



Plasticity of Human Adipose Lineage Cells Toward Endothelial Cells

Physiological and Therapeutic Perspectives

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Background—Adipose tissue development and remodeling are closely associated with the growth of vascular network. We hypothesized that adipose tissue may contain progenitor cells with angiogenic potential and that therapy based on adipose tissue-derived progenitor cells administration may constitute a promising cell therapy in patients with ischemic disease.

Methods and Results—In mice, cultured stromal-vascular fraction (SVF) cells from adipose tissue have a great proangiogenic potential, comparable to that of bone marrow mononuclear cells in the mouse ischemic hindlimb model. Similarly, cultured human SVF cells differentiate into endothelial cells, incorporate into vessels, and promote both postischemic neovascularization in nude mice and vessel-like structure formation in Matrigel plug. **In vitro, these cells represent a homogeneous population of CD34- and CD13-positive cells,** which can spontaneously express the endothelial cell markers CD31 and von Willebrand factor when cultured in semisolid medium. Interestingly, dedifferentiated mature human adipocytes have the potential to rapidly acquire the endothelial phenotype in vitro and to promote neovascularization in ischemic tissue and vessel-like structure formation in Matrigel plug, suggesting that cells of endothelial and adipocyte phenotypes may have a common precursor.

Conclusions—This study demonstrates, for the first time, that adipocytes and endothelial cells have a common progenitor. Such adipose lineage cells participate in vascular-like structure formation in Matrigel plug and enhance the neovascularization reaction in ischemic tissue. These results also highlight the concept that adipose lineage cells represent a suitable new cell source for therapeutic angiogenesis in ischemic disease. (*Circulation*. 2004;109:656-663.)

Key Words: angiogenesis ■ ischemia ■ adipose tissue ■ therapy, cell ■ progenitor cells

White adipose tissue has long attracted attention because of its great and reversible capacity for expansion, which appears to be permanent throughout adult life.¹ Adipose tissue enlargement is the result of adipocyte hypertrophy and the recruitment and differentiation of regenerative precursors located in the stromal-vascular fraction (SVF). **However, development of the capillary network is also required to ensure adipose tissue remodeling.**^{2,3} Indeed, a crucial link exists between adipose cells and the capillary network. First, the vascular pattern during embryonic development indicates that the formation of capillary convolutions is a decisive and specific phase in the development of fat lobules.⁴ Second, extensive vascularization is necessary for optimal functioning

of adipose tissue as a metabolic and endocrine tissue. Third, adipose lineage cells have been shown to release potent angiogenic factors such as monobutyl, vascular endothelial growth factor (VEGF), and leptin.^{5–8} Finally, antiangiogenic agents promote adipose tissue loss, underlining the functional interaction between adipogenesis and blood vessel formation.⁹

SVF represents a heterogeneous cell population surrounding adipocytes in fat tissue, including mature microvascular endothelial cells.^{10,11} Its culture was classically used to investigate preadipocyte differentiation into mature adipocytes. However, this fraction was also reported to be a very convenient and nonrestrictive source of pluripotent cells such

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as hematopoietic progenitors and spare mesodermal stem cells able to differentiate into osteogenic, chondrogenic, and myogenic lineages.^{12–14} Recently, the spectrum of differentiation potential was even extended to the neurogenic phenotype.¹⁵ We hypothesized that adipose tissue and especially SVF may also contain progenitor cells with angiogenic potential able to differentiate into mature endothelial cells and to participate in blood vessel formation.

In this study, we demonstrate for the first time that adipocytes and endothelial cells have a common progenitor. Such adipose lineage cells participate in vascular-like structure formation in Matrigel plug and enhance the neovascularization reaction in ischemic tissue.

Methods

Animals and Tissue Sampling

Seven-week-old male C57BL/6 or nu/nu mice (Harlan, France) were housed in a controlled environment (12 hours light/dark cycle at 21°C) with free access to water and standard chow diet. At the end of the experiments, mice were killed by cervical dislocation under CO₂ anesthesia. Inguinal adipose tissue or muscle was rapidly removed and processed for analyses as described below. All procedures were performed according to SELASA norms.

We obtained samples of human abdominal subcutaneous fat from patients undergoing abdominal dermolipectomy or nephrectomy in the Department of Plastic Surgery and Urology of Toulouse Rangueil Hospital (Toulouse, France). The patients gave their written informed consent and the Ethics Committee of the Hospital approved the study.

Cell Isolation and Culture

Mouse bone marrow cells were obtained by flushing the tibias and femurs. Low-density mononuclear cells were isolated by density gradient centrifugation with Ficoll, as previously described.¹⁶ Cells were isolated from adipose tissues according to Bjorntorp et al¹⁷ with minor modifications. Inguinal adipose tissue excised from mice and human adipose tissue from dermolipectomy were digested at 37°C in PBS containing 2% BSA and 2 mg/mL collagenase (Sigma) for 45 minutes. After elimination by filtration through 25 μ m filters of undigested fragments, mature adipocytes were separated from pellets of SVF cells by centrifugation (600g, 10 minutes). SVF cells were plated (30 000 cells/cm²) in DMEM-F12 10% newborn calf serum (NCS) medium. Six hours after plating, all nonadherent cells were removed by washing. SVF cells were then cultured for 3 days in the same medium before use, as previously described.¹⁷ The mature adipocyte fraction was also recovered, washed gently in DMEM-F12 10% NCS medium, and prepared as a 10⁶ cells/mL suspension. A 100- μ L aliquot of the cell suspension was layered onto a 25-mm Thermanox coverslip (Nunc) placed in a 35-mm culture dish. The first coverslip was covered by a second one, and after 15 minutes at room temperature, 1.5 mL of DMEM-F12 10% NCS was added. After 4 to 5 days, adherent cells with small lipid droplets (preadipocyte-like cells) appeared and developed a fibroblast-like morphology free of lipids.

For the VEGF assay, SVF cells were cultured for 3 days in DMEM-F12 10% NCS medium and then for 24 hours in 2 mL of DMEM-F12. The VEGF assay (radioimmunoassay, R&D Systems) was then performed on the collected supernatant and on 2 mL of cell-free medium referred to as control.

Methylcellulose Cultures

Dedifferentiated adipocyte or cultured SVF cells were trypsinized and plated at 7.10³ cells/mL in 1.5 mL methylcellulose (Methocult MG3534, StemCell Technologies, Vancouver). Cell culture was performed for 10 days to develop an endothelial-like morphology and fixed for immunostaining. This culture procedure was demon-

strated to be a useful and efficient reagent for preservation of cell function and was used to identify endothelial cell potential.¹⁸

Cell Phenotyping

Cells were stained in phosphate saline buffer containing 0.2% FCS and incubated with anti-mouse or anti-human monoclonal antibodies conjugated with fluorescein isothiocyanate, phycoerythrin, or peridinin chlorophyll protein for 30 minutes at 4°C. After washings, cells were analyzed on a fluorescence-activated cell sorter (FACS) (FACSCalibur, Becton Dickinson). Data acquisition and analysis were then performed (Cell Quest software, Becton Dickinson). All the antibodies were purchased from BD Biosciences, except CD144, which was obtained from Serotec.

Immunostaining

Cells in methylcellulose cultures were washed with PBS buffer and fixed in methanol/acetone for 20 minutes at –20°C. Slides were then blocked in PBS containing 1% BSA and incubated for 1 hour with either anti-human CD31 or anti-human/mouse von Willebrand factor (vWF) antibodies. Tissue samples and Matrigel plugs were processed as previously described.¹⁹ BCIP/NBT detection (Dako) was used to reveal CD31 and vWF antibodies, respectively.

Mouse Model of Ischemic Hindlimb

Animals were anesthetized by isoflurane inhalation. A ligature was placed on the right femoral artery as previously described.²⁰ Five hours after occlusion, 1 \times 10⁶ cells were administered by intramuscular injection in 3 different sites (gastrocnemius, gracilis, and quadriceps muscles, respectively, 25 μ L per injection) of the ischemic leg. Functional analyses were performed 15 days after the onset of ischemia.

Quantification of Angiogenesis

Vessel density was evaluated by high-definition microangiography at the end of the treatment period, as previously described.²⁰ The angiographic score was expressed as a percentage of pixels per image occupied by vessels in the quantification area. Microangiographic analysis was completed by assessment of capillary density by using antibody against total fibronectin.²⁰ Capillary density was analyzed in 3 different sections of gastrocnemius muscle. Five different fields were used in each section (HistoLab software, Microvision Instruments). Capillary/myocyte ratios were calculated, and the results were then expressed according to ischemic/nonischemic ratios.

To provide functional evidence for ischemia-induced changes in vascularization, laser Doppler perfusion imaging experiments were performed in mice, as previously described.²⁰

Angiogenesis Assay Using Matrigel Plug

Mice received 0.5 mL subcutaneous injection with Matrigel+1 \times 10⁶ SVF cells isolated either from mice or human tissue. On day 14, the mice were euthanized and analyses were performed as previously described.²¹

Statistical Analyses

Results were expressed as mean \pm SEM. One-way ANOVA was used to compare each parameter. Post hoc Bonferroni *t* test comparisons were then performed to identify which group differences account for the significant overall ANOVA.

Results

Mouse SVF Cells Promote Angiogenesis in Ischemic Muscle

The angiogenic potential of SVF derived from adipose tissue was first assessed with cultured mouse SVF cells and was compared with the proangiogenic potential of bone marrow-derived mononuclear cells (BM-MNC). SVF cells were

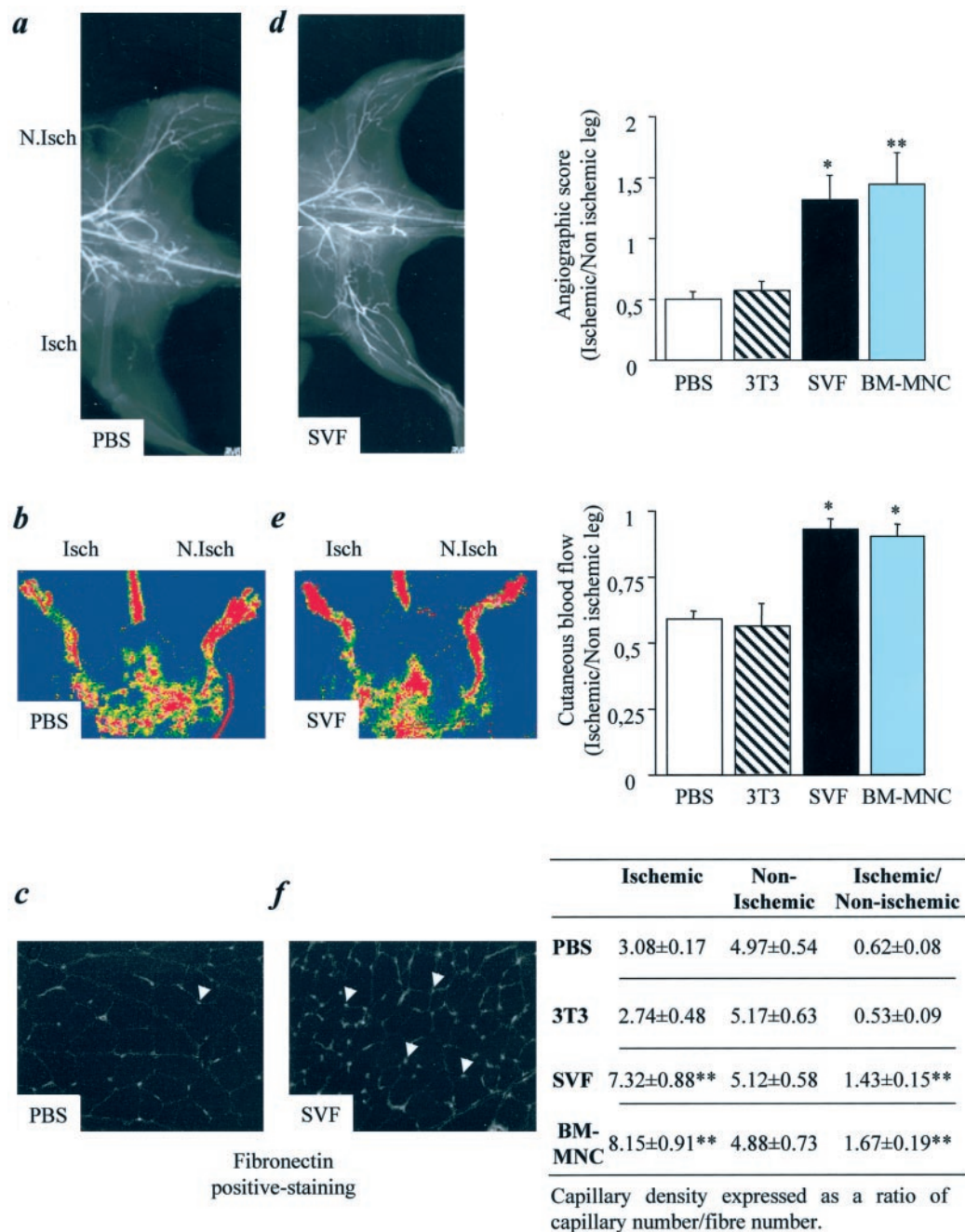


Figure 1. Cultured mouse SVF cells exhibit angiogenic properties after their injection in ischemic hindlimbs; comparison with BM-MNC. a and d, Representative microangiography of the right ischemic and left nonischemic hindlimbs 15 days after femoral artery occlusion. Red arrows indicate ligated ends of femoral arteries. b and e, Hindlimb blood flow monitored in vivo by laser Doppler perfusion imaging, 15 days after femoral artery occlusion. In color-coded images, normal perfusion is depicted in red, and marked reduction in blood flow of ischemic hindlimb is depicted in blue. c and f, Representative photomicrographs of ischemic muscle sections 15 days after femoral artery occlusion, hybridized with antibody directed against total fibronectin. Capillaries appear in white and myocytes in black. Values are mean \pm SEM, $n=6$ per group. PBS indicates PBS-treated mice with hindlimb ischemia; 3T3, mice with hindlimb ischemia receiving 3T3 cells; SVF, SVF-treated animals with hindlimb ischemia; and BM-MNC, mice with hindlimb ischemia receiving BM-MNC. ** $P<0.01$, *** $P<0.001$ vs PBS-treated mice.

prepared from inguinal adipose tissue and cultured for a limited culture of 3 days with no successive plating. Intramuscular transplantation of 1×10^6 freshly isolated SVF cells resulted in only a small increase in neovascularization (data not shown). After limited culture for 3 days, transplantation of 1×10^6 cultured SVF cells markedly improved tissue neovascularization in ischemic hindlimbs as revealed by a

2.6-fold increase in angiography score (Figure 1a and 1d, $P<0.01$), a 1.6-fold rise in Doppler tissue perfusion score (Figure 1b and 1e, $P<0.001$) and a 2.3-fold increase in capillary density (Figure 1c and 1f, $P<0.01$). The SVF-induced increase in ischemic/nonischemic capillary density ratio was confirmed by CD31 immunostaining (0.71 ± 0.09 versus 1.34 ± 0.18 in control and SVF-treated mice, respec-

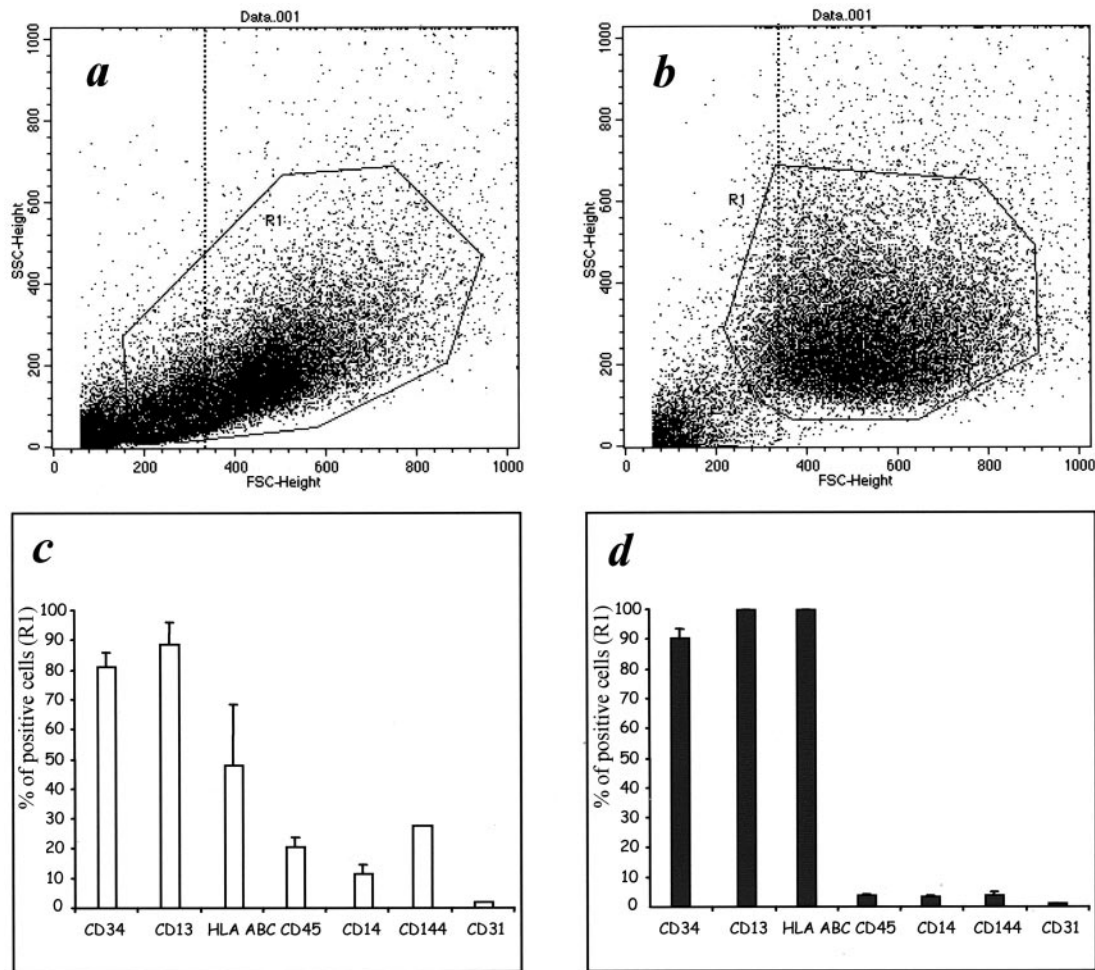


Figure 2. Culture of a heterogeneous population of freshly prepared human SVF promotes a homogeneous cell population with a high forward scatter. a and b, Scattergrams of freshly prepared and cultured human SVF cells. c and d, Panhematopoietic (CD45), monocyte/macrophage (CD14) and mature endothelial (CD144) antigens were identified in a heterogeneous population of freshly prepared SVF (c, open bars) markers and not in cultured SVF cells (d, filled bars), which expressed CD34, CD13, and HLA-ABC-positive markers.

tively, $P < 0.01$). This demonstrated that the SVF cells that emerged after a limited culture had a higher proangiogenic potential than freshly isolated SVF cells. Interestingly, administration of 1×10^6 cultured SVF cells enhanced the neovascularization process to a similar extent as that of 1×10^6 BM-MNC (Figure 1a through 1c). It is noteworthy that 3T3 administration did not modulate the neovascularization reaction (Figure 1). In addition, experiments with SVF cells purified from brown adipose tissue, well known to be more highly vascularized than white adipose tissue, were unsuccessful. Indeed, the ischemic/nonischemic ratio for angiographic score was 0.51 ± 0.07 versus 0.49 ± 0.06 ($P = 0.91$) and that of blood flow was 0.66 ± 0.05 versus 0.63 ± 0.06 ($P = 0.69$) in mice treated with SVF cells derived from brown adipose tissue compared with control animals, respectively (data not shown). This suggests that the high proangiogenic potential of cultured white adipose tissue SVF is not due to circulating endothelial precursors, but rather to cells resident in the fat tissue.

Phenotypic Characterization of Freshly Isolated SVF Cells and of SVF Cells Cultured for 72 Hours

We next analyzed the cellular phenotype of freshly isolated and cultured SVF cells in murine and human species by flow

cytometry. As similar results were obtained in both species, we pursued this study with human cells. In freshly prepared SVF cells from human subcutaneous adipose tissue, we detected several cell populations as shown on forward scatter (Figure 2a). Culture of these SVF cells for 3 days in previously described standard conditions led to the selection of the single cell population with the highest forward scatter (Figure 2b). The antigenic phenotype was consistent with this analysis; the great majority of the population (90% to 99%) was composed of undifferentiated cells expressing CD34, CD13, and HLA-ABC and negative for monocyte/macrophage, hematopoietic (CD45 and CD14), and differentiated endothelial cell markers (CD144 and CD31) (Figure 2c and 2d). Hence, an in vitro cell culture of 3 days appeared to increase the proportion of a unique population of cells with no surface markers of any differentiated cells and some surface antigens characteristic of immature cells with proangiogenic potential such as CD34-positive cells.

A Homogeneous Human SVF Cell Population Induces Neovascularization in Mouse Ischemic Muscle and Differentiates Into Endothelial Cells

We next assessed the efficiency of injected cultured human SVF cells on revascularization in immunodeficient nude

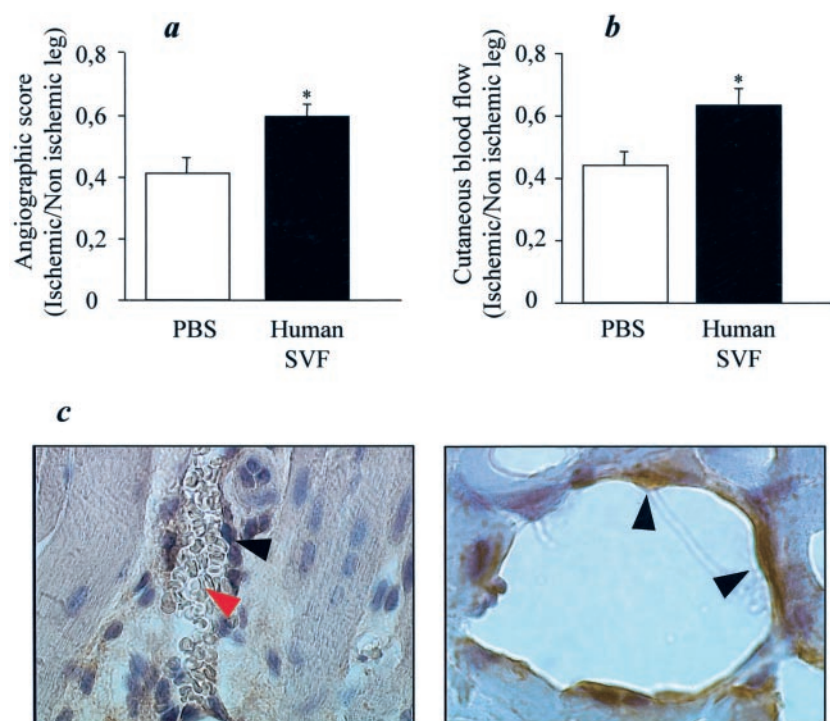


Figure 3. Cultured human SVF cells exhibit functional and antigenic properties of endothelial progenitor cells after their injection in ischemic hindlimbs. **a** and **b**, Cultured human SVF cells significantly improved angiography scores and blood flow monitored in vivo by laser Doppler perfusion imaging of the treated ischemic right hindlimbs compared with untreated nonischemic left hindlimbs ($*P<0.05$). **c**, Fifteen days after injection of cultured human SVF cells, antibody directed specifically against human CD31 isoform stained numerous CD31-positive cells (black arrowheads), which lined functional vessels with erythrocytes (red arrowhead). Magnification, $\times 1000$.

mice. As with mouse cells, the injection of 1×10^6 cultured human SVF cells after 15 days of hindlimb ischemia significantly improved angiographic score and cutaneous blood flow (by 1.6- and 1.5-fold, respectively, when compared with untreated ischemic nude mice; $P<0.01$) (Figure 3a and 3b). Two potential but nonexclusive mechanisms could explain this proangiogenic effect, as follows: either the release by SVF cells of angiogenic growth factors and/or a direct contribution of injected cells by their incorporation into regenerative vessels. Subsequently, we easily detected VEGF by radioimmunoassay, a potent angiogenic factor in the culture supernatant compared with cell-free medium (31 ± 8 versus 9 ± 6 ng/mL, respectively, $P<0.01$, $n=6$). Then, to assess the ability of SVF-derived cells to incorporate new blood vessels, we performed immunohistochemistry experiments using an antibody specific to human CD31, which did not cross-react with mouse tissue. Numerous CD31-positive cells lining regenerated vessels were revealed in SVF-injected hindlimbs (Figure 3c). No CD31⁺ cells were detected in noninjected contralateral hindlimbs. The detection of human CD31⁺ cells in mice ischemic leg strongly suggested that, in vivo, human SVF-derived cells can differentiate into endothelial cells and directly contribute to vascular regeneration.

Cultured Human SVF Cells Spontaneously Exhibit Endothelial Markers In Vitro and Differentiate Into Endothelial Cells in Subcutaneously Implanted Matrigel Plug

CD34 antigen expression in cultured SVF cells, associated with their efficient in vivo transformation into endothelial cells, raised the question of their differentiation potential. As previously described, cultured human SVF cells easily

differentiated into adipocytes in an adipogenic medium (Figure 4a). To test their in vitro endothelial differentiation potential, we first cultured these cells in a semisolid medium known to reveal endothelial potential in progenitors.¹⁸ In these conditions, cultured human SVF cells formed a network of branched tubelike structures (Figure 4b), and most of these cells were strongly stained by antibodies directed against CD31 and vWF (Figure 4c and 4d). To definitively determine the endothelial potential of cultured SVF cells, we subcutaneously injected these cells mixed with Matrigel.²¹ Within the Matrigel plug, cultured SVF cells formed numerous tubelike structures, and the presence of erythrocytes in the lumen demonstrated the existence of a functional vascular structure (Figure 4e and 4f). vWF antibodies positively stained the cells that lined these vessel-like structures (Figure 4g and 4h). Overall, these data demonstrated that cultured SVF cells could spontaneously differentiate into adipocytes and endothelial cells.

Unique Capacity of Mature Human Adipocytes to Dedifferentiate in Culture

The efficient emergence of endothelial as well as adipocyte cells from cultured SVF cells raised the question of the existence of a common precursor for both phenotypes. To address this issue, we used another approach previously described to obtain a homogeneous population of adipocyte precursor cells from adipose tissue.^{22–24} We isolated human mature adipocytes from tissue, obtaining a $>99\%$ pure floating population of cells. When cultured under the described conditions, adipocytes first lost their fatty acids and changed their morphology to a preadipocyte-like then to a fibroblast-like type of cell that could attach to the coverslip. This morphological change was associated with

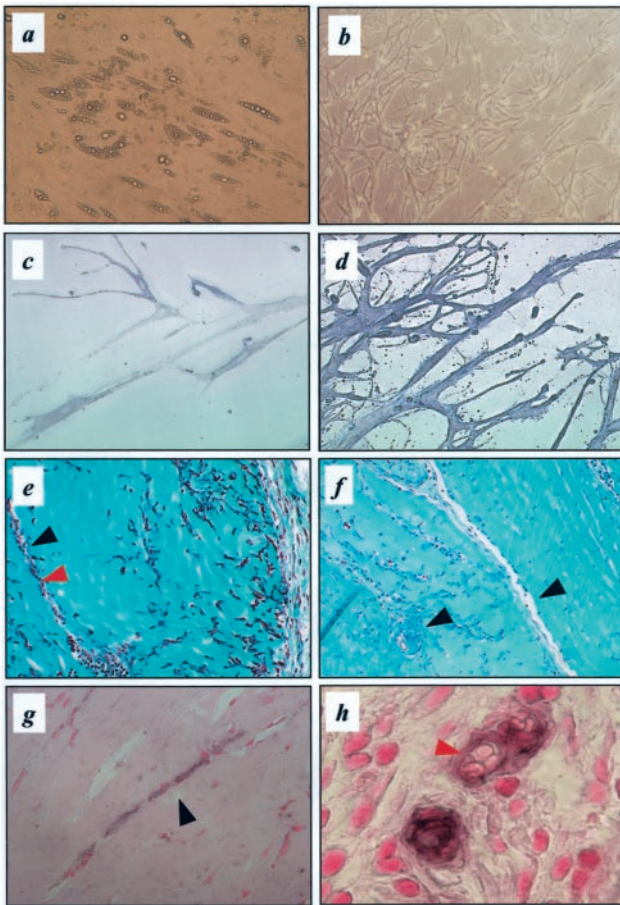


Figure 4. Cultured SVF cells differentiate into endothelial cells in vitro and in Matrigel plug implanted in vivo. a, In adipogenic medium, cultured SVF cells efficiently differentiate into adipocytes (magnification, $\times 200$). b, When cultured SVF cells were seeded in methylcellulose medium, they spontaneously formed branched alignments and tubelike structures ($\times 200$). c and d, Antibodies against human CD31 isoform and vWF, respectively, stained cultured SVF cells seeded in methylcellulose and forming branched alignments ($\times 600$). e and f, In Matrigel plug implanted in vivo, cultured SVF cells formed tubelike structures (black arrowheads) in which erythrocytes were observed (red arrowheads) ($\times 200$). g and h, In Matrigel plug, cultured SVF cells lining tubelike structures were strongly stained by antibody against vWF ($\times 400$ and $\times 1000$, respectively).

functional changes as the adipocytes also lost the enzymatic contents for lipolysis, lipogenesis, and molecular marker expression.²² These cells proliferated and again differentiated into adipocytes when cultured in standard liquid conditions (Figure 5a). When cultured in methylcellulose medium, the homogeneous population of human dedifferentiated adipose cells (hDDAC) formed branched alignments (Figure 5b) and coexpressed the same markers as the cultured SVF cells, including vWF (Figure 5c). As with cultured SVF cells, when hDDAC were injected together with Matrigel plug, they formed numerous tubelike structures that contained erythrocytes in their lumen, thus demonstrating the existence of a functional vascular structure (data not shown).

Dedifferentiated Human Adipocytes Elicit Neovascularization in Mouse Ischemic Muscle and Differentiate Into Endothelial Cells

hDDAC were then tested for their angiogenic potential in nude mice. They were injected in ischemic mouse muscle, and angiogenesis analysis was performed 15 days after ischemia. hDDAC were as efficient as cultured SVF cells in restoring vessel density in the ischemic hindlimb (Figure 5d and 5e). As with cultured SVF cells, numerous neovessels lined with CD31⁺ cells were identified in hDDAC-injected hindlimbs (Figure 5f).

Discussion

The main result of our study is that adipose lineage cells have the potential to differentiate into an endothelial cell phenotype and to participate in blood vessel formation in vivo.

Several hypotheses might be proposed to explain the presence of cells with proangiogenic potential in adipose tissue. These cells obtained from cultured adipose-derived cells may arise from circulating endothelial progenitor cells, which have been shown to mainly derive from monocyte/macrophages.^{25,26} A monocyte/macrophage origin can here be excluded because SVF cells are clearly negative for CD45 and CD14, panhematopoietic and macrophage antigens, respectively. One can also hypothesize that differentiation of endothelial cells from SVF could arise from multipotent adult stem cells (MAPC) present in various tissues.²⁷ Our culture conditions with limited cell culture and no successive plating do not support the selection of such MAPC and exclude this possibility. Therefore, it is likely that the SVF cells may be bipotent and can be considered as preadipocytes as well as endothelial progenitors. Several findings definitively support this hypothesis. Culture conditions of SVF cells lead to the emergence of a homogeneous CD34⁺ cell population (Gronthos et al²⁸ and present FACS analysis). This population of cells, traditionally used to study adipocyte differentiation, is able to spontaneously differentiate into endothelial cells in semisolid medium. In addition, human mature adipocytes that can dedifferentiate into immature cells have the potential to redifferentiate into mature adipocyte and, in both in vivo and in vitro assays, to rapidly acquire endothelial phenotype (expression of CD34, CD31, and vWF antigens) and functions. This suggests the existence of a common precursor for both the endothelial and adipocyte phenotypes (Figure 6). Identification of the pathways that regulate these plastic processes could lead to the development of new therapeutic strategies for control of adipose tissue development.⁹

The ability of adipocyte lineage cells to differentiate into vascular cells is very consistent with histological examination and with recent reports, which suggest that adipose tissue vessels are in a relatively immature state compared with other organs that display a lower plasticity.⁹ This statement is also in line with the cellular antigenic phenotype of cultured SVF cells or dedifferentiated adipocytes, expressing the hemangioblast marker CD34 but not

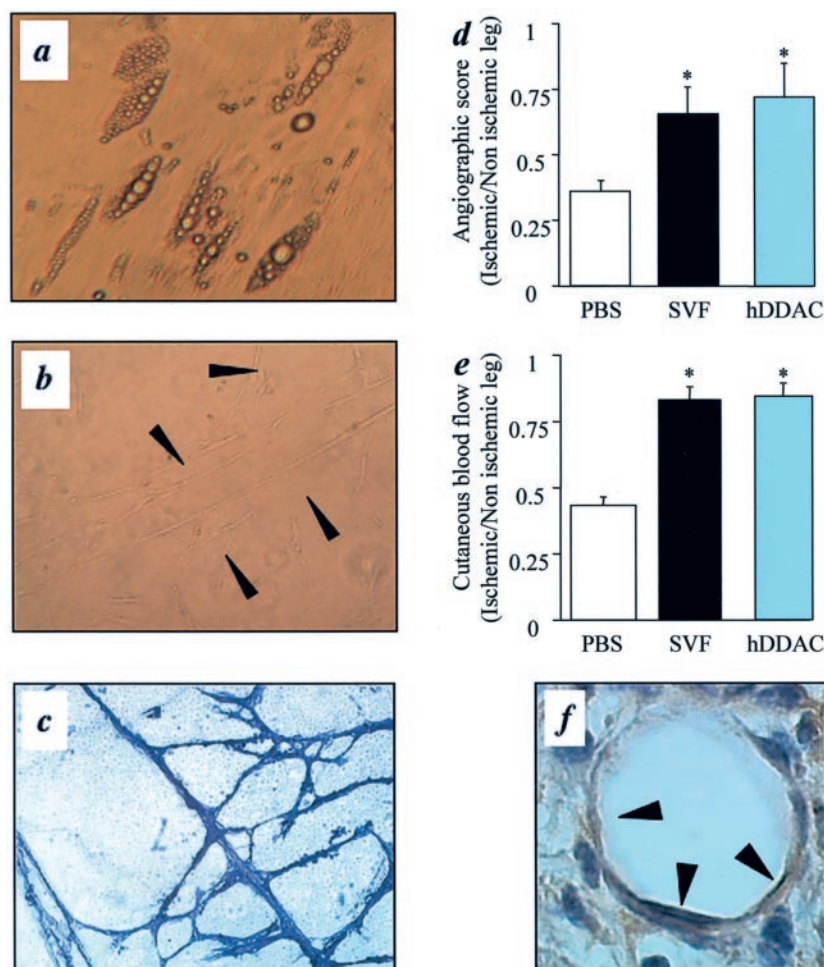


Figure 5. Mature adipocytes reverse to proliferative bipotent progenitors and have the ability to acquire an endothelial phenotype. a, hDDAC can proliferate and differentiate again into adipocytes when cultured in adipogenic medium (magnification, $\times 400$). b, hDDAC formed branched alignments and tubelike structures (arrowheads) when cultured in methylcellulose ($\times 400$). c, vWF antibody stained branched alignment and tubelike structures formed by dedifferentiated mature adipocytes in methylcellulose ($\times 400$). d and e, Proangiogenic properties of hDDAC after implantation in ischemic hindlimbs. hDDAC were as efficient as cultured SVF cells in restoring angiographic score and cutaneous blood flow of ischemic hindlimbs. Values are mean \pm SEM, $n=6$ per group. PBS indicates PBS-treated mice with hindlimb ischemia, $*P<0.05$. f, Antibody directed specifically against human CD31 isoform stained numerous CD31-positive cells (arrowheads), which lined vessels ($\times 1000$).

other characteristic markers of endothelial progenitor cells such as CD144. This could be interpreted as a noncommitted status for adipocyte progenitors or dedifferentiated adipocytes, which is favored by the culture conditions.

Therapeutic enhancement of neovascularization is one of the most important strategies needed to limit the complications of postischemic injury.²⁹ Considerable efforts have focused on the development of therapeutic strategies designed to increase vessel growth in the setting of ischemia. Particularly, transplantation of BM-MNC has been shown to stimulate neovascularization after experimental ischemic injury, resulting in long-term salvage and survival of viable tissue.^{30,31} The use of BM-MNC is now under intense investigation in humans, and the results of early small and uncontrolled studies point to a great potential for such therapy to limit disease progression.^{32–35} In the present work, we showed that the administration of adipose lineage cells can potentially affect revascularization to a similar extent as BM-MNC administration. As with BM-MNC, the proangiogenic properties of adipose lineage cells are consistent with their ability to differentiate into endothelial cells and to secrete growth factors such as VEGF and also their macrophage-like properties.^{5–8,36,37} However, we cannot rule out that part of the SVF-induced vessel growth might be related to the proangiogenic effect of resident microvascular endothelial cells. The fundamen-

tal scarcity of endothelial progenitors, combined with their putative functional impairment in pathological conditions, constitutes a major limitation of an endothelial progenitor-based strategy. Thus, the identification of suitable sources of angiogenic cells represents a challenge for therapeutic angiogenesis of ischemic tissues. The concept that adipose lineage cells might be used or recruited in the context of ischemia to induce neovessel formation is an attractive one, especially because of the ready availability of adipose tissue and of the capacity of these cells to expand *ex vivo*.

In conclusion, our results indicate that adipose lineage cells can function as bipotent progenitors for endothelial cells and adipocytes. This opens new perspectives on adipose tissue development and plasticity and highlights the way for angiogenic therapy based on the administration of adipose tissue-derived stem cells in the treatment of cardiovascular disease.

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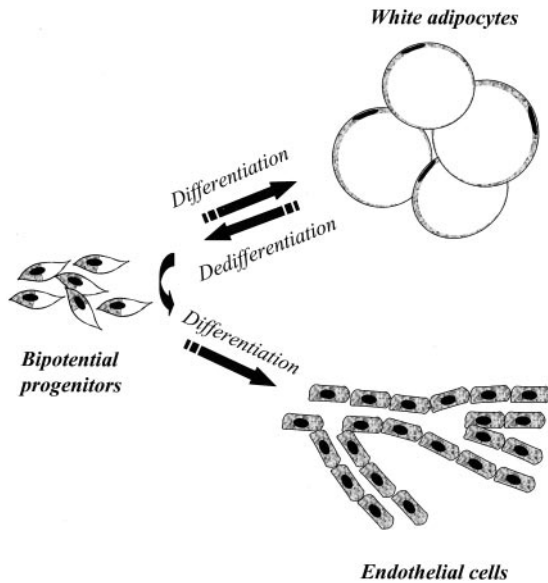


Figure 6. Plasticity of adipose lineage cells toward endothelial cells. Adipocyte progenitors have the ability to differentiate into adipocytes and to acquire a functional endothelial phenotype. Mature adipocytes can dedifferentiate and reverse to a bipotent phenotype. Identification of the pathways that regulate these processes could lead to the development of new therapeutic strategies for control of adipose tissue development and therapeutic angiogenesis.

References

- Ailhaud G, Grimaldi P, Negrel R. Cellular and molecular aspects of adipose tissue development. *Annu Rev Nutr.* 1992;12:207–233.
- Castellot JJ Jr, Karnovsky MJ, Spiegelman BM. Differentiation-dependent stimulation of neovascularization and endothelial cell chemotaxis by 3T3 adipocytes. *Proc Natl Acad Sci U S A.* 1982;79:5597–5601.
- Bouloumie A, Lolmede K, Sengenès C, et al. Angiogenesis in adipose tissue. *Ann Endocrinol (Paris).* 2002;63:91–95.
- Wassermann P. The development of adipose tissue. In: *Handbook of Physiology*. Washington, DC: American Physiological Society; 1965: 87–107.
- Dobson DE, Kambe A, Block E, et al. 1-Butyryl-glycerol: a novel angiogenesis factor secreted by differentiating adipocytes. *Cell.* 1990; 61:223–230.
- Claffey KP, Wilkison WO, Spiegelman BM. Vascular endothelial growth factor: regulation by cell differentiation and activated second messenger pathways. *J Biol Chem.* 1992;267:16317–16322.
- Bouloumie A, Drexler HC, Lafontan M, et al. Leptin, the product of Ob gene, promotes angiogenesis. *Circ Res.* 1998;83:1059–1066.
- Sierra-Honigsmann MR, Nath AK, Murakami C, et al. Biological action of leptin as an angiogenic factor. *Science.* 1998;281: 1683–1686.
- Rupnick MA, Panigrahy D, Zhang CY, et al. Adipose tissue mass can be regulated through the vasculature. *Proc Natl Acad Sci U S A.* 2002;99:10730–10735.
- Hutley LJ, Herington AC, Shurety W, et al. Human adipose tissue endothelial cells promote preadipocyte proliferation. *Am J Physiol Endocrinol Metab.* 2001;281:E1037–E1044.
- Frye CA, Patrick CW Jr. Isolation and culture of rat microvascular endothelial cells. *In Vitro Cell Dev Biol Anim.* 2002;38:208–212.
- Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell.* 2002;13:4279–4295.
- Erickson GR, Gimble JM, Franklin DM, et al. Chondrogenic potential of adipose tissue-derived stromal cells in vitro and in vivo. *Biochem Biophys Res Commun.* 2002;290:763–769.
- Cousin B, André M, Arnaud E, et al. Reconstitution of lethally irradiated mice by cells isolated from adipose tissue. *Biochem Biophys Res Commun.* 2003;301:1016–1022.
- Safford KM, Hicok KC, Safford SD, et al. Neurogenic differentiation of murine and human adipose-derived stromal cells. *Biochem Biophys Res Commun.* 2002;294:371–379.
- Mallat Z, Silvestre JS, Le Ricousse-Roussanne S, et al. Interleukin-18/interleukin-18 binding protein signaling modulates ischemia-induced neovascularization in mice hindlimb. *Circ Res.* 2002;91: 441–448.
- Björntorp P, Karlsson M, Pertoft H, et al. Isolation and characterization of cells from rat adipose tissue developing into adipocytes. *J Lipid Res.* 1978;19:316–324.
- Gehling UM, Ergun S, Schumacher U, et al. In vitro differentiation of endothelial cells from AC133-positive progenitor cells. *Blood.* 2000; 95:3106–3112.
- Nibbelink M, Moulin K, Arnaud E, et al. Brown fat UCP1 is specifically expressed in uterine longitudinal smooth muscle cells. *J Biol Chem.* 2001;276:47291–47295.
- Silvestre JS, Mallat Z, Tamarat R, et al. Regulation of matrix metalloproteinase activity in ischemic tissue by interleukin-10: role in ischemia-induced angiogenesis. *Circ Res.* 2001;89:259–264.
- Tamarat R, Silvestre JS, Durie M, et al. Angiotensin II angiogenic effect in vivo involves vascular endothelial growth factor- and inflammation-related pathways. *Lab Invest.* 2002;82:747–756.
- Negrel R, Grimaldi P, Ailhaud G. Establishment and characterization of fibroblast-like cell lines derived from adipocytes with the capacity to redifferentiate into adipocyte-like cells. *Methods Enzymol.* 1985; 109:377–385.
- Aoki S, Toda S, Sakemi T, et al. Coculture of endothelial cells and mature adipocytes actively promotes immature preadipocyte development in vitro. *Cell Struct Funct.* 2003;28:55–60.
- Shigematsu M, Watanabe H, Sugihara H. Proliferation and differentiation of unilocular fat cells in the bone marrow. *Cell Struct Funct.* 1999;24:89–100.
- Fernandez Pujol B, Lucibello FC, Gehling UM, et al. Endothelial-like cells derived from human CD14 positive monocytes. *Differentiation.* 2000;65:287–300.
- Rehman J, Li J, Orschell CM, et al. Peripheral blood “endothelial progenitor cells” are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation.* 2003;107:1164–1169.
- Jiang Y, Vaessen B, Lenvik T, et al. Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. *Exp Hematol.* 2002;30:896–904.
- Gronthos S, Franklin DM, Leddy HA, et al. Surface protein characterization of human adipose tissue-derived stromal cells. *J Cell Physiol.* 2001;189:54–63.
- Carmeliet P. Angiogenesis in health and disease. *Nat Med.* 2003;9: 653–660.
- Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature.* 2001;401:701–705.
- Kocher AA, Schuster MD, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med.* 2001;7:430–436.
- Assmus B, Schachinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation.* 2002;106:3009–3017.
- Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation.* 2002;106:1913–1918.
- Tateishi-Yuyama E, Matsubara H, Murohara T, et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet.* 2002;360:427–435.
- Tse HF, Kwong YL, Chan JK, et al. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet.* 2003;361:47–49.
- Cousin B, Munoz O, André M, et al. A role for preadipocytes as macrophage-like cells. *FASEB J.* 1999;13:305–312.
- Charriere G, Cousin B, Arnaud E, et al. Preadipocyte conversion to macrophage: evidence of plasticity. *J Biol Chem.* 2003;278: 9850–9855.