Basic Mechanisms Responsible for Osteolytic and Osteoblastic Bone Metastases

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Abstract

Certain solid tumors metastasize to bone and cause osteolysis and abnormal new bone formation. The respective phenotypes of dysregulated bone destruction and bone formation represent two ends of a spectrum, and most patients will have evidence of both. The mechanisms responsible for tumor growth in bone are complex and involve tumor stimulation of the osteoclast and the osteoblast as well as the response of the bone microenvironment. Furthermore, factors that increase bone resorption, independent of tumor, such as sex steroid deficiency, may contribute to this vicious cycle of tumor growth in bone. This article discusses mechanisms and therapeutic implications of osteolytic and osteoblastic bone metastases.

Breast Cancer: The Prototypic Osteolytic Tumor

Breast cancer commonly metastasizes to and destroys bone, causing pain and fracture. Tumors produce many factors that stimulate osteolysis: parathyroid hormone-related protein (PTHrP), interleukin (IL)-11, IL-8, IL-6, and receptor activator of nuclear factor-κB ligand (RANKL; refs. 4–9). Substantial data support a role for bone-derived transforming growth factor-β (TGF-β) and tumor-derived osteolytic factors, such as PTHrP, in a vicious cycle of local bone destruction in osteolytic metastases. Bone matrix stores several immobilized growth factors, particularly TGF-β, which is released in active form during osteoclastic resorption (10) and stimulates PTHrP production by tumor cells. PTHrP in turn mediates bone destruction by stimulating osteoclasts. A dominant-negative mutant of the type II TGF-β receptor inhibited TGF-β-induced PTHrP secretion in vitro and development of bone metastases in an MDA-MB-231 experimental metastasis model (5, 6). In addition, TGF-β regulates several genes that are responsible for enhanced bone metastases in MDA-MB-231: IL-11 and connective tissue growth factor (CTGF; refs. 8, 9). Collectively, these studies provided proof of principle to support a role for TGF-β blockade in the treatment of breast cancer bone metastases.

SD-208, a small-molecule inhibitor of TGF-β receptor I kinase, inhibits signaling downstream of the TGF-β receptor. The in vivo efficacy of SD-208 was evaluated in an experimental metastasis model using the breast carcinoma cell line MDA-MB-231. SD-208 reduced the development and progression of osteolytic bone metastases as assessed by computerized image analysis of radiographs and prolonged survival. To dissect the mechanism that regulates these in vivo therapeutic benefits, the effects of SD-208 were analyzed in cultured MDA-MB-231 cells. SD-208 did not affect cell proliferation. However, SD-208 inhibited TGF-β-induced smad2/3 phosphorylation as well as decreased production of TGF-β-stimulated osteolytic factors IL-11 and PTHrP and the growth-promoting factor CTGF. It also reduced TGF-β secretion from MDA-MB-231 cells, suggesting possible autocrine effects of TGF-β on the cancer cells. Taken together, these data indicate that therapeutic targeting of TGF-β may decrease the osteolytic bone metastases due to breast cancer by blocking tumor production of osteolytic and growth-promoting factors, such as IL-11, PTHrP, and CTGF.
Prostate cancer has a propensity to metastasize to bone and locally disrupt normal bone remodeling. Although such metastases have been classified as osteoblastic based on the radiographic appearance of the lesion, it is clear that bone resorption and bone formation are dysregulated. Recent clinical evidence indicates that both processes contribute to the metastatic phenotype even in the same patient. In fact, high concentrations of the bone resorption marker N-telopeptide were recently shown to predict a poor clinical outcome in men with prostate cancer and bone metastases (11, 12), and bone fractures predict poor survival in prostate cancer patients (13). Thus, an understanding of the mechanisms of tumor-induced bone formation and the role of the osteoclast in this process is critical to improve therapy. Mediators of osteoblastic disease are discussed herein as well as preclinical data to target these mediators in combination with antiresorptive therapy.

**Osteoblastic bone metastases: molecular mechanisms of ET-1 action on osteoblasts.** Tumor production of growth factors, such as platelet-derived growth factor, insulin-like growth factors, and adrenomedullin, has been implicated in osteoblastic bone metastases (2, 14–20). Recently, several groups have identified a role of the vasoactive peptide ET-1 in stimulating the new bone formation associated with osteoblastic metastases via the endothelin A receptor (ET\_A)R in mice and humans. An ET\_A antagonist (trasentan) prevented osteoblastic bone metastases in a mouse model and reduced skeletal morbidity in men with advanced prostate cancer (15–18). However, the molecular mechanisms through which ET-1 stimulates osteoblasts are unclear. Downstream targets of ET-1 in osteoblasts were identified by gene array analysis of RNA isolated from calvariae treated with or without ET-1 for 6 hours and 1, 4, and 7 days using mouse Affymetrix (Santa Clara, CA) GeneChips (22,600 genes). Up-regulated genes with possible roles in osteoblast function included secreted factors (IL-6, Wnt5a, TIMP-3, Cyr61, CTGF, and RANKL), signaling molecules (SGK), and transcription factors (TSC-22, C/EBP \(\delta\), TGIF, and Twist 2). ET-1 significantly down-regulated Dkk1, a secreted inhibitor of the Wnt signaling pathway recently implicated in suppressed bone formation of multiple myeloma (21). Of these factors, only IL-6, Cyr61, CTGF, TIMP-3, TGIF, RANKL, and Dkk1 were validated in primary osteoblasts. Dkk1 abrogated ET-1-induced osteoblast proliferation and new bone formation in calvarial organ cultures but did not inhibit basal osteoblast activity. Thus, ET-1 decreases Dkk1 expression and relieves tonic-negative regulation of osteoblast activity (22). In multiple myeloma bone disease, Dkk1 may depress bone formation. The opposite may occur in osteoblastic disease, where ET-1 stimulates osteoblast activity by decreasing autocrine production of the negative regulator Dkk1.

**Osteoblastic bone metastases: role of PTHrP fragments.** Prostate cancer metastases to the skeleton are frequently osteoblastic despite abundant expression of the osteolytic factor PTHrP. A proposed explanation for this paradox is that NH\_2-terminal fragments of PTHrP stimulate new bone formation by activating the ET\_A receptor. PTHrP 1 to 16 stimulated osteoblast proliferation and new bone formation in an *ex vivo* calvarial organ culture assay. The response was equivalent to that caused by equimolar ET-1, a 21-amino acid ligand for the ET\_A receptor that potently stimulates new bone formation. ET-1 residues 6 to 9 (LMDK) and PTHrP residues 8 to 11 (LHDK) share strong sequence homology that may explain the agonist properties of both peptides (23). The effects on new bone area and osteoblast number were blocked by ABT-627 (trasentan), an ET\_A antagonist, which did not block new bone formation stimulated by fibroblast growth factor-2. We found similarly strong anabolic responses to PTHrP 1 to 20 and 1 to 23, whereas PTHrP 1 to 34 instead caused extensive osteolysis. These data show that NH\_2-terminal peptides of PTHrP exert potent anabolic effects on bone via the ET\_A receptor. The NH\_2-terminal peptides do not bind to ET\_A receptors when overexpressed in mammalian cells, suggesting that an accessory receptor subunit is expressed in certain cell types, such as osteoblasts, making them responsive to these alternative ET\_A receptor ligands. The sequence of PTHrP 18 to 23, RRRFF, is cleaved by prostate-specific antigen. Proteolysis at this site frees residues 8 to 11 to bind to ET\_A receptors, providing a molecular explanation for the osteoblastic phenotype of PTHrP-positive prostate cancer bone metastases. The mimicry of ET-1 by PTHrP NH\_2-terminal peptides suggests that ET\_A antagonists could be effective in treating prostate cancer, both against the actions of endothelin itself and against the anabolic effects of PTHrP fragments.

**Osteoblastic bone metastases: role of osteoclastic bone resorption.** Osteoclastic bone resorption also contributes to the pathophysiology of osteoblastic metastases. Resorption markers are increased (11, 12), and bisphosphonate anti-resorptive drugs reduce skeletal morbidity in patients with osteoblastic disease (24). To determine if osteoclast activity contributes to skeletal morbidity in patients with prostate cancer, we used a preclinical animal model of osteoblastic prostate cancer to test the effect on tumor growth in bone of osteoblast inhibition with trasentan or osteoclast inhibition with bisphosphonate (zoledronic acid) as single agents or in combination with antiresorptive therapy.
Role of the bone microenvironment: increased bone turnover promotes tumor growth. The skeleton is the most common site of metastases in patients with advanced prostate cancer. Androgen ablation, a standard treatment for prostate cancer, increases osteoblastic bone resorption and bone loss (25, 26).

To determine if this provides a more fertile environment for bone metastasis, we developed two mouse models of metastasis to bone that mimic the clinical scenario of men rendered hypogonadal from treatment for prostate cancer. The first uses PC-3 prostate cancer cells that result in osteolytic bone metastases following intracardiac inoculation in nude mice. The second model, TSU-Pr1, is a bladder cancer cell line that results in mixed osteolytic-osteoblastic bone metastases. Hypogonadal mice had bone loss due to increased osteoclastic bone resorption and, when inoculated with the human prostate cancer cell line PC-3 or the human bladder cancer cell line TSU-Pr1, had accelerated bone metastases. Treatment with the zoledronic acid prevented bone loss due to androgen deprivation and also reduced metastases to bone. These data support the hypothesis that increased bone resorption due to androgen deprivation may result in a more fertile environment for the development of bone metastases. Bone resorption inhibitors, such as bisphosphonates, should prevent bone loss in hypogonadal men with advanced prostate cancer and may also decrease skeletal metastases.

Taken together, these data indicate that bone metastases are the result of complex interactions among tumor cells, bone cells, and the bone microenvironment. Targeting these interactions should lead to effective treatment of osteolytic bone metastases.

Open Discussion

Dr. Pearse: You are getting massive bone formation in your xenograft model. Do you think this new bone sequesters growth factors, such as IGF and TGF-β, to be released upon osteoclast resorption?

Dr. Guise: Wherever this bone formation occurs, there is usually an osteolytic response as well. So when you form abnormal, disorganized new bone, the body’s response is to remodel that bone, and you get increased osteoblastic activity.

Dr. Vessella: You can see osteolytic and osteoblastic events in the same section, but it is not concurrent. It is not that one lesion is osteolytic and the other lesion is osteoblastic. Within the same lesion you can see both types of activities, so it is a vicious cycle.

Dr. Roodman: What is the contribution of coupling in addition to this? These are not totally uncoupled like myeloma. Is there a contribution also simply to bone resorption followed by bone formation? Is part of the role of bisphosphonates to uncouple the process?

Dr. Guise: There is definitely a coupling response and the bisphosphonates uncouple that. When you get blastic activity, bone resorption follows. There may be additional contributions of tumor osteolytic factors as well.

Dr. Lipton: What about PDGF and VEGF?

Dr. Guise: There is some controversy regarding the role of angiogenesis and bone metastases, because bone is already a prevascular tissue; this is an understudied area but clearly an important one. VEGF stimulates not only angiogenesis but also osteoclastic bone resorption and osteoblast bone formation, so it could have multiple effects. Many of these angiogenic factors, such as PDGF and VEGF, and also osteolytic factors, such as IL-11 and IL-8, are regulated by HIF-1α, which is the hypoxia-induced factor and like TGF-β may be an upstream master switch to turn on many osteolytic factors. The angiogenesis aspects of bone metastasis have been understudied in a mechanistic way. We are creating a bone angiogenesis model to look at some of the mechanisms by which osteolytic and osteoblastic disease occur.

Dr. Clohisy: One of the things that has always bothered me about the osteoblastic models is that they don’t look like prostate metastases, they look more like osteosarcomas. Do you think we have finished developing classic models? How would you recommend we move forward?

Dr. Guise: What I showed you in terms of the prostate models are about as good as we have right now, and they are certainly not the best that we could have. We directly inject the tumor cells into the bone, which is why it probably looks like an osteosarcoma. We’ve tried to make it metastasize, like a real metastasis model, but we have a ways to go.

Dr. Weilbaecher: In patients who have prostate cancer bone metastasis, does it correlate that the higher the PSA the worse the metastasis? Do PSA neutralizing antibodies exist and could you use that in your models to confirm this mechanism?

Dr. Guise: This is a difficult project, because it’s hard to make the jump from in vitro to clinical significance. I don’t know, but those of you who see cancer patients may know if there is a correlation between PSA increase and the phenotype of bone metastases.

Dr. Smith: I don’t know if that has been specifically addressed. In men with hormone refractory prostate cancer with bone metastases, markers of osteoclast and osteoblast activity are highly correlated. It also appears that the most well-differentiated tumors tend to make the most PSA and the least well-differentiated tumors tend to form more lytic metastases.

Dr. Weilbaecher: Do neutralizing antibodies exist?

Dr. Vessella: Neutralizing antibodies to PSA at this point in time have not been able to affect what you see in bone. The PSA that is produced exists in the serum as a complex, and only at the local region is it an active enzyme. Apparently, we are not getting enough of the antibody into the site to neutralize the active
enzyme. It certainly drops the PSA level systemically, but it doesn’t get into the bone site enough to neutralize the active PSA.

Dr. Roodman: We are finding more and more factors, but do we have to target all of them to get rid of them or do we only have to target a couple because they are all acting at subpharmacologic levels?

Dr. Guise: It’s becoming so complex that just targeting one factor or figuring out which is the most important is difficult because all tumors are different. We need to understand what factors are upstream of many of these things. Just targeting the factors themselves is not going to be the whole answer. We are going to have to use different therapeutic modalities to target the cancer cells themselves.

Dr. Roodman: Why don’t we kill the osteoclasts and the osteoblasts and not worry about all of the signal pathways?

Dr. Guise: I agree with you totally. We have to think about this as we begin to understand or discover the complexity of the system.

References

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*Clin Cancer Res* 2006;12:6213s-6216s.

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