BoneKEy-Osteovision. 2005 October;2(10):16-19
http://www.bonekey-ibms.org/cgi/content/full/ibmske;2/10/16
DOI: 10.1138/20050179

COMMENTARIES

Sharing the Same Vascular Addressins for Osteotropic Behavior of Hematopoietic Progenitor Cells and Cancer

Charles J. Dimitroff

Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

Commentary on: Sipkins DA, Wei X, Wu JW, Runnels JM, Cote D, Means TK, Luster AD, Scadden DT, Lin CP. In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature.* 2005 Jun 16;435(7044):969-73.

The osteotropism of hematopoietic progenitor cells (HPC) is a process controlled by bone marrow endothelial cell (BMEC) factors. BMEC adhesion molecules, including endothelial E- and P-selectin, and a chemotaxis factor, chemokine stromal derived factor-1 (SDF-1), are expressed on BMEC surfaces and allow for the trafficking of circulating HPC to bone. These factors ultimately regulate the efficiency of hematopoiesis (1-5). More importantly, the regulated expression of HPC counter-receptors, namely, ligands for E- and P-selectin and the chemokine receptor CXCR4, help govern the bone-homing capacity (6-9) of HPC. These HPC surface receptors mediate, in part, the rolling activity, firm attachment, and diapedesis that are requisite for HPC to extravasate through BMEC and seed in bone (4-10). We and others have hypothesized that these adhesion and chemoattractant receptors are also critical for the metastasis (homing) of circulating tumor cells to bone.

In a recent publication by Sipkins *et al.*, observations from intravital microscopy and immunoimaging experiments have captured the essence and importance of BMEC factors in the bone-homing process (11). By utilizing fluorescence confocal/multiphoton microscopy and *in vivo* immunofluorescence imaging, the authors conducted progressive scanning and optical sectioning through the skull of live mice that were inoculated intravenously with fluorescently labeled HPC, leukemic cells, or solid tumor cells. Complementary imaging of mice that had been administered antibodies against BMEC adhesion molecules and chemokines was also performed to investigate the spatial localization of the expression of putative targeting molecules on BMEC and the localization of migrated HPC or tumor cells in bone. Intravascular adhesion, seeding, and engraftment (survival) of HPC or tumor cells in bone in juxtaposition to staining of BMEC adhesion and/or chemokine molecules were then visualized for qualitative and quantitative assessments. The authors convincingly show that intravascular rolling and extravasation occur in distinct microdomains or "hot spots" in the parasagittal vascular region and are coincident with the expression of E-selectin and SDF-1 on BMEC (11). The vascular microdomains demarcated by the expression of E-selectin and SDF-1 are also coextensive with the anatomic sites of seeding, growth, and/or engraftment of circulating normal HPC, leukemic cells, and even prostate tumor cells (11). These data solidify prior reports showing that BMEC E-selectin and SDF-1 are crucial for the rolling activity, seeding, and hematopoietic activity of HPC in bone (1-4). Furthermore, these data provide the first *in vivo* demonstration that bone metastasis occurs in specific microenvironmental niches that are characterized by the functional expression of vascular adhesion molecule, E-selectin, and the chemoattractant SDF-1.

BoneKEy-Osteovision. 2005 October;2(10):16-19
http://www.bonekey-ibms.org/cgi/content/full/ibmske;2/10/16
DOI: 10.1138/20050179

The results of Sipkins *et al.* also show that other BMEC adhesion molecules, including intercellular adhesion molecule-1, vascular cell adhesion molecule-1, platelet/endothelial cell adhesion molecule-1, and P-selectin, are expressed diffusely throughout the bone marrow vasculature (11). Although these adhesion molecules do not show the same restricted pattern of localization as E-selectin and SDF-1, their importance for the homing and extravasation process should not be ignored (1;2;7-9). In fact, in proof-of-principle experiments using E-selectin null mice, the bone-homing capacity of Nalm-6 pre-B acute lymphoblastic leukemia cells was reduced by <20% (11). The authors did not show, however, whether these cells express E-selectin ligand activity, which would help explain the modest reduction in homing capacity in E-selectin null mice. Overlapping functional activity of BMEC P-selectin, which also mediates tethering and rolling behavior, could compensate for E-selectin deficiency and, perhaps, be sufficient for extravasation and growth in bone. On the other hand, CXCR4 desensitization or CXCR4 blockade treatments prior to tumor cell injection causes a reduction of approximately 80% in homing to E-selectin$^+$/SDF-1$^+$ microvascular domains (11). This observation strongly suggests that the SDF-1–CXCR4 axis, which plays a key role in the retention of bone marrow cells in bone marrow parenchyma, is also the principal chemokine–chemokine receptor interaction initiating the exit of circulating tumor cells through BMEC into bone.

The studies by Sipkins *et al.* help merge the fields of tumor metastasis and leukocyte homing research and clearly support the hypothesis, which is shared by our laboratory, that interfering with tumor cell extravasation can be an efficacious mode of antimetastatic therapy (12). These studies challenge the notion that circulating tumor cells seed in metastatic sites by a nonspecific vascular lodgment phenomenon (13); rather, they suggest that the migration of tumor cells to bone may be mediated through a bone-specific homing mechanism. Much of the emphasis in tumor metastasis studies has been on elucidating the tumor-cell adhesion molecule and protease repertoire mediating adhesive, migratory, and growth-related activities within the parenchyma of a primary tumor or metastatic site. Although these efforts are paramount to our understanding of the pathogenesis of tumor metastasis and have provided multiple targets for anticancer or antimetastatic exploitation, recognition of mechanistic insights from the leukocyte homing paradigm will expand our current knowledge base and result in a renewed perspective on how and why tumor cells home in a tissue-specific manner.

Recent efforts highlight the conspicuous similarity between the bone-homing molecules expressed on breast and prostate tumor cells and those expressed on HPC. The chemokine receptor CXCR4 has been shown to be critical for the migration and growth of breast and prostate tumor cells in bone (14-16). In addition, we have shown that the leukocyte selectin ligand P-selectin glycoprotein ligand-1 (PSGL-1) is a major E-selectin glycoprotein ligand on metastatic prostate tumor cells (12). PSGL-1 is a well-characterized ligand for P-, E-, and L-selectin that was previously thought to be almost exclusively expressed on hematopoietic cells (17). In fact, PSGL-1 has been implicated as a candidate E-selectin ligand in the trafficking of HPC to bone (6;9;18). Evidence for the expression and selectin-binding function of PSGL-1 on prostate tumor cells (which characteristically metastasize to bone) underscores the importance of understanding its role in the bone metastasis of prostate and other osteotropic cancers.

In addition to similarities to the bone-homing molecule repertoire on HPC, prostate and breast tumor cells exhibit a distinctive rolling behavior on BMEC that mimics leukocyte rolling interactions (16;19;20). On prostate tumor cells metastatic to bone, *in vitro* flow chamber experiments suggest that these interactions are dependent on BMEC E-selectin (20). The fact that PSGL-1 has been found on prostate tumor cells implicates adhesive interactions between PSGL-1 and BMEC E-selectin as a potential mechanism of the bone metastasis process. However, *in*

vivo analysis of the rolling of prostate tumor cells on BMEC or other microvascular models, where both E- and P-selectin could contribute to the rolling activity, needs to be performed to fully appreciate the role of E- and/or P-selectin ligands in the homing of prostate tumor cells to bone. Intravital fluorescence confocal microscopy and *in vivo* immunoimaging technology utilized by Sipkins *et al.*, particularly in the context of E- and/or P-selectin null mice and PSGL-1 (+) or (-) tumor cells, could help validate the importance of these molecules in the osteotropism of prostate cancer.

In summary, observations from Sipkins *et al.* strengthen the premise that circulating tumor cells harness leukocyte homing receptors and resultant adhesive behaviors to facilitate tissue-specific metastasis. The osteotropism of tumor cells is undoubtedly also influenced by survival factors present in the bone marrow microenvironment. However, circulating tumor cells expressing the combination of bone-homing receptors and relevant counter-receptors or signaling pathways with bone marrow survival factors most likely represent the initiators of metastasis formation. Early research efforts identifying and characterizing bone-homing receptors and chemoattractant receptors on HPC should help guide tumor biologists in their research on potential mediators of bone metastasis. Acknowledging that bone metastasis could be mechanistically linked to the HPC bone-homing process may ultimately hasten strategies to develop bone-specific inhibitors for the control of tumor cells engineered to home to bone.

References

1. Frenette PS, Subbarao S, Mazo IB, von Andrian UH, Wagner DD. Endothelial selectins and vascular cell adhesion molecule-1 promote hematopoietic progenitor homing to bone marrow. *Proc Natl Acad Sci U S A*. 1998 Nov 24;95(24):14423-8.

2. Mazo IB, Gutierrez-Ramos JC, Frenette PS, Hynes RO, Wagner DD, von Andrian UH. Hematopoietic progenitor cell rolling in bone marrow microvessels: parallel contributions by endothelial selectins and vascular cell adhesion molecule 1. *J Exp Med*. 1998 Aug 3;188(3):465-74.

3. Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A, Ben-Hur H, Many A, Shultz L, Lider O, Alon R, Zipori D, Lapidot T. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science*. 1999 Feb 5;283(5403):845-8.

4. Peled A, Grabovsky V, Habler L, Sandbank J, Arenzana-Seisdedos F, Petit I, Ben-Hur H, Lapidot T, Alon R. The chemokine SDF-1 stimulates integrin-mediated arrest of CD34(+) cells on vascular endothelium under shear flow. *J Clin Invest*. 1999 Nov;104(9):1199-211.

5. Kollet O, Spiegel A, Peled A, Petit I, Byk T, Hershkoviz R, Guetta E, Barkai G, Nagler A, Lapidot T. Rapid and efficient homing of human CD34 (+)CD38 (-/low)CXCR4(+) stem and progenitor cells to the bone marrow and spleen of NOD/SCID and NOD/SCID/B2m(null) mice. *Blood*. 2001 May 15;97(10):3283-91.

6. Dimitroff CJ, Lee JY, Rafii S, Fuhlbrigge RC, Sackstein R. CD44 is a major E-selectin ligand on human hematopoietic progenitor cells. *J Cell Biol*. 2001 Jun 11;153(6):1277-86.

7. Xia L, McDaniel JM, Yago T, Doeden A, McEver RP. Surface fucosylation of human cord blood cells augments binding to P-selectin and E-selectin and enhances engraftment in bone marrow. *Blood*. 2004 Nov 15;104(10):3091-6.

8. Hidalgo A, Weiss LA, Frenette PS. Functional selectin ligands mediating human CD34(+) cell interactions with bone marrow endothelium are enhanced postnatally. *J Clin Invest*. 2002 Aug;110(4):559-69.

9. Katayama Y, Hidalgo A, Furie BC, Vestweber D, Furie B, Frenette PS. PSGL-1 participates in E-selectin-mediated progenitor homing to bone marrow: evidence for cooperation between E-selectin ligands and alpha 4 integrin. *Blood*. 2003 Sep 15;102(6):2060-7.

10. Naiyer AJ, Jo DY, Ahn J, Mohle R, Peichev M, Lam G, Silverstein RL, Moore MA, Rafii S. Stromal derived factor-1-induced chemokinesis of cord blood CD34(+) cells (long-term culture-initiating cells) through endothelial cells is mediated by E-selectin. *Blood*. 1999 Dec 15;94(12):4011-9.

11. Sipkins DA, Wei X, Wu JW, Runnels JM, Cote D, Means TK, Luster AD, Scadden DT, Lin CP. In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature*. 2005 Jun 16;435(7044):969-73.

12. Dimitroff CJ, Descheny L, Trujillo N, Kim R, Nguyen V, Huang W, Pienta KJ, Kutok JL, Rubin MA. Identification of leukocyte E-selectin ligands, P-selectin glycoprotein ligand-1 and E-selectin ligand-1, on human metastatic prostate tumor cells. *Cancer Res*. 2005 Jul 1;65(13):5750-60.

13. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer*. 2002 Aug;2(8):563-72.

14. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A. Involvement of chemokine receptors in breast cancer metastasis. *Nature*. 2001 Mar 1;410(6824):50-6.

15. Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. *Cancer Res*. 2002 Mar 15;62(6):1832-7.

16. Sun YX, Wang J, Shelburne CE, Lopatin DE, Chinnaiyan AM, Rubin MA, Pienta KJ, Taichman RS. Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) in vivo. *J Cell Biochem*. 2003 Jun 1;89(3):462-73.

17. Laszik Z, Jansen PJ, Cummings RD, Tedder TF, McEver RP, Moore KL. P-selectin glycoprotein ligand-1 is broadly expressed in cells of myeloid, lymphoid, and dendritic lineage and in some nonhematopoietic cells. *Blood*. 1996 Oct 15;88(8):3010-21.

18. Hidalgo A, Weiss LA, Frenette PS. Functional selectin ligands mediating human CD34(+) cell interactions with bone marrow endothelium are enhanced postnatally. *J Clin Invest*. 2002 Aug;110(4):559-69.

19. Glinsky VV, Glinsky GV, Glinskii OV, Huxley VH, Turk JR, Mossine VV, Deutscher SL, Pienta KJ, Quinn TP. Intravascular metastatic cancer cell homotypic aggregation at the sites of primary attachment to the endothelium. *Cancer Res*. 2003 Jul 1;63(13):3805-11.

20. Dimitroff CJ, Lechpammer M, Long-Woodward D, Kutok JL. Rolling of human bone-metastatic prostate tumor cells on human bone marrow endothelium under shear flow is mediated by E-selectin. *Cancer Res*. 2004 Aug 1;64(15):5261-9.