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Original Article

Comparison of Gene Expression of Umbilical Cord Vein and Bone Marrow–Derived Mesenchymal Stem Cells

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ABSTRACT

Mesenchymal stem cells (MSCs) give origin to the marrow stromal environment that supports hematopoiesis. These cells present a wide range of differentiation potentials and a complex relationship with hematopoietic stem cells (HSCs) and endothelial cells. In addition to bone marrow (BM), MSCs can be obtained from other sites in the adult or the fetus. We isolate MSCs from the umbilical cord (UC) veins that are morphologically and immunophenotpically similar to MSCs obtained from the BM. In culture, these cells are capable of differentiating in vitro into adipocytes, osteoblasts, and condrocytes. The gene expression profiles of BM-MSCs and of UC-MSCs were compared by serial analysis of gene expression, then validated by reverse transcription polymerase chain reaction of selected genes. The two lineages shared almost all of the first thousand most expressed transcripts, including vimentin, galectin 1, osteonectin, collagens, transgelins, annexin A2, and *MMP2*. Nevertheless, a set of genes related to antimicrobial activity and to osteogenesis was more expressed in BM-MSCs, whereas higher expression in UC-MSCs was observed for genes that participate in pathways related to matrix remodeling via metalloproteinases and angiogenesis. Finally, cultured endothelial cells, $CD34^+$ HSCs, MSCs, blood leukocytes, and bulk BM clustered together, separated from seven other normal nonhematopoietic tissues, on the basis of shared expressed genes. MSCs isolated from UC veins are functionally similar to BM-MSCs, but differentially expressed genes may reflect differences related to their sites of origin: BM-MSCs would be more committed to osteogenesis, whereas UC-MSCs would be more committed to angiogenesis.
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INTRODUCTION

Mesenchymal stem cells (MSCs) of the bone marrow (BM) give origin to the stromal environment that supports the hematopoiesis maintained by the hematopoietic stem cells (HSCs). They are multipotent precursors that are capable of differentiating into various cell types of mesodermal origin, including condrocytes, osteocytes, adipocytes, and stromal cells [1, 2], and they probably have a key role in hematopoiesis, both by cell–cell contacts and by secreted proteins.

Although the differentiation potential of adult stem cells was initially believed to be restricted to its tissue of origin, a great deal of work accumulated recently on the issue of stem

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cell plasticity. There are many reports on the ability of these precursor cells to originate differentiated cells of other organs and tissues, such as hepatic, renal, neural, and cardiac cells [3], although the interpretation is often controversial. Moreover, a matched-pair analysis showed that the co-infusion of HLA-identical BM donor–derived MSCs with the HSC graft in the allogeneic transplant setting increased the speed of myeloid engraftment, decreased graft-versus-host disease, and showed improvement of survival, compared with the patients who did not receive the co-infusion of MSCs [4]. Thus, the therapeutic potential of these cells is the focus of considerable interest. In addition to BM, MSCs can be obtained from other sites in the adult, fetus [5], amniotic fluid [6], or cord blood cells [7]. MSCs are also enriched in preterm cord blood, decreasing in number with gestational age [8]. Recently, many groups succeeded in isolating MSCs from umbilical cord (UC) blood [9–11], whereas controversial results were obtained by others who suggested that cord blood is not a source for MSCs [12, 13].

Instead of using the cord blood, Romanov et al. [13] and Covas et al. [14] obtain MSCs starting from cells detached from the UC vein, in a manner similar to that for initiating human umbilical vein endothelial cell (HUVEC) cultures. In vitro and in vivo observations indicate a complex relationship between MSCs of different origins with HSCs and endothelial cells [15–29]. One means of evaluating the functional relationship between these different cells is by comparing their gene expression profiles. We have recently described the global pattern of gene expression of BM-derived MSCs (obtained by serial analysis of gene expression [SAGE]) and pointed out similarities and differences with the CD34 hematopoietic precursors [30].

To extend the characterization of the MSCs derived from UC veins and to drive hypotheses concerning the presence of these cells in the UC, we compared the expression profiles obtained by SAGE of these cells to that of cultured BM MSCs. Their functional relationships with HSCs, endothelial cells, and other cells related and unrelated to hematopoiesis were evaluated by cluster analysis of the gene expression profiles.

Materials and Methods

Isolation and Culture of Human Umbilical Cord MSCs

The research protocol was approved by the institutional review board, and the samples were obtained after informed consent. The UC of a term delivery was internally washed with phosphate-buffered saline (PBS), then filled with 1% collagenase in PBS; the extremities were clamped and incubated for 20 minutes at 37°C. The collagenase solution with the detached cells was harvested, and the vein was washed twice again to gather the rest of the cells [14]. After centrifugation at 400 *g*, the pellet was resuspended in growth medium 199 (Sigma Chemical Corp., St. Louis) and cultured as previously described [14]. After expansion, the cells of the third passage were analyzed by flow cytometry (FACsort; BD Biosciences Pharmingen, San Jose, CA), and an aliquot of the culture was assayed for adipogenic, osteogenic, and condrocytic differentiation [2, 9, 31].

Flow Cytometry Analysis

The cells harvested were labeled directly with CD90-PE, CD51/61-PE, CD29-PE, CD49e-PE, CD49d-PE, CD44-FITC, CD45-FITC, CD54-PE, CD13-PE, CD14-PE, CD31-FITC, CD33-FITC, CD34-PE, CD36-FITC, CD133-PE, CD106-PE, HLADR-FITC or HLA class I-FITC (FITC, fluorescein isothiocyanate; PE, phycoerythrin) and analyzed on a FACSort (Becton, Dickinson, San Jose, CA) as previously described [30]. For KDR and cadherin 5, we used indirect labeling with FITC-conjugated goat anti-mouse immunoglobin.

SAGE Procedure

Total RNA was prepared from 4×10^7 cells obtained from a fresh culture using TRIzol LS Reagent (Invitrogen Corporation, Carlsbad, CA; Cat. No. 10296010) and treated with RQ1 RNase-Free Dnase (Promega Corporation, Madison, WI; Cat. No. M6101). Then 30 μg of total RNA was used for the SAGE procedure. SAGE was carried out using the I-SAGE Kit (Invitrogen Corporation; Cat. No. T5001-01) as previously described [30].

Tag frequency tables were obtained from sequences by the SAGE analysis software, with minimum tag count set to 1 and maximum ditag length set to 28 bp; the other parameters were set as default. The annotation was based on two specific mappings, SAGEmap (http://www.ncbi.nlm.nih.gov/SAGE/) and CGAP SAGE Genie (http://cgap.nci.nih.gov/SAGE).

For comparison, we used the data of a BM-derived MSC library [30]. The statistical analysis was carried out by the software SAGEstat [32], which implements a Z-test for the comparison of two SAGE libraries.

Clustering

In addition to our two (UC and BM) MSC libraries, 12 other libraries corresponding to normal human tissues were used to carry out the cluster analysis: bulk BM (our unpublished data); $CD34^+$ cells from BM [33]; HUVEC [34], kindly provided by the authors; and nine other libraries from normal human tissues—namely, leukocytes, brain, gastric epithelium, heart, microvascular endothelial cells, kidney, liver, and old muscle and young muscle, all of which are available at

the Gene Expression Omnibus site (http://www.ncbi.nlm.nih.gov/geo/), with their respective GEO accession numbers: 709, 763, 784, 1499, 706, 708, 785, 819, and 824.

Three different sets of tags were selected for clustering, consisting of the top-expressed 100, 500, and 1,000 tags of each of the 14 libraries. After excluding redundancy, those sets corresponded, respectively, to 544, 2,685, and 5,421 different tags. Tag counts of all the 14 libraries were normalized to a total of 200,000 and then were used to assemble the matrix for input to the software Cluster 3.0 developed by De Hoon and collaborators (http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster). No additional transformations or normalizations were performed for the cluster analysis.

Average linkage hierarchical clustering was performed with the three different sets of tags using three different metrics—namely, Euclidean (squared), Pearson (uncentered), and Spearman rank. K-median clustering was also performed using the three sets of selected tags, using Euclidean (squared) and Pearson (uncentered) metrics with the number of runs set to 1,000 and increasing numbers of K-clusters from two to six.

The software CIT (Clustering Identification Tool) [35] was used to search for the genes that best differentiate between the SAGE library clusters. The program was run with the number of permutations set to 10,000, the minimum mean cutoff parameter set to 0, and other parameters set as default.

Semiquantitative Evaluation by RT-PCR

Total RNA was obtained from seven human tissues. The transcription reaction was performed with 2 mg of total RNA, 0.5 mg of Oligo (dT) primer, and 200 units of superscript II Rnase H reverse transcriptase (RT) (Invitrogen Corporation) in a total volume of 20 m1, and 1/10 of the volume of the cDNA was used in the semiquantitative polymerase chain reaction (PCR). When the reaction was positive in the undiluted samples, the cDNA was serially diluted (1:2 to 1:128) before performing the PCR. Secreted protein, acidic, cysteine-rich (*SPARC*) expression was measured by real-time PCR with the Taqman approach (Applied Biosystems, Foster City, CA). The following specific primers were used:

COL1A1: 5'CGCTACTACCGGGCTGATGAT3' and 5'GTCCTTGGGGTTCTTGCTGATGTA3'
COL1A2: 5'AGGGCAACAGCAGGTTCACTTACA3' and 5'AGCGGGGGAAGGAGTTAATGAAAC3'
LGALIS: 5'CCACGGCGACGCCAACACCAT3' and 5'TGGGCTGGCTGATTTCAGTCAAAG3'
VIM: 5'TCTATCTTGCGCTCCTGAAAAACT3' and 5'AAACTTTCCCTCCCTGAACCTGAG3'
TPT1: 5'ATCCAGATGGCATGGTTGCTCTAT3' and 5'TGCCTCCACTCCAAATAAATCACA3'
TAGLN: 5'CTTTGGGCAGCTTGGCAGTGACCA3' and 5'CCAGCCCGCTTCTCCCTGCTTAG3'
TAGLN2: 5'AGCGGACGCTGATGAATCTGG3' and 5'TGGCTATGGGGAAGGGAATGTATT3'
MMP2: 5'CAGGCACTGGTGTTGGGGGAGAC3' and 5'CCATCGCTGCGGCCAGTATCAGTG3'
ANXA2: 5'GGTCTCCCGCAGTGAAGTGGACAT3' and 5'GGCCAGGCAATGCTTAGGCAACTA3'
S100A8.1: 5'GAATTTCCATGCCGTCTACAGG3' and 5'GCCACGCCCATCTTTATCACCAG3'
S100A8.2: 5'GGGCAAGTCCGTGGGCATCAT3' and 5'GCTACTCTTTGTGGCTTTCTTCAT3'
GAPDH: 5'TTAGCACCCCTGGCCAAGG3' and 5'CTTACTCCTTGGAGGCCATG3'
OSF2: 5'GACGGTCACTTCACACTCTTTG3' and 5'GTCACCGTCACATCCTATCTCA3'
S100A9.1: 5'AACCAGGGGGAATTCAAAGAGC3' and 5'CCTAGCCCCACAGCCAAGACAGTT3'
S100A9.2: 5'GTCGCAGCTGGAACGCAACA3' and 5'CCCGAGGCCTGGCTTATGGTG3'
CXCL6: 5'CCTGAAGAACGGGAAGC3' and 5'GACTGGGCAATTTTATGATG3'
BGNF: 5'CAAAGAGATCTCCCCTGACACCAC3' and 5'AGCCCGCTGAACACTCC3'
SPARC: 5'ACAAGCTCCACCTGGACTACATC3' and 5'GGGAATTCGGTCAGCTCAGA3'and probe 5'TTGCAAATACATCCCC3'

RESULTS

Characteristics of the Umbilical Cord MSC Population

With this approach, we have regularly obtained a cell population that assumes a spindle-shaped morphology in confluent wave-like layers in culture and can be replated several (20 or more) times. The cells harvested are negative for hematopoietic lineage markers (CD34, CD45, and CD133); for monocytic markers (CD14); and for endothelial markers such as KDR, cadherin-5, CD31, and CD133. As observed with other MSCs, the majority of cells were positive for CD13, CD29, CD44, CD54, CD90, and HLA class I, but negative for HLA class II (Table 1). Additionally, the sample used for SAGE was $CD49e^{+}$, $CD56/61^{-}$, and $CD49d^{-}$. When cultured with dexamethasone and ascorbic acid they undergo osteogenic differentiation, as demonstrated by alkaline phosphatase expression and positive calcium staining by the von Kossa reaction; in contrast, in culture with insulin, dexamethasone, and indomethacin, they originate adipocytes, which are identified by numerous vacuoles that stain positively with Sudan III. When cultured as a pellet in the bottom of the tube, they originate a mass of cells with condrocyte or condroblast fea-

Table 1. Immunophenotypic findings in three separate samples of mesenchymal stem cells obtained from the umbilical cord wall

Marker	Sample #1	Sample #2	Sample #3	Mean
CD13	99.2	90.9	91.8	93.9
CD14	0.1	0.1	0.0	0.06
CD29	99.6	96.6	97.7	97.9
CD31	0.2	4.3	0.9	2.4
CD33	0.0	0.0	0.0	0.0
CD34	0.6	2.5	0.8	1.1
CD36	0.1	0.7	0.1	0.3
CD44	92.8	93.2	70.9	85.6
CD45	0.0	0.2	0.0	0.06
CD54	98.2	63.8	55.5	72.5
CD90	99.7	96.8	98.3	98.2
CD106	50.0	27.7	25.5	34.4
CD133	—	3.7	—	3.7
KDR	0.7	3.7	1.6	2.2
Cadherin 5	0.7	3.7	1.0	1.8
HLA-Class I	97.2	94.9	91.7	94.6
HLA-DR	0.8	3.2	0.9	1.6

Results shown are percentage of positive cells.

tures such as rounded shape with a large vacuolated and basophilic cytoplasm on hematoxylin and eosin stains. The cells are disposed in nests intermingled by an extracellular matrix rich in type II and IV collagen (Fig. 1). Also, these cells stain positively for vimentin and S-100 protein. Thus, they exhibit distinguishing characteristics of the MSCs [2].

Gene Expression of Umbilical Cord MSCs

A total of 100,922 tags were obtained by sequencing. Excluding redundancy, these results correspond to 29,407 unique tags, of which 18,689 matched known genes or expressed sequence tags in the CGAP SAGE Genie mapping (85,080 total tags corresponding to 11,965 UniGene clusters); in contrast, 10,718 unique tags had no matches (15,842 total tags). The 50 most abundant transcripts of UC-MSCs are listed in Table 2. All the tags that appear in this list are found in the MSCs derived from BM [30], and 36 of those are also among the 50 most expressed tags in BM-MSCs, whereas all but three of the remaining are among the 100 most abundant in BM-MSCs.

A list of all the tags found in UC-MSC is at our Website: htpp://bit.fmrp.usp.br/uc-msc_tags/.

Corroboration of SAGE Results

Gene expression was measured semiquantitatively by RT-PCR or by real-time PCR in different tissues to validate SAGE results. The expressions of the transcripts *COL1A1*, *COL1A2*, *TPT1*, *SPARC*, *LGALS1*, *TAGLN2*, *VIM*, *MMP2*, *TAGLN*, and *ANXA2*, common to UC vein and BM-derived MSCs were all confirmed (Fig. 2). The higher levels of *CXCL6* and *CXCL8* in UC-MSC were also confirmed (Fig. 3). *CXCL6* was detected only in UC-MSCs up to 1/32 dilution: It showed 226 tags in UC-MSCs and was absent in BM-MSCs. There were 24 tags for *CXCL8* in UC-MSCs and none in BM-MSCs; the transcript was detected up to a dilution of 1/32 in UC vein MSCs and up to 1/4 dilution in BM-MSCs. The expression of the gene *SPARC* was measured by real-time PCR, and its level was at least 10 times higher in MSCs of both sources, as compared with the other tissues tested, which included bulk BM, $CD34^+$ HSCs, peripheral blood leukocytes (PBLs), liver, brain, and skeletal muscle. The expression level of *LGALS1*, *VIM*, *TPT1*, *TAGLN*, *TAGLN2*, *MMP2*, *COL1A1*, *COL1A2*, and *ANXA2* was also measured in the additional tissues mentioned above. The *TPT1* gene was detected in all the tissues tested, whereas the *TAGLN2* gene expression was observed only in the hematopoiesis-related tissues and was absent in muscle, brain, and liver. All the other genes (*COL1A1*, *COL1A2*, *LGALS1*, *VIM*, *TAGLN*, *MMP2*, and *ANXA2*) were positive mainly in the two MSC cell types, thus agreeing with the tag counts observed in the SAGE libraries of the different tissues (Fig. 2).

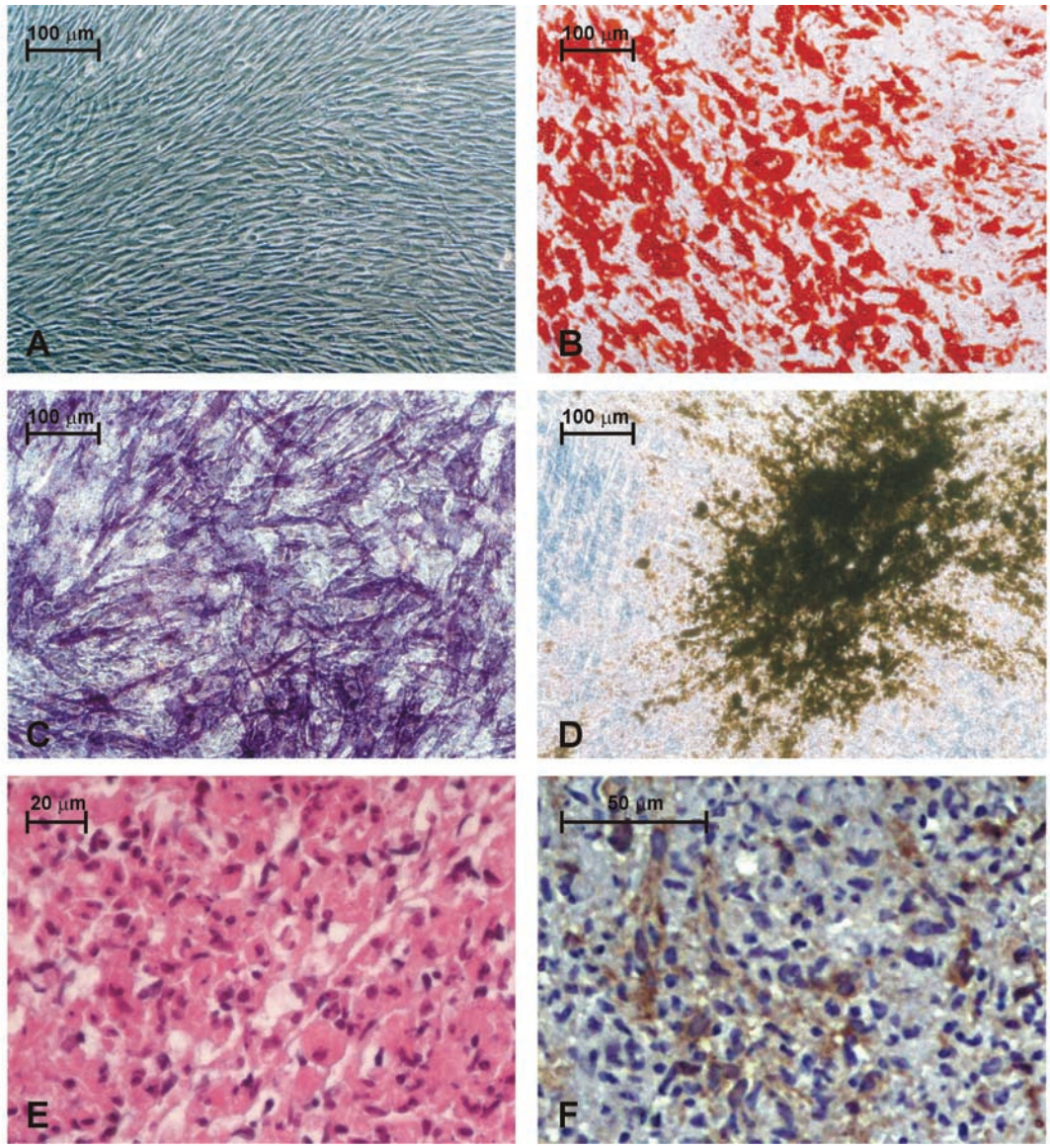

Figure 1. (A): A culture of MSCs obtained from the umbilical vein. **(B):** Sudan III staining of adipocytes derived from the MSCs. **(C, D):** Osteogenic differentiation of MSCs, shown by **(C)** positive staining for alkaline phosphatase and **(D)** calcium deposits demonstrated by the von Kossa reaction. **(E, F):** Chondrocyte differentiation of MSCs cultured as a pellet in the bottom of a 15-ml Falcon tube. Hematoxylin eosin–stained sections of the firm mass of cells recovered after 30 days showed cells with characteristic features of condrocytes or chondroblasts **(E)**; there are abundant collagen bundles in the extracellular matrix that stain with anti-collagen II **(F)**, anti-collagen IV, and vimentin (not shown). Abbreviation: MSC, mesenchymal stem cell.

Comparison of Umbilical Cord and Bone Marrow MSCs

Similarities

When the first thousand more abundant transcripts of each library are compared with the whole set of transcripts from the other library, only 8 tags found in UC veins are not found in BM (0.8 %), whereas 29 tags found in BM are not found in the UC (2.9 %). In addition, the Pearson's correlation coefficient, calculated on the basis of the normalized expression values of the first 1,000 transcripts of the two sources of MSCs (excluding the 37 exclusive tags) was .93. A comparison of the gene ontologies of the first thousand most abundant transcripts from each of the two libraries revealed differences in only two categories: response to external stimulus (19.30% in BM versus 8.86% in UC) and cell growth and/or maintenance (28.07% in BM versus 37.34% in UC). The expressions of *COL1A1*, *COL1A2*, *TPT1*, *SPARC*, *LGALS1* (all 5 among the top 50 in UC; Table 2), *VIM*, *MMP2*, *TAGLN* (among the top 50 in BM), *TAGLN2*, and *ANXA2* were validated by RT-PCR.

Table 2. First 50 most frequent tags in UC-MSCs: the numbers of tags (normalized for 200,000) in UC-MSCs are compared with BM-MSCs, and the CGAP (SAGEgenie) and NCBI SAGEMap mapping for each tag are shown

	No. of tags		UniGene Hs.[a]		
Tag	**UC-MSC**	**BM-MSC**	**CGAP**	**NCBI**	**Descriptions from CGAP and NCBI[b]**
GCCCCCAATA	2,434	1,121	407,909	—	**Lectin, galactoside-binding, soluble, 1 (galectin 1)**
ATGTGAAGAG	1,639	1,498	111,779	—	**Secreted protein, acidic, cysteine-rich (osteonectin)**
GAAAAATGGT	1,633	800	374,553	356,261	Laminin receptor 1 (ribosomal protein SA, 67kDa); transcribed sequence with strong similarity to protein sp:P08865 (*Homo sapiens*) RSP4_HUMAN 40S ribosomal protein SA
GCATAATAGG	1,609	951	381,123	22,982	Ribosomal protein L21; chromosome 21 open reading frame 80
GGGCTGGGGT	1,419	1,090	430,207	90,436	Ribosomal protein L29; sperm-associated antigen 7
TGGAAATGAC	1,373	2,566	172,928	193,076	**Collagen, type I, α 1**; GRB2-related adaptor protein 2
GAAGCAGGAC	1,365	1,222	170,622	—	Cofilin 1 (non-muscle)
TACCATCAAT	1,359	1,206	169,476	—	Glyceraldehyde-3-phosphate dehydrogenase
CTGGGTTAAT	1,203	1,107	381,184	334,534	Ribosomal protein S19; glucosamine (*N*-acetyl)-6-sulfatase (Sanfilippo disease IIID)
GAGGGAGTTT	1,199	1,134	356,342	—	Ribosomal protein L27a
CCCATCGTCC	1,132	1,377	417,764	No match	Transcribed sequence with strong similarity to protein prf: 0512543A (*H. sapiens*) 0512543A oxidase II, cytochrome (*H. sapiens*); no match
TTGGTCCTCT	1,092	815	381,172	381,171, 520,738[c]	Ribosomal protein L41; CDNA clone IMAGE: 6050358, partial cds; ribosomal protein L41
CCTAGCTGGA	1,076	728	356,331	177,285	Peptidylprolyl isomerase A (cyclophilin A); similar to peptidyl-Pro *cis trans* isomerase (LOC391532), mRNA
GGCTGGGGGC	1,027	625	408,943	352,407	Profilin 1; LOC388674 (LOC388674), mRNA
GTGTGTTTGT	949	1,434	421,496	—	Transforming growth factor, β-induced, 68 kDa
TAAGGAGCTG	943	539	355,957	—	Ribosomal protein S26
TAGGTTGTCT	923	732	374,596	—	**Tumor protein, translationally controlled 1**
TGTACCTGTA	922	656	446,608	—	Tubulin, α, ubiquitous
GGATTTGGCC	916	502	437,594	9,711; 259,326	Ribosomal protein, large P2; solute carrier family 35, member F2; cell cycle progression 8 protein
GGCAAGCCCC	866	510	448,396	187,577	Ribosomal protein L10a; SRY (sex-determining region Y)-box 21
AGGGCTTCCA	842	521	401,929	—	Ribosomal protein L10
TTGGTGAAGG	834	444	75,968	518,737	Thymosin, β-4, X-linked; thymosin-like 3
TGCACGTTTT	801	623	265,174	—	Ribosomal protein L32
GTGCTGAATG	745	374	77385	1,239	Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle; alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)
ATAATTCTTT	735	463	539	406,800	Ribosomal protein S29; transcribed sequences
AGCACCTCCA	725	658	75,309	—	Eukaryotic translation elongation factor 2
TTGGGGTTTC	648	650	448,738	167344	Ferritin, heavy polypeptide 1; vitelliform macular dystrophy (best disease, bestrophin)
TCAGATCTTT	602	412	446,628	196,953; 308,053	Ribosomal protein S4, X-linked; SNF2 histone linker PHD RING helicase; insulin-like growth factor 1 (somatomedin C)
TAATAAAGGT	600	541	512,675	—	Ribosomal protein S8
AGGAAAGCTG	577	317	408,018	406,485	Ribosomal protein L36; GGA binding partner
AGGCTACGGA	535	346	449,070	23,270	Ribosomal protein L13a; DKFZP566F2124 protein

(continued)

Table 2. (continued)

Tag	No. of tags		UniGene Hs.[a]		Descriptions from CGAP and NCBI[b]
	UC-MSC	BM-MSC	CGAP	NCBI	
GGGAAGCAGA	527	469	506,845	No match	F11 receptor; no match
GTGAAGGCAG	527	340	356,572	368,855	Ribosomal protein S3A; guanosine monophosphate reductase 2
TGCATCTGGT	519	214	310,769	—	Heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa)
GTAAGTGTAC	486	154	No match	No match	No match
ACATCATCGA	484	284	408,054	—	Ribosomal protein l12
CCAGAACAGA	482	399	400,295	—	Ribosomal protein l30
CGCCGCCGGC	478	169	182,825	—	Ribosomal protein l35
GTCTGGGGCT	466	220	406,504	—	**Transgelin 2**
GTTGTGGTTA	462	426	48,516	99,785	β_2 microglobulin; CDNA: FLJ21245 fis, clone COL01184
TTCAATAAAA	450	403	356,502	2,012	Ribosomal protein, large, P1; transcobalamin I (vitamin B12 binding protein, R binder family)
TGTGTTGAGA	444	718	439,552	406,283	Eukaryotic translation elongation factor 1 α1; MRNA expressed only in placental villi, clone SMAP83
GGGGAAATCG	440	379	446,574	—	Thymosin, β 10
GGACCACTGA	432	407	119,598	—	Ribosomal protein L3
GCAGCCATCC	428	309	356,371	—	Ribosomal protein L28
TTGTAATCGT	424	179	446,427	—	Ornithine decarboxylase antizyme 1
TTTGGTTTTC	424	724	232,115	281,117	Collagen, type I, α2; RAB22A, member RAS oncogene family
GTGAAACCCC	422	230	477,083	185,807; 323,949[c]	Platelet-activating factor acetylhydrolase 2, 40 kDa; component of oligomeric golgi complex 7; kangai 1 (suppression of tumorigenicity 6, prostate; CD82 antigen [R2 leukocyte antigen, antigen detected by monoclonal and antibody IA4])
CAATAAATGT	416	393	80,545	—	Ribosomal protein L37
ACCAAAAACC[d]	406	304	172,928	—	Collagen, type I, α1

[a]CGAP-SAGEgenie mapping indicates best gene for tag, whereas alternative UniGene clusters are shown in the NCBI-SAGEMap column. Dash (—) indicates no additional matches, besides CGAP-SAGEgenie.
[b]Transcripts in bold were selected for validation by reverse transcription polymerase chain reaction.
[c]SAGEMap does not include the UniGene cluster selected by CGAP as the best gene for the tag.
[d]Tag originated by internal priming of the COL1A1 transcript.
Abbreviations: BM, bone marrow; CGAP, Cancer Genome Anatomy Project; MSCs mesenchymal stem cells; NCBI, National Center for Biotechnology Information; SAGE, serial analysis of gene expression; UC, umbilical cord.

Differences

A set of 45 transcripts had at least 10-fold more abundant tags in BM-MSCs than in UC-MSCs ($p < .001$) and corresponded in most cases to tags not found in UC-MSCs. Conversely, there were 38 transcripts present at high levels in UC-MSCs that were absent or rare in BM-MSCs (Table 3). The higher expression of *CXCL6* and interleukin (IL)-8 (*CXCL8*) in UC was confirmed by RT-PCR, as was the higher expression of *BGN* in BM, although the difference was not as striking as that observed by SAGE (reaction positive up to 1:64 for UC and 1:128 for BM). The higher expression of *COL1A1* in BM and *LGALS1* in UC was also validated by RT-PCR, although the tags appearing in Table 3 are probably artifact tags generated from these highly expressed transcripts whose correct tags appear among the top 50 most frequent tags, both in BM and in UC. Semiquantitive RT-PCR did not confirm the difference observed for *OSF2* (equally positive in the two cell lineages up to 1:128) or for *S100A8* and *S100A9* (negative in both with two different primer sets).

Clustering

With a few exceptions, for all three sets of tags (top 100, 500, or 1,000) and metrics used for the hierarchical analysis, cultured endothelial cells, $CD34^+$ HSCs, MSCs, and bulk

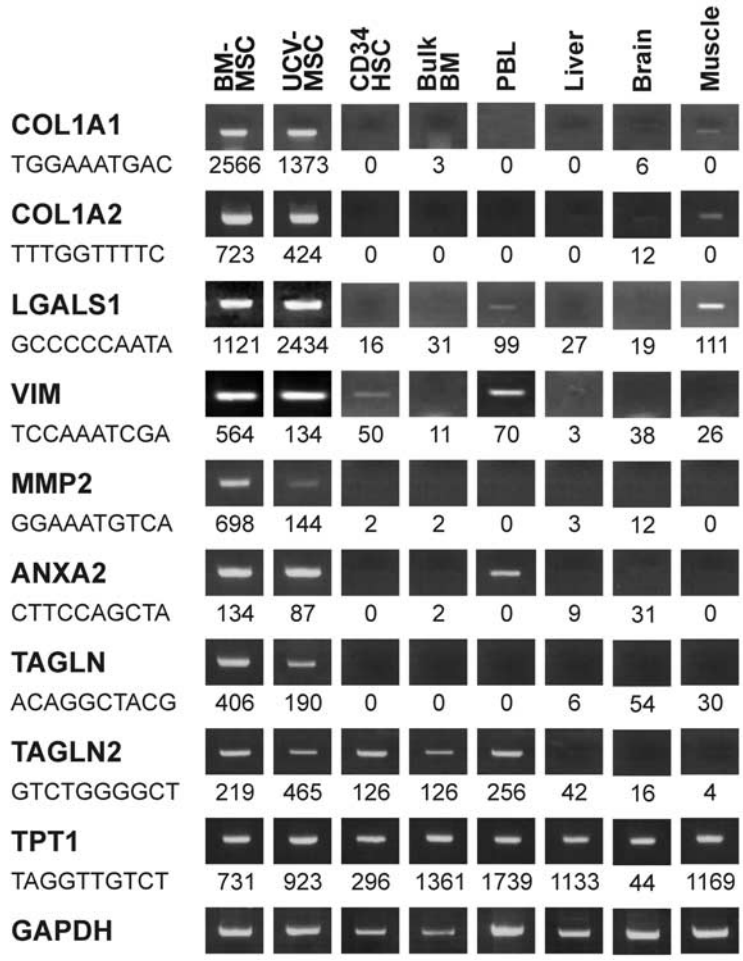

Figure 2. Comparison of gene expression by reverse transcription polymerase chain reaction for nine genes in the MSCs obtained from two different sources and in six additional tissues. Underneath each band, the normalized number of tags obtained by us BM-MSCs and UCV-MSCs or from the literature is indicated. The expression of *GAPDH* was used as reference for evaluating the quality of mRNA. Abbreviations: BM, bone marrow; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; HSC, hematopoietic stem cell; MSC, mesenchymal stem cell; PBL, peripheral blood leukocytes; UCV, umbilical cord vein.

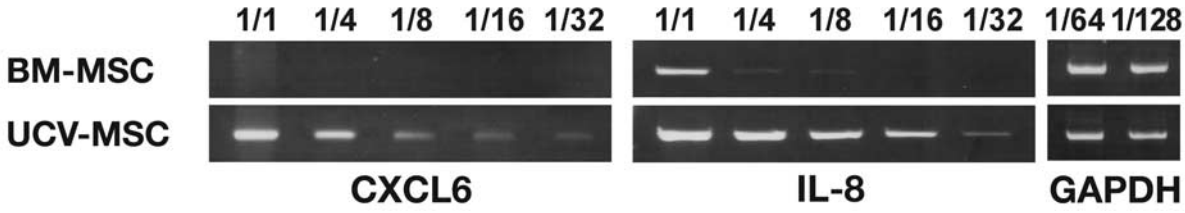

Figure 3. Semiquantitative evaluation of mRNA abundance by reverse transcription PCR. Total RNA was reverse transcribed into cDNA and diluted 1/1 to 1/32, followed by a 30-cycle PCR with specific primers located in different exons. At the left is shown the reaction for chemokine *CXCL6*, at the center the reaction for *IL-8*, and at the right the control for *GAPDH* (only the 1/64 and 1/128 reactions are shown). The expression of the two genes is more abundant for UCV-MSCs than for BM-MSCs, in agreement with the results of serial analysis of gene expression. Abbreviations: BM, bone marrow; *CXCL6*, C-X-C motif ligand 6; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *IL-8*, interleukin-8; MSC, mesenchymal stem cell; PCR, polymerase chain reaction; UCV, umbilical cord vein.

BM clustered together, separated from the hematopoiesis-unrelated tissues. PBLs also clustered together with the hematopoiesis-related tissues with all three tag sets, except for Euclidean metrics. K-median clustering corroborated this structure as in general, cultured endothelial cells, $CD34^+$ HSCs, and MSCs clustered together. The dendrogram obtained by uncentered Pearson's correlation with the top 500 tag set (Fig. 4) illustrates the overall relationship between hematopoiesis-related tissues.

Discrimination Analysis

The software CIT identified a set of 350 tags that best differentiate the clusters of hematopoiesis-related from the hematopoiesis-unrelated cells. There were 39 unique tags (Table 4) that were at least 4-fold more abundant in hematopoiesis-related cells, present with counts of at least 10 tags. Those tags represent genes with higher expression among the hematopoietic-related tissues as compared with nonrelated. Their gene ontology categories include genes associated with cell motility, communication, cell death, cell growth and/or maintenance, morphogenesis, and response to external stimulus, among others. The higher or exclusive expression of *VIM*, *SPARC*, *LGALS1*, *ANXA2*, and *TAGLN2* in hematopoiesis-related tissues or in MSCs was confirmed by RT-PCR (Fig. 2). The lower or absent expression of albumin, actin α-1, desmin, and clusterin in hematopoiesis-related cells (including MSCs) was confirmed by RT-PCR, in comparison with high expression in other tissues: liver (*ALB*), muscle (*ACTA1* and *DES*), and brain (*CLU*) (data not shown).

Discussion

MSCs can be obtained from BM and from other sites in the adult or the fetus. We have previuosly demonstrated that cultures with morphological features, immunophenotypic markers, and differentiation ability similar to BM-MSCs can be isolated from the UC wall [14], and in the present work we demonstrate that the gene expression profiles of the MSCs from the two sources are very similar. Among the top-expressed genes of cells of both origins are transforming growth factor–α induced, transgelin (or *SM22α*), cofilin1, vimentin, galectin 1, laminin receptor 1, and profilin 1.

The similarities between cultured MSCs derived from BM and from the UC vein at the transcriptional level definitively places UC vein–derived MSCs as a new potential and more accessible source for obtaining these cells. One of the concerns of cord blood transplants is the delayed hematopoietic recovery compared with BM transplants [36, 37], and probably the co-infusion of MSCs derived from UC veins with the UC blood graft may improve engraftment [4, 38]. Other promising potential applications for these cells is their use in co-cultures with cord blood HSCs to potentiate their expansion, mediated by chemokines and ILs secreted by MSCs [39]. The expression of the chemokines *CXCL1*, *CXCL6*, and *CXCL8* exclusively by UC-derived MSCs, as demonstrated here, may increase propagation of hematopoietic precursors in co-culture settings.

Nevertheless, some differences were observed between the two expression profiles. Among the genes that were exclusively or expressed at higher levels by BM-derived cells are lysozime and defensins, recognized for their antimicrobial activity, and *PRSS11*, a protease with an insulin-like growth factor binding domain. Other genes expressed at higher levels in BM-derived MSCs include biglycan, *TSC22*, CD44, and vitronectin, which may be involved in osteogenesis [40–48]. In fact, all of the integrin ligands implicated in the adherence of osteoblasts to the matrix are expressed at higher levels in MSCs of BM origin, including type 1 collagen, fibronectin, laminin, and vitronectin.

The genes expressed exclusively or at higher levels in the UC vein–derived MSCs include *CXCL6* (*GCP-2*), *IL-8* (or *CXCL8*), IL-1 receptor-like ligand (or *IL1RL1LG*), *MMP1* (interstitial collagenase), *ITGA3* (CD49C), *CXCL1* (*GROa* or *MGSA*), and *PTX3* (pentaxin related). All these genes are part of interconnected pathways related to angiogenesis mediated by *IL-1*, tumor necrosis factor alpha (*TNF-α*) and other intermediary molecules that may be involved in matrix remodeling by metalloproteinases. Our data demonstrate that type 1 IL-1 receptor (*IL1R1*) and its associated kinase (*IRAK1*) are expressed in MSCs. *IL-1-α*, *IL-8*, and *CXCL1* are members of the same family; they mediate angiogenesis and tumor invasion and cause reduction in the expression of interstitial collagen, as observed by us in UC-MSC [49–54]. Either IL-1 or TNF upregulates *IL-8*, *CXCL1*, and *CXCL6* [49, 52–55]. CXCL1 can bind only the CXCR2 receptor, whereas IL-8 and CXCL6 bind both CXCR1 and CXCR2 receptors [52, 56].

Although MSCs of both origins are highly similar, these differences could be functionally related to the origin of the MSCs, indicating that MSCs derived from BM are more committed to the osteoblastic and adipocytic lineages, whereas MSCs derived from the UC would be more committed to angiogenesis. If confirmed, this would imply that MSCs from a specific source may be more efficient for a particular therapeutic target; for instance, UC-MSCs could be more appropriate for the treatments aiming at increasing revascularization than would be the use of BM-MSCs [57]. These differences, however, should be viewed cautiously because the expression analysis was based on cultured cells, and although UC vein–derived MSCs were analyzed in the third passage culture in media similar to the BM-derived MSCs, they were obtained from primary HUVEC cultures,

Table 3. Differentially expressed transcripts in BM-MSCs and UC-MSCs

CGAP[a]	Description[b]	Count BM-MSCs	Count UC-MSCs	Fold BM/UC
Higher expression in BM-derived MSCs				
Hs.416073	**S100 calcium-binding protein A8 (calgranulin A)**	387		387
Hs.112405	**S100 calcium-binding protein A9 (calgranulin B)**	167		167
Hs.511887	Defensin, α 1, myeloid-related sequence	97		97
Hs.449630	Hemoglobin, α 2	66		66
Hs.274485	Major histocompatibility complex, class I, C	60		60
Hs.372009	CDNA FLJ42951 fis, clone BRSTN2007765	53		53
Hs.436441	Lamin A/C	51		51
Hs.114611	Chromosome 7 open reading frame 10	49		49
Hs.155376	Hemoglobin, β	35		35
Hs.234734	Lysozyme (renal amyloidosis)	35		35
Hs.13349	Neurofascin	35		35
Hs.393201	ARP2 actin-related protein 2 homolog (yeast)	33		33
Hs.294176	Defensin, α 3, neutrophil-specific	33		33
Hs.4980	LIM domain binding 2	31		31
Hs.172928[c]	Collagen, type I, α 1	31		31
Hs.26146	Down syndrome critical region gene 3	27		27
Hs.136348	**Periostin, osteoblast-specific factor**	97	4	25
Hs.307494	Glutamate receptor, ionotropic, kainate 2	21		21
Hs.114360	Transforming growth factor β–stimulated protein TSC-22	37	2	19
Hs.450230	Insulin-like growth factor binding protein 3	35	2	18
Hs.75111	Protease, serine, 11 (IGF binding)	29	2	15
Hs.821	Biglycan	140	10	14
Hs.155223	Stanniocalcin 2	27	2	14
Hs.343586	Zinc finger protein 36, C3H type, homolog (mouse)	27	2	14
Hs.284283	Butyrophilin, subfamily 3, member A1	49	4	12
Higher expression in UC-derived MSCs				**Fold UC/BM**
Hs.164021	**Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)**		226	226
Hs.512693	FLJ20859 gene		63	63
Hs.83169	Matrix metalloproteinase 1 (interstitial collagenase)	2	113	58
Hs.789	Chemokine (c-x-c motif) ligand 1 (melanoma growth stimulating activity, α)		48	48
Hs.406013	Keratin 18	2	67	35
Hs.369785	Hypothetical protein mgc2749		32	32
Hs.356123	Keratin 8	6	170	29
Hs.470110	Transcribed sequences		28	28
Hs.624	**Interleukin-8**		24	24
Hs.24301	Polymerase (rna) II (dna directed) polypeptide E, 25kda		24	24
Hs.17936	DKFZP434H132 protein		24	24
Hs.514018	CDNA: FLJ22209 fis, clone HRC01496		24	24
Hs.30332	Glutamine-fructose-6-phosphate transaminase 2		24	24
Hs.438231	Tissue factor pathway inhibitor 2		22	22
Hs.7258	Hypothetical protein FLJ22021		22	22

(continued)

Table 3. (continued)

CGAP[a]	Description[b]	Count BM-MSCs	Count UC-MSCs	Fold BM/UC
Higher expression in UC-derived MSCs				**Fold UC/BM**
Hs.12289	CDC42 effector protein (Rho GTPase binding) 2		22	22
Hs.2050	Pentaxin-related gene, rapidly induced by IL-1 β		22	22
Hs.438231	Tissue factor pathway inhibitor 2		22	22
Hs.446628	Ribosomal protein S4, X-linked		22	22
Hs.407909[d]	Lectin, galactoside-binding, soluble, 1 (galectin 1)		20	20
Hs.429896	Alcohol dehydrogenase 6 (class V)		20	20
Hs.123094	Sal-like 1 (*Drosophila*)		20	20
Hs.334798	Eukaryotic translation elongation factor 1 Δ (guanine nucleotide exchange protein)		20	20
Hs.438231	Tissue factor pathway inhibitor 2		20	20
Hs.265829	Integrin, α 3 (antigen CD49C, α 3 subunit of VLA-3 receptor)	2	38	19
Hs.403989	Actin, γ2, smooth muscle, enteric	6	97	17
Hs.449321	discoiDin, CUB and LCCL domain containing 1	2	32	16
Hs.183803	Heat shock protein 75	2	30	15
Hs.300463	Aconitase 2, mitochondrial	2	26	13
Hs.110855	Solute carrier family 20 (phosphate transporter), member 1	4	48	12
Hs.446686	Interleukin 1 receptor-like 1 ligand	4	40	10
Hs.355935	Mitochondrial ribosomal protein L52	12	117	10

Transcripts correspond to tags with at least 10-fold higher levels in one type of MSC in comparison with the other (p value < .001). Out of 83 tags selected by these criteria, the 58 that mapped to a known gene or expressed sequence tag are shown.
[a]The best UniGene cluster for the tag is indicated in the CGAP column.
[b]Transcripts in bold were selected for validation by reverse transcription polymerase chain.
[c]The *COL1A1* tag of this table is probably derived by incomplete digestion of the transcript that is present among the 50 most abundant transcripts; nevertheless, the best tag also shows a significant difference.
[d]The *LGALS1* tag is a 1-nt variant of the correct tag for this transcript, which appears among the 50 most abundant transcripts, and is probably derived by an artifact; nevertheless, the correct tag also shows a significant difference.
Abbreviations: BM, bone marrow; CGAP, Cancer Genome Anatomy Project; MSCs, mesenchymal stem cells; UC, umbilical cord.

which were supplemented by many growth factors that might cause part of the differences observed.

The relationships of MSCs with HSCs and endothelial cells are more complex, because these three cell types seem related not only functionally but also by the ontogenesis, since they may have common ancestors or the capacity to differentiate into the others' mature population. There is evidence for a common precursor for HSCs and MSCs [58, 59] and for trilineage hematopoietic recovery of totally irradiated dog transplanted with $CD34^-$ fibroblast-like stem cells [60]. Cord blood $CD34^+$ cells can give rise to adherent layers with endothelial characteristics [61, 62]. A subpopulation of $CD34^+$ identified as hemangioblasts that feed into the hematopoietic and endothelial precursors has been isolated from the adult BM, cord blood, and fetal liver [63, 64], whereas a mesodermal progenitor cell that is capable of differentiating into osteoblasts, chondrocytes, adipocytes,

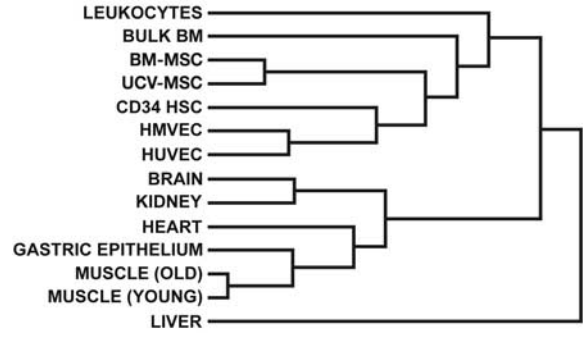

Figure 4. Dendrogram generated by hierarchical clustering (uncentered Pearson's correlation, average linkage). Clustering was carried out with the first 500 most frequent tags of each of 14 libraries obtained from normal human tissues. Abbreviations: BM, bone marrow; HMVEC, microvascular endothelial cell; HSC, hematopoietic stem cell; HUVEC, human umbilical vein endothelial cell; MSC, mesenchymal stem cell; UCV, umbilical cord vein.

Table 4. Transcripts expressed at higher levels in the cluster of hematopoiesis-related cells

		UniGene Hs.[a]		
Tag	**Fold**	**CGAP**	**NCBI**	**CGAP SAGEgenie Description[b]**
TCCAAATCGA	19	435,800	—	**Vimentin**
ATGTGAAGAG	14	111,779	—	**Secreted protein, acidic, cysteine-rich (osteonectin)**
GCCCCCAATA	12	407,909	—	**Lectin, galactoside-binding, soluble, 1 (galectin 1)**
GGCTGGTCTG	11	446,688	—	Hypothetical protein MGC4677
CTGAGTCTCC	9	77,269	—	Guanine nucleotide binding protein (G protein), α-inhibiting activity polypeptide 2
CTGCCAAGTT	9	75,873	—	Zyxin
ATTTGTCCCA	9	57,301	—	High mobility group AT-hook 1
CCCCGCCAAG	7	169,718	—	Calponin 2
GAAGCAGGAC	7	170,622	—	Cofilin 1 (non-muscle)
GGCTGGGGGC	7	408,943	352,407	Profilin 1 / LOC388674 (LOC388674), mRNA
TGTGTTGAGA	7	439,552	406,283	Eukaryotic translation elongation factor 1 α 1; MRNA expressed only in placental villi, clone SMAP83
CTAGCCTCAC	6	14,376	—	Actin, γ 1
GCACAAGAAG	6	19,340	—	MRNA; cDNA DKFZp564D0164 (from clone DKFZp564D0164)
CCTAGCTGGA	6	356,331	177,285	Peptidylprolyl isomerase A (cyclophilin A); similar to peptidyl-Pro cis trans isomerase (LOC391532), mRNA
CCCCAGCCAG	6	387,576	196,176	Ribosomal protein S3; enoyl coenzyme A hydratase 1, peroxisomal
CTGGGTTAAT	6	381,184	334,534	Ribosomal protein S19; glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)
CATCTTCACC	6	512,676	No match	Ribosomal protein S25; no match
TGTACCTGTA	5	446,608	—	Tubulin, α, ubiquitous
ATCAAGGGTG	5	412,370	—	Ribosomal protein L9
AGAAAGATGT	5	287,558	—	Annexin A1
TTGGCAGCCC	5	No match	356,342	No match; ribosomal protein L27a
GGCAGAGGAC	5	118,638	30,656	Nonmetastatic cells 1, protein (NM23A) expressed in KIAA0528 gene product
GTCTGGGGCT	5	406,504	—	**Transgelin 2**
AAGGACCTTT	5	109,051	—	SH3 domain binding glutamic acid-rich protein like 3
ATGGCAAGGG	5	270,232	25,425[c]	Hypothetical protein MGC40157; FLJ35696 protein
GGACCACTGA	5	119,598	—	Ribosomal protein L3
AGAAATACCA	5	380,933	—	Similar to ribosomal protein L22
ATTGTTTATG	5	181,163	380,159	High-mobility group nucleosomal binding domain 2; KIAA1393
GTAGCAGGTG	5	140,452	8,728	Mannose-6-phosphate receptor binding protein 1; filamin-binding LIM protein-1
TTGGTGAAGG	4	75,968	518,737	Thymosin, β 4, X-linked; thymosin-like 3
GCCATAAAAT	4	1,908	—	Proteoglycan 1, secretory granule
TCTGGTTTGT	4	446,574	194,110, 74,137[c]	Thymosin, β 10; hypothetical protein PR02730; transmembrane traffic-ing protein
TCACCCACAC	4	406,300	—	Ribosomal protein L23
GTGAAGGCAG	4	356,572	368,855	Ribosomal protein S3A; guanosine monophosphate reductase 2
GGGGAAATCG	4	446,574	—	Thymosin, β 10
TAGGGCAATC	4	380,973	—	SMT3 suppressor of mif two 3 homolog 2 (yeast)
CAGGAGGAGT	4	308,709	213,029	Glucose regulated protein, 58 kDa; phosphatidylinositol glycan, class O
GAAAAATGGT	4	374,553	356,261	Laminin receptor 1 (ribosomal protein SA, 67 kDa); transcribed sequence with strong similarity to protein sp:P08865 RSP4_HUMAN 40S ribosomal protein SA
TTCTATTTCA	4	170,328	—	Moesin

Transcripts with counts of at least 10 tags and at least fourfold more abundant in hematopoiesis-related tissues were selected among 350 tags obtained by the discrimination analysis

[a]CGAP-SAGEgenie mapping indicates best gene for tag, whereas alternative UniGene clusters are shown in the NCBI-SAGEMap column. A dash (—) indicates no additional matches, besides CGAP-SAGEgenie.

[b]Transcripts in bold were selected for validation by reverse transcription polymerase chain reaction.

[c]SAGEMap does not include the UniGene cluster selected by CGAP as the best gene for tag.

Abbreviations: CGAP, Cancer Genome Anatomy Project; NCBI, National Center for Biotechnology Information; SAGE, serial analysis of gene expression.

stroma cells, skeletal myoblasts, and endothelial cells has been purified from the postnatal human marrow [25]. Finally, transplanted HSCs have been demonstrated to differentiate into endothelial cells [65]. The comparison of the gene expression profiles that we adopted in the present study is one means of evaluating the relationship of cells from various tissues. The cluster analysis strongly indicates that endothelial cells, $CD34^+$ HSCs, and MSCs share a close relationship based on the expressed transcripts, and this relationship may reflect their common ontogeny. Alternatively, clustering on the basis of the expression profiles would indicate only the activation of similar sets of genes owing to closer functional roles.

The genes expressed at a similar high level in MSC and endothelial cells as compared with other tissues (*SPARC*, *LGALS1*, *ZYX, CFL1*, *PFN1*, *SOC*, *MSN*, *TIMP2*, *TM4SF1*, *TSMB10*, *TGFB1*, *FLNA*, and *FLNA*) indicate a common machinery involved with the structural organization of the cytoskeleton and with the connection of matrix and cell–cell external signals with the intracellular signaling pathways [66–72]. Additionally, transcripts of other genes were abundant in all the five hematopoiesis-related tissues, in contrast with hematopoiesis-unrelated tissues, among which are *VIM*, *GNAI2*, *HMGA1*, *CNN2*, *EEF1A1*, *ACTG1*, *K-α-1*, *ANXA1*, *TAGLN2*, *SMT3H2*, and *LAMR1*. Although heterogeneous, most of these genes are related to the cytoskeletal organization, cell–cell and cell–matrix interactions, cell motility, and proliferation [73–82].

Our results show that the three lineages of precursors related to hematopoiesis share the high expression of a considerable number of transcripts, parts of common differentiation pathways present in these cells. It seems reasonable to suggest that the most abundantly expressed transcripts are, in fact, shared by most of the cells in the culture instead of being expressed by a small subset of cells. This would mean that the phenotypical expression as MSC, HSC, or endothelial cell does not imply such a drastic change of the cell programming as would the differentiation into muscle or brain cells. In the latter case, this transdifferentiation would probably be the result of a more profound change of a subset of cells.

Although informative, the view provided by our work is still restricted and needs to be complemented with data from other approaches. The results of the gene expression evaluation support the similarities of the cells obtained from the two sources of MSCs observed with morphological, immunophenotypical, and in vitro differentiation studies; at the same time, the results reveal a difference that is probably related to the local specialization of the cells to participate in the osteogenic or the angiogenic processes.

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