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One NOTCH Further: Jagged 1 in Bone Metastasis

Jianning Tao,[1] Ayelet Erez,[1] and Brendan Lee[1,2,*]
[1]Department of Molecular and Human Genetics
[2]Howard Hughes Medical Institute
Baylor College of Medicine, Houston, Texas 77030, USA
*Correspondence: blee@bcm.edu
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The outgrowth of metastatic cells to bone depends on the interaction between multiple intrinsic and host factors. In this issue of *Cancer Cell*, Sethi and colleagues report Notch signaling in bone cells as responsible for promoting this outgrowth and provide evidence for a beneficial treatment effect of NOTCH inhibitors.

Metastasis, the last and most devastating stage of tumor progression, remains the cause of 90% death in cancer patients. The development of metastases requires a series of sequential rate-limiting steps through which malignant tumor cells from the primary site invade into blood and lymphatic vasculature, survive in the circulation, lodge at distant organs, and outgrow. The revised "seed and soil" theory, originally proposed by Steven Paget a century ago, hypothesizes that the outcome of metastasis depends on crosstalk between predetermined cancer cells (the "seeds") and specific organ microenvironments (the "soil"), which release homeostatic factors (Fidler and Poste, 2008). In the soil organ, the seed cancer cells can enter either latent phase or outgrowth phase.

The skeletal system is recognized as the habitat of the hematopoietic stem cell as well as the most common metastatic site for breast cancer. Recently, a positive feedback loop causing a "vicious cycle" has been identified, in which the outgrowth phase of the bone metastases is determined by a bidirectional interaction between the cancer cells and the bone microenvironment. This crosstalk involves growth factors and cytokines derived from both host and cancer cells (Figure 1) (Kang et al., 2003; Zhang et al., 2009).

Seventy percent of breast cancer patients are affected by bone metastasis, manifested by skeletal-related events such as severe bone pain and pathological fractures (Mundy, 2002). There is much evidence that metastatic cancer cells usurp the normal process of bone remodeling through stimulation of both osteoclasts that resorb bone and osteoblasts that deposit bone, and the net outcome of lesions depends on the relative contribution of each cell type. In the outgrowth (or osteolytic) phase, multiple growth factors, including transforming growth factor β (TGFβ) and insulin-like growth factor 1 (IGF1), are released from the degraded bone matrix. TGFβ and IGF1 both enhance the growth of cancer cells and stimulate them to produce several cytokines, including parathyroid hormone-related protein (PTHrP), connective tissue growth factor (CTGF), and interleukin 11 (IL11). PTHrP and IL11 regulate the expression of osteoclastogenic factors receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG) in osteoblasts, whereas CTGF mediates both angiogenesis and invasion (Massague, 2008). Additional cells, such as bone borrow-derived stromal, endothelial, and hematopoietic cells, have all been shown to contribute to the development of the macrometastases and the production of prometastatic factors (Joyce and Pollard, 2009). On the other hand, the processes by which metastatic cancer cells directly communicate with various types of cells in the bone and bone marrow remains an enigma in the field of bone metastasis. Undoubtedly, fully answering such questions will provide the insight necessary for the development of effective therapies against bone metastasis.

Sethi et al. (2011) now provide both experimental and preclinical evidence that the Notch ligand Jagged1 plays a critical role in the promotion of bone metastatic outgrowth of breast cancer. Using a bioinformatic approach that correlates the gene expression pattern of Notch signaling pathway components (ligands, receptors, and downstream targets) to bone metastasis, the authors indentified a unique upregulation of Jagged1, which was highly correlated with human breast cancer metastases to bone. To investigate the functional role of Jagged1 in the development of bone metastasis, the authors applied two different types of Jagged1-expressing human breast cancer cell lines in a xenograft mouse model. In the strongly bone tropic cell lines with high levels of Jagged1 expression, stable knockdown of Jagged1 resulted in a reduction of

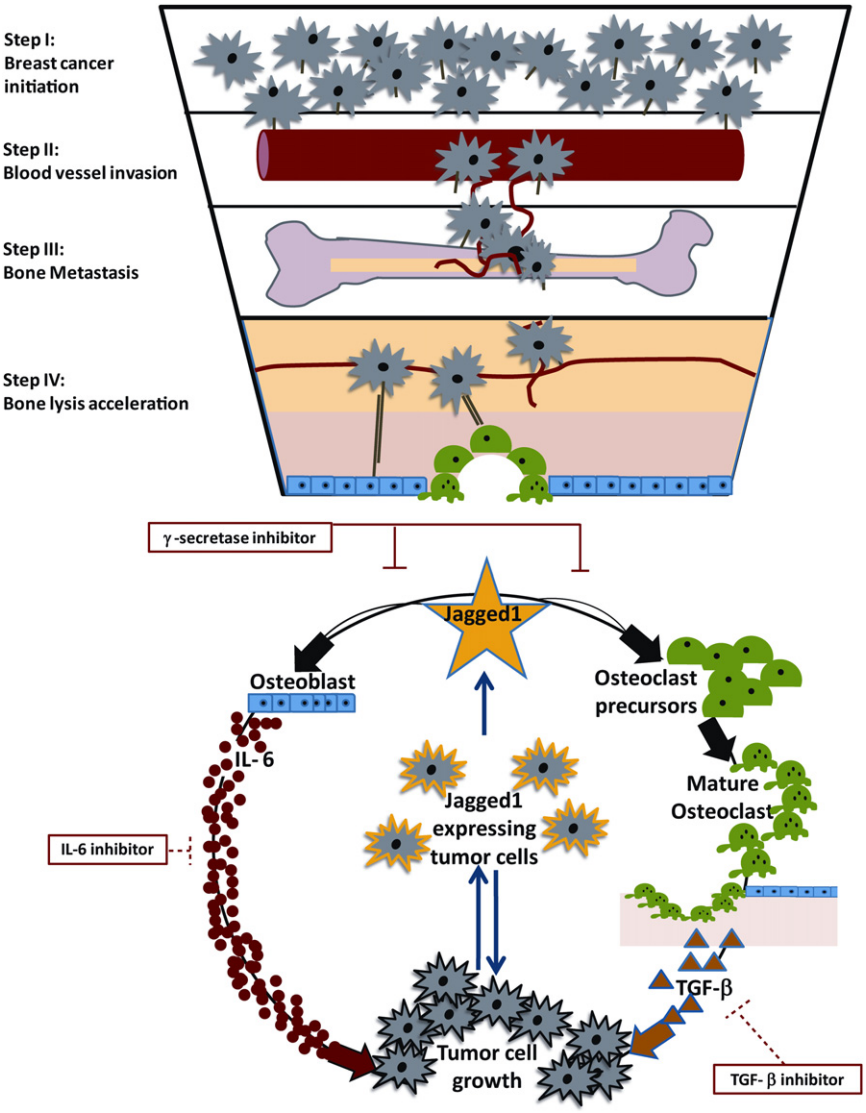

Figure 1. The Role of Jagged1 in the "Vicious Cycle" of Breast Cancer Osteolytic Metastases
The upper panel schematically describes the steps required for tumor progression from cancer initiation to osteolytic metastases. After cancer initiation, the cells invade the blood vessel, home in the bone marrow, and proceed with the last phase, which is the osteolytic outgrowth. The lower panel shows a new view of the "vicious cycle," which is the focus of the article by Sethi et al (2011). Here, the breast cancer cells expressing Jagged1 directly interact with osteoblasts and preosteoclasts, resulting in the release of active IL6 and TGFβ, respectively, promoting the tumor outgrowth. This positive feedback/crosstalk between the breast cancer cells (the "seed") and the bone cells (the "soil") is crucial for the development of breast cancer bone macrometastases. The inhibitors of Notch signaling, IL6, and TGFbeta, which have been extensively used in this study, are demonstrated in this figure, as they might be beneficial clinically in the future.

tumor outgrowth of bone lesions, while in the weakly bone tropic cell lines with low expression of Jagged1, overexpression of Jagged1 resulted in a significant increase tumor outgrowth of bone lesions. In both cases, there was no change in cells' proliferative and invasive abilities, either in culture or as primary tumors, suggesting that Jagged1 is necessary and sufficient to promote osteolytic bone metastasis.

TGFβ is a central component of the "vicious cycle" in bone metastatic outgrowth (Massague, 2008). Using gene-set enrichment analysis, the authors found that Jagged1 is one of ten core genes that respond to TGFβ, among them IL11 and CTGF, which have been previously identified as targets of TGFβ in metastasis (Kang et al., 2003). Indeed, Jagged1 mRNA and protein levels in cultured breast cancer cells significantly increased in the presence of excess active TGFβ, mimicking the in vivo pathological environment of osteolytic bone metastases. To confirm this finding, Sethi et al. (2011) applied pharmacological inhibitors and an inducible Smad4 knockdown system to block TGFβ signaling in vitro and in vivo, verifying that Jagged1 is an essential target of the TGF-β pathway. Notably, Jagged1 overexpression restored the ability of Smad4 knockdown cells to generate osteolytic bone metastasis. Those findings establish Jagged1 as a critical component in this "vicious cycle."

The authors observed severe osteolytic phenotype in the Jagged1-mediated bone metastases, which is attributable to elevated osteoclastic activity. The classic view suggests that metastasized cancer cells indirectly regulate osteoclastic activity via PTHrP-induced expression of osteoblast-derived RANKL and OPG (Mundy, 2002). Augmented RANKL to OPG ratio is responsible for one of the major determinants of pathological osteoclastic activity and bone-destructive function. Using a co-culture system that contains both tumor cells and preosteoclastic cells, Sethi et al. (2011) demonstrated that Jagged1-expressing cells directly interact with preosteoclast to increase this activity by accelerating osteoclast differentiation and maturation. In contrast, the authors show that RANKL and OPG levels are not changed when osteoblasts are co-cultured with tumor cells. Furthermore, the pharmacological inhibitor of Notch signaling suppresses Jagged1-mediated Notch activity. Although this finding still needs to be confirmed in vivo, this new mechanism advances our understanding of the processes that underlie osteolytic bone metastasis.

A persistent outgrowth of bone metastases likely depends on many intrinsic tumor cell-specific and bone/bone marrow-specific factors. To identify factors in the bone microenvironment that promote outgrowth of metastatic breast cancer, the authors performed a series of elegant experiments using a co-culture system that contains both tumor cells and osteoblasts, the major secretory cell of the skeletal system. They first found that cultured tumor cells acquired a growth advantage via activating Notch pathway in osteoblasts.

Next, they performed microarray profiling of the co-cultured osteoblasts and found IL6, a proproliferative cytokine involved in bone metastasis (Ara et al., 2009), among the upregulated genes. Using a combination of strategies, the authors nicely demonstrate that IL6 is a downstream target of the Jagged1-Notch-Rbpj-Hey1 cascade, and is released from osteoblasts to promote tumor proliferation.

Notch signaling is a central pathway for regulating cell-cell interaction during embryonic development and is also involved in the pathogenesis of skeletal diseases (Tao et al., 2010). Hence, it is not unexpected that pharmacological inhibitors that block Notch pathway signaling will have a beneficial effect in both co-cultured Jagged1-expressing tumor cells and bone cells, as well as in the mouse model. However, a remaining clinically relevant question is whether these inhibitors can inhibit the outgrowth of bone metastasis in patients. Another important area that is critical for clinical translation would be the effect of Notch inhibition in other components of the skeletal system (Engin et. al., 2008), as well as elsewhere in the body.

In summary, the work presented by Sethi et al. (2011) identified a novel "seed and soil" crosstalk mediated by the TGFβ-Jagged1-Notch-IL6 signaling network that promotes the outgrowth of bone metastasis. The knowledge gained in this study contributes to our understanding of the pathogenesis of bone metastases and aids in finding therapy against it. In addition, it opens doors for many remaining unanswered questions. Does Jagged1 activate Notch signaling in other bone marrow-residing cells? How does Notch pathway interplay with other factors involved in bone metastasis? Will the use of Notch inhibitors together with inhibitors of IL6 or/and TGFβ be synergistic in halting bone metastasis?

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HiJAKing the Methylosome in Myeloproliferative Disorders

Radek C. Skoda[1,*] and Jürg Schwaller[1]
[1]Department of Biomedicine, University Hospital Basel, Hebelstrasse 20, 4031 Basel, Switzerland
*Correspondence: radek.skoda@unibas.ch
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***JAK2* gain-of-function mutations have been shown to cause myeloproliferative neoplasms. In this issue of *Cancer Cell*, Liu et al. (2011) demonstrate that these JAK2 mutants, but not wild-type JAK2, directly phosphorylate PRMT5 and inhibit its arginine methyltransferase activity, establishing a link between mutant JAK2 and histone arginine methylation.**

Janus kinase 2 (JAK2) is a ubiquitously expressed intracellular tyrosine kinase that associates with the cytoplasmic domains of hematopoietic cytokine receptors and becomes activated upon these receptors binding to their cognate ligands. Activating mutations in *JAK2* have been found in the majority of patients with myeloproliferative neoplasms (MPN), which represent clonal hematopoietic stem cell diseases characterized by increased proliferation of the erythroid, megakaryocytic, or myeloid lineages. The vast majority of patients with MPN (about 80%) carry mutations in *JAK2* codon 617 that exchanges a valine with a phenylalanine (*JAK2*-V617F) (Skoda, 2007). This mutated JAK2-V617F is an activated tyrosine kinase that renders hematopoietic cells hypersensitive for signals from upstream cytokine receptors (Epo, Tpo, and G-CSF) and phosphorylates (STAT3 and STAT5), as well as other downstream signaling proteins. In a minority of patients (less than 3% of MPNs), *JAK2* mutations have been found in codon 539 (e.g., *JAK2*-K539L) or in neighboring codons, leading to a variant of MPN with selectively increased numbers of erythroid cells. The mutant JAK2 proteins were shown to activate proliferation, inhibit apoptosis, and interfere with genome stability. However, all