

Mesenchymal Stem Cells Can Be Differentiated Into Endothelial Cells In Vitro

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ABSTRACT

Human bone marrow-derived mesenchymal stem cells (MSCs) have the potential to differentiate into mesenchymal tissues like osteocytes, chondrocytes, and adipocytes in vivo and in vitro. The aim of this study was to investigate the in vitro differentiation of MSCs into cells of the endothelial lineage. MSCs were generated out of mononuclear bone marrow cells from healthy donors separated by density gradient centrifugation. Cells were characterized by flow cytometry using a panel of monoclonal antibodies and were tested for their potential to differentiate along different mesenchymal lineages. Isolated MSCs were positive for the markers CD105, CD73, CD166, CD90, and CD44 and negative for typical hematopoietic and endothelial markers. They were able to differentiate into adipocytes and osteocytes after cultivation in respective media. Differentiation into

endothelial-like cells was induced by cultivation of confluent cells in the presence of 2% fetal calf serum and 50 ng/ml vascular endothelial growth factor. Laser scanning cytometry analysis of the confluent cells in situ showed a strong increase of expression of endothelial-specific markers like KDR and FLT-1, and immunofluorescence analysis showed typical expression of the von Willebrand factor. The functional behavior of the differentiated cells was tested with an in vitro angiogenesis test kit where cells formed characteristic capillary-like structures. We could show the differentiation of expanded adult human MSCs into cells with phenotypic and functional features of endothelial cells. These predifferentiated cells provide new options for engineering of artificial tissues based on autologous MSCs and vascularized engineered tissues. *Stem Cells* 2004;22:377-384

INTRODUCTION

Adult bone marrow contains at least two types of stem cells: hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), sometimes also referred to as marrow stromal cells [1]. Both cell types can be isolated from the mononuclear cell fraction of bone marrow aspirates, and

HSCs can be further enriched by immunomagnetic isolation based on specific surface antigens like CD34 or CD133. MSCs lack a unique surface antigen that could be used for positive selection, and therefore the general strategy for the enrichment of MSCs is based on the adherence of cells to plastic dishes in medium with low serum [1-3].

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In vitro MSCs grow as a homogenous population of adherent cells and express a set of marker proteins on their surface, including CD105 and CD73 [1-3], sometimes also referred to as SH2 and SH3, [4, 5], CD44, CD90, and CD29 [1-5]. Since these markers are not specific for MSCs, they are mainly characterized by their ability to differentiate into multiple mesenchymal lineages, including osteocytes, chondrocytes, adipocytes, and skeletal muscle cells under controlled in vitro conditions [2]. In vivo, human MSCs transplanted into fetal sheep are able to differentiate into cells of various tissue [6], and transplantation studies in several mouse models also showed an engraftment at ischemic lesions [7, 8]. Therefore, MSCs fulfill all criteria of true stem cells, i.e., self-renewal, multilineage differentiation, and in vivo reconstitution of tissue [9].

Progenitor cells for endothelial cells have been identified both in peripheral blood and in bone marrow. Peripheral blood endothelial progenitor cells (EPCs) can be isolated by magnetic bead selection on the basis of the CD34 antigen, and they were found to be positive for CD34, CD133, and vascular endothelial growth factor (VEGF) receptor 2, sometimes also referred to as KDR or FLK1 [10]. EPCs originate in the bone marrow and can be mobilized either endogenously by tissue ischemia or exogenously by cytokine stimulation or HMG-CoA reductase inhibitors [11, 12]. CD133-selected cells from peripheral blood were also shown to have the capacity to differentiate into endothelial cells under defined conditions [13, 14].

Multipotent adult progenitor cells (MAPCs) were isolated from bone marrow by depleting hematopoietic cells from the bone marrow cell fraction and plating the resulting cells. MAPCs are positive for the VEGF receptors KDR and FLT1 and dimly positive for CD44 and CD133 [15, 16]. Besides their ability to differentiate in numerous mesenchymal tissues, they were also shown to differentiate in endothelial and neuronal cells in vitro and in vivo [16, 17], indicating a greater developmental potential of MAPCs compared with MSCs.

The aim of our study was to test in vitro if bone marrow MSCs isolated by density centrifugation and positive for the markers CD105 (SH2) and CD73 (SH4) were capable of differentiation into endothelial cells. We have therefore established a protocol based on low-serum culture supplemented with VEGF. We can show that under these conditions, MSCs acquire several features of mature endothelium, including the expression of VEGF receptors, VE-cadherin, VCAM-1, and von Willebrand factor (vWF). They show also an enhanced ability to form capillary structures in semisolid medium. MSCs may therefore be an alternative source for endothelial progenitors for clinical therapies like tissue replacement or vascularization of artificial organs. In addition, the in vitro differentiation of

MSCs might be a useful model for the elucidation of the role of VEGF for differentiation and maturation of endothelial cells.

MATERIALS AND METHODS

Isolation and Culture of Bone Marrow-Derived Human MSCs

Bone marrow samples were collected from healthy donors (age 22-49 years) at The Bone Marrow Transplantation Center of the University Hospital Carl Gustav Carus, Dresden, after obtaining informed consent. The study was approved by the local institutional review board. MSCs were isolated and cultured according to modifications of previously reported methods [1-3]. Briefly, 5-7 ml bone marrow aspirate was diluted 1:5 in phosphate-buffered saline (PBS) containing 0.5% human serum albumin (HSA, Braun; Melsungen, Germany). A 20-ml aliquot was layered over a Percoll solution ($d = 1.073$ g/ml, Biochrom; Berlin, Germany, <http://www.biochrom.de>) and centrifuged at 900 *g* for 30 minutes at room temperature. Mononuclear cells at the interface were recovered, pressed through a 100- μ m nylon cell strainer (Becton Dickinson; Franklin Lakes, NJ; <http://www.bd.com>) and washed twice in PBS 0.5% HSA. All cells were seeded into 75-cm² flasks containing Dulbecco's-modified Eagle's Medium-Low Glucose (DMEM-LG, GIBCO Invitrogen; Carlsbad, CA; <http://www.invitrogen.com>) supplemented with GlutaMAX-I^m 2 mM (L-alanyl-L-glutamin; GIBCO Invitrogen), penicillin 10 U/ml, streptomycin 100 μ g/ml (both from Biochrom), and 10% fetal calf serum (FCS). MSC cultures grew at 37°C in 5% CO₂. Nonadherent cells were removed after 24 hours by washing with PBS 0.5% HSA. The medium was changed subsequently every 4 days. Two weeks later the culture reached 90% confluency. MSCs were recovered using 0.25% Trypsin-EDTA (Sigma; St. Louis, MO; <http://www.sigmaaldrich.com>) and replated at a density of 5,000-6,000 cells per cm² of surface area as passage 1 (P₁) cells [3].

Differentiation Into Osteocytes and Adipocytes

For osteogenic differentiation, a 70% subconfluent culture of MSCs from passages P⁰-P² was used. Cells were incubated in osteogenic medium with 10⁻⁷ M dexamethasone, 0.2 mM ascorbic acid, and 10 mM β -glycerophosphate (all Sigma). The medium was replaced twice a week. After day 15 in differentiation medium, cell colonies displayed bone-like nodular aggregates of matrix mineralization. The mineral deposition could be visualized by Kossa staining for calcium [1]. Briefly, cell layers were fixed with 10% formalin (Sigma) for 30 minutes, incubated with 2% silver nitrate solution (weight/volume [w/v], Sigma) for 15 minutes in the dark, and developed with 1% pyrogallol (Merck; Darmstadt, Germany; <http://pb.merck.de>). The layer was washed thoroughly with deionized water. The alkaline

phosphatase in osteogenic differentiated cells was determined by using the histochemical, semiquantitative kit for alkaline phosphatase (Sigma). For adipogenic differentiation, MesenCult medium with adipogenic stimulatory supplements (StemCell Technologies; Vancouver, Canada, <http://www.stemcell.com>) was used. The medium was replaced every 3-4 days for 21 days.

Endothelial Cell Differentiation

Confluent cells were cultivated in the presence of 2% FCS and 50 ng/ml VEGF (Promocell; Heidelberg, Germany; <http://www.promocell.de>) for 7 days. Medium was changed every 2 days.

Immunohistochemistry

For von Willebrand staining, cells were fixed with methanol at -20°C for 10 minutes and rinsed with PBS. Samples were incubated with an antibody against vWF (DAKO; Hamburg, Germany; <http://www.dakocytomation.com>) for 30 minutes, rinsed with PBS, and incubated with a labeled secondary goat anti-mouse antibody (Dianova; Hamburg, Germany; <http://www.dianova.de>).

Flow Cytometry

Cells were trypsinized, washed with PBS, and incubated with antibodies against CD34, CD45, CD44, CD73, CD90, VE-cadherin, VCAM-1 (all Becton-Dickinson), CD105 (Serotec; Cambridge, UK; <http://www.serotec.co.uk>), CD117 (Dianova), KDR and FLT-1 (both Sigma), and CD133-1 (Miltenyi Biotech; Bergisch Gladbach, Germany; <http://www.miltenyibiotec.com>). Analysis was performed with a FACScalibur flow cytometer (Becton Dickinson).

Laser Scanning Cytometry Analysis of MSCs

Cells were grown in chamber slides (Nunc; Wiesbaden, Germany; <http://www.nalgenunc.com>); stained with antibodies against KDR, FLT-1, VCAM-1, and VE-cadherin (Sigma); rinsed with PBS; and stained with a secondary goat anti-mouse antibody labeled with fluorescein isothiocyanate (FITC; Dianova). After that, cells were again rinsed with PBS and fixed with 4% paraformaldehyde (Sigma) in PBS for 15 minutes at room temperature. Cells were again rinsed with PBS and permeabilized with 0.1% Triton (Sigma) in PBS for 5 minutes at room temperature. Subsequently, cells were rinsed with PBS and stained with 0.1 μ g/ml TO-PRO-3 Iodide (Molecular Probes; Eugene, OR; <http://www.probes.com>) in PBS for 1 hour. Finally, cells were rinsed and mounted on a microscopic slide and subsequently analyzed on a LSC 2 laser scanning cytometer (CompuCyte; Cambridge, MA; <http://www.compuCyte.com>) with an air-cooled 15-mW 488-nm argon-ion laser for FITC

excitation and with a 25-mW 633-nm helium-neon laser for TO-PRO-3 Iodide excitation using a 20 \times objective. FITC signals were detected through a 505-530-nm green filter and TO-PRO-3 signal through a 650-nm long-pass filter. Contouring of cells was achieved by nuclear staining with TO-PRO-3 Iodide. Photomultiplier tube (PMT) settings for PMT voltage, offset, and gain were 30%, 2070, and 255, respectively, for green and 15%, 2070, and 255, respectively for far-red. As minimal area 10 μ m² were defined and 12 pixels were added to cover the whole cell. Data were acquired and analyzed with WinCyte acquisition software (CompuCyte).

In Vitro Angiogenesis

Analysis of capillary formation was performed using the in vitro angiogenesis kit (Chemicon; Temecula, CA; <http://www.chemicon.com>) according to the manufacturer's instructions. Fifty microliters of gel matrix solution were applied into one well of a 96-well plate and incubated for 1 hour at 37°C. Cells were then trypsinized and 5 \times 10³ cells were suspended in 50 μ l of the DMEM containing various concentrations of VEGF and plated onto the gel matrix and incubated for 2 hours. Cells were counted by eye for the formation of capillary structures. The percentages of formed capillaries were calculated from two independent experiments.

RESULTS

Cell Culture of MSCs

MSCs were isolated according to standard techniques for the isolation of mononuclear cells from bone marrow using density gradient centrifugation. Phase contrast microscopy from cells in passage P⁰ demonstrated a fibroblast-like, spindle-shaped morphology (Fig. 1A). In later passages (>P⁵), the spindle-shaped cells began to display a broadened, flat morphology. Therefore, endothelial experiments were performed only on cells from P¹ to P⁵. Cells were tested with flow cytometry for the presence or absence of characteristic hematopoietic and endothelial markers. MSCs typically expressed the antigens CD105 and CD73, which are also recognized by the SH2 and SH3 antibodies described by Pittenger *et al.* [2]. Furthermore, cells expressed CD166, CD90, and CD44 (Fig. 2A). They were negative for typical lymphocytic markers like CD45 and CD14 and for the early hematopoietic markers CD34 and CD133. Undifferentiated MSCs neither expressed VEGF receptors KDR and FLT-1 nor VE cadherin and VCAM-1 (Fig. 2B).

The ability of the MSCs to differentiate into osteocytes and adipocytes was tested in all cultures from various donors. When cultured in osteogenic medium for 15 days,

Figure 1. A) MSCs with a 60× magnification. B) 30×. C) After differentiation into osteocytes after von Kossa staining. D) After adipogenic differentiation.

the morphology changed: A) on day 1, a nearly confluent spindle-shaped layer; B) day 5-7, cells are positive for APase (data not shown) and form nodular aggregates, and C) day 12-15, cells began to mineralize their matrix and were positive for Kossa staining (Fig. 1C). They were also able to differentiate into adipocytes, and cells accumulated different amounts of lipid vacuoles (Fig. 1D) after cultivation in adipogenic medium.

Differentiation of MSCs Into Endothelial-Like Cells

We introduced differentiation into endothelial-like cells by cultivating confluent MSCs in the presence of 2% FCS and 50 ng/ml VEGF for 7 days. Cell morphology showed no difference compared with undifferentiated MSCs. Immunohistochemical staining for vWF was chosen for the basal characterization of endothelial-like cells. Undifferentiated MSCs showed almost no specific staining for vWF, but after 7 days of cultivation the overall fluorescence intensity of the differentiated MSCs was markedly enhanced. Also, Weibel-Palade bodies were visible in differentiated MSCs (Fig. 3A). fluorescence-activated cell sorter (FACS) analysis confirmed the expression of vWF (Fig. 3B).

The ability to form capillaries in semisolid medium was tested with an in vitro angiogenesis kit. Undifferentiated and differentiated MSCs were trypsinized and seeded on top of the ECmatrix gel solution. Cells were cultivated in the presence of two different concentrations

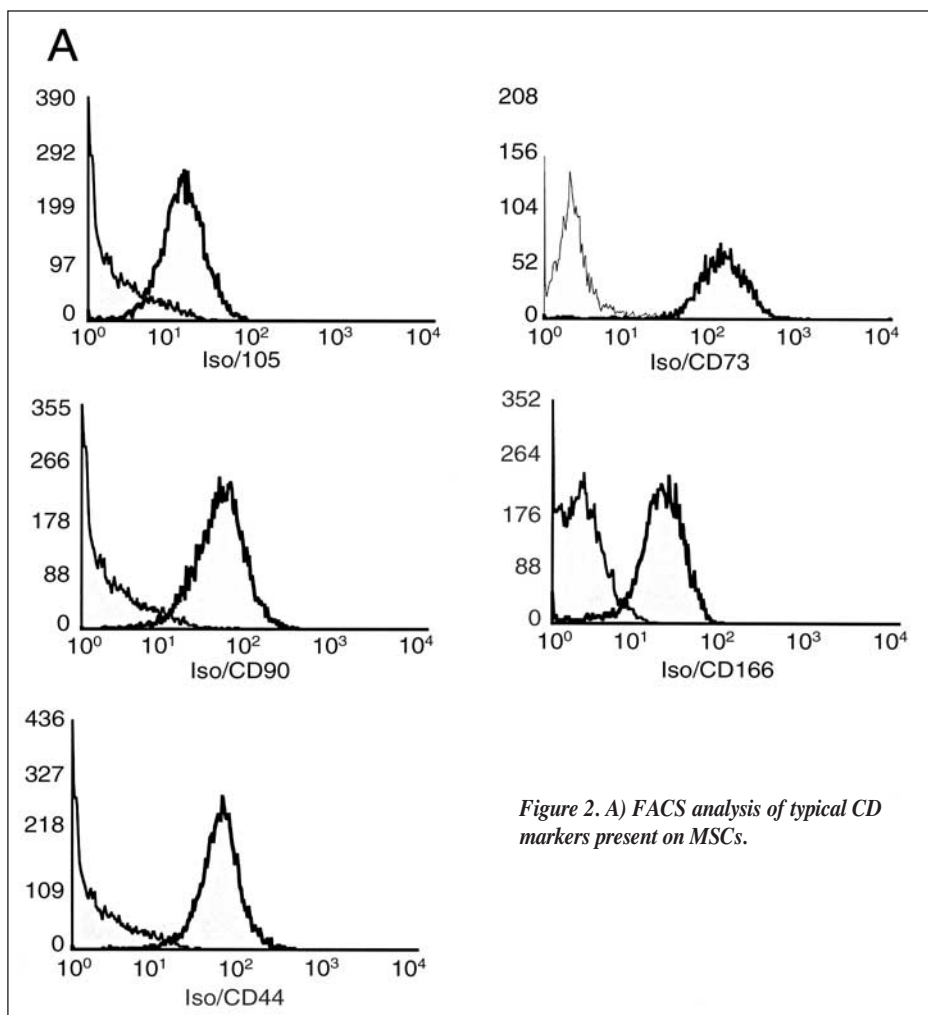
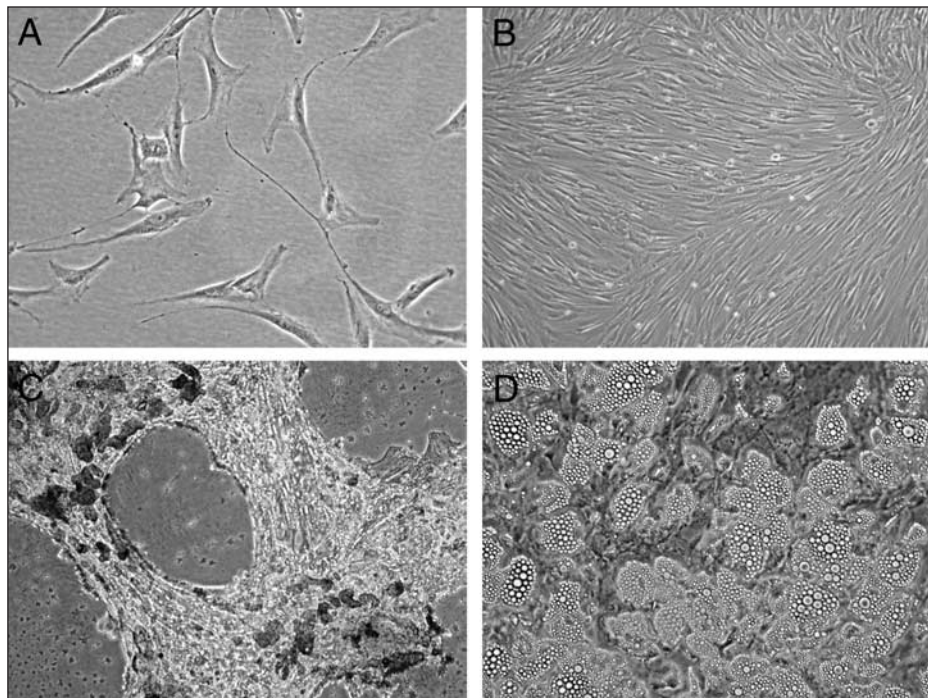


Figure 2. A) FACS analysis of typical CD markers present on MSCs.

Figure 2. B) FACS analysis of typical hematopoietic and endothelial markers which are not expressed by MSCs.

of VEGF and once without VEGF. The undifferentiated MSCs showed very few capillaries after 2 hours and most of the cells stayed round in the medium. When cultivated in the presence of VEGF, more tube-like structures were visible. Undifferentiated MSCs showed a substantial formation of capillary structures when cultivated for 2 hours in the presence of 50 ng/ml VEGF (Fig. 4). After differentiation more than 80% of MSCs form capillary structures both in the presence and in the absence of VEGF (Table 1).

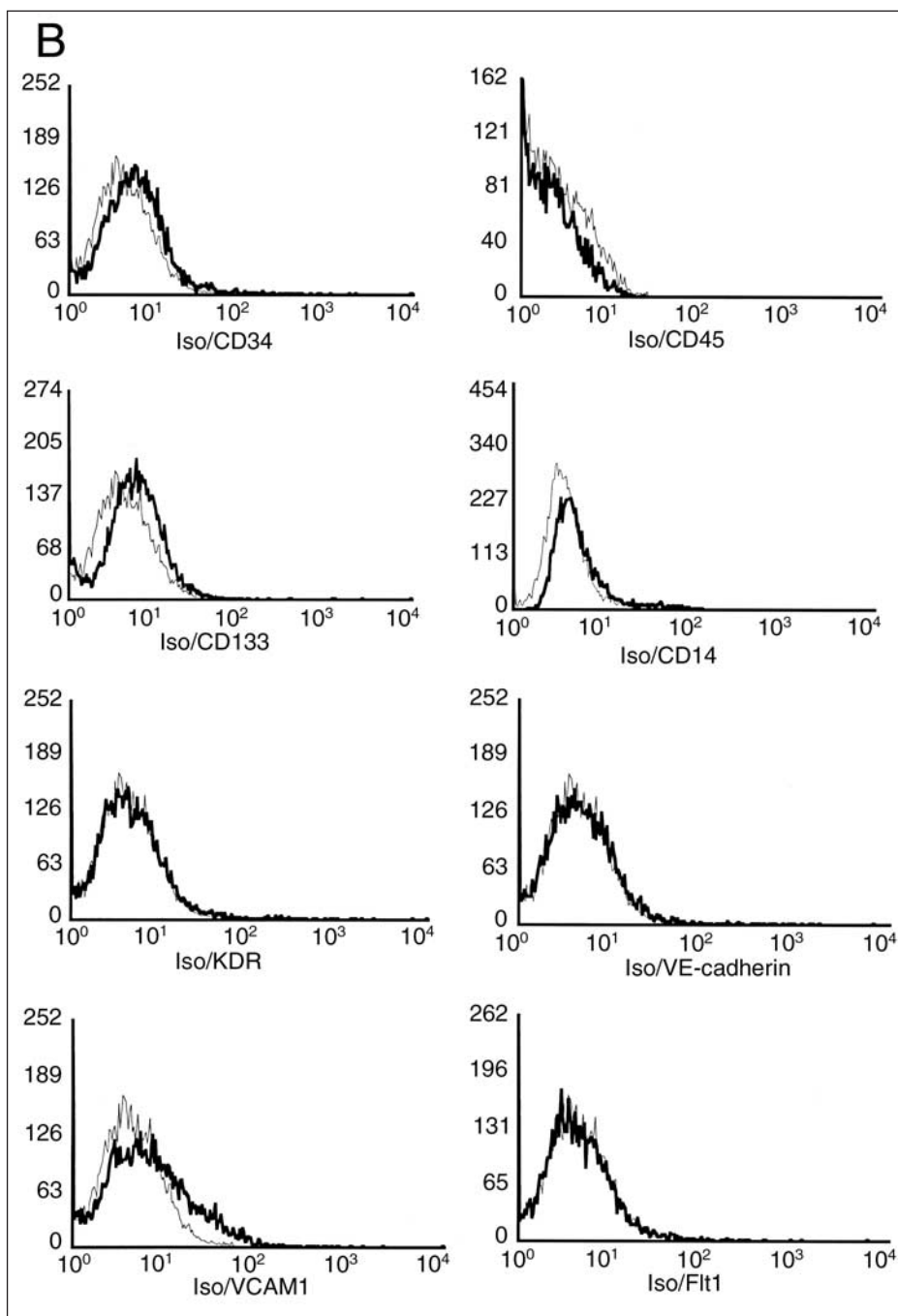
For the quantification of endothelial-specific marker expression *in situ* after immunophenotyping, a laser-scanning cytometry (LSC)-based protocol was introduced. LSC is an alternative method to flow cytometry, especially for the analysis of small cell numbers. For contouring of cells, nuclear staining with the DNA stain TO-PRO-3 Iodide was chosen, and specific cellular antigens were stained with an FITC-labeled antibody. LSC analysis of differentiated MSCs showed expression of the VEGF receptors KDR and FLT-1 and also VE-cadherin and VCAM-1 (Fig. 5). These data show a substantial increase of expression of endothelial-specific marker molecules on MSCs after differentiation with VEGF. Taken together, our data indicate that cultivating in the presence of VEGF leads to a substantial expression of endothelial-specific markers on MSCs.

DISCUSSION

The relative ease of isolating MSCs from bone marrow and the great plasticity of the cells make them ideal tools for an autologous or allogeneic cell therapy. Clinical trials for the treatment of osteogenesis imperfecta [18], metachromatic leukodystrophy, and Hurler syndrome [19] have proven the therapeutic relevance of transplanted MSCs. The use of

autologous vascular endothelial progenitor cells seems attractive for the development of engineered vessels as well as for the vascularization of engineered tissues, and may also be useful to augment vessel growth in ischemic tissue [5, 9].

Our study shows for the first time that human bone marrow-derived CD105⁺ CD73⁺ MSCs are capable of differentiating into endothelial cells *in vitro*, which make them attractive candidates for the development of autologous tissue grafts. Serial analysis of gene expression (SAGE) revealed that single cell-derived colonies of MSCs expressed mRNAs of multiple cell lineages, including characteristic epithelial



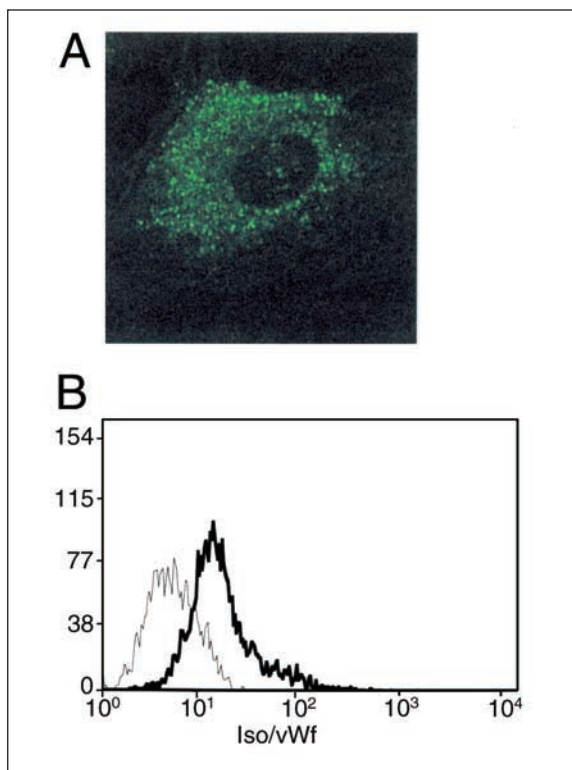
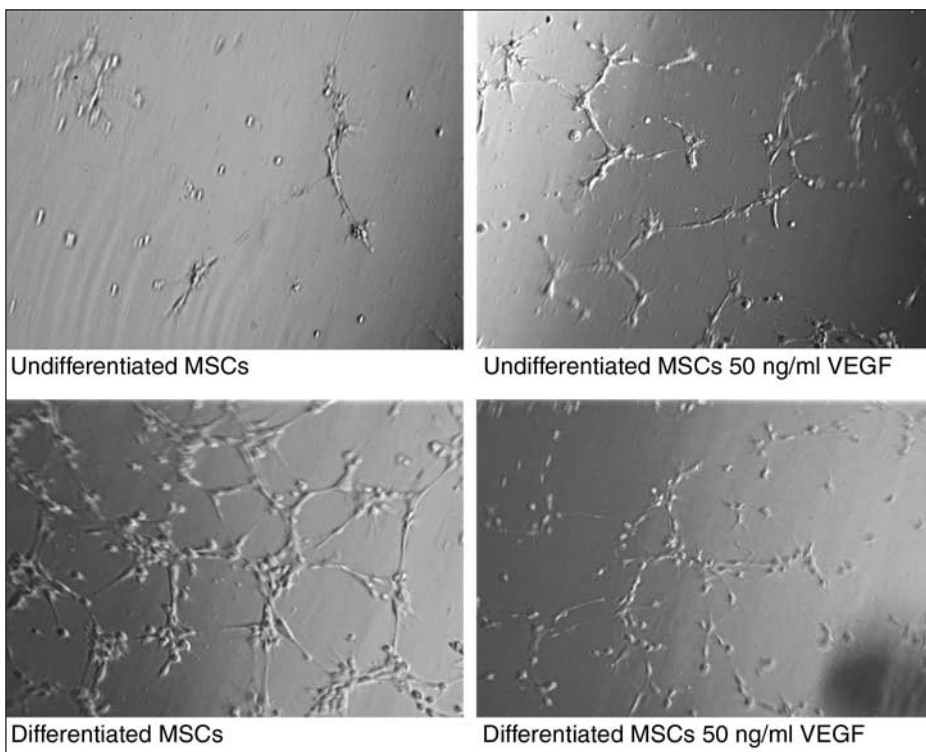


Figure 3. A) von Willebrand staining of differentiated MSCs. B) FACS analysis of differentiated MSCs stained with vWf.

and endothelial molecules like Epican and Keratins 8 and 10 [20]. These data suggest that the in vitro differentiation potential of MSCs is not restricted to mesodermal lineages but also transdifferentiation of MSCs into other lineages like endothelial could be realized in vitro and in vivo.

The formation of endothelial tissue (vasculogenesis) is a process in which the embryo angioblasts are differentiated from mesodermal cells and organized to form a primitive vascular network [21]. Angiogenesis, the formation of new blood vessels by sprouting from pre-existing vessels, occurs in many situations such as embryonic development and pathological conditions like tissue ischemia. Although the molecular mechanisms responsible

Figure 4. Light microscopic analysis of differentiated and undifferentiated MSC on semisolid medium in presence and absence of VEGF.



for vasculogenesis and angiogenesis are currently not fully understood, the pivotal role of VEGF for both processes is evident [22, 23]. Hence, VEGF is part of all cocktails for the in vitro differentiation of either endothelial progenitor cells or hematopoietic stem cells into endothelial cells in vitro [10, 13-15].

Several populations of bone marrow-derived cells have the potential to differentiate into endothelial-like cells. CD133⁺ HSCs cultivated at high cellular density and in the presence of endothelial growth factors like VEGF were shown to acquire endothelial features [13, 14], and CD34⁺ HSCs isolated from peripheral blood can differentiate into endothelial cells in vitro [10] and contribute to vascularization in animal models [7].

A distinct population of adult stem cells called MAPCs were described by the Verfaillie group [15]. They are capable of differentiating into endothelial cells in vivo and in vitro [16, 17], but this subset of multipotent cells is probably a different population than the MSCs isolated by plastic adherence. All cited model systems were derived of KDR-positive cells or at least KDR-dim cells, whereas MSCs in our system were KDR negative and CD133 negative, which clearly separates them from the MAPCs used by the Verfaillie group and also from EPCs [10, 12]. The major advantage of MSCs is the vast number of cells that can be achieved from one bone marrow aspirate. MSCs were shown to be genetically stable over many passages [2].

One major criticism of studies describing plasticity of bone marrow stem cells is the heterogeneity of the cell population.

Table 1. Percentage of capillar-like cells after 2 hours of cultivation on semisolid medium

	Undifferentiated MSCs	Differentiated MSCs
- VEGF	38 ± 1	85 ± 7
+ VEGF	64 ± 24	86 ± 1

Differentiated and undifferentiated MSCs were cultivated in presence or absence of 50 ng/ml VEGF.

Although we cannot rule out the possible existence of sub-populations of committed cells, it appears unlikely since cells do not proliferate during the differentiation in presence of 2% FCS and VEGF. Differentiation experiments with single-cell-derived MSCs will ultimately prove the plasticity of MSCs.

In our differentiation system MSCs acquire major characteristics of mature endothelial-like expression of vWF, VEGF receptors 1 and 2 (FLT-1 and KDR), VE-cadherin, and VCAM-1. Cells do not express CD31 and CD34 after a 7-day differentiation, which indicates that these markers are obviously later expressed in endothelial maturation. Elongation of differentiation time will probably also lead to an upregulation of these markers.

After differentiation, the formation of capillary-like structures in semisolid medium was markedly enhanced when cells were cultivated without VEGF. Recent studies have shown that murine stroma cells can also be differentiated into vasculature-forming cells under hypoxic conditions or when genetically transduced to express VEGF [24, 25]. We also found that MSCs form tube-like structures when cultivated in semisolid medium; the presence of VEGF markedly enhanced this behavior. Interestingly, the numbers of capillary-like cells in this assay were strongly enhanced in predifferentiated MSCs. Hypoxia upregulates several genes involved in angiogenesis like basic fibroblast growth factor, VEGF, the

VEGF receptors KDR and FLT-1, and components of the plasminogen system [26]. Differentiation of MSCs with VEGF also upregulates the expression of the VEGF receptors KDR and FLT-1, which play a major role in angiogenesis in vivo and contribute together with matrix-metalloproteases to the formation of capillary-like structures in vitro.

Our findings may support the development of tissue-engineered vascular grafts based on autologous MSCs. Differentiated MSCs could also be beneficial in the engineering of complex tissues, where vascularization of the tissue is an essential feature for the successful engraftment. Clinical studies will have to prove whether the systemic application of predifferentiated endothelial MSCs may have positive effects in patients with small vessel diseases. In addition, tissues derived from autologous MSCs might engraft easier when the blood supply can be improved by vascularization of artificial bone, cartilage, or other tissues.

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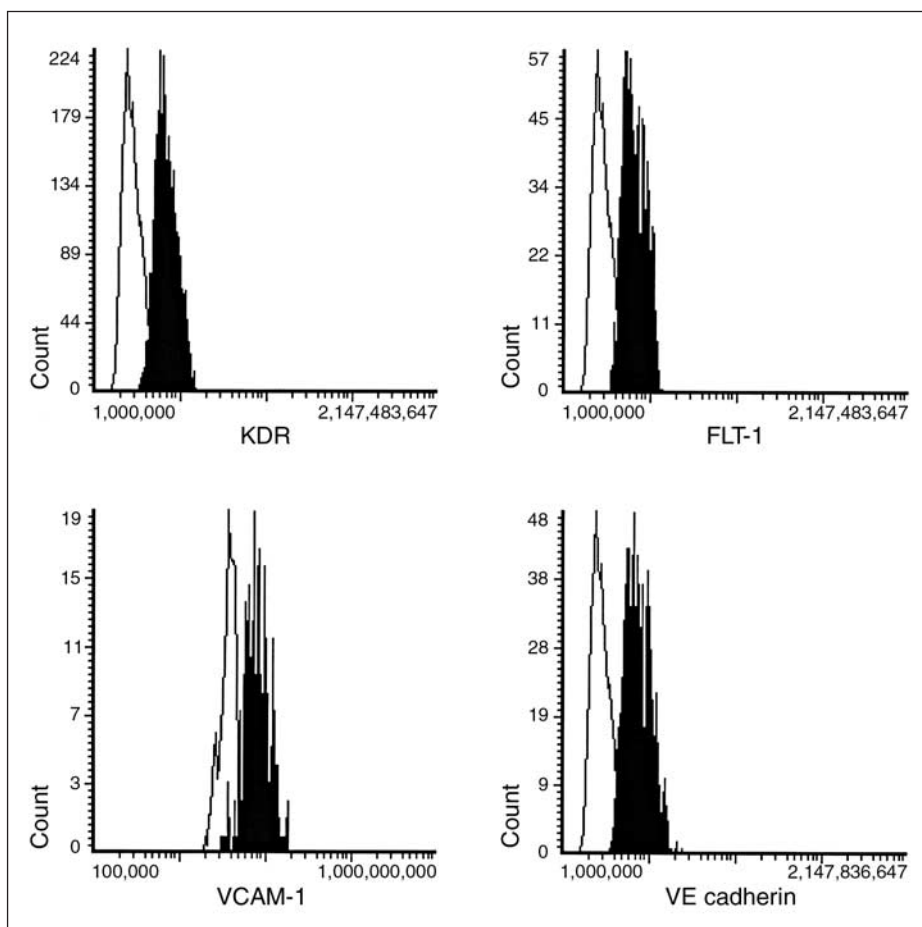


Figure 5. LSC analysis for the expression of endothel-specific markers after differentiation of MSCs.

REFERENCES

- 1 Majumdar MK, Thiede MA, Mosca JD et al. Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSC) and stromal cells. *J Cell Physiol* 1998;176:57-66.
- 2 Pittenger MF, Mackay AM, Beck SC et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143-147.
- 3 Phinney DG. Building a consensus regarding the nature and origin of mesenchymal stem cells. *J Cell Biochem* 2002;38(suppl):7-12.
- 4 Barry FP, Boynton RE, Haynesworth S et al. The monoclonal antibody SH-2, raised against human mesenchymal stem cells, recognizes an epitope on endoglin (CD105). *Biochem Biophys Res Commun* 1999;265:134-139.
- 5 Barry F, Boynton R, Murphy M et al. The SH-3 and SH-4 antibodies recognize distinct epitopes on CD73 from human mesenchymal stem cells. *Biochem Biophys Res Commun* 2001;289:519-524.
- 6 Liechty KW, MacKenzie TC, Shaaban AF et al. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. *Nat Med* 2000;6:1282-1286.
- 7 Kalka C, Masuda H, Takahashi T et al. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. *Proc Natl Acad Sci USA* 2000;97:3422-3427.
- 8 Orlic D, Kajstura J, Chimenti S et al. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;410:701-705.
- 9 Verfaillie CM. Adult stem cells: assessing the case for pluripotency. *Trends Cell Biol* 2002;12:502-508.
- 10 Asahara T, Murohara T, Sullivan A et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275:964-967.
- 11 Llevadot J, Murasawa S, Kureishi Y et al. HMG-CoA reductase inhibitor mobilizes bone marrow-derived endothelial progenitor cells. *J Clin Invest* 2001;108:399-405.
- 12 Dimmeler S, Aicher A, Vasa M et al. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J Clin Invest* 2001;108:391-397.
- 13 Gehling UM, Ergun S, Schumacher U et al. In vitro differentiation of endothelial cells from AC133-positive progenitor cells. *Blood* 2000;95:3106-3112.
- 14 Quirici N, Soligo D, Caneva L et al. Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells. *Br J Haematol* 2001;115:186-194.
- 15 Reyes M, Lund T, Lenvik T et al. Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. *Blood* 2001;98:2615-2625.
- 16 Reyes M, Dudek A, Jahagirdar B et al. Origin of endothelial progenitors in human postnatal bone marrow. *J Clin Invest* 2002;109:337-346.
- 17 Jiang Y, Jahagirdar BN, Reinhardt RL et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002;418:41-49.
- 18 Horwitz EM, Prockop DJ, Fitzpatrick LA et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med* 1999;5:309-313.
- 19 Koc ON, Day J, Nieder M et al. Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). *Bone Marrow Transplant* 2002;30:215-222.
- 20 Tremain N, Korkko J, Ibberson D et al. MicroSAGE analysis of 2,353 expressed genes in a single cell-derived colony of undifferentiated human mesenchymal stem cells reveals mRNAs of multiple cell lineages. *STEM CELLS* 2001;19:408-418.
- 21 Jain RK. Molecular regulation of vessel maturation. *Nat Med* 2003;9:685-693.
- 22 Carmeliet P, Ferreira V, Breier G et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* 1996;380:435-439.
- 23 Ferrara N, Carver-Moore K, Chen H et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 1996;380:439-442.
- 24 Annabi B, Lee YT, Turcotte S et al. Hypoxia promotes murine bone-marrow-derived stromal cell migration and tube formation. *STEM CELLS* 2003;21:337-347.
- 25 Kupatt C, Hinkel R, Vachenaier R et al. VEGF165 transfection decreases postischemic NF-kappa B-dependent myocardial reperfusion injury in vivo: role of eNOS phosphorylation. *FASEB J* 2003;17:705-707.
- 26 Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. *Nat Med* 2003;9:677-684.