interest is high initial content of stem cells in the fat compared to other tissues. We can conclude that the bone marrow is not the main source of MSC in human organism and that subcutaneous fat is an alternative source of MSC for clinical purposes, which agrees with previous data [4,10].

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