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Research Article

Novel markers of mesenchymal stem cells defined by genome-wide gene expression analysis of stromal cells from different sources

Nikolas Kaltz[a], *Jochen Ringe*[b], *Christina Holzwarth*[a], *Pierre Charbord*[c], *Markus Niemeyer*[d], *Volker R. Jacobs*[d], *Christian Peschel*[a], *Thomas Häupl*[b], *Robert A.J. Oostendorp*[a,*]

[a]*3rd Department of Internal Medicine, Klinikum rechts der Isar, Technische Universität München, München, Germany*
[b]*Department of Rheumatology and Clinical Immunology, Tissue Engineering Laboratory and BCRT, Charité-Universitätsmedizin Berlin, Berlin, Germany*
[c]*INSERM U972, Hôpital Paul Brousse, Villejuif, France*
[d]*Department of Gynaecology and Obstetrics, Klinikum rechts der Isar, Technische Universität München, München, Germany*

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ABSTRACT

Mesenchymal stem cells (MSC) represent a mixture of different cell types, of which only a minority is therapeutically relevant. Surface markers specifically identifying non-differentiated MSC from their differentiated progeny have not been described in sufficient detail. We here compare the gene expression profile of the in vivo bone-forming bone marrow-derived MSC (BM-MSC) with non-bone-forming umbilical vein stromal cells (UVSC) and other non-MSC. Clustering analysis shows that UVSC are a lineage homogeneous cell population, clearly distinct from MSC, other mesenchymal lineages and hematopoietic cells. We find that 89 transcripts of membrane-associated proteins are represented more in cultured BM-MSC than in UVSC. These include previously identified molecules, but also novel markers like NOTCH3, JAG1, and ITGA11. We show that the latter three molecules are also expressed on fibroblast colony-forming units (CFU-F). Both NOTCH3 and ITGA11, but not JAG1, further enrich for CFU-F when combined with CD146, a known marker of cells with MSC activity in vivo. Differentiation studies show that NOTCH3+ and CD146+ NOTCH3+ cells sorted from cultured BM-MSC are capable of adipogenic and osteogenic progeny, while ITGA11-expressing cells mainly show an osteogenic differentiation profile with limited adipogenic differentiation. Our observations may facilitate the study of lineage relationships in MSC as well as facilitate the development of more homogeneous cell populations for mesenchymal cell therapy.

Introduction

Mesenchymal stem cells (MSC) are the multipotential progenitors that give rise to skeletal cells (osteoblasts, chondrocytes, hematopoietic-supportive stromal cells) and adipocytes. While the multipotential nature of these cells is undisputed, their "stemness" is controversial. Currently, widespread use of MSC as stem cells for regenerative medicine is hampered by several issues. First, there are currently no markers which can be used to conclusively detect or prospectively isolate human multipotent MSC. Second, as in the

* *Corresponding author.* Laboratory of Stem Cell Physiology, III, Medizinische Klinik und Poliklinik, Klinikum rechts der Isar, Ismaningerstrasse 22, 81675 Munchen, Germany. Fax: +49 89 4140 6057.
E-mail address: oostendorp@lrz.tum.de (R.A.J. Oostendorp).

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hematopoietic cell system, no culture conditions have been described which can maintain multipotency over time. Third, because of the lack of knowledge about the relation between multi-, bi- and unipotent cells, directed differentiation of MSC is currently unlikely to give homogeneous cell populations useful for regenerative purposes.

We recently described the characterization of bone marrow-derived MSC (BM-MSC) and stromal cells derived from umbilical veins (UVSC) [1–4]. Our study showed that cells of these morphologically similar populations show different patterns of differentiation. The differentiation potential in the adipogenic or osteogenic lineage was particularly inefficient in UVSC as compared to BM-MSC. Poor osteogenic performance was confirmed in *in vivo* experiments which demonstrated that only the BM-MSC form bone, undergo adipogenesis, and recruit the hematopoietic microenvironment in an in vivo xenograft model [2].

In the current study, the gene expression patterns of BM-MSC capable of MSC activity in vivo [1,2] and UVSC was compared to other adherent and hematopoietic cell types. By the inclusion of UVSC as an additional non-MSC population, we now refine our previous studies and we describe novel markers for enrichment of fibroblastoid colony-forming units (CFU-F) with adipogenic and osteogenic potential.

Materials and methods

Isolation and culture of stromal cells

Human umbilical cords were obtained after parental consent from clinically normal pregnancies and caesarean sections. Bone marrow was obtained from normal donors after informed consent. All clinical procedures were approved by the Committee on Medical Ethics of the Klinikum rechts der Isar. Umbilical vein stromal cells (UVSC), and bone marrow stromal cells (BM-MSC) cells were isolated and cultured as described [1,2]. For array and PCR experiments as well as expansion purposes, adherent cells were harvested at seventy percent confluency.

RNA extraction, gene expression profiling and real-time RT-PCR

Total RNA was extracted from eight BM-MSC samples and eight UVSC samples. Two separate global gene expression experiments were performed. One in which five first passage (p1)/p2 UVSC samples were compared to six previously described p1 BM-MSC samples, and a second experiment in which three p2 UVSC samples were compared to two p2 BM-MSC samples. Hybridization on HG-U133 Plus 2.0 microarrays (Affymetrix, Santa Clara, CA) was performed as described elsewhere [1]. Hierarchical clustering as well as principal components analyses were performed using Genesis software (http://www.genesis-softwareonline.com/).

Microarray data are available in the Gene Expression Omnibus (GEO) at http://www.ncbi.nlm.nih.gov/geo/ with accession number GSE9894 [1] and http://www.bioretis.de.

RT and real-time PCR amplification

PolyT-primed cDNA was amplified using gene-specific primers (see Supplementary Table 1) and amplified as described previously [2]. In the Figures, normalization is shown relative to the large ribosomal protein p0 housekeeping gene (RPLP0).

Cell sorting and CFU-F assays

Adherent BM-MSC and UVSC were detached using Collagenase Type II and stained with the antibodies indicated (Supplementary Table 2). Cells were sorted on a MoFlo high performance cell sorter (Beckmann-Coulter, Krefeld, Germany). For fibroblastoid colony-forming (CFU-F) assays, cultured BM-MSC or UVSC, and sorted cells were plated at a density of 100–1000 cells/cm^2. Adherent colonies were counted after 10 days as described previously [1].

Differentiation assays

Sorted cells were expanded until forming a monolayer as described above. Adipogenic and osteogenic differentiation were performed on confluent monolayers of sorted and unfractionated BM-MSC as described previously [1,2]. Adipocytic fat vacuoles were visualized using staining of paraformaldehyde fixed cells with Nile O red. Osteogenic mineralization was demonstrated using von Kossa staining. As a result of this staining, the calcium deposits appear in black due to local co-deposition of metallic silver.

Results

Gene expression analysis of BM-MSC and UVSC

We performed two independent gene expression analysis experiments as described in Materials and Methods. The first experiment included the BM-MSC samples shown to be multipotent in vivo [2]. The second experiment was a small scale experiment for confirmation purposes. In an initial round of analysis, we assessed possible lineage relations between the BM-MSC, UVSC, synovial fibroblasts (SFb), periosteal cells (POC) and hematopoietic lineages. For this purpose, we analysed a 142 transcript selection of surface molecules, predominantly CD markers (reference 1, Supplementary Table 3). Hierarchical clustering revealed that the UVSC clustered very closely to the BM-MSC, but differed from the other cell populations analysed (Fig. 1a and Supplementary Fig. 1). Further principal component analysis showed that the UVSC clustered as a discrete population away from all other populations analysed, confirming that UVSC indeed represent a distinct population of stromal cells (Fig. 1b).

We next extended the comparison between BM-MSC and UVSC to all molecules which were membrane-associated according to the gene ontology information on cellular components. In this comparison, 473 tags were significantly overrepresented more than 2-fold in BM-MSC in both experiments performed. Of these, 102 correspond to 89 membrane-associated gene products (Supplementary Table 4). Differentially expressed genes include known markers of multipotent cells, such as VCAM1 [5], and ALPL [6]. In addition, many molecules involved in extracellular matrix were expressed by BM-MSC, including HAS1, GALNT5, CSPG2, COL14A1, and POSTN. There were 182 tags significantly overrepresented more than 2-fold in UVSC of both experiments, of which 43 tags corresponded to 29 membrane-associated gene products (Supplementary Table 5). These molecules included surface markers such as the endothelial marker FLT1 and the myogenic precursor marker NCAM1 (CD56). Also, the expression of DSC2, DSC3, and DSG2 in UVSC reveals the potential of desmosome formation.