Role of bone marrow-derived cells in tumor angiogenesis and treatment

The role of bone marrow-derived cells in tumor neovascularization is currently the subject of intense research and debate. Two recent studies (De Palma et al., 2003 and Garcia-Barros et al., 2003) offer novel yet somewhat conflicting evidence for the role of these cells in tumor growth and neovascularization. These results have significant implications for tumor biology and treatment. At the same time, they raise many questions, which must be addressed for translating these important findings into new, improved treatment strategies.

Our understanding of the formation of tumor vessels by vasculogenesis is in its infancy compared to our understanding of vessel formation by angiogenesis. Angiogenesis involves migration and proliferation of endothelial cells of existing vessels. Postnatal vasculogenesis is thought to involve endothelial progenitors, a subset of bone marrow-derived cells (BMDCs, Figure 1). Two recent reports—De Palma et al. (2003) and Garcia-Barros et al. (2003)—offer conflicting data on the incorporation of BMDCs in tumor vessels using some of the same tumor models. These findings are significant because of their clinical implications and timely because of the exciting developments in the field of stem cell biology. However, they raise many important questions about the biology of BMDCs.

In 1997, Isner and coworkers reported the existence of putative endothelial progenitor cells—angioblasts—in adults, and proposed the bone marrow as a source of these cells (Asahara et al., 1997). Initially, these ideas stirred intense controversy and generated great skepticism. Six years later, the existence of endothelial progenitor cells and their involvement in vessel formation is widely accepted, but the controversy has shifted to their role in solid tumors. Emerging initially as an unexpected contribution of the bone marrow to tumor endothelium (Takahashi et al., 1999), the focus shifted to the molecular characterization of BMDCs. Rafii and coworkers defined endothelial progenitor cells as VEGFR2- (vascular endothelial growth factor receptor 2) positive BMDCs. However, these authors reported that VEGFR1-positive BMDCs also home to tumors, contributing indirectly to neovascularization. They provided genetic evidence for the critical role of bone marrow in tumor neovascularization by rescuing tumor growth and angiogenesis in *Id* mutant mice—which have defective angiogenesis—by transplanting bone marrow from wild-type/nonmutant mice (Lyden et al., 2001). Simultaneously, Carmeliet and coworkers reported rescuing pathological angiogenesis in placental growth factor (PlGF) null mice by transplanting bone marrow from nonmutant mice (Carmeliet et al., 2001).

In the May 16 issue of *Science*, Garcia-Barros et al. (2003) offer compelling evidence for the critical role of BMDCs in solid tumor growth in nonmutant mice. Bone marrow cells transplanted from Rosa-26 mice—which express β-galactosidase—formed about half of the tumor-vascular endothelium. These authors propose endothelial apoptosis as a key determinant of the tumor response to ionizing radiation treatment. By knocking out the *Acid Sphingomyelinase* gene, responsible for the endothelial apoptosis, the authors show that a transplant of *Acid Sphingomyelinase* null BMDCs was sufficient to reduce significantly the radiation sensitivity of the tumors.

The findings of De Palma et al. (2003), published online on May 12 in *Nature Medicine*, also attest to the critical role played by BMDCs in the neovascularization and growth of mouse tumor xenografts. However, despite using multiple and efficient vector constructs for gene transfer of the green fluorescence protein (GFP), and constitutive GFP-bone marrow cells transplants, these authors report the absence of GFP-positive cells in tumor endothelium. This unexpected result was consis-

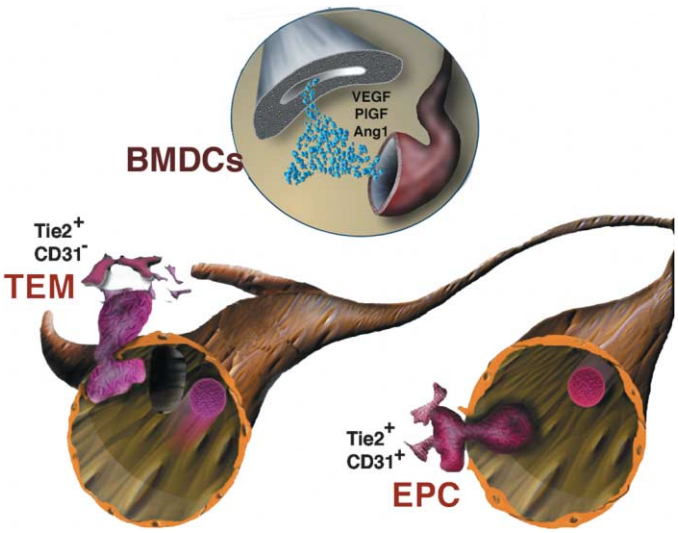

Figure 1. Partiicipation of bone marrow-derived cells in tumor neovascularization

Tumors overexpress angiogenic growth factors such as VEGF, PlGF, and Ang1. These growth factors augment mobilization of bone marrow cells, which in turn facilitate the tumor neovascularization. These cell populations include VEGFR2+ endothelial progenitor cells (EPCs) that purportedly form tumor endothelium, and Tie2+ mononuclear cells (TEMs), which control indirectly the angiogenesis during tumor growth and liver regeneration. Two recent reports offer novel insights into the role of BMDCs in tumor angiogenesis. However, the data on BMDC incorporation in angiogenic vessels are conflicting, even for the same tumor cell lines. It is also not clear if these TEMs are the VEGFR1+ hematopoietic precursors previously described by Hattori et al. (2002) as the stem cells responsible for hematopoietic reconstitution. Whereas the molecular definition of these cells, the extent and kinetics of their incorporation into vessel wall, and the mechanisms involved are largely unknown, these findings have multiple therapeutic implications and warrant urgent and careful mechanistic and phenotypic characterization. Figure courtesy of Dr. Lance L. Munn.

tently observed in three different tumor lines, including B16 melanoma—a line used by Garcia-Barros et al. in their studies. The reasons for these diametrically opposite results are not known. Of interest, De Palma et al. discovered a new subset of BMDCs that are involved in postnatal angiogenesis. Expression of GFP under an endothelial-specific (Tie2) promoter/enhancer revealed that bone marrow contribution to the tumor vessels in their setting relied not on the endothelial progenitor cells, but rather on a hematopoietic (i.e., CD45- and CD11b-positive, but CD31-negative) cell population—Tie2-expressing mononuclear cells (TEMs). These TEMs home specifically to the tumors and contribute indirectly to neovascularization; hence, they offer an ideal platform for cell-based gene delivery to solid tumors.

The new data of De Palma et al. broaden the view on the involvement of the bone marrow in tumor angiogenesis (Figure 1) and highlight the limits of our current understanding of the relationship between cell subsets and physiologic function. For example, Rafii and coworkers proposed the notion that the VEGFR1-positive stem cells mobilized by angiogenic factors such as VEGF and PlGF may actually be responsible for rescuing hematopoiesis following chemo- or radiation-induced injury of the bone marrow (Hattori et al., 2002). It is not clear whether the TEMs share features with cell types such as VEGFR1-positive BMDCs. On a related subject, work from Verfaillie's laboratory established ex vivo the existence of multipotent adult progenitor/stem cells in the bone marrow (Jiang et al., 2002). However, there is a strong debate on the extent of hematopoietic stem cell multipotency and transdifferentiation capacity (Wagers et al., 2002). These controversies call for urgent characterization of the molecular definition of various subsets of BMDCs and mechanisms involved in their function.

Conflicting data notwithstanding, these findings have important clinical implications for physiological and pathological angiogenesis. The possibility of selective gene delivery and/or autologous cell transplants that would target pathologic angiogenesis specifically and efficiently is of great interest to immunologists and gene therapists alike. Equally important, understanding the biology of the tumor-endothelial response to radiation is critical for improving the existing therapies and designing more efficient strategies. Lastly, research on BMDCs has an impact beyond the field of oncology. A recent clinical trial showed that infusion of autologous progenitor cells has great promise in alleviating limb ischemia (Tateishi-Yuyama et al., 2002). Tissue engineering and regenerative medicine await further insight into the biology of these cells. Generating new functional vasculature based on progenitor cells could prove crucial for these fields.

Despite the consensus on the important role of BMDCs, several critical questions remain unanswered. Why is the extent of incorporation of endothelial progenitor cells in these two studies different? Is the extent of incorporation dependent on factors such as tumor size, tumor type, site, host, or genetic marker? Is it similar in transplanted versus spontaneous tumors? Which adhesion molecules are involved in cell homing? Do the growth factors produced by tumors mobilize these cells and also upregulate adhesion molecules on the angiogenic endothelium (Melder et al., 1996)? Do these cells adhere to the vessel luminally or reside abluminally, and thus provide appropriate growth factors? Or do they incorporate in the vessel wall? If so, what are the kinetics of cell incorporation? Do these cells proliferate after they are incorporated? Do they extravasate and transdifferentiate into mural cells after they are incorporated? How specific is their contribution to tumor vessels? What is the role of these cells in response to cancer treatment with antiangiogenic therapy, chemotherapy, radiation therapy, or combination therapy? Can progenitor cell kinetics in the circulation of patients be used as an independent marker for diagnosis or prognosis? Intravital microscopy coupled with the use of transgenic technologies and mathematical modeling has the potential to address several of these questions (Jain et al., 2002; Stoll et al., 2003). Comprehensive correlative studies in clinical trials can also provide valuable insight into these issues in human disease. Eventually, these integrated findings will allow the safe and efficient translation from bench to the bedside.

Rakesh K. Jain* and
Dan G. Duda

E.L. Steele Lab for Tumor Biology
Department of Radiation Oncology
Massachusetts General Hospital and
Harvard Medical School
Boston, Massachusetts 02114
*E-mail: jain@steele.mgh.harvard.edu

Selected reading

Asahara, T., Murohara, T., Sullivan, A., Silver, M., van der Zee, R., Li, T., Witzenbichler, B., Schatteman, G., and Isner, J.M. (1997). Science *275*, 964–967.

Carmeliet, P., Moons, L., Luttun, A., Vincenti, V., Compernolle, V., De Mol, M., Wu, Y., Bono, F., Devy, L., Beck, H., et al. (2001). Nat. Med. *7*, 575–583.

De Palma, M., Venneri, M.A., Roca, C., and Naldini, L. (2003). Nat. Med. *9*, 789–795.

Garcia-Barros, M., Paris, F., Cordon-Cardo, C., Lyden, D., Rafii, S., Haimovitz-Friedman, A., Fuks, Z., and Kolesnick, R. (2003). Science *300*, 1155–1159.

Hattori, K., Heissig, B., Wu, Y., Dias, S., Tejada, R., Ferris, B., Hicklin, D.J., Zhu, Z., Bohlen, P., Witte, L., et al. (2002). Nat. Med. *8*, 841–849.

Jain, R.K., Munn, L.L., and Fukumura, D. (2002). Nat. Rev. Cancer. *2*, 266–276.

Jiang, Y., Jahagirdar, B.N., Reinhardt, R.L., Schwartz, R.E., Keene, C.D., Ortiz-Gonzalez, X.R., Reyes, M., Lenvik, T., Lund, T., Blackstad, M., et al. (2002). Nature *418*, 41–49.

Lyden, D., Hattori, K., Dias, S., Costa, C., Blaikie, P., Butros, L., Chadburn, A., Heissig, B., Marks, W., Witte, L., et al. (2001). Nat. Med. *7*, 1194–1201.

Melder, R.J., Koenig, G.C., Witwer, B.P., Safabakhsh, N., Munn, L.L., and Jain, R.K. (1996). Nat. Med. *2*, 992–997.

Stoll, B.R., Migliorini, C., Kadambi, A., Munn, L.L., and Jain, R.K. (2003). Blood Published online May 29, 2003. 10.1182/blood-2003-02-0365.

Takahashi, T., Kalka, C., Masuda, H., Chen, D., Silver, M., Kearney, M., Magner, M., Isner, J.M., and Asahara, T. (1999). Nat. Med. *5*, 434–438.

Tateishi-Yuyama, E., Matsubara, H., Murohara, T., Ikeda, U., Shintani, S., Masaki, H., Amano, K., Kishimoto, Y., Yoshimoto, K., Akashi, H., et al. (2002). Lancet *360*, 427–435.

Wagers, A.J., Sherwood, R.I., Christensen, J.L., and Weissman, I.L. (2002). Science *297*, 2256–2259.