COMMENTARIES

Thromboembolism to Metastasis: The Platelet-Lysophosphatidic Acid Connection

Theresa A. Guise

University of Virginia, Charlottesville, VA, USA

March 2005

Commentary on:
- Boucharaba A, Serre CM, Gres S, Saulnier-Blache JS, Bordet JC, Guglielmi J, Clezardin P, Peyruchaud O. Platelet-derived lysophosphatidic acid supports the progression of osteolytic bone metastases in breast cancer. *J Clin Invest.* 2004 Dec;114(12):1714-25.
- Gupta GP, Massague J. Platelets and metastasis revisited: a novel fatty link. *J Clin Invest.* 2004 Dec;114(12):1691-3.

The hypercoagulable state has long been linked to cancer and cancer metastasis. This association was first described by Trousseau in the nineteenth century, and supporting data have been gleaned from animal models of tumor metastasis. Only in recent years have mechanisms been put forth to dissect the role of coagulation and, specifically, the platelet as facilitators of metastasis. Recently, Boucharaba *et al.* (1) and Gupta and Massague (2) added to this growing body of evidence by implicating platelet-produced lysophosphatidic acid (LPA) in osteolysis associated with breast cancer metastasis to bone.

Since the time of Trousseau, there has been no doubt that hypercoagulable states are associated with metastasis. Patients with cancer have increased platelet adhesion to fibrinogen, compared with healthy controls. Furthermore, adhesion is greater in patients with metastasis, compared with those with localized disease (3). How does this promote metastasis? The initiation of metastasis is believed to be a rare and inefficient process. Initial detachment,

extravasation, angiogenesis, migration, chemotaxis, attachment, and evasion of host defense mechanisms comprise the multistep process necessary for cancer cell metastasis to any site (4). Platelets seem to facilitate these general mechanisms of metastasis in several ways. First, platelets protect against immune-mediated pathways of tumor cell clearance by impeding natural killer (NK) cell elimination of tumor cells (5). Platelet aggregation may cloak tumor antigens, thereby preventing immune-mediated attack. Second, tumor cell-bound platelets may facilitate adhesion to areas of disrupted vascular endothelium. Platelets facilitate the spreading of tumor cells to vasculature (6) and the adhesion of cancer cells to leukocytes and endothelial cells. Third, aggregating platelets on tumor cells could provide paracrine growth and survival factors, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Platelet-produced thrombospondin-1 may modulate angiogenesis by regulating endothelial cell apoptosis, protease expression, and VEGF expression (7). Fourth, tumor-associated platelet aggregates or thrombi may mechanically lodge in distant organ microvasculature, allowing tumor entry and metastasis (8). These mechanisms can be either thrombin dependent or independent and suggest that even partial platelet function may provide benefit against metastasis (9). Furthermore, cancer cells (particularly MDA-MB-231) used in the study by Boucharaba *et al.*, cause platelet aggregation (10).

Bone metastasis-specific effects of platelets have also been demonstrated. Bakewell *et*

12

al. (8) showed that platelet and osteoclast β_3 integrins are critical for the metastasis of B16 melanoma to bone. A critical role for platelet $\alpha_{IIb}\beta_3$ in tumor trafficking and entry into bone was demonstrated, and the results indicate that antiplatelet therapy may be beneficial in preventing the metastasis of solid tumors.

Boucharaba *et al.* (1) described another role for platelets in metastasis to bone, specifically, as a source of LPA. LPA is a bioactive water-soluble phospholipid that stimulates cell proliferation, migration, survival, and endothelial permeability by acting through specific G-protein-coupled receptors. It consists of a single fatty acyl chain, glycerol backbone, and free phosphate group. LPA is produced extracellularly from lysophosphatidylcholine by autotoxin (ATX), a ubiquitous exophosphodiesterase that was originally identified as an autocrine motility factor. ATX expression is increased by two growth factors, bone morphogenetic protein 2 (BMP-2) and fibroblast growth factor 2 (FGF-2), as well as by retinoic acid and Wnt signaling. There are at least four LPA receptors (LPA1, LPA2, LPA3, and LPA4) that couple to multiple signaling pathways, which include those initiated by the small GTPases (i.e., Ras, Rho, and Rac). Both LPA receptors and ATX are aberrantly expressed in several cancers. LPA stimulates the production of VEGF, interleukin 6 (IL-6), and IL-8, which can act as paracrine growth factors for cancer cells and alter the tumor microenvironment by increasing neovascularization (11).

Platelets are the major endogenous source of LPA. Herein lies the link between platelets, LPA, breast cancer growth, and osteolysis. First, Boucharaba *et al.* (1) demonstrated that functional LPA receptors are present in breast cancer. Using reverse transcription PCR, the researchers showed that human breast cancer and fibroadenomas expressed LPA1, LPA2, and LPA3, as did seven of eight human breast cancer lines. LPA stimulated *in vitro* tumor growth in lines that expressed LPA receptors, but not in breast cancer line SK-BR-3, which lacks receptors. Next, they overexpressed LPA1 in a bone-tropic variant of the MDA-MB-231 breast cancer line, MDA-BO2, using a tet-off-regulated promoter system in which overexpression is induced by withdrawal of the repressor doxycycline. In two different stable MDA-BO2/LPA1 clones, LPA (but not other growth factors) stimulated *in vitro* growth in the absence of doxycyline. *In vivo* growth as subcutaneous tumors and osteolytic bone metastasis were greater in mice inoculated with MDA-BO2/LPA1 via subcutaneous and intravenous routes, respectively, when LPA1 expression was turned on. Thus, there seemed to be an endogenous source of local production of bioactive LPA. However, the tumor cells did not produce LPA or ATX, the enzyme required for LPA production.

Because platelets are a major source of endogenous LPA, and tumor-induced platelet aggregation is important for tumor cell dissemination, the authors went on to show that MDA-MB-231, MDA-BO2, and MDA-BO2/LPA1 induced platelet aggregation and stimulated release of LPA from activated platelets and that in the absence of doxycycline, supernatants from tumor-induced platelet aggregation promoted proliferation of MDA-BO2/LPA1, which was abrogated by phospholipase B, the LPA degrading enzyme. These key experiments indicate that the above-mentioned tumor cells stimulate bioactive LPA from platelets, a process that is mitogenic for breast cancer cells. To determine the significance of this *in vitro* finding, an inhibitor of platelet aggregation, the $\alpha_{IIb}\beta_3$ integrin antagonist (integrilin), was administered to mice bearing osteolytic lesions caused by MDA-BO2/LPA1. Bakewell *et al.* (8) previously showed that this antagonist prevented the development of bone metastasis caused by B16 melanoma cells. In MDA-BO2/LPA1 mice, integrilin induced thrombocytopenia and a 70% reduction in circulating LPA. Remarkably, this was associated with a 50% reduction in osteolytic lesion area in the integrilin-treated mice, compared with control mice. Moreover, this effect was not restricted to breast cancer, as a similar result was obtained when the authors used Chinese hamster ovarian cells that overexpressed $\beta3$ integrin (CHO-$\beta3$). This tumor causes osteolytic lesions *in vivo* and

13

expresses LPA1, but not LPA2 or LPA3. Collectively, these data indicate that blood platelets are the source of endogenous LPA and that blocking platelet activity reduces LPA and bone metastasis.

These results provide an explanation for the increased tumor growth, but not for the profound increase in osteoclastic bone resorption observed in bone metastasis from MDA-BO2/LPA1 mice that did not receive doxycycline. Thus, it seems likely that LPA could induce tumor production of bone-resorbing cytokines. IL-6 and IL-8 are obvious candidates, as LPA has been shown to stimulate their production from ovarian and breast cancer (12). Furthermore, IL-8 has been implicated in bone metastasis caused by breast cancer (13) and prostate cancer (14-16). Indeed, LPA induced a profound increase in both IL-6 and IL-8 in the absence of doxycycline, an effect that was abrogated by phospholipase B.

Platelets have been implicated in bone metastasis, and LPA, in cancer growth and metastasis, but Boucharaba et al. (1) were the first to show a coordinated effort between tumor activation of platelets, LPA-induced mitogenesis, and cytokine production in the development and progression of osteolytic bone metastasis caused by breast cancer. However intriguing, the findings do not show a direct role for LPA signaling in this process, as no attempt was made to block LPA action by either knock-down of LPA1 expression or using pharmacologic inhibition of LPA1. Nonetheless, important mechanistic and therapeutic insight can be gleaned from these studies. Based on the work of Boucharaba et al. and other studies, the authors of this and the accompanying editorial (1,2) proposed a model to explain the propensity of breast cancer to metastasize and grow in bone. Tumor cells that enter the circulation induce platelet aggregation, which results in a protective cloak for the tumor cells to resist NK cell-mediated tumor killing and resistance to shear stress. The tumor-platelet complex adheres to and extravasates through bone marrow endothelium. Housed in bone, the activated platelets produce LPA and factors

that stimulate angiogenesis, such as VEGF and thrombospondin-1. LPA acts on tumor cells to stimulate proliferation and production of the osteolytic cytokines IL-6 and IL-8, which act in concert with other tumor-produced osteolytic factors, such as PTH-related protein (PTHrP) and IL-11. Bone-derived growth factors, such as transforming growth factor β (TGF-β) and insulin-like growth factors (IGFs), which are released as a consequence of osteoclastic bone resorption, stimulate tumor growth and the production of more osteolytic factors. The resulting morbidity is well-known: bone pain, fracture, and the inability to cure the breast cancer.

These findings are highly relevant to our understanding of bone metastasis caused by breast and other solid tumors. Boucharaba et al. (1) have added another factor (LPA) and cell type (platelet) to the vicious cycle of bone metastasis. Importantly, the findings shed new insight into the mechanisms of tumor growth in bone, but also into the general mechanisms responsible for tumor metastasis to any site. Equally important are the new questions raised by this study. Will inhibition of LPA or LPA receptors be effective to treat and ultimately prevent metastasis? Will inhibition of LPA signaling alter normal physiologic processes? The phenotype of LPA receptor null mice indicates a role in the developing fetus, but it is not clear whether this family of receptors mediates normal functions in the adult (11). What are the roles of other LPA receptors in cancer metastasis? Will integrilin inhibition of platelet activity be effective to block metastasis? Boucharaba et al. (1) showed a marked reduction in platelet number in mice after integrilin treatment. Bakewell et al. (8) did not report platelet counts in integrilin-treated mice. However, neither Boucharaba et al. (1) nor Bakewell et al. (8) described excessive bleeding, which would prohibit integrilin use in humans with cancer. Would other anticoagulation modalities, such as low-molecular weight heparin, work as well (17)? The ideal candidate treatment must have specificity for the pathological tumor cell-platelet interaction without altering normal platelet hemostasis. Does LPA stimulate tumor production of other bone-active

14

cytokines, such as PTHrP or IL-11? LPA can increase production of the osteoblast stimulatory factor endothelin-1 by endothelial cells, which are often in close proximity to tumor and bone cells at metastatic sites (18). The molecule can also increase the production of other bone-stimulatory proteins, such as adrenomedullin (19) and connective tissue growth factor (20), the latter of which is part of a recently described bone metastasis signature of MDA-MB-231 breast cancer (21). Does LPA synergize with bone-derived growth factors, such as TGF-β and IGFs, to enhance production of osteolytic factors or tumor proliferation? Do bone-derived growth factors stimulate tumor production of ATX, the enzyme responsible for LPA synthesis? Do these growth factor signaling pathways crosstalk with LPA signaling? What are the direct effects of LPA on osteoblasts and osteoclasts? Does LPA alter the receptor activator of NF-κB (RANK)-RANK ligand-osteoprotegerin pathway or the Wnt signaling pathway, both with emerging roles in cancer biology? The answers to these and other questions will provide a better understanding of the bone-specific effects of platelet-LPA interactions in metastasis. Although the findings of Boucharaba et al. (1) are by no means exclusive to bone metastasis, they enrich our understanding of the complex milieu of the bone microenvironment in metastatic disease. New therapy directed against LPA and/or platelets will bolster and likely complement the current and less than optimal armamentarium to treat metastasis to bone and other sites.

References

1. Boucharaba A, Serre CM, Gres S, Saulnier-Blache JS, Bordet JC, Guglielmi J, Clezardin P, Peyruchaud O. Platelet-derived lysophosphatidic acid supports the progression of osteolytic bone metastases in breast cancer. *J Clin Invest.* 2004 Dec;114(12):1714-25.

2. Gupta GP, Massague J. Platelets and metastasis revisited: a novel fatty link. *J Clin Invest.* 2004 Dec;114(12):1691-3.

3. Caine GJ, Nadar SK, Lip GY, Stonelake PS, Blann AD. Platelet adhesion in breast cancer: development and application of a novel assay. *Blood Coagul Fibrinolysis.* 2004 Sep;15(6):513-8.

4. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat Rev Cancer.* 2003 Jun;3(6):453-8.

5. Palumbo JS, Talmage KE, Massari JV, La Jeunesse CM, Flick MJ, Kombrinck KW, Jirouskova M, Degen JL. Platelets and fibrin(ogen) increase metastatic potential by impeding natural killer cell-mediated elimination of tumor cells. *Blood.* 2005 Jan 1;105(1):178-85.

6. Im JH, Fu W, Wang H, Bhatia SK, Hammer DA, Kowalska MA, Muschel RJ. Coagulation facilitates tumor cell spreading in the pulmonary vasculature during early metastatic colony formation. *Cancer Res.* 2004 Dec 1;64(23):8613-9.

7. Sargiannidou I, Qiu C, Tuszynski GP. Mechanisms of thrombospondin-1-mediated metastasis and angiogenesis. *Semin Thromb Hemost.* 2004 Feb;30(1):127-36.

8. Bakewell SJ, Nestor P, Prasad S, Tomasson MH, Dowland N, Mehrotra M, Scarborough R, Kanter J, Abe K, Phillips D, Weilbaecher KN. Platelet and osteoclast beta3 integrins are critical for bone metastasis. *Proc Natl Acad Sci U S A.* 2003 Nov 25;100(24):14205-10.

9. Camerer E, Qazi AA, Duong DN, Cornelissen I, Advincula R, Coughlin SR. Platelets, protease-activated receptors, and fibrinogen

in hematogenous metastasis. *Blood.* 2004 Jul 15;104(2):397-401.

10. Belloc C, Lu H, Soria C, Fridman R, Legrand Y, Menashi S. The effect of platelets on invasiveness and protease production of human mammary tumor cells. *Int J Cancer.* 1995 Jan 27;60(3):413-7.

11. Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid in cancer. *Nat Rev Cancer.* 2003 Aug;3(8):582-91.

12. Fang X, Yu S, Bast RC, Liu S, Xu HJ, Hu SX, LaPushin R, Claret FX, Aggarwal BB, Lu Y, Mills GB. Mechanisms for lysophosphatidic acid-induced cytokine production in ovarian cancer cells. *J Biol Chem.* 2004 Mar 5;279(10):9653-61.

13. Bendre MS, Gaddy-Kurten D, Mon-Foote T, Akel NS, Skinner RA, Nicholas RW, Suva LJ. Expression of interleukin 8 and not parathyroid hormone-related protein by human breast cancer cells correlates with bone metastasis in vivo. *Cancer Res.* 2002 Oct 1;62(19):5571-9.

14. Uehara H, Troncoso P, Johnston D, Bucana CD, Dinney C, Dong Z, Fidler IJ, Pettaway CA. Expression of interleukin-8 gene in radical prostatectomy specimens is associated with advanced pathologic stage. Prostate. 2005 Jan 13; [Epub ahead of print]

15. Deftos LJ, Barken I, Burton DW, Hoffman RM, Geller J. Direct evidence that PTHrP expression promotes prostate cancer progression in bone. *Biochem Biophys Res Commun.* 2005 Feb 11;327(2):468-72.

16. Lehrer S, Diamond EJ, Mamkine B, Stone NN, Stock RG. Serum interleukin-8 is elevated in men with prostate cancer and bone metastases. *Technol Cancer Res Treat.* 2004 Oct;3(5):411.

17. Castelli R, Porro F, Tarsia P. The heparins and cancer: review of clinical trials and biological properties. *Vasc Med.* 2004 May;9(3):205-13.

18. Yakubu MA, Leffler CW. Regulation of ET-1 biosynthesis in cerebral microvascular endothelial cells by vasoactive agents and PKC. *Am J Physiol.* 1999 Feb;276(2 Pt 1):C300-5.

19. Yang JH, Jiang W, Pan CS, Qi YF, Wu QZ, Pang YZ, Tang CS. Effects of adrenomedullin on cell proliferation in rat adventitia induced by lysophosphatidic acid. *Regul Pept.* 2004 Sep 15;121(1-3):49-56.

20. Muehlich S, Schneider N, Hinkmann F, Garlichs CD, Goppelt-Struebe M. Induction of connective tissue growth factor (CTGF) in human endothelial cells by lysophosphatidic acid, sphingosine-1-phosphate, and platelets. *Atherosclerosis.* 2004 Aug;175(2):261-8.

21. Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordon-Cardo C, Guise TA, Massague J. A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell.* 2003 Jun;3(6):537-49.