Bone metastases are the most common skeletal manifestation of malignancy. Certain cancers have a propensity to metastasize to bone and cause destruction or new bone formation. The osteolytic or osteoblastic phenotypes are due to the action of tumor-produced factors to stimulate either osteoclast or osteoblast activity and disrupt normal bone remodeling. Thus, the tenacity with which certain tumors grow in bone depends on tumor cell-bone cell interactions as well as the fertile soil of the bone microenvironment. In both metastatic skeletal phenotypes, these tumor cell-bone cell interactions constitute a vicious cycle in which tumor cells stimulate the respective bone cells to cause either bone destruction or new bone formation. As a consequence, the bone microenvironment provides the tumor cells with growth factors which further fuel tumor growth in bone.

Osteolytic metastasis

Role of tumor-produced PTHrP and bone-derived TGFβ:

Osteolytic bone destruction by metastatic cancer is mediated by the osteoclast. Substantial data support major roles for bone-derived TGFβ and tumor-derived parathyroid hormone-related protein (PTHrP) in the vicious cycle of local bone destruction which characterizes osteolytic metastases. Tumor-produced PTHrP stimulates osteoclastic bone resorption to result in the bone destruction associated with breast cancer metastases. Neutralizing antibodies to PTHrP not only reduced osteoclastic bone resorption but also inhibited the development of metastases to bone by the human breast cancer cell line, MDA-MB-231. Transforming growth factor (TGFβ), stored in bone matrix and released locally in active form during osteoclastic resorption stimulates PTHrP production by tumor cells. A dominant-negative TGFβ type II receptor (TβRIIΔcyt) stably expressed in MDA-MB-231 breast cancer line rendered the cells unresponsive to TGFβ, inhibited TGFβ-induced PTHrP secretion and the development of bone metastases in a mouse model. This dominant-negative type II blockade was reversed by a constitutively active TGFβ type I receptor (TβRI(T204D)). Furthermore, transfection of the cDNA for PTHrP into the original dominant negative MDA-MB-231 line also resulted in increased PTHrP production and accelerated bone metastases. These published data establish that TGFβ in bone can promote osteolysis by increasing PTHrP secretion from breast cancer cells. They do not, however, exclude contributions from other TGFβ-responsive tumor factors.

PTHRP is the effector of TGFβ:

To determine if PTHrP is the major mediator of TGFβ-induced osteolysis, mice were inoculated with an MDA-MB-231 clonal line overexpressing the constitutively active type I TGFβ receptor, TβRI(T204D), and treated with neutralizing PTHrP antibody or control IgG. The mice treated with PTHrP antibody had significantly lower tumor burden than the control mice, suggesting that the major downstream effector of TGFβ in the development and progression of bone metastases was PTHrP.

TGFβ increases PTHrP via Smad and MAP kinase pathways:

TGFβ increases PTHrP expression by stabilizing mRNA as well as by transcriptional mechanisms. The signaling pathways through which TGFβ increases PTHrP secretion in breast cancer cells have not been defined, although intracellular mediators known as Smads are indispensable for many of the responses to TGFβ and have also been reported being part of TGFβ regulation of PTHrP in MDA-MB-231. However, there is also accumulating evidence that TGFβ signals through other pathways. TGFβ activates MAP kinase pathway components Ras, Erk1/2, and JNKs. TAK1 (TGFβ-activated kinase-1), and its upstream activator TAB1 (TAK1 binding protein) mediate some responses to TGFβ family members. Activation by TGFβ of p38 MAP kinase, which is downstream of TAK1, has also been reported. Recently, a MAP kinase kinase-independent activation of p38α by TAB1-dependent mechanism was demonstrated.
negative Smads were stably overexpressed in MDA-MB-231 breast cancer cells, and the changes in PTHrP production were measured. The data supported both Smad-dependent and independent mechanism for the TGFβ stimulation of PTHrP production by breast cancer cells. Specific protein kinase inhibitors were used to determine the Smad-independent signaling of TGFβ to promote PTHrP production. This study indicated that the MAP kinase pathway, and specifically the p38 component, is a major component of this Smad-independent signaling by TGFβ and provides new molecular targets for anti-osteolytic therapy.

**Extracellular calcium stimulates tumor production of PTHrP:**

Osteoclastic resorption of bone can not only release high concentrations of stored growth factors into the bone microenvironment, but also increased concentrations of ionized calcium and phosphate, from the dissolution of the hydroxyapatite of mineralized bone. The calcium-sensing receptor is a G-protein coupled seven transmembrane domain receptor which enables cells expressing it to respond to small variations in the concentration of extracellular calcium. Recent reports establish a role of calcium-sensing receptor signaling in regulating tumor secretion of PTHrP. In both studies, extracellular calcium as well as the polycation neomycin stimulated tumor production of PTHrP by the human MDA-MB-231 breast and PC-3 prostate cancer cells. The combination of calcium and TGFβ increased PTHrP more than either alone. Furthermore, cell-surface expression of the calcium-sensing receptor was detected in both lines. Thus, high ionized calcium concentrations at sites of osteolysis may contribute to the vicious cycle by increasing PTHrP production and thence further osteolysis.

**PTHRP stimulates osteoclastic bone resorption through stromal cells and RANK Ligand:**

PTHRP does not stimulate osteoclastic bone resorption directly; osteoblasts are required for this process. A possible mediator of the effect of tumor-produced PTHrP to stimulate osteoclastic bone resorption is receptor activator of NF kappa B ligand (RANKL). Thomas et al. determined that the breast cancer lines MDA-MB-231, MCF-7, and T47D and primary breast tumor cells did not express RANK ligand but did express osteoprotegerin (OPG) and RANK. MCF-7, MDA-MB-231 and T47D cells failed to support osteoclast formation in co-culture experiments, consistent with their lack of expression of RANK ligand. MCF-7 cells overexpressing PTHrP were added to co-cultures of murine osteoblasts and hematopoietic cells. Osteoclast formation resulted without the addition of osteotropic agents, whereas co-cultures with MCF-7 or MCF-7 cells transfected with empty vector required exogenous agents. When MCF-7 cells overexpressing PTHrP were cultured with murine osteoblasts, osteoblastic RANK ligand mRNA levels were enhanced and osteoblastic OPG mRNA levels diminished, as determined with PCR primers specific for the mouse factors. MCF-7 parental cells had no effect on these mRNA levels when cultured with osteoblastic cells. MCF-7 cells which overexpress PTHrP, tested in a nude mouse model, caused significantly more bone metastases which was associated with increased osteoclast formation, plasma PTHrP concentrations and hypercalcemia compared with parental or empty vector controls.

**Involvement of osteolytic factors other than PTHrP in metastasis:**

A variety of factors are now known to stimulate osteoclastic bone resorption through increasing the expression of RANK ligand on the surface of cells in the osteoblastic lineage. Some of these also decrease OPG expression from the same cells. Several of these are candidates to be produced by tumor cells in bone. Of particular potential are interleukins 6 and 11, which bind to similar receptors that share the common signaling subunit gp130. These two interleukins are secreted by breast cancer cell lines, such as MDA-MB-231, and may be regulated by TGFβ in a manner similar to the regulation of PTHrP. Similar to PTHrP, overexpression of IL-11 in MDA-MB-231 breast cancer cells increased osteolytic metastases in the mouse bone metastases model. The data obtained with PTHrP-neutralizing antibodies in mice bearing MDA-MB-231 bone metastases indicate that PTHrP is the primary osteolytic factor responsible for bone destruction. The other factors, such as interleukin 11, may enhance the end-organ effects of PTHrP, as has already been shown for IL-6. They could also play a central part in stimulating osteolysis by metastatic cancer cells which are PTHrP-negative.

**Osteoblastic metastases**

On the other end of the spectrum are osteoblastic bone metastases. The cell responsible for the aberrant new bone formation is the osteoblast, but the mechanisms by which tumor cells stimulate the new bone formation are still unclear. Many tumor-associated factors have been proposed as stimulators of the disorganized new bone formation at sites of metastases, including insulin-like growth factors (IGF)-1 and –2, TGFβ, prostate-specific antigen (PSA), urokinase-type plasminogen activator (uPA), fibroblast growth factors (FGF)-1 and -2, bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF) and endothelin-1 (ET-1). Some of these factors, such as IGF-1 and -2, TGFβ, BMPs, PDGF, ET-1 and FGFs directly stimulate osteoblast activity. Others, such as the proteases PSA and UPA, have indirect effects by activating latent TGFβ or cleaving IGFs from inhibitory binding proteins such as IGF binding protein 3.

**Role of tumor-produced ET-1**

Accumulating evidence suggests a central role for ET-1 in the pathogenesis of osteoblastic metastases. ET-1 is a potent vasoconstrictor which belongs to a family of three 21-amino-
acid peptides. The endothelins mediate their effects through endothelin A (ETA) and endothelin B (ETB) receptors\textsuperscript{11-14}. ETA receptors bind ET-1 with 10 times greater affinity than ET-3, while the B receptor binds all three endothelins with equal affinity. Most of the activities of ET-1 are mediated via ETA receptor. The endothelin axis was originally identified in vascular endothelial cells and has a major role in hypertension, but it is clearly important in bone and cancer. Evidence of this stems from the fact that ET-1 is also produced by breast and prostate cancer and is a potent stimulator of osteoblast proliferation. Plasma concentrations of ET-1 are increased in men with advanced prostate cancer\textsuperscript{15}. We identified 3 breast cancer lines that cause osteoblastic metastases in female nude mice and provide evidence that tumor-produced endothelin-1 (ET-1) mediates the osteoblastic response. Tumor conditioned media, as well as exogenous produced endothelin-1 (ET-1) mediates the osteoblastic response. Mice inoculated with the ZR-75-1 breast cancer line and treated with a selective ETA receptor antagonist (ABT-627) had significantly fewer osteoblastic bone metastases and less tumor burden compared with untreated mice. In contrast, there was no effect of ABT-627 on osteolytic bone metastases caused by ET-1-negative breast cancer, MDA-MB-231. ABT-627 had no effect growth in vitro or at the orthotopic site of ZR-75-1 or MDA-MB-231 cells. Collectively, the data suggest that tumor-produced ET-1 mediates osteoblastic bone metastases by stimulating osteoblast proliferation and new bone formation. ETA receptor blockade may be useful for prevention and the treatment of osteoblastic bone metastases due to breast or prostate cancer.

Evidence from other models implicates tumor-produced platelet-derived growth factor as another mediator of osteoblastic metastases\textsuperscript{16}. Taken together, these examples indicate that tumor cells selectively interact with the bone microenvironment. Understanding these interactions at the molecular level has identified novel targets for therapeutic intervention aimed at both osteolytic and osteoblastic metastases.

References