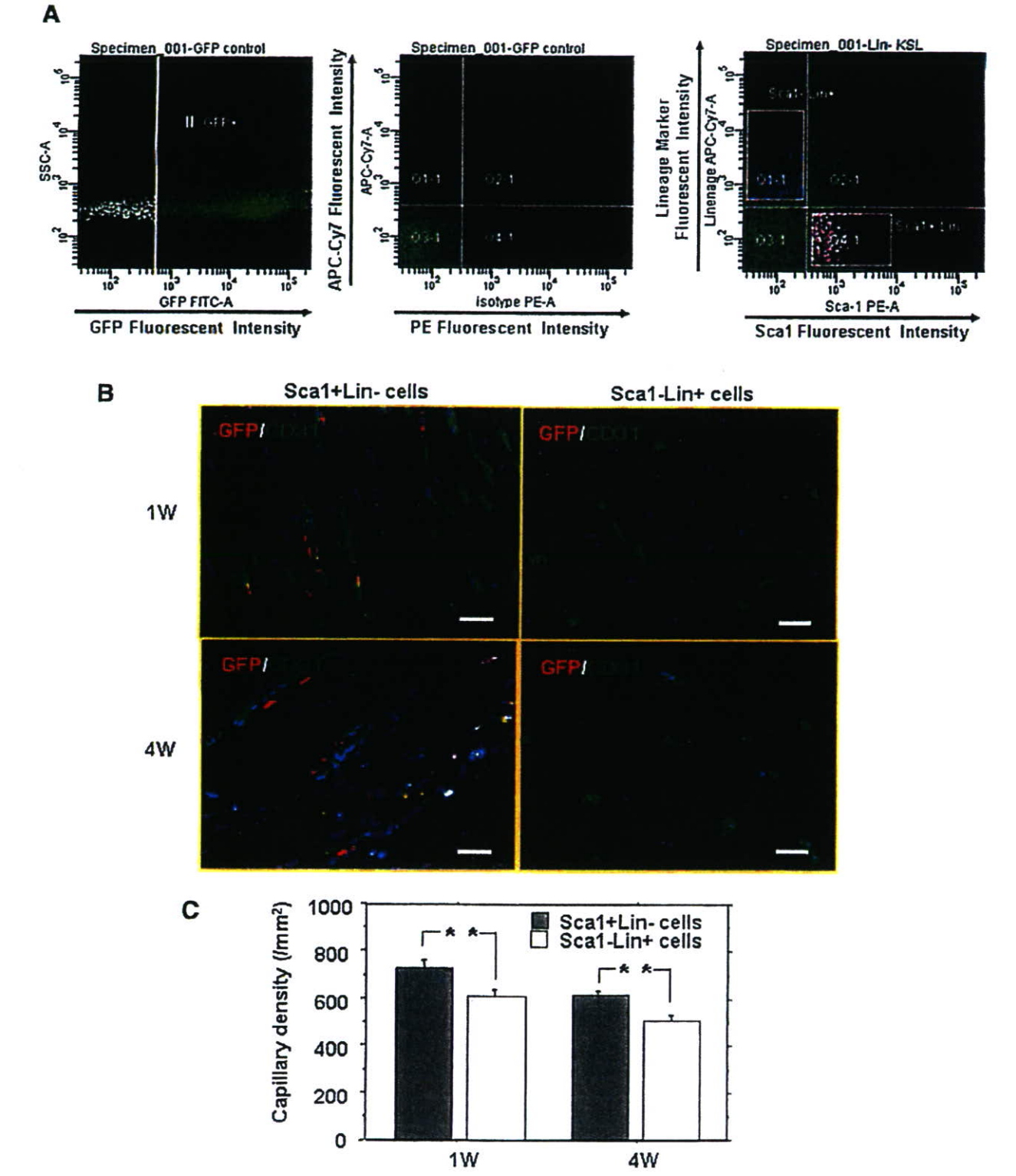

Fig. 4. Contribution of PB Sca1+Lin− cells to fracture healing. A: Representative example of FACSAria sorting of PB-GFP+/Sca1+/Lin− or GFP+/Sca1−/Lin+ cells from fractured GFP mice. Green color population indicated GFP+ cells in PB of GFP transgenic mice (Left part). Middle part shows PE- and APC-Cy7-isotype control. After GFP+ cell selection, PB-Sca1+/Lin− cell (Q4-1: white) or Sca1−Lin+ cell population (Q1-1: blue) were sorted (Right part). B: Double immunostaining for GFP (red) and CD31 (green) with tissue samples 1 and 4 weeks post-fracture and intravenous transplantation of GFP+/Sca1+/Lin− or GFP+/Sca1−/Lin+ cells. More abundant distribution of the double positive cells were identified at the fracture site following PB GFP+/Sca1+/Lin− cell transplantation compared with Sca1−/Lin+ cell infused group. Blue fluorescence indicates DAPI for nuclear staining. Scale bars: 20 μm. C: Whole vascularization assessed by CD31-positive capillary density was significantly enhanced in animals receiving Sca1+Lin− cells compared with Sca1−Lin+ group at weeks 1 and 4. n = 5 in each group. $^{**}P < 0.01$.

EPCs, suggesting other mechanisms such as paracrine effect of the BM-derived EPCs on resident EPCs and ECs, vasculogenesis by local EPCs or angiogenesis by the resident ECs. Our previous study demonstrated that transplanted CD34+ cells secreted angiogenic factors including VEGF, FGF2, and HGF may be also involved in bone healing at the fracture site, and that the inhibition of angiogenesis by soluble Flt1 (VEGF antagonist) suppressed not only angiogenesis/vasculogenesis but also intrinsic osteogenesis, indicating that angiogenic factors released by the transplanted CD34+ cells, at least in part, contribute to fracture healing in paracrine manner (Matsumoto et al., 2006).

The endochondral ossification and callus formation was advanced following the early phase of fracture healing process, suggesting that the initial stage of neovascularization by EPCs is considered to be crucial for complete fracture healing in the late phase. These pathophysiological findings suggest the demand of EPCs at the fracture sites and the therapeutic usefulness in future clinical application. In conclusion, neovascularization in the early phase of fracture healing is modulated by mobilization of BM-derived EPCs into circulation and their incorporation into fracture site, leading to a contribution to bone healing.

Acknowledgments

We thank Yumiko Masukawa and Yukako Ueno for their secretarial assistance. We also would like to thank Ms. Janina Tubby for her editing assistance in preparing for this manuscript.

Literature Cited

Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. 1997. Isolation of putative progenitor endothelial cells for angiogenesis. Science 275:964–967.

Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM. 1999. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 85:221–228.

Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM. 2002. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 106:3009–3017.

Brighton CT, Hunt RM. 1997. Early histologic and ultrastructural changes in microvessels of periosteal callus. J Orthop Trauma 11:244–253.

Britten MB, Abolmaali ND, Assmus B, Lehmann R, Honold J, Schmitt J, Vogl TJ, Martin H, Schachinger V, Dimmeler S, Zeiher AM. 2003. Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI): Mechanistic insights from serial contrast-enhanced magnetic resonance imaging. Circulation 108:2212–2218.

Colnot CI, Helms JA. 2001. A molecular analysis of matrix remodeling and angiogenesis during long bone development. Mech Dev 100:245–250.

Eghbali-Fatourechi GZ, Lamsam J, Fraser D, Nagel D, Riggs BL, Khosla S. 2005. Circulating osteoblast-lineage cells in humans. N Engl J Med 352:1959–1966.

Eghbali-Fatourechi GZ, Modder UI, Charatcharoenwitthaya N, Sanyal A, Undale AH, Clowes JA, Tarara JE, Khosla S. 2007. Characterization of circulating osteoblast lineage cells in humans. Bone 40:1370–1377.

Ford JL, Robinson DE, Scammell BE. 2004. Endochondral ossification in fracture callus during long bone repair: The localisation of 'cavity-lining cells' within the cartilage. J Orthop Res 22:368–375.

Harper J, Kalgsbrun M. 1999. Cartilage to bone–angiogenesis leads the way. Nat Med 5:617–618.

Iwasaki H, Kawamoto A, Ishikawa M, Oyamada A, Nakamori S, Nishimura H, Sadamoto K, Horii M, Matsumoto T, Murasawa S, Shibata T, Suehiro S, Asahara T. 2006. Dose-dependent contribution of CD34-positive cell transplantation to concurrent vasculogenesis and cardiomyogenesis for functional regenerative recovery after myocardial infarction. Circulation 113:1311–1325.

Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, Kearney M, Li T, Isner JM, Asahara T. 2000. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. Proc Natl Acad Sci USA 97:3422–3427.

Karsenty G, Wagner EF. 2002. Reaching a genetic and molecular understanding of skeletal development. Dev Cell 2:389–406.

Kawamoto A, Gwon HC, Iwaguro H, Yamaguchi JI, Uchida S, Masuda H, Silver M, Ma H, Kearney M, Isner JM, Asahara T. 2001. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. Circulation 103:634–637.

Kawamoto A, Tkebuchava T, Yamaguchi J, Nishimura H, Yoon YS, Milliken C, Uchida S, Masuo O, Iwaguro H, Ma H, Hanley A, Silver M, Kearney M, Losordo DW, Isner JM, Asahara T. 2003. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. Circulation 107:461–468.

Kernek CB, Wray JB. 1973. Cellular proliferation in the formation of fracture callus in the rat tibia. Clin Orthop Relat Res: 197–209.

Khosla S, Eghbali-Fatourechi GZ. 2006. Circulating cells with osteogenic potential. Ann NY Acad Sci 1068:489–497.

Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S. 2001. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function [see comment]. Nat Med 7:430–436.

Laing AJ, Dillon JP, Condon ET, Street JT, Wang JH, McGuinness AJ, Redmond HP. 2007. Mobilization of endothelial precursor cells: Systemic vascular response to musculoskeletal trauma. J Orthop Res 25:44–50.

Linden M, Sirsjo A, Lindbom L, Nilsson G, Gidlof A. 1995. Laser-Doppler perfusion imaging of microvascular blood flow in rabbit tenuissimus muscle. Am J Physiol 269:H1496–H1500.

Manigrasso MB, O'Connor JP. 2004. Characterization of a closed femur fracture model in mice. J Orthop Trauma 18:687–695.

Marsh D. 1998. Concepts of fracture union, delayed union, and nonunion. Clin Orthop Relat Res 355:S22–S30.

Matsumoto T, Kawamoto A, Kuroda R, Ishikawa M, Mifune Y, Iwasaki H, Miwa M, Horii M, Hayashi S, Oyamada A, Nishimura H, Murasawa S, Doita M, Kurosaka M, Asahara T. 2006. Therapeutic potential of vasculogenesis and osteogenesis promoted by peripheral blood CD34-positive cells for functional bone healing. Am J Pathol 169:1440–1457.

Murohara T, Ikeda H, Duan J, Shintani S, Sasaki K, Eguchi H, Onitsuka I, Matsui K, Imaizumi T. 2000. Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. J Clin Invest 105:1527–1536.

Otani A, Kinder K, Ewalt K, Otero FJ, Schimmel P, Friedlander M. 2002. Bone marrow-derived stem cells target retinal astrocytes and can promote or inhibit retinal angiogenesis. Nat Med 8:1004–1010.

Pardanaud L, Yassine F, Dieterlen-Lievre F. 1989. Relationship between vasculogenesis, angiogenesis and haemopoiesis during avian ontogeny. Development 105:473–485.

Rafii S, Lyden D. 2003. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat Med 9:702–712.

Risau W, Sariola H, Zerwes HG, Sasse J, Ekblom P, Kemler R, Doetschman T. 1988. Vasculogenesis and angiogenesis in embryonic-stem-cell-derived embryoid bodies. Development 102:471–478.

Rodriguez-Merchan EC, Forriol F. 2004. Nonunion: General principles and experimental data. Clin Orthop Relat Res 419:4–12.

Sahara M, Sata M, Matsuzaki Y, Tanaka K, Morita T, Hirata Y, Okano H, Nagai R. 2005. Comparison of various bone marrow fractions in the ability to participate in vascular remodeling after mechanical injury. Stem Cells 23:874–878.

Sato T, Laver JH, Ogawa M. 1999. Reversible expression of CD34 by murine hematopoietic stem cells. Blood 94:2548–2554.

Sivan-Loukianova E, Awad OA, Stepanovic V, Bickenbach J, Schatteman GC. 2003. CD34+ blood cells accelerate vascularization and healing of diabetic mouse skin wounds. J Vasc Res 40:368–377.

Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Stern DM, Naritomi H, Matsuyama T. 2004. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. J Clin Invest 114:330–338.

Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, Asahara T. 1999. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 5:434–438.

Wardell K, Jakobsson A, Nilsson GE. 1993. Laser Doppler perfusion imaging by dynamic light scattering. IEEE Trans Biomed Eng 40:309–316.

Werner N, Junk S, Laufs U, Link A, Walenta K, Bohm M, Nickenig G. 2003. Intravenous transfusion of endothelial progenitor cells reduces neointima formation after vascular injury. Circ Res 93:e17–e24.