Implantation of Adipose-Derived Regenerative Cells Enhances Ischemia-Induced Angiogenesis

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Objective—Therapeutic angiogenesis using autologous stem/progenitor cells represents a novel strategy for severe ischemic diseases. Recent reports indicated that adipose tissues could supply adipose-derived regenerative cells (ADRCs). Accordingly, we examined whether implantation of ADRCs would augment ischemia-induced angiogenesis.

Method and Results—Adipose tissue was obtained from C57BL/6J mice, and ADRCs were isolated using standard methods. ADRCs expressed stromal cell–derived factor 1 (SDF-1) mRNA and proteins. Hind limb ischemia was induced and culture-expanded ADRCs, PBS, or mature adipocytes (MAs) as control cells were injected into the ischemic muscles. At 3 weeks, the ADRC group had a greater laser Doppler blood perfusion index and a higher capillary density compared to the controls. Implantation of ADRCs increased circulating endothelial progenitor cells (EPCs). SDF-1 mRNA abundance at ischemic tissues and serum SDF-1 levels were greater in the ADRC group than in the control group. Finally, intraperitoneal injection of an anti–SDF-1 neutralizing antibody reduced the number of circulating EPCs and therapeutic efficacies of ADRCs.

Conclusions—Adipose tissue would be a valuable source for cell-based therapeutic angiogenesis. Moreover, chemokine SDF-1 may play a pivotal role in the ADRCs-mediated angiogenesis at least in part by facilitating mobilization of EPCs.

Key Words: angiogenesis ■ adipose-derived regenerative cells ■ progenitor cells ■ chemokine

When tissue is exposed to severe ischemia, new blood vessels develop into the ischemic foci to prevent tissue necrosis. Because circulating endothelial progenitor cells (EPCs) have been shown to participate in postnatal neovascularization after mobilization from the bone marrow (BM),1,2 we have performed basic and clinical studies related to therapeutic angiogenesis using EPCs or BM cells.3–5 We have performed therapeutic angiogenesis using autologous BM mononuclear cell (BM-MNCs) implantation into the ischemic muscles in patients with critical limb ischemia (TACT).6–8 Although the safety and efficiency of the TACT protocol have been established, we recently reported that patients with very severe peripheral artery occlusive disease had poor responses to the TACT procedure.7 Moreover, recent data indicated that patients with severe obstructive vascular disease or multiple coronary risk factors had diminished functions of EPCs and poor responses to angiogenic cell therapy.9–12 Thus, alternative source of stem/progenitor cells for therapeutic angiogenesis has been searched extensively.

Recently, several investigators have reported that adipose tissues contain multipotent mesenchymal cells termed adipose-derived regenerative cells (ADRCs), which have an ability to regenerate damaged tissues.13–15 However, little is known as to how implantation of ADRCs would induce angiogenesis in ischemic tissues. It has been known that ADRCs secrete multiple angiogenic growth factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF).13,14 Such growth factors would mobilize EPCs from the BM into peripheral blood (PB) and finally to ischemic tissues. However, there is limited evidence regarding the effects of in vivo implantation of ADRCs on EPC kinetics during ischemia-induced angiogenesis.

Accordingly, we examined whether implantation of ADRCs might augment angiogenesis, collateral vessel formation, and mobilization of EPCs in a mouse model of hind limb ischemia.

Methods

Isolation of Mouse ADRCs

All protocols were approved by the Institutional Animal Care and Use Committee of Nagoya University School of Medicine. ADRCs were isolated from inguinal fat pads of C57BL/6j mice (n=32) and of GFP-transgenic mice with C57BL/6j background (n=3; kindly provided by Dr M. Okabe at Osaka University) as described...
Adipocyte Differentiation Assay
Adipogenic differentiation of ADRCs was introduced as previously described (please see supplemental materials).15 Adipogenic differentiation was confirmed by Oil Red O staining. To examine whether ADRCs can give rise to EPCs or mature endothelial cells (ECs), ADRCs were cultured in EBM-2 (endothelial cell basal medium; Clonetix) supplemented with EGM-2 MV. At day 7, attaching cells were stained with incorporation of 1,1′-diiododecyl-1 to 3,3′,3′-tetramethylindolo-carboxyanine perchlorate-labeled acetylated LDL (Di-ac-LDL, Biomedical Technology Inc) and binding of fluorescein isothiocyanate (FITC)-labeled Bandeiraea simplicifolia lectin 1 (FITC-BS-1 lectine, Vector Laboratories), and antiplatelet endothelial cell adhesion molecule-1 (PECAM-1 or CD31) monoclonal antibody (mAb) (Becton Dickinson) and anti α-smooth muscle actin (α-SMA) mAb (Abcam).16 After immunofluorescence staining, nuclei were stained with DAPI (Invitrogen).

Characterization of ADRCs by Flow Cytometry
A total of $5 \times 10^5$ cells were incubated for 30 minutes at 4°C with mAbs against Ly-6A/E (Scal), CD31, CD34, c-kit, flk-1, and Lin (BD Biosciences). To characterize the phenotypes of ADRCs, fluorescence-activated-cell sorter (FACS) analysis was performed using the FACS Caliber instrument (Becton Dickinson) and Cell Quest software (BD Biosciences).

Real-Time Reverse Transcribe-Polymerase Chain Reaction Analysis
Total RNA was isolated from cultured ADRCs and differentiated MAs using TRZol Reagent (Invitrogen Life Technologies). Total RNA from the frozen tissues with liquid nitrogen (LN2) obtained at days 0, 3, and 7 after hind limb ischemia was extracted using FastPrep System (BIO 101). Real-time RT-PCR analysis of the VEGF, SDF-1, and GAPDH mRNAs was performed using 2 μg total RNA on Mx3000P Real-Time PCR System (Stratagene) using manufacturer’s instruction (Applied Biosystem).17 mRNA levels were expressed relative to the levels of GAPDH. Further information is described in the supplemental materials.

ELISA
Conditioned medium were obtained from cultured ADRCs and MAs after 72 hours at final concentration of fresh DMEM/10%FBS. Concentrations of SDF-1α and VEGF proteins in the media were determined by ELISA (mouse CXCL12/SDF-1α Quantikine ELISA kit and mouse VEGF ELISA kit, R&D Systems) according to manufacturer’s instruction. Plasma levels of SDF-1α and VEGF at days 0, 3, and 7 after hind limb ischemia were also measured.

Mouse Model of Unilateral Hind Limb Ischemia
Unilateral hind limb ischemia was induced in male C57BL/6j mice (6 to 10 weeks old, n=32; Nihon Crea) as described previously.20 No mice died during the experimentation. Mice were randomly divided into 2 groups. The control group (n=8) received phosphate-buffered saline (PBS). The ADRC group (n=8) received ADRCs ($1 \times 10^5$ cells per animal), and the MA group (n=6) received mature adipocytes (MA) ($1 \times 10^6$ cells per animal) implanted at 3 predetermined points of the ischemic muscles at postoperative day 1. After treatment, angiogenesis and collateral vessel formation in the ischemic tissues were analyzed by microscopic observation of intracellular lipid droplets and positive staining with Oil Red O (supplemental Figure IAd). Immunocytochemistry revealed that these adherent cells were positive for α-SMA (supplemental Figure IAc), but cells cultured in endothelial differentiation medium (EGM2-MV) were negative for CD31 (supplemental Figure IAf), Di-ac-LDL incorporation and FITC-BS1 lectin binding (supplemental Figure IAg), indicating that EGM2 endothelial culture condition could not lead ADRCs differentiate into endothelial lineage. Moreover, FACS analysis of culture-expanded ADRCs were positive for Sca-1 but not for CD31, CD34, c-kit, Lin, and flk-1, markers of differentiated cells (supplemental Figure IAh).

Angiogenic Cytokine Production by ADRCs
We next examined the expression of VEGF and SDF-1 mRNAs in cultured ADRCs and MAs by real-time RT-PCR. Abundance of VEGF mRNA of ADRCs was not significantly different from that of MAs. However, abundance of SDF-1 mRNA of ADRCs was significantly greater (2.7-fold, n=4,
Augmentation of Ischemia-Induced Neovascularization by ADRC Implantation

We examined whether in vivo implantation of ADRCs could augment ischemia-induced angiogenesis using a mouse model of hind limb ischemia. Representative images of laser Doppler blood perfusion image (LDPI) system are shown in supplemental Figure IIA. A greater degree of blood perfusion was observed in the ischemic limb at postoperative day 3 in the ADRCs-implanted mice compared to control or MA-implanted mice. Figure 2A shows summarized data of the ischemic/model of hind limb ratio. Although a marked recovery of blood perfusion was observed in the ADRCs-implanted group, the LDPI ratio remained low in the other 2 groups (*P<0.05, †P<0.01 versus control). Interestingly, blood flow recovery in mice of the MA-implanted group showed a weaker recovery of LDPI ratio compared to saline-injected control animals.

We also measured the capillary density in histological sections harvested from the ischemic tissues. Representative photomicrographs are shown in supplemental Figure IIB. Quantitative analysis revealed that the capillary density at the ischemic skeletal muscles was significantly greater in the ADRCs group compared to the other 2 groups (n=5 for each group, *P<0.05, **P<0.01 versus control; Figure 2B). Again capillary density in the MA-implanted group was lower as compared to the control group (Figure 2B), consistent with the data of LDPI ratio.

Effects of ADRCs Implantation on Circulating and BM EPCs in the Hind Limb Ischemia Model

To assess EPC kinetics, we performed culture assay of PB and BM-MNCs by double staining for DiI-acLDL incorporation and BS-1 lectin binding. Representative images of cultured EPCs are shown in supplemental Figure IIIA. A greater number of EPCs in the BM was observed at postoperative day 3 in the ADRCs group compared to the control group (2.8-fold increase in the ADRCs group versus 1.7-fold increase in the control group, n=6 for each group, P<0.05; Figure 3A left). In the PB, the number of EPCs increased at days 3 and 7 (1.6-fold increase at day 3 in the ADRCs group versus 1.2-fold increase at day 3 in the control group, P<0.01; and 2.7-fold increase at day 7 in the ADRCs group versus 1.6-fold increase at day 7 in the control group, n=6 to 8 for each group, P<0.05; Figure 3A right). These findings were corroborated with the data of FACS analysis of BM and PB samples collected at same time points, indicating that the number of Sca-1+/Flk-1+ cells was consistently greater in the ADRCs-implanted mice than in the PBS treated animals (Figure 3B). Representative images of FACS analysis are shown in supplemental Figure IIIB.
Implanted ADRCs Augmented Secretion of Angiogenic Cytokines From Ischemic Tissues

We investigated whether ADRC implantation upregulates SDF-1 and VEGF mRNA expression in ischemic hind limb muscles. At postoperative day 3, SDF-1 mRNA abundance increased significantly (2.8-fold, n/H11005 7 to 8 for each group, P/H11021 0.05) in the ADRCs group compared to the control group (Figure 4A left). At days 3 and 7, VEGF mRNA abundance also increased significantly (1.6-fold, n/H11005 7 to 8 for each group, P/H11021 0.01) in the ADRCs group compared to the control group (Figure 4A right). Plasma SDF-1 (n/H11005 4 to 5 for each group, P/H11021 0.01) and VEGF (n/H11005 4 to 5 for each group, P/H11021 0.01) protein levels were also increased at day 3 in the ADRCs group compared to the control group (Figure 4B).

Furthermore, to confirm whether implanted ADRCs secrete SDF-1 or VEGF proteins in the ischemic tissues, frozen sections from ischemic tissues of mice that received GFP-transgenic mice–derived ADRCs were stained with anti-SDF-1 or anti-VEGF mAbs. Some of these cells were positive for SDF-1 (supplemental Figure IVA) in the ischemic tissues at day 3. Although the most of these cells were negative for VEGF, VEGF was detected in the cytoplasm of skeletal myofibers nearby GFP positive cells in the ischemic muscles (supplemental Figure IVB). The number of cells stained positive for either SDF-1 or VEGF were significantly lower in the control group compared to the ADRCs group (supplemental Figure IVA and IVB).

Location of Implanted ADRCs at Chronic Phase

We examined whether in vivo implanted ADRCs could survive and differentiate into ECs at chronic phase using mice receiving GFP-transgenic mice-derived ADRCs. GFP positive cells were found in the ischemic area at post operative day 28 (supplemental Figure VA) and some of these cells seemed to be incorporated into Rhodamine-BS-1 lectin positive capillaries. (supplemental Figure VB and VC). Immunofluorescence staining revealed that some of the GFP-positive cells resided nearby vascular structures and capillaries stained with CD31 (supplemental Figure VD), and some of these cells were stained with anti-CD31 (supplemental Figure VE). In addition, these cells were positive for CD 140b, a pericyte maker (supplemental Figure VF). These results indicated that implanted ADRCs might contribute at least in part to vascular formation as pericytes in chronic phase. Furthermore, we could not detect formation of any tumors in transplanted animals until at least day 60 (n/H11005 3, data not shown).

SDF-1α Is Required for ADRCs Implantation-Induced Neovascularization

Intraperitoneal injection of an anti–SDF-1 neutralizing mAb significantly suppressed the angiogenesis mediated by ADRC implantation to the level equal to animals without ADRCs. Representative images of LDPI are shown in supplemental Figure VA. The LDPI ratio revealed that the blood flow recovery was significantly suppressed with the anti–SDF-1 mAb treatment (n/H11005 4 to 5, *P<0.05, †P<0.01 vs control; Figure 5A). Furthermore, the anti-SDF-1 mAb significantly reduced numbers of circulating EPCs at postoperative day 7 as assessed by culture assay and FACS analysis (n/H11005 5 to 7 for each group, *P<0.05, †P<0.01 versus control; Figure 5B).
Discussion

Major findings in the present study are as follows: (1) Cultured ADRCs expressed mesenchymal markers but not endothelial lineage markers in vitro. (2) ADRCs could differentiate into mature adipocytes (MAs), but these cells gave rise to neither EPCs nor mature ECs in vitro. (3) Direct local implantation of ADRCs but not MAs into ischemic hind limb muscles significantly augmented neovascularization. The angiogenic actions of ADRCs was markedly suppressed by an anti–SDF-1 neutralizing mAb treatment. Finally, (4) ADRC implantation increased SDF-1 release from ischemic tissues, which mobilized EPCs in vivo.

Adipose tissue mainly comprises 2 classes of cell population: one is mature adipocytes (MAs), and the other is stromal cells called “stromal vascular fraction” (SVF). Studies have shown that SVF contains multi-potent mesenchymal cells that differentiate into various lineage cells including fibroblasts, pericytes, osteoblasts, and myocytes. We previously demonstrated that ADRCs could be isolated from small amount of human subcutaneous adipose tissue. Although some studies reported that adipose-derived cells could differentiate into ECs or EPCs, we could not confirm differentiation of ADRCs into endothelial lineages in the present study. The reason of this discrepancy is unknown, however Miranville and coworkers used low-serum medium supplemented with VEGF and insulin-like growth factor-1 (IGF-1). The difference of such culture condition may affect maturation of ADRCs into ECs. Nevertheless, a recent study suggested that human ADRCs failed to differentiate into ECs even under culture with EGM-2, being consistent with our findings. In addition, we could not confirm differentiation of ADRCs into ECs in vivo. Implanted ADRCs expressed no endothelial maker, but implanted ADRCs were positive for CD140b and colocalized with vessels like pericytes. These results suggest that ADRCs might not have capability to differentiate into ECs but smooth muscle lineage cells. Similarly, several recent studies showed that ADRCs differentiated into pericytes in vitro and in vivo.

Nakagimi and coworkers and Sumi and coworkers reported that implantation of adipose-derived mesenchymal cells induced angiogenesis via secretion of angiogenic cytokines. In the present study, we also found that implantation of ADRCs significantly augmented angiogenesis in a mouse model of hind limb ischemia. Our data indicate that implantation of ADRCs induced angiogenesis not by an endothelial differentiation but by chemokines such as SDF-1. Interestingly, we found that implantation of MAs into skeletal muscles even worsened angiogenesis compared to saline-injected control mice. We found that ADRCs expressed SDF-1, and the abundance of mRNA and protein were significantly greater in ADRCs than in MAs. In contrast, mRNA and protein abundance of VEGF did not differ between ADRCs and MAs. These results suggest that SDF-1 secreted from ADRCs may at least in part account for the difference in the angiogenic potency between ADRCs and MAs. In addition, recent studies indicated that MAs release other adipocytokines including tumor necrosis factor (TNF)-α and interleukin (IL)-6. These potentially deleterious inflammatory cytokines might have negatively affected angiogenesis by MA implantation observed in the present study.

SDF-1 is a member of CXC chemokines originally isolated from murine BM stromal cells. CXCR4 is the receptor for SDF-1 and is a coreceptor for HIV type 1 infection. SDF-1/CXCR4 interaction regulates multiple physiological processes including embryonic development and organ homeostasis. Interestingly, SDF-1 is considered as one of the key regulators of EPCs trafficking from BM into PB. Thus, SDF-1 has been shown to augment neovascularization by acceleration of EPC recruitment into ischemic tissues. In addition, VEGF is one of powerful angiogenic cytokines that can mobilize EPCs from BM and inhibit EPC apoptosis. In the mouse ischemic hind limb model, VEGF-A–mediated angiogenesis partly depends on the activation of the SDF-1/CXCR4 pathway. Taken together, SDF-1 plays a pivotal role for the cell therapy–mediated angiogenesis. In fact, therapeutic efficacies and mobilization of EPCs of ADRCs implantation was markedly suppressed by i.p. injection of an anti–SDF-1 neutralizing mAb in the present study.

Regarding clinical trial of angiogenesis, we have reported the safety and efficiency of therapeutic angiogenesis using autologous BM-MNCs (TACT). However, there are some patients who do not respond well to this procedure. Zeiher and coworkers showed that EPC mobilization and functions were reduced in patients with ischemic cardiomyopathy compared to nonischemic subjects, indicating that there may be a limited efficacy of the implantation of autologous BM cells for angiogenesis. Our current study indicates that autologous ADRCs are good alternatives to BM or circulating progenitor cells to induce angiogenesis.

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Disclosures

None.

References


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Supplemental Methods

Isolation and culture of mouse ADRCs

Under general anesthesia with pentobarbital sodium (50 mg/kg i.p.), mice ADRCs were isolated from inguinal fat pads (0.1-0.2g) obtained from C57BL/6J mice (n=32) and GFP-transgenic mice with C57BL/6J background (n=3). Adipose tissues were minced and digested with 2mg/mL type I collagenase (Wako, Japan). After filtration through a 40 μm filter (BD Falcon, Bedford, MA), mature adipocytes and stromal vascular fractions (SVFs) were separated by centrifugation (1,200 rpm for 5 min). SVFs were then cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS) and antibiotic/antimycotic solution (GIBCO). When adherent cells have reached confluence (P0), these attaching ADRCs were reseeded in the similar medium at a concentration of 2.0 x 10^3 cells/cm^2. For most experiments, 1st and 2nd passages of ADRCs were used.

Real-time RT-PCR

Real-time RT-PCR analysis of the VEGF, SDF-1 and GAPDH mRNAs was performed using 2μg total RNA on Mx3000P Real-Time PCR System using SYBR Green I and following conditions: 95°C for 10min followed by 40 cycles at 95°C for 15sec and 60°C for 45sec. Primers used were as follows: 5’-
CTGTAACGATGAAGCCCTGGAG-3’ and 5’-TGTTGAGGTTTGATCCGCAT-3’ for mouse VEGF-A; 5’-GCTCTGCATCAGTGACGGTA-3’ and 5’-
ATTTCGGGTCAATGCACACT-3’ for mouse SDF-1; and 5’-
CCCTTATTGACCTCAACTACATGGT-3’ and 5’-
GAGGGGCCATCCACAGTCTTCTG-3’ for mouse GAPDH genes.

**Laser Doppler blood flowmetry and capillary density**

We evaluated blood flow of ischemic thigh area using a laser Doppler blood perfusion image (LDPI) system (moorLDI, Moor Instruments) before surgery and at postoperative days 3, 7, 14 and 21. Quantitative values were expressed as the ratio to the non-ischemic limb. The effect of cell implantation or PBS injection on neovascularization was also assessed using light microscopy by measuring the number of capillaries stained for anti-CD31 mAb (PECAM-1: Becton Dickinson) in the sections taken from the ischemic muscles at postoperative day 21. Frozen sections with 5μm in thickness were prepared and stained for anti-CD31 mAb (PECAM-1: Becton Dickinson) to detect capillary ECs. The number of capillary ECs were counted under light microscopy (×200). Five fields from the 3 different muscle samples of each animal were randomly selected for the capillary density analysis. The data were presented as capillary/muscle fiber ratio.
Supplemental Figure Legend

Figure I. A, Characterization of cultured ADRCs., Phase contrast micrographs of adherent ADRCs 24hr (a) and 1week (b) after plating (_100). c, Phase contrast micrograph of adipogenic differentiated ADRCs involving lipid-filled droplets (_400). Adipogenic differentiated ADRCs stained with Oil Red O (_400). e, ADRCs were stained with anti-a-SMA antibody and Alexa 594 (red), counterstained with DAPI (_100). Endothelial differentiation was not confirmed by immunostaining with anti-PECAM mAb and Alexa 488 (f) (green) and double positive cells staining with DiI-Ac-LDL and FITC-BS1 lectine (g). B, Flow cytometry analysis of cultured ADRCs (P2) showing anti-Sca-1, CD34, c-kit, Lin, CD31, or flk-1antibody (n=3). R3 area shows respective isotype control for each antibody, and also positive signals for each antibody were shown in R4 area.

Figure II. Effects of cell implantation on blood flow recovery in hind limb ischemia. Cultured ADRCs (1_10^6 cells, 100µl, n=8), mature adipocytes (MA, n=6) or PBS as a control (n=8) were injected into the left thigh muscle at postoperative day1. A, Representative images of LDPI. B, Representative microscopic photographs of capillary
density in the ischemic muscles at 21 days after surgery (_200). Capillaries in ischemic muscles were immunostained with anti-CD31 mAb.

**Figure III.** A, Representative fluorescence microscopy of DiI-Ac-LDL and FITC-BS1 lectin double positive EPCs in BM (left) and circulating PB (right) at days 3 and 7 after surgery. B, Representative images of EPCs (Sca-1 and flk-1 positive cells in MNCs) in BM (left) and circulating PB (right).

**Figure IV.** A, SDF-1 (red) was expressed on implanted ADRCs (green) located adjacent to skeletal myocytes at day 3 (x400). B, VEGF (red) was mainly expressed in cytoplasm of skeletal myocytes, not GFP-positive ADRCs (green) in ischemic muscles at day 3 (x400). C, SDF-1 positive cells (red) were lower in ischemic skeletal myocytes injected PBS at day 3 (x400). D, VEGF expression (red) was lower in cytoplasm of ischemic skeletal muscle injected PBS at day 3 (x400).

**Figure V.** A, Implanted GFP-positive ADRCs (green) were found in the ischemic area at postoperative day 28 (x200). B, Vessels are visualized with rhodamine-BS-1 lectin injection (red) (x200) C, Merged image shows that GFP positive cells are co-
localized with rhodamine-BS-1 lectin positive capillaries (white arrows) (x200).

However, this does not indicate which cell types ADRCs differentiated into.  

D, Implanted GFP-positive ADRCs resided nearby vessels (white arrow) but the cells did not merge with CD31-positive endothelial cells (red), indicating that ADRCs did not give rise to endothelium. (x400).  

E, Implanted GFP-positive ADRCs were located like pericytes nearby capillaries (white arrow). Again ADRCs did not merge with CD31-positive endothelium.  

F, Many implanted GFP-positive ADRCs were positive for CD140b, a marker for pericytes (white arrow heads), indicating that implanted ADRCs could differentiate into pericyte-like vascular smooth muscle cells (x400).

**Figure VI.** Representative images of LDPI after implantation of ADRCs with anti-SDF1 mAb, implantation of ADRCs with control rat IgG and PBS with anti-SDF1 mAb at postoperative day 28.
Supplementary figure
A

bone marrow

peripheral blood

PBS 3d  ADRC 3d  PBS 7d  ADRC 7d

PBS 3d  ADRC 3d  PBS 7d  ADRC 7d

B

flk-1

Sca-1

Control day3  ADRC day3  Control day7  ADRC day7

Supplementary figure
**A**

H&E | GFP | SDF-1 | DAPI | Merged
--- | --- | --- | --- | ---

**B**

H&E | GFP | VEGF | DAPI | Merged
--- | --- | --- | --- | ---

**C**

GFP | SDF-1 | DAPI | Merged
--- | --- | --- | ---

**D**

GFP | VEGF | DAPI | Merged
--- | --- | --- | ---

Supplementary figure
Supplementary figure
ADRC + nonspecific rat IgG
ADRC + anti-SDF-1 Ab
PBS + anti-SDF-1 Ab