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Research Article

Mesenchymal stem cells can be obtained from the human saphena vein ☆

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Abstract

Mesenchymal stem cells (MSC) can be isolated from many sites adults and the fetus. Cells with osteoblastic, chondrogenic, leiomiogenic and stromogenic potentials have been obtained from the bovine artery wall, and we now show that MSC can be isolated also from the adult human vein wall. Cells detached from internal surface of the saphenous vein are cultured in vitro for 2–3 weeks and replated weekly. The culture forms a semi-confluent layer of spindle-shaped cells that are CD13+, CD29+, CD44+, CD34−, CD45−, CD14−, CD133−, CD31−, CD33−, CD54+, CD106−, CD90+, KDR−, cadherin-5−, HLA class I+ and HLA-DR− and differentiate in vitro into osteoblasts, chondrocytes and adipocytes. Gene expression, when compared with seven other normal tissues, shows strong similarity with MSC obtained from other sources. Three genes more expressed in saphenous MSC than in the other two MSC are related to angiogenesis, and the expression of two of them is shared by endothelial cells. These results demonstrate that the human vein wall contains mesenchymal cells with morphologic features, immunophenotypic markers, gene expression profile and differentiation potential that are similar to MSC obtained from the bone marrow and from the umbilical vein.

Keywords: Mesenchymal stem cell; Gene expression; Vascular cells; Angiogenesis

Introduction

Mesenchymal stem cells (MSC) are multipotent precursors capable of differentiating into various cell types of mesodermal origin including chondrocytes, osteocytes, adipocytes, myocytes and bone marrow stromal cells [1,2].

Although the differentiation potential of adult stem cells was initially believed to be restricted to its tissue of origin, recently, there have been 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. Bone marrow mesenchymal cells may also have a key role in hematopoiesis, both by cell–cell contacts and by secreted proteins, and their coinfusion with hematopoietic stem cells may facilitate engraftment and reduce graft-versus-host disease after bone marrow transplantation [4]. Thus, the therapeutic potential of these cells is the focus of considerable interest.

MSC can be obtained from various sites in the adult, fetus [5], amniotic fluid [6], placenta or cord blood cells [7–9]. Recently, many groups succeeded in isolating MSC from umbilical cord (UC) blood [10–12], whereas controversial results were obtained by others who suggested that cord

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blood is not a source for MSC [13,14]. We and Romanov et al. [14,15] obtained MSC starting from cells detached from the UC vein and demonstrated that their gene expression is very similar, although not identical, to that of BM MSC.

The presence of precursor mesenchymal cells was recently demonstrated in the bovine aorta [16]. Described as calcifying vascular cells, their potential to differentiate along osteoblastic, chondrogenic, leiomiogenic and stromogenic pathways was demonstrated in vitro. Owing to the similarities of these cells with mesenchymal cells obtained from other sources, we investigated the possibility of obtaining mesenchymal stem cells from the adult human vascular wall.

Material and methods

Isolation and culture of MSC from the human saphenous vein

Segments of 15 to 20 cm of varicose saphenous veins obtained during saphenectomy were maintained in phosphate-buffered saline (PBS) until processed. The segments were internally washed with PBS, filled with 0.5% collagenase in PBS, and the extremities were clamped and incubated for an hour 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 [15]. Each segment yields ca. 1×10^5 cells.

The cells collected were centrifuged at $400 \times g$, and the pellet was resuspended in α-minimum essential medium (α-MEM Gibco-BRL, Gaithersburg, MD, USA) supplemented with 20% fetal calf serum (Hy Clone, Logan, UT, USA), 2 mmol/l L-glutamine (Gibco-BRL, Gaithersburg, MD, USA) and 100 U penicillin/streptomycin (SIGMA). A total of 10^6 cells were seeded into a 25 cm^2 culture flask (Corning, Cambridge, MA, USA) at the concentration of 10^5 cells per ml. Colonies of MSC could be observed after 1 week when the medium with the non-adherent cells was removed and replaced. After 3 weeks with weekly medium changes, the growth of spindle-shaped fibroblastoid cells was predominant, and at this time, the cells were detached with trypsin (5 mg/ml), washed in PBS and replated into 75 cm^2 culture flask (Corning, Cambridge, MA, USA) for expansion with the specific medium. After expansion, the cells of the third passage were analyzed by flow cytometry (FACSort, BD, San Jose, CA, USA), and an aliquot of the culture was replated for adipogenic, osteogenic and chondrocytic differentiation, as previously described [2,10,17].

Flow cytometry analysis

The cells harvested were labeled with CD90-PE, CD14-PE, CD54-PE, CD29-PE, CD34-PE, CD-106-PE, CD133-PE, CD44-FITC, CD33-FITC, CD45-FITC, CD13-FITC, CD31-FITC, KDR, cadherin-5, HLADR-FITC and HLA ClassI-FITC and analyzed on a FACSort (Becton Dickinson; San Jose, CA) as previously described [18].

Gene expression analysis during adipogenic differentiation

Total RNA was isolated using Trizol (Invitrogen, Life Technologies, Carlsbad, CA, USA) following the manufacturer's instruction. RT-PCR was done using SuperScript™One-step RT-PCR. The RT-PCR reaction was carried out in a 25 μl final volume, which contained 2 μg of total RNA, 0.2 mM of each dNTP, 1.2 mM $MgSO_4$, 0,5 μl of RT/Platinum *Taq* mix and 10 μM of each primer. The primers sequences for β-actin were: sense—5′ gccctgaggcactcttcca 3′ and reverse—5′ ccagggcagtgatctccttct 3′. For the PPARγ2 were: sense—5′ gttatgggtgaaactctgggag 3′ and reverse—5′ ggagatgca ggctccactttg 3′. RT and PCR cycles were carried out for 30 min at 50°C and 2 min at 94°C followed by 35 cycles of 40 s at 94°C, 50 s at 59°C and 1 min at 72°C, with an additional 10 min incubation at 72°C after completion of the cycles. The amplified fragments were analyzed by 1.5% agarose gel electrophoresis, ethidium bromide stained.

Semiquantitative evaluation by RT-PCR

Total RNA was obtained from seven human tissues. The transcription reaction was performed with 2 μg of total RNA, 0.5 μg of Oligo(dT) primer and 200 U of Superscript II Rnase H Reverse Transcriptase (Invitrogen Corporation, Carlsbad, CA, USA) in a total volume of 20 μl, and 1/10 of the volume of the cDNA was used in the semiquantitative PCR. Primers for each gene were designed in two different exons so that any amplification product of eventual contaminant genomic DNA has a different size. When the reaction was positive in the undiluted samples, the cDNA was serially diluted (1:2 to 1:128) before performing the PCR. The expression of four genes was measured by real-time PCR with the Taqman approach (Applied Biosystems, Foster City, CA): SPARC (secreted protein acidic cysteine-rich or osteonectin), PGK1 (phosphoglycerate kinase 1), CCND1 (cyclin D1) and MGLL (monoglyceride lipase). The results are expressed as fold change over the value obtained for bulk (unfractionated) bone marrow, on the basis of $2^{-\Delta\Delta Ct}$ [19].

Results

The adherent cells that grow in the culture after seeding cells detached from the saphena vein wall are morphologically similar to the mesenchymal stem cells cultured from adherent cells obtained from the human bone marrow. The cells are long-lived in culture and have a significant capacity for expansion. We have tested them for up to 12 passages without loosing the proliferative capacity. After the third replating, they were negative for hematopoietic lineage

Table 1
Immunophenotypic characteristics of the mesenchymal stem cells obtained from the human saphenous vein

Marker	Percent positive cells		
	Sample #1	Sample #2	Sample #3
CD13	97.63	99.61	93.25
CD14	0.86	1.82	0.32
CD29	97.16	99.39	94.4
CD31	0.2	5.65	11.27
CD33	0.0	0.0	0.0
CD34	0.9	4.12	8.87
CD36	0.35	0.26	0.13
CD44	87.59	88.8	26.16
CD45	0.09	0.01	0.05
CD54	74.08	73.32	54.07
CD90	96.91	97.22	94.95
CD106	1.36	12.15	1.77
CD133	1.49	2.92	0.22
CD144 (cadherin-5)	3.31	4.02	4.69
HLA class I	94.21	97.86	88.65
HLA class II	2.37	0.7	0.0
KDR	2.24	0.84	0.79

markers (CD34, CD45 and CD133), monocytic (CD14), and for endothelial markers such as KDR, cadherin-5, CD31 and CD133 (Table 1). As other mesenchymal stem cells, the majority of cells were positive for CD13, CD29, CD90, HLA class I, CD44 and CD54. Flow cytometry of cells at the 8th passage showed a stable phenotype. Freshly collected cells were negative for MSC markers (CD73 and CD105) and for endothelial and hematopoietic cells markers

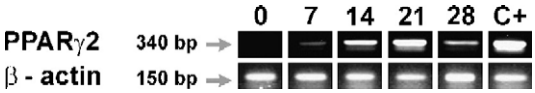

Fig. 2. Evaluation of expression of PPARG2 by RT-PCR during differentiation of cultured human mesenchymal stem cells into adipocytes. For comparison, expression of β-actin is also displayed. C+ represents result obtained with RNA extract from a suspension of human adipocytes aspirated during lipoaspiration surgery.

(CD90, CD34, CD45, KDR) in two experiments. When cultured with dexamethasone and ascorbic acid, they underwent osteogenic differentiation, as demonstrated by positive alkaline phosphatase reaction and positive calcium staining by the von Kossa reaction, whereas in culture with insulin, dexamethasone and indomethacin, they originated adipocytes, identified by vacuoles that stained positively with Sudan III (Fig. 1). Differentiation into adipocytes was accompanied by the appearance at the 7th culture day of the expression of PPARG2 (peroxisome proliferator-activated receptor gamma) gene, which increased in the following days (Fig. 2). This member of the nuclear hormone receptor subfamily of transcription factors has been demonstrated to be highly expressed in adipogenic cells and absent in undifferentiated mesenchymal cells [2]. When cultured under hypoxic conditions, they originated chondroblasts and chondrocytes that stained strongly for vimentin, with abundant extracellular collagen 2 and 4.

Real-time PCR quantification of SPARC demonstrated abundant expression of SPARC in the three MSC lineages,

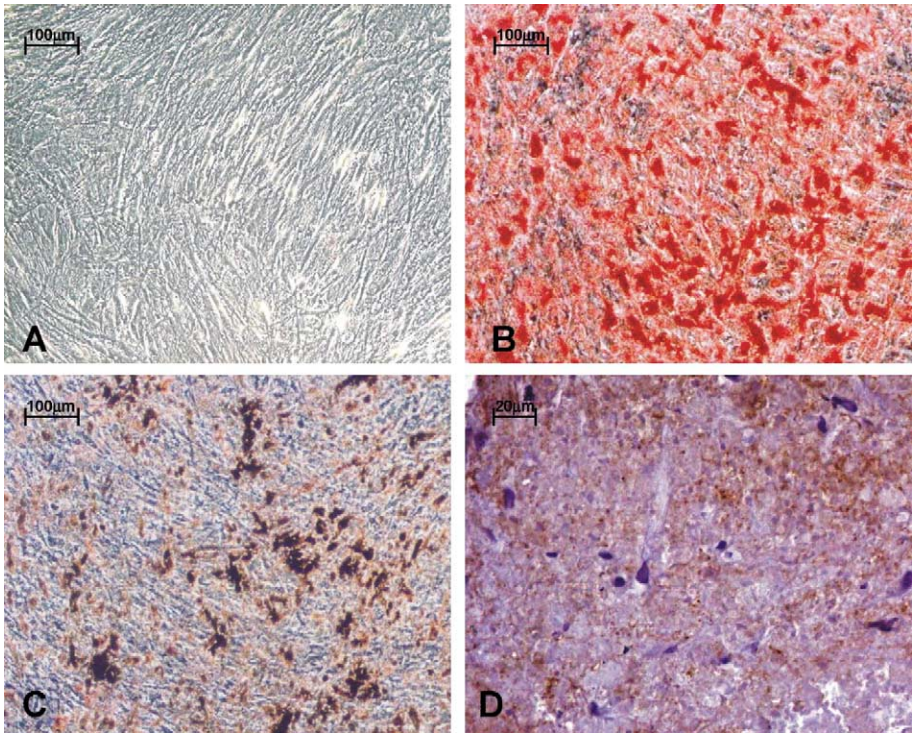

Fig. 1. (A) A culture of MSC obtained from the human saphena vein. (B) Sudan III staining of adipocytes derived from the MSC. (C) Osteogenic differentiation of MSC, shown by positive staining for calcium deposits demonstrated by the von Kossa reaction. (D) Chondrocyte differentiation of MSC cultured as a pellet in the bottom of a 15-ml Falcon tube: there are abundant collagen bundles in the extracellular matrix that stain with anti-collagen II.

Table 2
Quantification of mRNA for four genes by real-time PCR (rt-PCR)

Tissue	SPARC		PGK1	CCND1	MGLL
	rt-PCR[a]	Tags			
Bulk bone marrow (reference)	1.0	11	1.0	1.00	1.0
Umbilical cord vein MSC	49.9	1722	0.6	34.1	0.5
Bone marrow MSC	47.2	1595	0.4	174.2	0.7
Saphena CMS	45.2	nd	0.3	52.3	0.9
HUVEC	4.7	nd	0.3	156.0	2.3
CD34+	3.7	10	nd	nd	nd
PB Leukocytes	3.1	37	nd	nd	nd
Liver	3.0	33	1.2	347.3	23.2
Brain	0.2	10	0.3	2.7	0.9
Muscle	4.0	14	1.9	70.5	22.7

Expression is reported as fold change in reference to bulk bone marrow normalized with GAPDH expression. For osteonectin (SPARC), the gene expression evaluated by rt-PCR is compared with the number of tags obtained from SAGE libraries of these same tissues.
nd: not done.
[a] Fold change of mRNA related to normal bone marrow bulk normalized with GAPD. Real-time reaction was performed as described in User Bulletin #2, Applied Biosystems.

well above all the other tissues examined, in agreement with results of gene expression obtained by SAGE analysis (Table 2). The three MSC lineages also showed low PGK1 and MGLL expression.

Semiquantitative RT-PCR expression analysis for 19 genes demonstrated higher expression in the three MSC lineages for COL1A1, COL1A2, LGAL1S, VIM, TAGLN, TAGLN2, ANXA2, MMP2, TPT1 and INHB (Table 3). The expression pattern of HUVEC has similarities with the MSC lineages, but there are clear differences, such as the absence of expression of COL1A1, COL1A2 and TAGLN.

Discussion

From cells detached from the internal surface of human saphena vein, we obtain, by culture cells that exhibit morphological characteristics, immunophenotypic markers and differentiation capabilities of the MSC [2]. The cells are long-lived in culture and have a significant capacity for expansion. They are negative for hematopoietic lineage, monocytic and endothelial markers and positive for markers observed in other mesenchymal stem cells.

In two previous studies, we demonstrated that osteonectin (or SPARC) is the most abundantly expressed mRNA in MSC from the bone marrow [18] and the second (after galectin 1) in MSC from the umbilical vein (RA Panepucci, unpublished data, 2004). Table 2 shows that the levels of osteonectin expression in the mesenchymal cells obtained from the adult human vein are equivalent to that obtained from the two other sources of these cells and significantly different from the cells from seven other tissues. Altogether, the gene expression of the mesenchymal cells from the adult vein is very similar to that of the mesenchymal cells obtained either from the bone marrow or the umbilical cord vein, on the basis of 22 additional genes, and differs from the patterns observed for bulk unfractionated bone marrow, human umbilical vein endothelial cells (HUVEC), liver, skeletal muscle and brain. Interestingly, two genes that are more expressed in the saphenous than in the other two mesenchymal cells, interleukin 8 and matrix-metalloproteinase 2 or gelatinase A, are related to angiogenesis [20–23]. The expression of both genes is shared with endothelial cells from the umbilical cord vein, thus strengthening the notion that they are of particular importance in vascular genesis and physiology. EGR1 (early growth response 1) has been

Table 3
Comparison of mRNA abundance in MSC from the saphenous vein with six other tissues by RT-PCR

Gene	MSC bone marrow	MSC umbilical vein	MSC saphena	HUVEC	Bone marrow	Liver	Skeletal muscle	Brain
COL1A1	1:64	1:64	1:64	–	–	–	–	–
COL1A2	1:64	1:32	1:64	–	–	–	1:1	–
LGAL1S	1:64	1:64	1:64	1:64	1:8	1:8	1:64	1:16
VIM	1:64	1:64	1:64	1:64	1:16	–	1:16	1:64
TAGLN	1:64	1:16	1:2	–	–	–	–	–
TAGLN2	1:8	1:1	1:16	1:16	1:1	–	–	1:8
ANXA2	1:16	1:4	1:32	1:16	–	–	–	1:1
MMP2	1:8	1:8	1:32	1:8	–	–	–	–
CLU	1:1	–	1:8	1:4	1:64	1:2	–	1:64
EGR1	1:2	1:2	1:32	1:2	–	–	–	1:32
IL8	1:1	1:32	1:64	1:64	nd	nd	nd	1:16
TPT1	1:16	1:8	1:32	1:8	1:16	1:2	–	1:8
INHB	1:64	1:64	1:64	1:16	1:1	1:1	–	1:4
CXCL6	–	1:8	–	–	–	nd	nd	–
SELL	–	–	–	–	1:16	–	–	1:1
NCAM2	–	–	1:2	–	–	–	–	1:4
MMP9	–	–	–	–	1:4	–	–	–
HLA-DRB3	–	–	–	–	1:16	1:1	1:1	1:16
CXCR3	–	–	–	–	1:1	–	–	1:1

–: negative in undiluted sample.
nd: not done.

shown to be the responsible for the activation of peroxisome proliferator-activated receptors (particularly PPARγ1) in vascular smooth muscle cells [24].

We conclude that mesenchymal stem cells morphologically and functionally similar to those obtained from the bone marrow and the umbilical vein can be isolated in vitro from adult human veins. This finding may have practical implications in view of the suggested plasticity of these cells and their application in cell therapies. The growing list of sites from which MSC can be derived suggests that these cells are ubiquitous in the organism, a property that may be related to its wide potential for differentiation. Additionally, in agreement with others, we observe slight functional differences between MSC obtained from different sources. These differences might be explored advantageously, combined with the MSC plasticity, if the cells are to be used in cell therapy.

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