Cancer Cell
Previews

Breast Cancer Src Activity: Bad to the Bone

Dennis C. Sgroi[1,2,*]
[1]Molecular Pathology Research Unit and Center for Cancer Research, Massachusetts General Hospital
[2]Department of Pathology
Harvard Medical School, Boston, MA 02129, USA
*Correspondence: dsgroi@partners.org
DOI 10.1016/j.ccr.2009.06.010

Bone metastases are a major cause of breast cancer morbidity and mortality. In this issue of *Cancer Cell*, Zhang and colleagues identify a Src activation expression signature associated with late-onset breast cancer bone metastases and provide evidence for Src as a key mediator of survival signals in latent bone metastases.

Metastasis, the disseminated spread and growth of malignant cells to distant organs, is the final and most devastating stage of human breast cancer progression. Bone represents the most common organ involved by metastatic breast cancer, and metastases to this site occur in approximately 30% of all women diagnosed with invasive breast cancer. Once breast cancer has metastasized to bone, the disease often inflicts marked morbidity and is ultimately incurable.

The development of bone metastases from a primary invasive breast cancer involves a complex sequence of interdependent events that include extravasation of tumor cells into the blood or lymphatic vasculature, survival within the circulation, arrest and adhesion at the distant site, and intravasation and seeding of the bone marrow microenvironment (Gupta and Massague, 2006). Once metastatic cells have successfully seeded the bone marrow, three potential outcomes may be distinguished (Brackstone et al., 2007): (1) the tumor cells can die or be eliminated by an effective immune response or therapeutic intervention; (2) the malignant cells may survive and remain clinically dormant; or (3) the tumor cells can survive and enter into a "vicious cycle" of bone resorption and tumor outgrowth that gives rise to clinically significant osteolytic macrometastases (Kang et al., 2003, 2005; Mundy, 2002). Although proliferating metastatic cells may be of primary clinical concern, clinically dormant tumor cells and micrometastases also have significant clinical importance as they can and do frequently serve as the source of "latent" metastatic breast disease.

In this issue of *Cancer Cell*, Zhang et al. (2009) provide both clinical and experimental evidence that Src plays a critical role in the establishment of latent bone metastases in breast cancer. Using a bioinformatic approach that investigated the association of various signaling pathway-specific gene expression patterns with breast cancer outcome, the authors identified a Src activity gene expression signature (designated as the Src responsive signature, or SRS) that was highly associated with late-onset breast cancer bone metastases. In a multivariate analysis, SRS was found to be independent of the distinct molecular subtypes of human breast cancer and independent of cell proliferation, the common biological principle driving the prognostic performance of most previously discovered breast cancer gene expression signatures. Most notably, the SRS was found independent of estrogen receptor (ER) status, the conventional clinicopathological parameter currently most closely associated with breast cancer bone metastases.

To address the role of Src in the bone metastatic process, the authors employed the use of two SRS-expressing human breast cancer cell lines that possess either aggressive or indolent metastatic bone tropism in a xenograft mouse model. In the cell line possessing aggressive metastatic bone tropism, stable knockdown of Src resulted in a significantly decreased rate of tumor outgrowth of bone lesions that was most apparent in the latter stages of this model, while knockdown of other Src family kinases did not impact bone metastatic activity. In the indolent model of bone metastatic disease, knockdown of Src resulted in near complete loss of bone metastatic activity. Src knockdown did not alter lung or lymph node metastatic activity supporting a specific role for Src in bone metastasis.

Src has the potential to impact one or more steps in the highly complex bone metastatic process. In addition to its effect on increasing cell proliferation, Src demonstrates pleiotropic functional activity that includes cellular differentiation, adhesion, migration, invasion, and survival. Zhang et al. showed that the potential pro-proliferative effect of Src do not account for the bone metastatic outgrowth in their model system by demonstrating that stable knockdown of Src did not decrease the intrinsic proliferative activity of the cells in both in vitro- and in vivo-based assays. These results suggested that Src may play a role in the initial seeding of or in the sustained survival of metastatic cells in the bone microenvironment. To better understand the role of Src as it relates to these two different aspects of the metastatic cascade, the authors performed a series of elegant genetic and pharmacological studies (using dasatinib, a Src tyrosine kinase inhibitor) that demonstrate Src is dispensable for bone marrow seeding but rate-limiting for the survival and sustained outgrowth of indolent breast cancer cells in the bone marrow microenvironment.

Like early stage primary breast cancer, the successful survival and outgrowth of bone metastases likely develops from intrinsic tumor epithelial-specific and tumor stromal microenvironment-specific (i.e., bone marrow-specific) factors. In order to identify cell survival factors in the bone marrow microenvironment that contribute to metastatic breast cancer outgrowth, Zhang et al. performed comparative microarray gene expression analysis of metastatic breast cancer samples derived from human bone, lung, brain, and liver. Seventeen secreted factors were differentially upregulated in

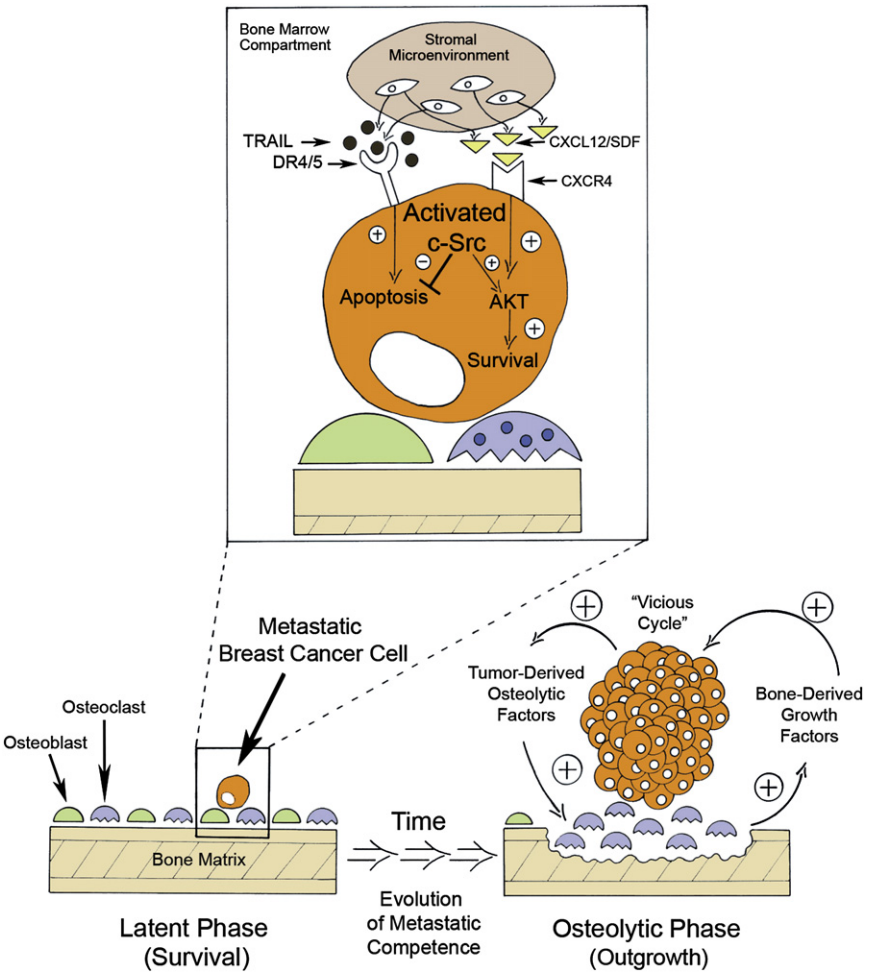

Figure 1. The Role of Src in Breast Cancer Bone Metastases

Metastatic colonization of a distant organ can be viewed as a two step process consisting of a "latent" phase and a final "osteolytic" outgrowth phase. Activated Src plays a critical role in the initiation and maintenance of latent phase by priming cells to respond to the bone-derived factors CXCL12/SDF and TRAIL. Specifically, activated Src mediates CXCL12/SDF-induced activation of the AKT survival pathway and mediates resistance to TRAIL-induced apoptosis. The dual survival effect of Src generates a state of tumor cell latency, and over a variably protracted length of time these latent tumor cells achieve full metastatic competence required for progression to the "vicious cycle" of the osteolytic outgrowth phase.

the human bone metastatic samples including the chemokine CXCL12/SDF1 (a survival factor for cells expressing the receptor CXCR4) and the cytokine TRAIL (a proapoptotic ligand that binds to the DR4/5 receptor). The authors demonstrated a similar cytokine expression profile in metastatic tumor samples obtained from their xenograft model of breast cancer bone metastases. Zhang at al. also explored the functional implication of these observations through Src knockdown and rescue experiments in their SRS-expressing bone metastatic cell lines and demonstrated that Src is required for CXCL12/SDF-mediated cell survival and abrogates TRAIL-mediated apoptosis.

Taken together, the findings of Zhang et al. provide strong clinical and experimental evidence that Src activation plays a critical role in bone-specific metastatic breast cancer cell survival and in the establishment of latent breast cancer bone metastases. These findings set the stage for the development of novel therapeutic strategies directed at eradicating breast cancer metastases to bone. Because metastatic bone colonization consists of an initial latent phase mediated by a Src survival response and a "vicious cycle" outgrowth phase mediated by complex molecular signaling between bone osteoclasts and tumor cells (Figure 1), one could envision therapeutic interventions aimed at interrupting one or both phases of colonization. Currently, strategies targeting the "vicious cycle" of bone metastases include the use of bisphosphonates, potent inhibitors of osteoclasts. Emerging clinical data support this approach because bisphosphonates have been shown to improve disease free survival in early-stage breast cancer patients (Gnant et al., 2009). However, now that Src inhibitors, including dasatinib (Finn, 2008), have become available for clinical testing, one could foresee using such agents as a means to interrupt survival of latent tumor cells before such cells acquire competency to progress to the outgrowth phase. Additionally, one can envision the potential use of the SRS bone relapse gene expression signature as a tool to guide adjuvant Src inhibitor therapy. The use of these tantalizing mechanistically driven strategies could have a major impact on breast cancer, and testing these approaches should be a priority for future clinical trials.

In summary, the finding presented by Zhang et al. highlight the synergistic power of combining clinical and basic science knowledge to dissect the cellular and molecular components of a long-standing and an exceptionally relevant clinical problem, latent breast cancer metastases. The knowledge gained from this elegant work generates significant optimism regarding the development of successful novel therapeutic strategies for women suffering from the devastating effects of breast cancer.

REFERENCES

Brackstone, M., Townson, J.L., and Chambers, A.F. (2007). Breast Cancer Res. *9*, 208.

Finn, R.S. (2008). Ann. Oncol. *19*, 1379–1386.

Gnant, M., Mlineritsch, B., Schippinger, W., Luschin-Ebengreuth, G., Postlberger, S., Menzel, C., Jakesz, R., Seifert, M., Hubalek, M., Bjelic-Radisic, V., et al. (2009). N. Engl. J. Med. *360*, 679–691.

Gupta, G.P., and Massague, J. (2006). Cell *127*, 679–695.

Kang, Y., He, W., Tulley, S., Gupta, G.P., Serganova, I., Chen, C.R., Manova-Todorova, K., Blasberg, R., Gerald, W.L., and Massague, J. (2005). Proc. Natl. Acad. Sci. USA *102*, 13909–13914.

Kang, Y., Siegel, P.M., Shu, W., Drobnjak, M., Kakonen, S.M., Cordon-Cardo, C., Guise, T.A., and Massague, J. (2003). Cancer Cell *3*, 537–549.

Mundy, G.R. (2002). Nat. Rev. Cancer *2*, 584–593.

Zhang, X., Wang, Q., Gerald, W., Hudis, C.A., Norton, L., Smid, M., Foekens, J.A., and Massague, J. (2009). Cancer Cell *16*, this issue, 67–78.