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# Secretion of Angiogenic and Antiapoptotic Factors by Human Adipose Stromal Cells

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***Background***—The delivery of autologous cells to increase angiogenesis is emerging as a treatment option for patients with cardiovascular disease but may be limited by the accessibility of sufficient cell numbers. The beneficial effects of delivered cells appear to be related to their pluripotency and ability to secrete growth factors. We examined nonadipocyte stromal cells from human subcutaneous fat as a novel source of therapeutic cells.

***Methods and Results***—Adipose stromal cells (ASCs) were isolated from human subcutaneous adipose tissue and characterized by flow cytometry. ASCs secreted $1203\pm254$ pg of vascular endothelial growth factor (VEGF) per $10^6$ cells, $12\,280\pm2944$ pg of hepatocyte growth factor per $10^6$ cells, and $1247\pm346$ pg of transforming growth factor-$\beta$ per $10^6$ cells. When ASCs were cultured in hypoxic conditions, VEGF secretion increased 5-fold to $5980\pm1066$ pg/$10^6$ cells ($P=0.0016$). The secretion of VEGF could also be augmented 200-fold by transfection of ASCs with a plasmid encoding VEGF ($P<0.05$). Conditioned media obtained from hypoxic ASCs significantly increased endothelial cell growth ($P<0.001$) and reduced endothelial cell apoptosis ($P<0.05$). Nude mice with ischemic hindlimbs demonstrated marked perfusion improvement when treated with human ASCs ($P<0.05$).

***Conclusions***—Our experiments delineate the angiogenic and antiapoptotic potential of easily accessible subcutaneous adipose stromal cells by demonstrating the secretion of multiple potentially synergistic proangiogenic growth factors. These findings suggest that autologous delivery of either native or transduced subcutaneous ASCs, which are regulated by hypoxia, may be a novel therapeutic option to enhance angiogenesis or achieve cardiovascular protection. (***Circulation***. 2004;109:1292-1298.)

**Key Words:** endothelium ■ angiogenesis ■ cells

The delivery of autologous stem cells and progenitor cells into ischemic tissue of patients with atherosclerotic disease to augment angiogenesis and protect cardiac tissue is emerging as a novel therapeutic option.[1–3] Early clinical trials suggest that angiogenesis and cardioprotection induced by cell therapy may become a viable clinical option.[4–7] There is also a growing recognition that a significant proportion of the beneficial effects of cell therapy may be due to the secretion of multiple, possibly complementary angiogenic and antiapoptotic growth factors.[8,9] Animal studies suggest that the beneficial effect of cell therapy on angiogenesis is dose dependent.[10] Most of the clinical trials have used bone marrow cells, which are only available in limited numbers and cannot be easily expanded.

The discovery that nonadipocyte stromal cells in the fat tissue can be easily harvested and expanded in vitro[11] and secrete the angiogenic growth factor hepatocyte growth factor (HGF)[12] suggested the fat tissue as a novel source for cells to be used in cardiovascular cell therapy. We therefore examined the angiogenic potential of adipose stromal cells (ASCs) by investigating their ability to secrete bioactive levels of the angiogenic and antiapoptotic growth factors granulocyte-macrophage colony stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), and transforming growth factor-$\beta$ (TGF-$\beta$) and their ability to serve as autologous cell vectors for cell-based cardiovascular gene therapy.

## Methods

### Isolation and Culture of Human Adipose Stromal Cells

Human subcutaneous adipose tissue samples were obtained from lipoaspiration/liposuction procedures and digested in collagenase

type I solution (Worthington Biochemical) under gentle agitation for 1 hour at 37°C, filtered with 500-$\mu$m and 250-$\mu$m Nitex filters, and centrifuged at 200$g$ for 5 minutes to separate the stromal cell fraction (pellet) from adipocytes. The ASC fraction was treated with red blood cell lysis buffer for 5 minutes at 37°C, then centrifuged at 300$g$ for 5 minutes. The supernatant was discarded, and the cell pellet was resuspended in endothelial growth medium-2 MV (EGM-2-MV, Cambrex), which consists of endothelial basal medium-2 (EBM-2), 5% fetal bovine serum (FBS), and the supplemental growth factors VEGF, bFGF, epidermal growth factor, and insulin-like growth factor.

## Flow Cytometric Characterization of Human Adipose Stromal Cells

ASCs (100 $\mu$L) were labeled for 20 to 30 minutes on ice with manufacturer-recommended concentrations with the mouse anti-human monoclonal fluorescent antibody for CD34, CD45, CD144, or the isotype control (both Becton Dickinson). Cells were subsequently washed with PBS, paraformaldehyde fixed (Tousimis), and analyzed on a FACS-Calibur instrument (Becton-Dickinson).

## Harvest of Conditioned Media From Human Adipose Stromal Cells Cultured in Normoxia and Hypoxia

Human ASCs (hASCs) were cultured and expanded on tissue culture plates in EGM-2-MV medium and used for the experiments at passages 0 through 2. At 90% confluence, ASCs were switched to EBM-2/5% FBS. On the following day, the medium was replaced with fresh EBM-2/5% FBS, and ASCs were placed in either normoxic (21% $O_2$) or hypoxic (1% $O_2$) conditions for 72 hours. At the end of the incubation period, the conditioned media from ASCs were collected, and cell numbers were determined by a hemacytometer.

## Determination of Angiogenic and Antiapoptotic Growth Factors in hASC-Conditioned Media

The 72-hour conditioned media of hASCs were analyzed for the angiogenic or antiapoptotic growth factors GM-CSF, VEGF, HGF, bFGF, and TGF-$\beta$ by Pierce Technology with a Searchlight multiplex ELISA array. Data are expressed as mean$\pm$SEM picograms of the secreted factor per $10^6$ cells at the time of harvest.

## Measurement of the Effect of Hypoxia on Growth Factor mRNA

RNA was isolated from ASCs cultured in normoxic or hypoxic conditions for 24 hours. Total cellular RNA was isolated (RNase Miniprep Kit (Qiagen) followed by a reverse transcription with the Omniscript RT kit (Qiagen). Levels of growth factor mRNA expression were analyzed with the ABI 7700 sequence detection system with the SYBR Green PCR Master Mix. The following oligonucleotides were used as primers: VEGF: 5'-primer (5'-TACCTCCACC-ATGCCAAGTG-3') and 3'-primer (5'-TGATGATTCDTGCCCTC-CTCC-3'); GM-SCF: 5'-primer (5'-CTTGACCATGATGGCCA-GC-3') and 3'-primer (5'-CTGGGTTGCACAGGAAGTTTC-3'); HGF: 5'-primer (5'-GATGGCCAGCCGAGGC-3') and 3'-primer (5'-TCAGCCCATGTTTTAATTGCA-3'); bFGF: 5'-primer (5'-GACGGCAGAGTTGACGG-3') and 3'-primer (5'-CTCTCTCTT-CTGCTTGAAGTTGTAGC-3'); TGF-$\beta$: 5'-primer (5'-GCTGAGC-GCTTTTCTGATCCT-3') and 3'-primer (5'-CGAGTGTGCTGC-AGGTAGACA-3'); and $\beta$-actin: 5'-primer (5'-CACCCTGAAGTA-CCCCATCG-3') and 3'-primer (5'-TGCCAGATTTTCTCCATG-TCG-3').

Polymerase chain reactions (PCRs) were performed in a final volume of 25-$\mu$L reaction mix that contained 10 ng of cDNA product, 200 nmol/L of each probe. Thermal cycling parameters were 10 minutes at 95°C for AmpliTaq Gold activation followed by 40 cycles of 15 seconds at 95°C for denaturation and 1 minute at 60°C for anneal/extend. The $\beta$-actin mRNA level was used for sample standardization.

## Evaluation of Bioactivity of hASC Conditioned Media

Human microvascular endothelial cells (Cambrex, Baltimore, Md) at passages 7 or less were seeded at a density of 10 000 or 20 000 cells per well in a 12-well plate and then switched for 24 hours to EBM-2/5% FBS medium, which limits cell growth, because of the absence of supplemental growth factors. On the following day, the EBM-2/5% FBS medium was then replaced with either fresh EBM-2/5% FBS medium or 50% fresh EBM-2/5% FBS medium and 50% conditioned EBM-2/5% FBS medium that had been harvested previously from normoxic and hypoxic ASCs. The endothelial cell numbers on day 4 were determined per hemacytometer, and the data are expressed as mean$\pm$SEM percent of seeded cells.

To assess the effects of ASC-conditioned medium on endothelial cell apoptosis, human aortic endothelial cells at passages 7 or less (Cambrex, Baltimore, Md) were grown in 8-well chamber slides (Nalgene Nunc International, Laboratory Tek brand product) for 3 days and then incubated for 24 hours with 3 $\mu$g/mL cycloheximide (Sigma) and 10 ng/mL tumor necrosis factor-$\alpha$ (Sigma) in the presence or absence of conditioned media obtained from ASCs cultured in normoxic or hypoxic conditions. The cells were then fixed with 1% paraformaldehyde in PBS for 10 minutes at room temperature, postfixed in ethanol acetic acid 2:1 for 5 minutes at −20°C, and stained with the ApopTag Plus fluorescein in situ apoptosis detection kit (Chemicon International). After the fluorescein staining, cells were mounted under a glass coverslip with a mounting medium that contained 0.5 $\mu$g/mL DAPI. A fluorescence microscope was used to count the apoptotic cells. Data are expressed as mean$\pm$SEM percentage of apoptotic cells.

## Animal Studies

Animals were cared for in accordance with guidelines published by National Institutes of Health, and all study procedures were approved by the Indiana University Institutional Animal Care and Use Committee.

## Delivery of hASCs Transfected With Green Fluorescent Protein Into NOD/SCID Mice

hASCs at passage 1 were infected with Ad5-GFP (green fluorescent protein) virus ($10^7$ viral particles/mL medium) in EBM-2/5% FBS overnight and cultured for an additional 2 days before injection. The transfection efficiency of ASCs for Ad5-GFP was >95%, as assessed by flow cytometry. Cells were injected locally into the tibialis anterior muscles of NOD/SCID mice.[13] Animals were killed at different time points, and the treated muscles were harvested and cryopreserved in OCT media. Frozen tissue sections were used for histological evaluation of cell distribution.

## Measuring Survival of Cells In Vivo

ASCs were labeled by addition of 30 $\mu$g/mL DAPI in EBM-2/5% FBS,[14] followed by washing with medium only. The tibialis anterior muscles of NOD/SCID mice (n=3) were injected with $10^5$ DAPI-labeled cells (20 $\mu$L). One week after injection, tissues were harvested and frozen in OCT compound for subsequent cryosectioning. The entire muscle was sectioned serially at 5-$\mu$m thickness, mounted on slides, and then microscopically visualized with epifluorescence. The number of identifiable DAPI-positive cells in every tenth section was determined. The total number of cells present in the each muscle was extrapolated, and the percentage of surviving cells was calculated.

## Mouse Hindlimb Ischemia Model

Unilateral hindlimb ischemia was created in 8-week-old nude mice as described previously.[15,16] The animals were anesthetized with 2.5% tribromoethanol, after which an incision was made at the midline of the left hindlimb. The femoral artery and its branches were ligated, beginning at the inguinal ligament to the bifurcation of saphenous and popliteal arteries, followed by excision of the region between the ligatures.

One day after creation of unilateral hindlimb ischemia, either $5\times10^5$ ASCs suspended in 200 $\mu$L of EBM-2/5%FBS or just 200 $\mu$L
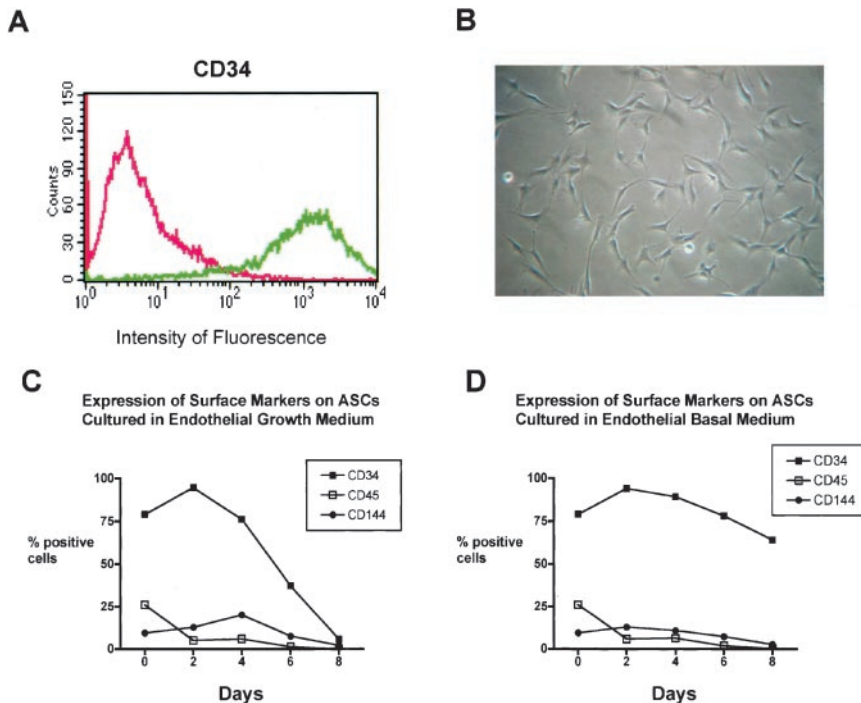
**Figure 1.** A, Flow cytometry histogram of freshly isolated ASCs showing anti-CD34 binding (green line) and comparing it to isotype control antibody (pink). Phase contrast micrographs of plated ASCs demonstrate that cells are adherent (B). Measurement of expression of stem/progenitor cell marker CD34, leukocyte marker CD45, and endothelial marker CD144 (VE-cadherin) on cultured ASCs cultured in EGM-2MV (C) and EBM-2/5% FBS medium (D) over time.

of the medium were injected through the tail vein. Three groups of mice were treated. One group received cells plated for 1 to 2 days; the second group received EGM-2MV–cultured passage 1 cells; and the last group received medium only.

Blood flow restoration in ischemic limbs after treatment was evaluated by laser doppler imaging (Moor Instruments) as described previously[16] with minor modifications. Briefly, animals were anesthetized by isoflurane inhalation and placed on a heating pad set at 37°C. Data were collected from the plantar surfaces of both limbs. To account for variability between measurements, such as ambient light and temperature, the results are expressed as the ratio of perfusion in ischemic (left) and intact (right) limbs. Measurements were performed on days 1, 5, and 10.

### Statistical Analysis

Data are expressed as mean±SEM when possible. Comparisons between hypoxia and normoxia were performed with a paired *t* test. Comparisons of multiple groups were done with ANOVA with corrections for multiple comparisons. In vivo changes in perfusion by laser Doppler imaging were analyzed by between-group repeated-measures ANOVA. All analyses were performed with Prism 4 and Instat Software (both by Graphpad).

## Results

### Characterization of hASCs

Freshly isolated hASCs expressed the stem/progenitor marker CD34 (Figure 1A). hASCs were adherent (Figure 1B) and showed significant expansion in culture (data not shown). The average number of ASCs isolated was 73 200±24 700 cells (n=7) per gram of lipoaspiration fat. The mean size of ASCs was 12.2±0.4 $\mu$m after they were plated for 1 to 2 days and 14.6±0.3 $\mu$m after they were cultured and passaged. Flow cytometric characterization of hASCs in culture revealed that the percentage of cells positive for CD34 expression decreased markedly after 1 week of culture for cells expanded in growth factor–rich EGM-2MV medium (Figure 1C) and only minimally for cells grown in EBM-2 medium (Figure 1D). Although there was some presence of CD45-

positive leukocytes at the time of isolation, these cells were nearly undetectable after 1 week of culture (Figures 1C and 1D). Endothelial cells, as characterized by the expression of VE-cadherin (CD144) were present at the time of isolation (5% to 15% positive cells in various preparations). These endothelial cells were likely to be microvascular endothelial cells contained in the adipose tissue.

### Regulation of Growth Factor Secretion by Hypoxia

Over a 72-hour period in basal medium with 5% FBS but without supplemental growth factors under normoxic conditions, hASCs secreted significant amounts of VEGF (1203±254 pg/$10^6$ cells), HGF (12 280±2944 pg/$10^6$ cells), and TGF-$\beta$ (1247±346 pg/$10^6$ cells) and only minimal amounts of GM-CSF (84±15 pg/$10^6$ cells) or bFGF (124±13 pg/$10^6$ cells). When hASCs were cultured in hypoxic conditions, there was a consistent and prominent augmentation observed in the secretion of VEGF (Figure 2), which increased nearly 5-fold from 1203±254 to 5980±1066 pg/$10^6$ cells ($P=0.0016$, paired *t* test, n=7).

The enhancement of protein expression by hypoxia was mirrored by commensurate increases in mRNA (Figure 3). Quantitative reverse transcription-PCR analysis of total RNA indicated that the increase in VEGF mRNA was the most significant (7.4-fold; $P<0.01$ versus HGF, GM-CSF, and TGF-$\beta$ and $P<0.05$ versus bFGF; n=5).

### Effects of hASC Supernatants on Endothelial Cell Proliferation and Endothelial Cell Survival

Because the proliferation and survival of endothelial cells is an important aspect of angiogenesis,[17] we measured the effects of ASC-conditioned media on human endothelial cell growth and survival (Figures 4 and 5). The number of endothelial cells cultured for 4 days in ASC-conditioned basal medium was significantly higher than that of endothe-

## Secretion of Growth Factors by ASCs over 72 hours
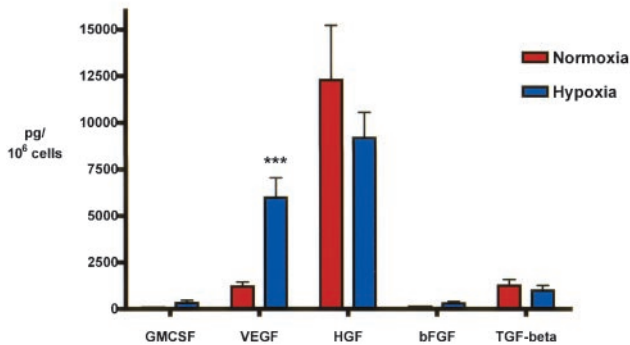


**Figure 2.** Secretion of GM-CSF, VEGF, HGF, bFGF and TGF-$\beta$ by ASCs cultured in normoxic (red) or hypoxic (blue) conditions over 72 hours was measured by ELISA and is presented as mean±SEM picograms of secreted factor normalized to $10^6$ cells at time of harvest. Growth factor production in normoxia and hypoxia was compared with paired *t* test (***$P<0.005$).

lial cells in unconditioned basal medium (115±11% versus 32±4% of seeded endothelial cells; $P<0.001$). Conditioned media from hypoxic ASCs resulted in even higher endothelial cell numbers (162±14% of seeded endothelial cells; $P<0.05$ versus endothelial cells in media from normoxic ASCs). Endothelial cells cultured in media from hypoxic ASCs showed significantly lower apoptosis rates ($P<0.05$ versus endothelial cells in basal media), whereas normoxic ASC media showed a trend toward reduced apoptosis of human endothelial cells without reaching statistical significance.

### Delivery and Survival of hASCs In Vivo

To assess whether ASCs could survive delivery into tissue, hASCs were transduced with a GFP-expressing adenovirus for tracking purposes and injected into mouse hindlimbs of

## Hypoxia Selectively Increases VEGF Expression
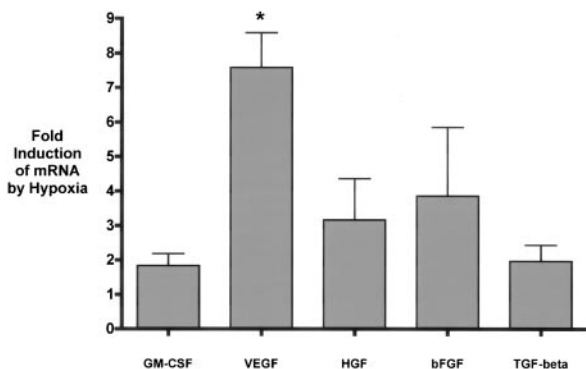


**Figure 3.** Amount of growth factor mRNA in ASCs after 24 hours of normoxia or hypoxia was measured with quantitative reverse transcription–PCR and normalized to $\beta$-actin. Hypoxia:normoxia ratio of normalized growth factor RNA is shown as mean±SEM and represents degree of induction (x-fold induction) of each growth factor by hypoxia. Induction for VEGF was most significant (*$P<0.01$ for VEGF vs HGF, TGF-$\beta$, and GM-CSF, and $P<0.05$ for VEGF vs bFGF; statistical comparisons were performed with ANOVA with correction for multiple comparisons).
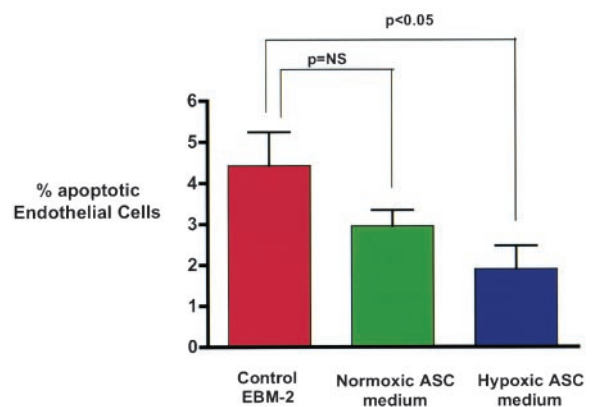
## ASC Conditioned Medium Enhances Endothelial Cell Growth
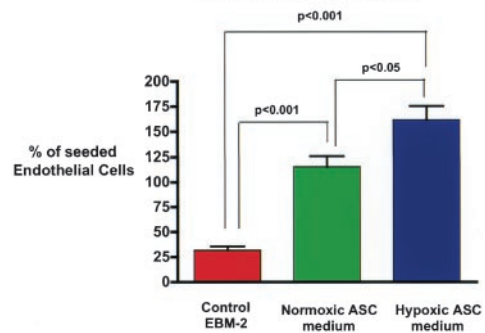


**Figure 4.** Effects of conditioned medium on endothelial cell growth are shown as percentage of cells on Day 4 versus initial cell number on Day 0 (mean±SEM). Numbers of endothelial cells exposed to EBM-2/5% FBS medium only (red) are much lower than those exposed to half EBM-2/5% FBS and half normoxic ASC-conditioned medium (green) or half EBM-2/5% FBS and half hypoxic ASC-conditioned medium (blue). Statistical comparisons were performed with ANOVA with correction for multiple comparisons.

NOD/SCID mice. Nearly all ASCs were positive for GFP after transduction (Figure 6A). One day after injection into the tibialis anterior muscle, hASCs were typically present in the form of clusters at the injection sites (Figure 6B). One week after the injection, GFP-expressing ASCs had migrated between the myotubes of the muscle and exhibited viability as evidenced by the expression of GFP (Figure 6C). To determine an approximate rate of survival, DAPI-labeled cells were injected into the muscle. One week after injection, 28±2% (n=3) of injected cells could be identified on serial sections of the muscle.

## Hypoxic ASC Medium Enhances Endothelial Cell Survival



**Figure 5.** Effects of conditioned medium on endothelial cell survival are shown as mean±SEM percentage of apoptotic endothelial cells (of total adherent endothelial cells) after 24 hours of apoptosis induction. Percentage of apoptotic endothelial cells only exposed to EBM-2/5% FBS medium (red) is higher than percentage of those exposed to half EBM-2/5% FBS and half normoxic ASC-conditioned medium (green) or half EBM-2/5% FBS and half hypoxic ASC-conditioned medium (blue). Statistical comparisons were performed with ANOVA with correction for multiple comparisons.
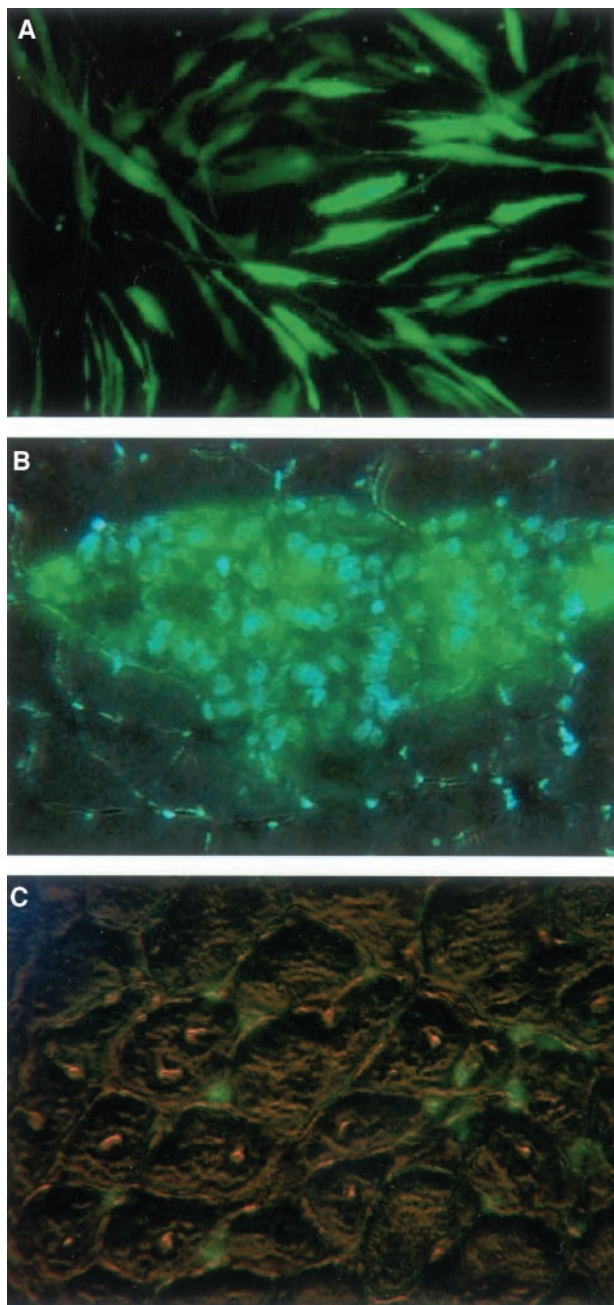
**Figure 6.** A, GFP-expressing cultured ASCs at 1 day after transduction with Ad5-GFP vector (10× magnification). B, GFP-expressing ASCs were injected into mouse tibialis anterior (TA) muscle and visualized microscopically (40× magnification) 1 day after injection. DAPI staining (blue fluorescing nuclei) was performed to improve cell detection. C, Engrafted GFP-expressing ASCs in the TA muscle were visualized at 1 week after injection (40× magnification).

## In Vivo Proangiogenic Effects of hASCs

After unilateral ligation of hindlimbs in nude mice, the mice were treated with either control media or $5 \times 10^5$ hASCs. Representative images of control-treated and ASC-treated hindlimbs are shown in Figures 7A and 7B, suggesting marked improvement of perfusion in ASC-treated mice. Quantitative analysis performed by laser Doppler imaging confirmed that freshly plated ASCs (Figure 7C) and cultured

passage 1 ASCs (Figure 7D) were both able to markedly enhance perfusion in the ischemic hindlimb compared with control-injected mice ($P<0.05$ by repeated-measures ANOVA). The significant improvement was already observed on day 5 after treatment and was sustained until day 10, when the experiment was terminated because of the developing severe hindlimb necrosis in the control-treated animals.

## Discussion

This study identifies the stromal cell fraction in human subcutaneous fat as a novel source for autologous cell therapy in cardiovascular disease. ASCs secrete multiple angiogenic and antiapoptotic growth factors at levels that are bioactive. Because the proliferation of endothelial cells and the inhibition of their apoptosis is critical for the growth of new blood vessels,[17] the secretion of these growth factors by ASCs delivered to ischemic tissue is likely to promote angiogenesis. The experiments in our study using the ischemic hindlimb model confirm that ASCs have a marked proangiogenic potential in vivo.

The main benefit of using ASCs is that they can be easily harvested from patients in a simple, minimally invasive lipoaspiration procedure and then be readily available for autologous cell therapy. Our experience with lipoaspiration indicates that it typically yields between 50 and $400 \times 10^6$ cells, which can then be further expanded at least 10-fold within 1 week. Because cells plated for 1 to 2 days appear to have a proangiogenic potency in vivo similar to that of long-term cultured ASCs, both options could be used in the clinical setting, depending on the time constraints of the clinical situation.

### Adaptive Growth Factor–Secreting Cells

The administration of single angiogenic growth factors to augment neovascularization in patients with atherosclerotic disease has shown only modest success.[18,19] One possible reason for this may be that complex processes such as angiogenesis and collateral growth are likely to require multiple growth factors acting in concert.[20] It is in this context that the secretion of angiogenic and antiapoptotic growth factors such as VEGF and HGF, which are known to act in a synergistic manner,[21] by ASCs makes them a valuable tool for angiogenesis therapy. The additional ability of ASCs to respond to a stimulus such as hypoxia could allow them to adapt to the environment into which they are placed (ie, after delivery to the ischemic myocardium of patients), by modulating the production of VEGF in response to ischemia when the nascent vasculature is forming. Hypoxic regulation of ASC growth factor secretion may also be important in regulating angiogenesis in the context of adipogenesis.[22]

The transfectability of ASCs shown in the present study also allows supplementation of native growth factor secretion by markedly increasing the amount of secreted factors such as VEGF (see supplemental data) or the addition of factors that are not part of the baseline secretion profile. A previous study[23] using processed lipoaspirated cells, which are isolated in a fashion parallel to that for ASCs, demonstrated that such
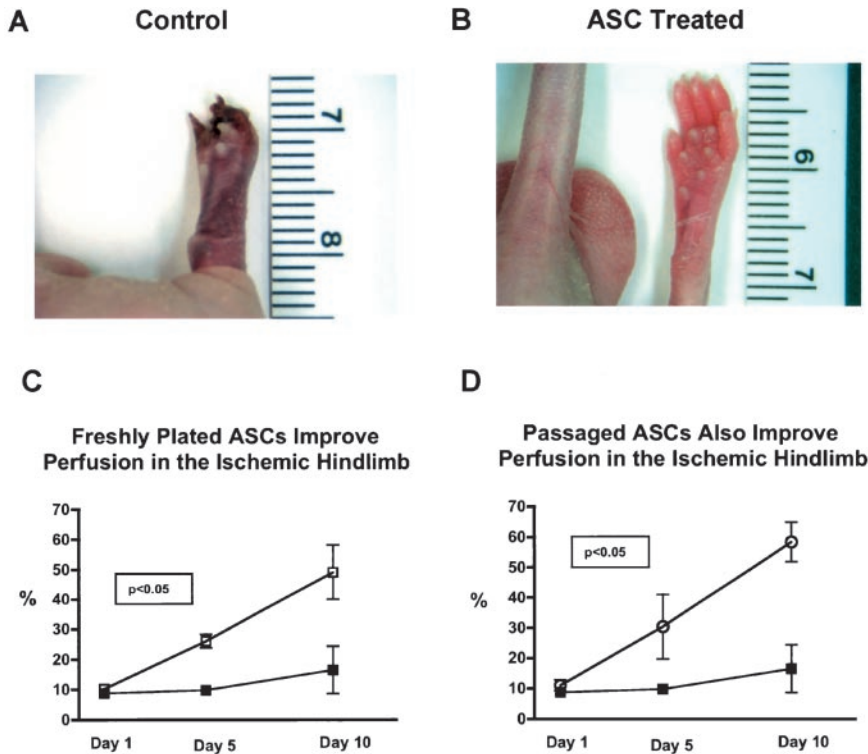
**Figure 7.** Hindlimb ischemia was induced by femoral artery ligation in nude mice, and animals were treated with either control medium, $5 \times 10^5$ ASCs plated for 1 to 2 days, or $5 \times 10^5$ cultured and passaged ASCs. Representative photos of control medium (A) or ASC-treated (B) ischemic hindlimbs on day 5 are shown. Quantitative analysis with laser Doppler imaging was performed, and data are presented as percentage of perfusion in ischemic leg compared with nonischemic leg over time on days 1, 5, and 10. Injection with either ASCs plated for 1 to 2 days (C) or long-term cultured ASCs was compared with injection with control medium. Analysis was performed with between-group repeated-measures ANOVA. Both ASC types were significantly more effective than control medium in restoring blood flow to ischemic hindlimb.

cells could be marked with lentiviral vectors but did not show secretion of an angiogenic growth factor such as VEGF.

One key advantage of using cell-based gene therapy is that the cells encoding for the gene may retain their ability to migrate and respond to environmental cues after cell delivery. The large numbers of autologous ASCs that can be easily harvested and transfected could therefore allow for feasible implementation of cell-based gene therapy.

## ASC Pluripotency as a Complement to Growth Factor Secretion

In addition to growth factor secretion ("manager" function), cells that may provide therapeutic effects to vascular structures could do so via vascular differentiation and subsequent vascular incorporation ("building block" function). ASCs represent the nonadipocyte cell fraction of adipose tissue and consist of a heterogeneous cell population that includes preadipocytes and vascular cells.[24] A number of studies have shown that the ASC fraction contains pluripotent cells that can differentiate along multiple cell lineages, including myogenic, osteogenic, neurogenic, and hematopoietic pathways.[25–28] The present data confirmed previous findings that ASCs express the surface marker CD34,[25,26,29] which is found on hematopoietic stem and progenitor cells as well as some endothelial cell populations. Whether the expression of CD34 is related to their pluripotency and which subfraction of ASCs contains the pluripotent cells still need to be determined. The present findings regarding the therapeutic potential of ASCs are independent of their pluripotency and degree of vascular incorporation, but it is tempting to speculate that their angiogenic effects may be further augmented if they also differentiate into vascular cells.

In summary, hASCs are a novel source of cells for autologous cell therapy in the setting of cardiovascular disease. In addition to the secretion of growth factors by ASCs, future studies should also explore whether pluripotent cells within the ASC fraction can be directed toward differentiation into cardiovascular cells and thus complement their ability to secrete growth factors. Future studies could also address the mechanisms by which ASCs home to the sites of angiogenesis and compare their efficacy with other sources of autologous cells, such as peripheral blood or bone marrow. Finally, the therapeutic potential of native cells or transduced cells to achieve neovascularization and cardiovascular protection will need to be tested in controlled clinical trials.

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