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Perspective Article

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Interactions between tumor and bone alter the phenotypes of both

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The majority of patients dying from cancer of the breast or prostate have metastases to the skeleton. The affinity of these and several other solid tumors for this metastatic site is the consequence of the special microenvironment provided by bone. Stephen Paget in 1889 proposed the seed and soil hypothesis: bone provides the fertile soil in which certain cancer cell seeds prefer to grow[1]. We now appreciate that mineralized bone matrix is a rich storehouse of growth factors[2], which are mobilized by osteoclastic bone resorption. The released growth factors enrich the local microenvironment. A main effect of the factors appears to be alteration of the tumor cell phenotype rather than an increased growth rate. Tumor cells in turn secrete additional factors that act upon bone cells, causing the responses that characterize the osteolytic and osteoblastic metastases. Local interactions between tumor cells and bone form a vicious cycle, which underlies the development of skeletal metastases[3,4]. Interactions between tumor cells and bone cells change the phenotypes of both.

The basic mechanisms of cancer metastases to specific sites have been controversial. Cancer cells *in vivo* continue to mutate. The ability of cancer cells to metastasize is characteristic of advanced disease and could occur only after the gradual accumulation of a necessary set of pro-metastatic mutations[5]. However, recent experiments suggest that a constellation of expressed genes necessary for metastasis to bone pre-exists within the primary breast tumor[6]. Thus, bone-specific metastases are the consequence of a selection of variants from a heterogeneous population of cells within the primary tumor, plus changes in gene expression induced by bone factors. Cells of the osteoblastic lineage appear to be the main targets of tumor-secreted factors. Bone-derived transforming growth factor-β (TGFβ) is a major factor regulator of tumor cell behavior in bone.

There are no convenient animal models where primary tumors reproducibly metastasize to bone. Many of the results described here use an animal model in which human tumor cells are inoculated into immuno-deficient mice. Injection into the venous circulation most often results in tumor cell entrapment within the capillary beds of the lung or liver. However, careful tumor administration directly into the left cardiac ventricle can result in 100% incidence of bone metastases with many tumor cell lines. Osteolytic lesions are detected by X-ray as early as 3 weeks and can be quantified by image analysis. Osteoblastic lesions may take up to 6 months to develop in nude mice, and the lesions cannot be quantified radiographically.

Osteolytic metastases are characteristic of breast cancer. The most prominent cause of bone destruction is parathyroid hormone-related protein, PTHrP, which is secreted by many cancer types and, when systemically elevated, is responsible for humoral hypercalcemia of malignancy (HHM). Breast cancer cells that secrete PTHrP in concentrations insufficient to induce HHM still cause extensive osteolytic bone destruction in nude mice. Bone lesions and tumor burden can be significantly decreased, and survival increased, by treatment with PTHrP-neutralizing antibody[7]. The antibody has been humanized and is in clinical trials against HHM. PTHrP cannot be the only factor responsible for bone metastases, and a number of other proteins play either contributory or PTHrP-independent roles[3]. Candidate factors that may contribute to PTHrP-induced osteolytic lesions are interleukin (IL)-11, macrophage colony-stimulating factor (M-CSF), and vascular endothelial growth factor (VEGF)[8]. A number of PTHrP-independent factors have also been reported, included IL-8[9].

Kang et al.[6] compared less- and more-metastatic variants of a breast cancer cell line by gene expression profiling. They

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identified mRNAs whose expression strongly correlated with increased bone metastasis. Five of the mRNAs encoded IL-11, matrix metalloproteinase (MMP)-1, osteopontin, connective tissue growth factor (CTGF), and CXCR-4. MMP-1 is an interstitial collagenase made by osteoblasts. It cleaves collagen at a site resistant to osteoclastic enzyme hydrolysis and may be rate-limiting in normal bone resorption[10]. Osteopontin plays a complex role in metastasis, including modulation of anti-tumor immune responses[11]. CTGF is a potent osteoblast-stimulatory factor[12], as well as being expressed by tumor cells. CXCR-4 is the receptor for the chemokine SDF-1 and functions in the attraction of breast cancer cells to specific metastatic sites including, but not limited to, bone[13]. Kang et al. found that the pro-metastatic gene set was coordinately increased in cells, which pre-existed in the original cell population. The authors attempted to convert low-metastatic MDA-MB-231 breast cancer cell line clones into ones highly metastatic to bone by overexpressing each of the five individual factors. They found that conversion of the cells to a phenotype of aggressive metastasis to bone required co-transduction of combinations of four of the five factors. The results strongly support a multi-factorial mechanism underlying organ-specific metastases.

Many osteolytic factors act via osteoblasts and stimulate osteoclastic bone resorption indirectly, rather than acting directly on cells of the osteoclast lineage[14]. This has been shown for PTHrP, which induces osteoblastic expression of RANK ligand[15]. IL-11 can act similarly, while M-CSF and VEGF serve as co-factors for the RANK ligand-stimulated differentiation of hemopoietic precursors into active osteoclasts.

Osteoblastic metastases are characteristic of prostate cancer and also occur in 15% of breast cancer bone metastases. The tumor-induced lesions are characterized by disorganized new bone formation and are accompanied by increased bone resorption. A number of candidate factors made by tumor cells could stimulate osteoblasts, but progress has lagged in the area until recently. Endothelin-1 (ET-1), a 21-amino acid vasoactive peptide is a potent stimulator of new bone formation. It is secreted by tumor cells and can cause osteoblastic metastases in the nude mouse model. Metastases are effectively blocked with a selective antagonist of the endothelin A receptor[16,17]. This orally active antagonist is in clinical trials in men with advanced metastatic prostate cancer[18]. The vicious cycle model predicts that osteoblasts, osteoclasts and tumor cells cooperate to cause the pathology of bone metastases. The endothelin receptor antagonist blocks the activation of osteoblasts by tumor-produced ET-1. It also decreases osteoclastic bone resorption, as indicated by decreases in markers of resorption seen in the patient trials. Conversely, bisphosphonates effectively reduce skeletal-related events in prostate cancer[19]. These observations support a role for the vicious cycle in patients.

Other factors responsible for osteoblastic metastases remain to be identified. Such factors need to meet two initial criteria: 1) ability to stimulate osteoblastic new bone formation, and 2) expression by cancer cells. The bone morphogenetic proteins (BMPs) are obvious candidates, but a causal role in bone metastases has not been demonstrated. CTGF, identified in the experiments of Kang et al.[6] is another factor that stimulates osteoblasts[12]. Adrenomedullin is a 52-amino acid vasoactive peptide with potent bone-stimulatory actions[20]. It is made by many cancers[21], and we have recent data that it increases bone metastases *in vivo.*

Mixed osteolytic/osteoblastic metastases are characteristic of both breast and prostate cancers. The effect of combined expression of osteolytic and osteoblastic factors on bone has not been studied, so the net response of bone at the metastatic site is unpredictable. As noted above, osteolytic factors such as PTHrP and IL-11 act on osteoblasts to increase expression of RANK ligand. We tested the effects of introducing the osteoblastic factor, ET-1, into the PTHrP-secreting MDA-MB-231 breast cancer cell line. Instead of converting the bone response from osteolytic to osteoblastic, the bone-destructive effects were enhanced by ET-1. Some of this effect may be caused by autocrine responses of the tumor cells to ET-1. We believe that osteoblastic factors can stimulate osteoblast proliferation, increasing the population of early osteoblasts[22]. The enlarged pool of early osteoblasts responds to osteolytic factors by increased expression of RANK ligand[23].

Another puzzling question has been the role of PTHrP in osteoblastic metastases, especially those due to prostate cancers, which nearly always express PTHrP. A partial explanation was provided by the observation that prostate-specific antigen (PSA) is a serine protease, which cleaves PTHrP after phenylalanine residues 22 and 23[24,25]. The resulting fragment fails to activate the classical PTH/PTHrP receptor. This is not the end of the story. It was later observed that the inactive fragment PTHrP1-16 increased cyclic AMP in cardiomyocytes by binding to the endothelin A receptor. Binding was attributed to a 4 amino acid near-identity between the two peptides[26]. We have extended these observations to bone. PTHrP1-23 is a potent stimulator of calvarial new bone formation at concentrations as low as 1nM. The results suggest that PSA proteolysis of PTHrP, rather than inactivating it, converts the protein from an osteolytic factor to a potent osteoblastic one.

The effects of bone-derived factors on tumor cells remain understudied. Van der Pluijm et al.[8] elegantly demonstrated that several mRNAs are increased in bone versus non-bone sites of human breast cancer metastases in nude mice. RNA abundances were determined by species-specific RT-PCR. PTHrP, VEGFs and M-CSF were increased specifically in bone, while several mouse markers of host angiogenesis were similarly increased. These experiments did not identify the factor(s) responsible for the bone-specific mRNA induction. Hauschka et al.[2] found that insulin-like growth factors (IGFs) -II, then -I, were the most abundant factors in bone matrix, followed by TGFβ, after which were lower concentrations of BMPs, fibroblast growth factors -1 and -2, and platelet-derived growth factor. Of these, only TGFβ has

been shown to play a direct role in stimulating tumor cells. TGFβ is growth-inhibitory in the early stages of tumorigenesis. Advanced cancers lose growth inhibition but retain TGFβ regulation of metastasis-promoting genes[27], such as CTGF and IL-11, identified by Kang et al.[6], and PTHrP[28]. In the MDA-MB-231 model of breast cancer metastasis to bone, detailed experiments showed that tumor cell expression of PTHrP is the major target of TGFβ and that TGFβ is the most important regulator of PTHrP[29]. These experiments also showed that dual pathways in the tumor cells, through p38 MAP kinase and through the Smad proteins, transmit TGFβ signaling to the nucleus. Osteoclastic bone resorption specifically activates TGFβ from its stored form in bone matrix[30]. This step may be another point at which the efficacy of bisphosphonates against bone metastases is exerted[19].

Future directions in the field include the continuing identification of candidate osteolytic and osteoblastic factors. Many of those already identified require substantial further testing *in vivo* to determine whether they are valid targets for therapeutic intervention. They include IL-11, CTGF, adrenomedullin, and CXCR4. The roles of the abundant, bone-derived IGFs I and II need to be tested for their contributions to metastases to bone. The relation between bone metastases and angiogenesis remains to be clarified. On a broader scale are the clinical consequences of bone metastases, in particular severe bone pain and systemic weight loss (cachexia). ET-1 for example is nociceptive. Mechanisms of bone pain are specific and under active investigation[31]. It seems likely that bone metastases release unknown factors into the circulation, which stimulate wasting of skeletal muscle[32]. These syndromes are of great consequence to the patients who suffer from cancers of the breast and prostate, which are incurable once they become housed in bone[4]. Tumor-bone interactions, and the secreted factors which mediate them, offer targets for future therapeutic intervention to ameliorate or perhaps prevent skeletal metastases.

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