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Comparative Analysis of Secretome of Human Umbilical Cord- and Bone Marrow-Derived Multipotent Mesenchymal Stromal Cells

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Production of cytokines and growth factors by cultured human umbilical cord tissue- and bone marrow-derived multipotent mesenchymal stromal cells was measured by multiplex analysis. In most cases, the concentrations of bioactive factors in the culture medium conditioned by umbilical cord-derived cells was ten- to hundred-times higher than in the medium conditioned by bone marrow-derived cells. These results suggest that both multipotent mesenchymal stromal cells from the umbilical cord and cell-free products can have more pronounced therapeutic effect in comparison with mesenchymal stromal cells obtained from "adult" sources.

Key Words: *multipotent mesenchymal stromal cells; umbilical cord; bone marrow; secretome*

Tissues of perinatal origin attract now much attention due to the prospects of using multipotent mesenchymal stem cells (MSC) in the therapy of a wide range of acquired and hereditary disorders. Hundreds of experimental and clinical studies are aimed at studying biological properties of MSC derived from the placenta, umbilical cord, and amniotic fluid, their safety and effectiveness in various fields of regenerative medicine: cardiology, neurology, angiology, traumatology and orthopedics, in the therapy of the liver, kidneys, reproductive system diseases, *etc*. [7,8,10,13,15,17].

Due to the ability of MSC to differentiate into cells of various tissues, they can directly participate in the reparative regeneration of damaged organs and restore tissue defects [8,14]. At the same time, a number of studies show that after the systemic (intravenous) administration only a small numbers of MSC reach the target tissue, while after local delivery, they are rapidly eliminated from the site of injection [9]. Therefore, the effects of MSC can be achieved due to other mechanisms including the so-called "paracrine" regulation [11,18-20].

Like most other cell types, MSC can synthesize and secrete into the extracellular space a wide range of biologically active compounds and structures: cytokines, growth factors, peptides, microparticles, as well as various components of extracellular matrix, shared under one common term – "secretome" [11,17,19]. Both the secretome itself and each of its individual components are considered now as a potential therapeutic tool for regenerative medicine [8].

Despite the fact that MSC could be successfully isolated from many postnatal tissues (vascular wall, liver, skin, peripheral, umbilical and menstrual blood, and even from the pulp of naturally exfoliated deciduous teeth) [7,12,16,21-23], the most promising sources (in terms of clinical application) were considered to be bone marrow and adipose tissue. Isolation and effective expansion of MSC from other tissues are difficult due to their extremely low concentration (less than one thousandth of a percent in the case of umbilical cord blood in full-term pregnancy), or difficulties in obtaining these tissues in sufficient quantities. In this regard, perinatal tissues have a number of

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advantages. Their collection (in particular, umbilical cord) does not require surgical procedure and is usually carried out after childbirth. The content of MSC in the umbilical cord tissue significantly surpasses that in adult tissues: ~5×10^4 cells/cm umbilical cord *vs.* 5×10^3 cells/g of adipose tissue and 0.01% in bone marrow mononuclear fraction [17]. Moreover, biological properties of "young" cells (proliferative activity, multidirectional differentiation capacity, *etc*.) favorably distinguish them from adult MSC, which have already exhausted a significant part of their inherited potential. No unambiguous data on other fundamental differences between MSC from the umbilical cord and from adult sources, in particular, in their synthetic and secretory activity, are available.

The aim of this study was to analyze the secretion of bioactive molecules by MSC isolated from umbilical cord tissue and cells obtained from the bone marrow of adult donors.

MATERIALS AND METHODS

MSC isolation and culturing. The procedures of MSC isolation from human umbilical cord tissue (UC-MSC) and bone marrow (BM-MSC) have been described in our previous publications in detail [1-6]. Both types of cells were cultured in DMEM/F-12 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin (all substanses were from GIBCO Invitrogen) and 10% fetal bovine serum (HyClone). We used cell cultures derived from three umbilical cord tissue samples (passage 2-4 cultures) and from three bone marrow aspirates from different donors (passage 3 cultures). To avoid differences associated with individual characteristics of media, sera, and other components, the cells were cultured under absolutely identical conditions using the same lots of reagents.

Preparation of the conditioned medium. Confluent MSC cultures were washed with Hanks balanced salt solution and fresh culture medium was added. In 48 h, the cells were used in experiments [24], while conditioned media were collected, centrifuged for 20 min at 3000 rpm to precipitate cellular debris, aliquoted, frozen, and stored in the vapor phase of liquid nitrogen at a temperature below -160°C. The duration of storage varied from 2 months to 1 year.

Analysis of MSC secretion products. The concentration of MSC secreted molecules was measured in thawed aliquots of conditioned media using commercial kits Bio-Plex Pro Human Cytokine Group I Assay (Eotaxin, FGF-basic, G-CSF, GM-CSF, IFNγ, IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17α, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-BB, RANTES, TNFα, and VEGF) and Bio-Plex Pro Human Cytokine Group II Assay (CTACK, GRO-α, HGF, IFNα2, IL-1α, IL-2RA, IL-3, IL-12(p40), IL-16, LIF, MCP-3, M-CSF, MIG/CXCL9, NGF-β, SCF, SCGF-β, SDF-1α+β/CXCL12, TNFβ, and TRAIL) (Bio-Rad) on Bio Plex 200 System (Bio-Rad) in accordance with manufacturer's instructions. To prevent measurement errors, all samples were analyzed in one experiment. The growth medium not conditioned by MSC was used as the control.

Statistical analysis. In view of limited number of analyzed samples and variability of the studied parameters between the cell cultures, non-parametric Mann—Whitney *U* test was used (Statistica 7.0). The differences were significant at $p<0.05$.

RESULTS

As expected, control growth media did not contain significant amounts of the analyzed molecules. The exceptions were IL-12(p40) (40-92 pg/ml), GRO-α (41-180 pg/ml), SCGF-β (71-276 pg/ml), and FGF (33-47 pg/ml); the concentration of other substances did not exceed 10-25 pg/ml.

In comparison with the control, the concentrations of some cytokine in BM-MSC conditioned media varied from 25-100 pg/ml (IFNγ, IL-12, IL-16, M-CSF, MIF, and TNFβ) to 100-1500 pg/ml (MCP-1, SCGF-β, SDF-1A, VEGF, and TRAIL) (Table 1); production of other biological factors (CTACK, GRO-α, IL-1α, IL-1β, IL-2, IL-2RA, IL-12(p40), IL-17, IL-18, IP-10, TGF-β, FGF, and RANTES) did not differ from control values. IL-6 was found to be the most abundant molecule (3389±2458 pg/ml). The detection of some other components in BM-MSC-conditioned media (*e.g.* IFNγ) was more likely due to the presence of hematopoietic cells in some MSC cultures at early passages.

Analysis of media conditioned by UC-MSC showed more impressive results. Despite rather low concentration of the majority of analyzed molecules (<100 pg/ml, though significantly higher than in the control media), the concentration of some cytokines (IL-2RA, IL-3, LIF, MCP-1, MCP-3, M-CSF, SDF-1α, and TRAIL) attained 1000 pg/ml and even 1000-5000 pg/ml (G-CSF, HGF, IL-12p40, IL-16, and MIF). The most abundant cytokines and growth factors in UC-MSC-conditioned media were GRO-α, IL-6, IL-8, and SCGF-β (concentration range up to 5000-65,000 pg/ml).

Thus, UC-MSC are characterized by significantly higher secretory activity than BM-MSC. In some cases, the mean concentrations for some molecules (IFNα2, IL-1α, IL-2RA, IL-3, IL-6, IL-12(p40), IL-16, LIF, M-CSF, SCGF-β, and RANTES) differed by 5 to 10 times and much more. and by some parameters