J Musculoskel Neuron Interact 2004; 4(3):308-318

Review Article

Hylonome

Tumor-bone cellular interactions in skeletal metastases

J.M. Chirgwin, K.S. Mohammad, T.A. Guise

Division of Endocrinology, Department of Medicine, University of Virginia, Charlottesville, USA

Abstract

Human tumor cells inoculated into the arterial circulation of immunocompromised mice can reliably cause bone metastases, reproducing many of the clinical features seen in patients. Animal models permit the identification of tumor-produced factors, which act on bone cells, and of bone-derived factors. Local interactions stimulated by these factors drive a vicious cycle between tumor and bone that perpetuates skeletal metastases. Bone metastases can be osteolytic, osteoblastic, or mixed. Parathyroid hormone-related protein, PTHrP, is a common osteolytic factor, while vascular endothelial growth factor and interleukins 8 and 11 also contribute. Osteoblastic metastases can be caused by tumor-secreted endothelin-1, ET-1. Other potential osteoblastic factors include bone morphogenetic proteins, platelet-derived growth factor, connective tissue growth factor, stanniocalcin, N-terminal fragments of PTHrP, and adrenomedullin. Osteoblasts are the main regulators of osteoclasts, and stimulation of osteoblast proliferation can increase osteoclast formation and activity. Thus, combined expression of osteoblastic and osteolytic factors can lead to mixed metastases or to increased osteolysis. Prostate-specific antigen is a protease, which can cleave PTHrP and thus change the balance of osteolytic versus osteoblastic responses to metastatic tumor cells. Bone itself stimulates tumor by releasing insulin-like growth factors and transforming growth factor-β. Secreted factors transmit the interactions between tumor and bone. They provide novel targets for therapeutic interactions to break the vicious cycle of bone metastases. Clinically approved bisphosphonate anti-resorptive drugs reduce the release of active factors stored in bone, and PTHrP-neutralizing antibody, inhibitors of the RANK ligand pathway, and ET-1 receptor antagonist are in clinical trials. These adjuvant therapies act on bone cells, rather than the tumor cells. Recent gene array experiments identify additional factors, which may in the future prove to be clinically important targets.

Keywords: Cancer, Breast, Prostate, Bone Metastasis, Parathyroid Hormone-Related Protein, Endothelin, Adrenomedullin, Transforming Growth Factor-β, Prostate-Specific Antigen

Introduction

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. We now appreciate that mineralized bone matrix is a rich storehouse of growth factors[1], 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[2,3]. 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[4]. However, recent experiments suggest that a constellation of expressed genes necessary for metastasis to bone pre-exists within the primary breast tumor[5]. Thus, bone-specific metastases are the consequence of a selection

The authors have no conflict of interest.

Corresponding author: John M. Chirgwin, Ph.D. Division of Endocrinology, PO Box 801421, Department of Medicine, University of Virginia, Charlottesville, VA 22908, USA
E-mail: jc3qb@virginia.edu

Accepted 11 February 2004

of variants from a heterogeneous population of cells within the primary tumor, plus changes in gene expression induced by bone factors[6]. 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 (Table 1). 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

Destructive bone lesions 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)[7]. Breast and lung 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[8,9]. The antibody has been humanized and is in clinical trials against HHM and bone metastases.

PTHrP was originally suspected to play a role in osteolytic metastases based on its known role as a stimulator of hypercalcemia[7], plus its high expression by tumor cells in bone versus soft tissue sites in patients[10,11]. These results suggested that PTHrP expression by the primary tumor might be prognostic of metastases to bone. This is not the case. In a prospective study, PTHrP expression by the primary tumor was an independent prognostic marker of improved survival and *decreased* metastasis to bone[12]. PTHrP is a complex, multifunctional protein and appears to play independent roles in primary and metastatic cells. It is likely that the expression of PTHrP by breast cancer cells in bone is the result of induction of its gene by factors in bone, such as TGFβ[13].

PTHrP cannot be the only factor responsible for bone metastases, and a number of other proteins play either contributory or PTHrP-independent roles[2]. 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)[14]. PTHrP-independent factors have also been reported, including IL-8, which can directly stimulate the osteoclast[15,16].

Kang et al. compared less- and more-metastatic variants of a breast cancer cell line by gene expression profiling[5]. They 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[17]. Osteopontin plays a complex role in metastasis, including modulation of anti-tumor immune responses[18] and is differentially regulated in tumor cells metastatic to bone versus other sites[19]. CTGF is a potent osteoblast-stimulatory factor[20], 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[21]. Kang et al.[5] found that the pro-metastatic gene set was coordinately increased in cells that 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[22]. This has been shown for PTHrP, which induces osteoblastic expression of RANK ligand[23]. 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

Metastases with net formation of disorganized new bone are characteristic of prostate cancer[24] and also occur in 15% of breast cancer bone metastases. The tumor-induced lesions are characterized by formation of new but abnormal and disorganized new bone, and they 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[25] and can cause osteoblastic metastases in the nude mouse model. Metastases are effectively blocked with a selective antagonist of the endothelin A receptor[26,27]. This orally active antagonist is in clinical trials in men with advanced metastatic prostate cancer[28-30]. 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 resorp-

Cell Line	Bone Metastases	PTHrP	IL-6[F]	IL-11[F]	VEGF	Other Osteolytic Factors	ET-1	AM	Other Blastic Factors
Breast									
ZR-75-1	OB	0	0	0	99±1.5		81±5		
MCF-7	OB	0	3.3±0.2	0	180±2		19±2	+[A]	PDGF[B]
					[171±20][M]				
T47D	OB	0			[68±12][M]		227±12	+[A]	
BT483	M	0					22±6		
MDA-	OL	0.54±0.1	360	240±3	1258±34	IL-8[C]	0	+	CTGF[D]
MB-231					[1021±23][M]				
BT549	OL	0.44±0.12					0		
MDA-	OL	0.29±0.12			[91±7][M]		0		
MB-435s									
HS578T	N	0.46±0.05					3±2		
MDA-	N	0.4±0.4					0		
MB-436									
MDA-	N	0					0		
MB-361									
Prostate									
DU-145	N	0.75±0.08					23±2x	78±10[K]	
							[4±0.3][J]		
LNCaP	N	0.08±0.08			470[L]		0	0[K]	
							[1.2±0.2][J]		
PC-3	OL	12±0.8			<25[L]		0	+[E]	
							[5±0.3][J]	71±9[K]	
Lung									
RWGT2F	OL	64±2	117±2	0	1233±14				
SBC-5G	OL	+							
A549	OL	+[H]					+	+	
HARAI	OL	+							
Ovary									
CHO-K1	OL	+							

Table 1. Cancer Cell Lines and Phenotypes of Bone Metastases. PTHrP in pM/10^5 cells/48 hr by Nichols Institute 2-site IRMA. ET-1, IL-6, IL-11, VEGF in pg/ml/10^5 cells/48 hr by R&D Systems immunoassays. OB = osteoblastic, OL = osteolytic, M = mixed, N = none, when cells are inoculated into the arterial circulation of nude mice via the left cardiac ventricle. Most data from Yin et al.[27]. [A]AM = adrenomedullin (Miller et al.)[40]. [B]PDGF = platelet-derived growth factor B chain (Yi et al.)[97] [C]IL-8 = interleukin-8 (Bendre et al.)[15]; [D]CTGF = connective tissue growth factor (Kang et al.)[5]; [E]AM = adrenomedullin (Abasolo et al.)[43]; [F](Gallwitz et al.)[81]; [G](Miki et al.)[92]; [H](Hastings et al.)[42]; [I](Iguchi et al.)[9]; [J](Chiao et al.)[93]; [K]Fmol/mg protein (Rocchi et al.)[41]; [L]pg/mg protein (Dall'Era et al.)[94]; [M]Arbitrary mRNA units (Scott et al.)[95]. Remaining data, indicated by pluses, are unpublished from the authors' laboratory. Note that some of these cells (MCF-7 and MDA-MB-231, for examples), vary in PTHrP production depending on source of the cell line. A number of cell lines, scored N here, cause bone responses when injected directly into the marrow cavity of the tibia or femur[96]. Most of the cell lines are of human origin.

tion seen in the patient trials. Conversely, bisphosphonates effectively reduce skeletal-related events in prostate cancer[31]. These observations support an important 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.[5] is another factor that stimulates osteoblasts[20,30]. It is a normal product of hypertrophic chondrocytes and is the second of three closely related gene products CCN1-3, which are selectively expressed in certain tumor types[33]. It is possible that CCN1 and CCN3 may contribute to bone metastases. CCN3 is expressed by prostate cancers[34], while CCN1, cyr61, is expressed by breast[35] and other cancers.

Adrenomedullin (AM) is a 52-amino acid vasoactive peptide with potent bone-stimulatory actions *in vitro*[36,37] and *in vivo*[38,91]. It is made by many cancers[39], including breast[40] and prostate[41]. We have recent data that it increases bone metastases *in vivo*. We tested the A549 human lung adenocarcinoma cell for its ability to form bone metastases when inoculated into the left cardiac ventricle. Animals developed osteolytic metastases after five weeks. These cells express PTHrP[42], ET-1, and AM. AM secretion was reduced 50% by stable expression of AM siRNA. These cells, made by Dr Alfredo Martinez at the National Cancer Institute, caused fewer metastases and increased survival, compared to empty vector-transfected A549 cells (unpublished results). In experiments the human prostate cancer cell line PC-3 was transfected with an AM expression DNA, resulting in a greater than ten-fold increase in AM secretion. These cells showed slower growth *in vitro* and as subcutaneous tumors[43]; this response is opposite to that found with many other tumors, where AM is an autocrine growth stimulatory and pro-angiogenic factor[39]. However, when these same PC-3 subclones were inoculated into nude mice, animal survival was less than half that of mice receiving control PC-3 cells. Mice with AM-overexpressing tumors showed accelerated osteolytic lesions and also adjacent areas of osteoblastic new bone formation (unpublished).

Another factor that could play a role in osteoblastic metastases is stanniocalcin, a polypeptide that is produced by cancer cells[44,45] and can stimulate new bone formation[46,47].

Mixed osteolytic/osteoblastic metastases

Mixed lesions 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[48]. The enlarged pool of early osteoblasts responds to osteolytic factors by increased expression of RANK ligand[49]. A similar mechanism may be involved in the metastases caused by A549 and PC-3 cells, described in the preceding paragraph. Both cell lines secrete PTHrP and adrenomedullin, and A549s make ET-1 as well (unpublished). Thus, expression of an osteoblastic factor may not simply convert an osteolytic tumor cell line into one that causes osteoblastic metastases.

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[50,51]. 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 contraction of cardiac myocytes apparently by binding to the endothelin A receptor. Binding was attributed to a 4 amino acid near-identity between the two peptides[52]. We have extended these observations to bone. PTHrP1-23 is a potent stimulator of calvarial new bone formation at concentrations as low as 1nM (unpublished results), although this polypeptide has no detectable affinity for the type 1 PTH receptor. New bone formation was blocked by the endothelin A receptor antagonist, ABT-627. The results suggest that PSA proteolysis of PTHrP, rather than inactivating it, converts the protein from an osteolytic factor to a potent osteoblastic one. These preliminary results are *in vitro* and require confirmation of their physiological significance *in vivo*. PTHrP can also be cleaved by the neutral endopeptidases, neprilysin, which is expressed on the surface of prostate and bone cells[53]; so N-terminal fragments of PTHrP could play a role in normal bone metabolism.

Actions of bone on tumor cells

The effects of bone-derived factors on tumor cells remain understudied. Van der Pluijm et al. elegantly demonstrated that several mRNAs are increased in bone versus non-bone sites of human breast cancer metastases in nude mice[14]. 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.[1] found that insulin-like growth factors (IGFs) -2, then -1, were the most abundant factors in bone matrix, followed by TGFβ, after which were lower concentrations of

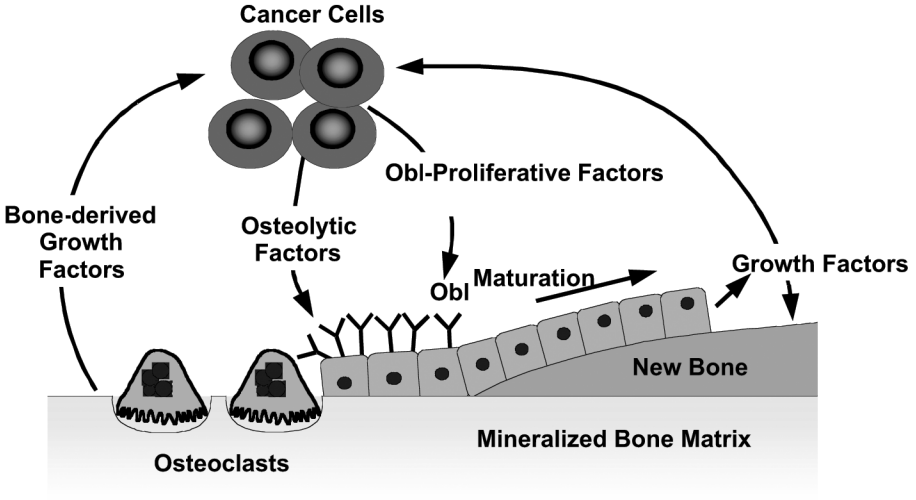

Figure 1. Tumor stimulation of osteoblasts can increase both new bone formation and resorption. Tumor products, such as endothelin-1 and adrenomedullin, stimulate osteoblast (Obl) proliferation. Immature osteoblasts respond to osteolytic cytokines, such as parathyroid hormone and interleukin-11, by expressing RANK ligand (Y). RANK ligand stimulates bone resorption by osteoclasts, which releases growth factors, such as transforming growth factor-β, from mineralized matrix. Mature osteoblasts synthesize growth factors, which are incorporated into bone and also enrich the local microenvironment. Growth factors stimulate tumor cells. Osteoblasts lose RANK ligand expression during maturation. The balance of osteoblast proliferation versus maturation, plus tumor production of factors like PTHrP, determines whether bone metastases are osteoblastic, osteolytic, or mixed. The new bone synthesized in osteoblastic metastases is disorganized and of poor mechanical quantity.

BMPs, fibroblast growth factors -1 and -2, and platelet-derived growth factor[1]. 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[54], such as CTGF and IL-11, identified by Kang et al.[5], and PTHrP[13,55]. 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[56]. 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[57]. This step may be another point at which the efficacy of bisphosphonates against bone metastases is exerted[31].

It is likely that in patients, tumor cells secrete sets of multiple proteins with actions on bone cells[5], and these sets may vary between metastatic sites within individuals. The list of the factors contributing to bone metastases is already large (Table 1) and will continue to grow. The effects of multiple factors and their relative expression levels probably change during the course of growth of a metastatic lesion, and the responses of the host cells at the metastatic site will also change over time. Many of the factors isolated from bone matrix[1] have been generically named as growth factors. However, tumor cell proliferation is generally growth factor-independent, and such proliferation is not rate-limiting for the progress of bone metastases. Experiments with inhibitors of bone responses to tumor cells (but which do not directly target the tumor cells) effectively decrease tumor burden[8,27,58,59]. These results suggest that paracrine interactions between tumor cells and bone cells are central regulators of skeletal metastases, and that the regulation is not via control of cell proliferation.

The vicious cycle

Animal models have established that bone metastases involve a vicious cycle between tumor cells and the skeleton (Figure 1). The cycle is driven by four obvious contributors: the tumor cells, bone-forming osteoblasts, bone-destroying osteoclasts, and organic bone matrix. Osteoclast formation and activity is controlled by the osteoblast, adding complexity to the vicious cycle. The mineralized matrix of bone provides a rich store of growth factors, such as insulin-like and transforming growth factors[1]. These factors are synthesized by osteoblasts and released by osteoclasts. The factors reach high local concentrations in the bone microenvironment and can act on tumor cells to encourage metastatic growth. The products released from resorbing bone attract tumor cells[60].

In turn, breast cancers secrete many factors that act on bone cells. It is likely that at sites of osteoblastic metastases, the tumor cells continue to secrete osteolytic factors, such as parathyroid hormone-related protein, which stimulate bone resorption. Therapies targeting the vicious cycle can decrease metastases by lowering the concentrations of growth factors in bone. It is characteristic of skeletal metastases that the properties of the bone are altered, contributing to the clinical pathology seen in patients. As disease progresses, the bone and the bone cells probably become more and more abnormal, under the continuing influence of tumor-secreted factors. It should be borne in mind that the images of metastases seen in animal models represent a much-simplified version of the clinical picture. The animal models are of much shorter duration and involve homogeneous tumor lines, whereas bone metastases in patients are much more heterogeneous, even within individual patients[61].

Roles of other cells in bone to skeletal metastases

It is an oversimplification to consider only osteoblasts and osteoclasts as the interacting partners of tumor cells in the vicious cycle of cancer bone metastases. Vascular endothelial cells are a major fourth cellular component. Most of the tumor-produced factors discussed above are vasoactive and many are pro-angiogenic, including endothelin[62], adrenomedullin[63], IL-8[64], the CCN proteins[33,65], stanniocalcin[66], as well as VEGF. PTHrP may be anti-angiogenic[67]. These same angiogenic factors are also increased by the hypoxic response in tumor cells, including endothelin[68], adrenomedullin[69], the CCN proteins[70,71], and stanniocalcin[72]. To further complicate the situation, many of these factors are both regulated by and regulators of VEGF, and many are also TGFβ-regulated genes. Thus, there must be an extremely complex cross-talk between endothelial cells, bone cells, and tumor cells at the metastatic site. Making sense of these interactions will require the development of novel and subtle experimental strategies.

Endothelial cells in bone differ from the cells lining blood vessels in other organs, which could contribute to the bone-tropism of certain tumors[73]. Just as tumor-secreted factors can alter the phenotypes of bone cells in the vicinity of metastases, such factors will probably also alter the behavior of endothelial cells. Van der Pluijm et al. observed specific changes in host vascular markers in bone versus non-bone metastatic sites[14]. Di Raimondo et al. similarly detected angiogenic factors at higher concentrations in bone marrow plasma than in the peripheral circulation of patients with multiple myeloma[74], suggesting the specific induction of angiogenic factor expression within the bone microenvironment of patients with cancer in the skeleton. Tumor cells are also likely to perturb the lineages of cells in bone. Bone marrow is a major site of stem cells, including the stem cells of the bone stromal lineage[75], and cells in bone such as pericytes could alter their differentiation under the influence of tumor-produced factors. Thus, tumor cells could alter the supply of osteoblastic precursors and also alter the differentiation of hematopoietic precursors into osteoclasts[22]. The animal model that is the focus of this article relies on T-cell deficient nude mice, which ignores the contribution in patients of the immune system to both normal bone cell function[76], as well as to cell-mediated immune surveillance and killing of tumor cells.

Clinical applications

The bisphosphonates are a class of drugs that resemble pyrophosphate. The replacement of the central oxygen of pyrophosphate by a carbon in the bisphosphonate backbone results in resistance to hydrolysis and confers high affinity for bone mineral, which is the basis for bisphosphonate-radionuclide conjugates as diagnostic bone-scanning agents. The bisphosphonates have high affinity for bone, where they can persist for years[77]. They are released at high concentrations in areas of active bone remodeling and are absorbed by nearby cells. They inhibit cells by several mechanisms, including the stimulation of apoptosis. Bisphosphonates are effective in animal models and the clinic. Whether bisphosphonates have significant effects *in vivo* on tumor cells or angiogenesis[78], especially at non-bone sites where their concentrations are low, is controversial.

An inhibitor of intracellular src signaling, when modified by the addition of a pair of phosphonate groups, inhibited osteoclastic bone resorption[79]. This approach could add bone specificity to existing chemotherapeutic and adjuvant compounds. Recombinant osteoprotegerin decreased osteolytic destruction and tumor burden in bone, without affecting metastases to soft-tissue sites in an animal model[59], and decreased cancer bone pain[80]. Recombinant osteoprotegerin entered clinical trials but is likely to be superceded by neutralizing antibodies against RANK ligand.

A recent study identified 6-thioguanine and 6-thioguanosine as effective inhibitors of PTHrP transcription. These agents have long been used against leukaemias and several inflammatory disorders. They were effective in animal models of humoral hypercalcemia of malignancy and breast cancer bone metastases[81]. Endothelin receptor antagonists are in extensive clinical trials, and their efficacy against osteoblastic metastasis is discussed above. Most of these adjuvant treatments target bone cells, but it may be possible to block the effects on tumors of bone-derived factors such as TGFβ[82].

Treatment options

Bisphosphonate drugs are currently approved for skeletal metastases due to multiple myeloma and solid tumors of the breast and prostate. In the last case, an anti-resorptive is effective against what are predominantly osteoblastic metastases. In fact, osteoblastic metastases are accompanied by active bone remodeling, and prostate cancer patients with bone metastases have markers of bone resorption higher than those seen in patients with osteolytic disease[83].

Bisphosphonates are also effective in animal models of osteoblastic bone metastases[84]. The results are consistent with the importance of the vicious cycle, outlined above and in Figure 1, to both osteolytic and osteoblastic diseases. The role of a vicious cycle in osteoblastic metastases is also supported by the observation that when patients with advanced metastatic prostate cancer were treated with the endothelin A receptor antagonist ABT-627, atrasentan, markers of bone resorption were decreased[29,30]. Trials are now underway to test whether combining a bisphosphonate drug with an endothelin receptor antagonist is more effective than the single agent treatments.

Future directions and limitations

Areas of active research 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-8, IL-11, CTGF, adrenomedullin, stanniocalcin, and CXCR-4. Angiogenesis is of obvious central importance in bone metastases. Many of the factors made by tumor cells are increased by hypoxia and in turn are angiogenic, in addition to having stimulatory actions on bone cells.

The roles of the abundant, bone-derived insulin-like growth factors need to be tested for their contributions to metastases to bone. IGF-2 is the most abundant bioactive factor in the mineralized matrix[1], but its role in skeletal metastasis, along with those of IGF-1, has been little studied[85]. The effects of these molecules, through activation of the type 1 IGF-1 receptor on tumor cells and bone cells, are likely to be complex and extend beyond stimulation of growth. Actions of secreted IGFs are controlled by the IGF binding proteins[86-88]. The extremely complex relationship between bone metastases and angiogenesis, discussed above, remains to be clarified.

The animal models, which are the focus of this article, have been limited to a modest number of cultured cell lines (Table 1). This is a strength in terms of defining molecular mechanisms, but it is clearly a weakness for understanding the more diverse and complex situations encountered in the clinic. We believe that this weakness is not effectively addressed by repeating the animal model experiments with more cell lines, even if funding were available for such confirmatory studies. A goal of the animal models is to provide platforms for pre-clinical testing of therapeutic interventions. If the animal models provide useful guidance in improving the care of patients with skeletal metastases, then they will have served their purpose well.

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[80]. It seems likely that bone metastases release unknown factors into the circulation, which stimulate wasting of skeletal muscle[89]. 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[3]. It is now appreciated that the standard treatments for patients with cancers of the breast and prostate result in bone loss. Not only does this result in skeletal morbidity for the patients, but increased bone turnover could enhance metastases to bone by stimulating the vicious cycle. Patients with treatment-related bone loss should benefit from therapy with bisphosphonate antiresorptive drugs[90]. Tumor-bone interactions, and the secreted factors which mediate them, offer targets for future therapeutic intervention to ameliorate or perhaps prevent skeletal metastases.

Acknowledgements

Work in the authors' laboratory was supported by funding from the Gerald D. Aurbach Endowment and the Mellon Institute of the University of Virginia Cancer Center, and research grants from the NIH (R01 CA69158) and the U.S. Army (DAMD17-99-1-9401) to TAG and the Veterans Administration Research Service (Merit Award) and the U.S. Army (DAMD17-98-1-8245 and DAMD17-02-1-0586) to JMC. Mr. Cliff Martin assisted in the preparation of the figure.

References

1. Hauschka PV, Mavrakos AE, Iafrati MD, Doleman SE, Klagsbrun M. Growth factors in bone matrix. Isolation of multiple types by affinity chromatography on heparin-Sepharose. J Biol Chem 1986; 261:12665-12674.
2. Chirgwin JM, Guise TA. Molecular mechanisms of tumor-bone interactions in osteolytic metastases. Crit Rev Eukaryot Gene Exp 2000; 10:159-178.
3. Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. Nat Rev Cancer 2002; 2:584-593.
4. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer 2003; 3:1-6.
5. Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordon-Cardo C, Guise TA, Massague J. A multigenic program mediating breast cancer metastasis to bone. Cancer Cell 2003; 3:537-549.
6. Hynes RO. Metastatic potential: generic predisposition of the primary tumor or rare, metastatic variants – or both? Cell 2003; 113:821-823.
7. Suva LJ, Winslow GA, Wettenhall RE, Hammonds RG, Moseley JM, Diefenbach-Jagger H, Rodda CP, Kemp BE, Rodriguez H, Chen EY. A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression. Science 1987; 237:893-896.
8. Guise TA, Yin JJ, Taylor SD, Kumagai Y, Dallas M, Boyce BF, Yoneda T, Mundy GR. Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteoly-

sis. J Clin Invest 1996; 98:1544-1549.
9. Iguchi H, Tanaka S, Ozawa Y, Kashiwakuma T, Kimura T, Hiraga T, Ozawa H, Kono A. An experimental model of bone metastasis by human lung cancer cells: the role of parathyroid hormone-related protein in bone metastasis. Cancer Res 1996; 56:4040-4043.
10. Southby J, Kissin MW, Danks JA, Hayman JA, Moseley JM, Henderson MA, Bennett RC, Martin TJ. Immunohistochemical localization of parathyroid hormone-related protein in human breast cancer. Cancer Res 1990; 50:7710-7716.
11. Powell GJ, Southby J, Danks JA, Stillwell RG, Hayman JA, Henderson MA, Bennett RC, Martin TJ. Localization of parathyroid hormone-related protein in breast cancer metastases: increased incidence in bone compared with other sites. Cancer Res 1991; 51:3059-3061.
12. Henderson M, Danks J, Moseley J, Slavin J, Harris T, McKinlay M, Hopper J, Martin T. Parathyroid hormone-related protein production by breast cancers, improved survival, and reduced bone metastases. J Natl Cancer Inst 2001; 93:234-237.
13. Guise TA, Chirgwin JM. Transforming growth factor-beta in osteolytic breast cancer bone metastases. Clin Orthop 2003; 415(Suppl):S32-38.
14. Van der Pluijm G, Sijmons B, Vloedgraven H, Deckers M, Papapoulos S, Lowik C. Monitoring metastatic behavior of human tumor cells in mice with species-specific polymerase chain reaction: elevated expression of angiogenesis and bone resorption stimulators by breast cancer in bone metastases. J Bone Miner Res 2001; 16:1077-1091.
15. Bendre MS, Gaddy-Kurten D, Mon-Foote T, Akel NS, Skinner RA, Nicholas RW, Suva LJ. Expression of interleukin 8 and not parathyroid hormone-related protein by human breast cancer cells correlates with bone metastasis *in vivo*. Cancer Res 2002; 62:5571-5579.
16. Bendre MS, Montague DC, Peery T, Akel NS, Gaddy D, Suva LJ. Interleukin-8 stimulation of osteoclastogenesis and bone resorption is a mechanism for the increased osteolysis of metastatic bone disease. Bone 2003; 33:28-37.
17. Zhao W, Byrne MH, Boyce BF, Krane SM. Bone resorption induced by parathyroid hormone is strikingly diminished in collagenase-resistant mutant mice. J Clin Invest 1999; 103:517-524.
18. Weber GF. The metastasis gene osteopontin: a candidate target for cancer therapy. Biochim Biophys Acta 2001; 1552:61-85.
19. Carlinfante G, Vassilioul D, Svensson O, Wendel M, Heinegard D, Andersson G. Differential expression of osteopontin and bone sialoprotein in bone metastasis of breast and prostate carcinoma. Clin Exp Metastasis 2003; 20:437-444.
20. Safadi FF, Xu J, Smock SL, Kanaan RA, Selim AH, Odgren PR, Marks SC Jr, Owen TA, Popoff SN. Expression of connective tissue growth factor in bone: its role in osteoblast proliferation and differentiation *in vitro* and bone formation *in vivo*. J Cell Physiol 2003; 196:51-62.
21. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001; 410:50-56.
22. Roodman GD. Biology of osteoclast activation in cancer. J Clin Oncol 2001; 19:3562-3571.
23. Thomas RJ, Guise TA, Yin JJ, Elliott J, Horwood NJ, Martin TJ, Gillespie MT. Breast cancer cells interact with osteoblasts to support osteoclast formation. Endocrinology 1999; 140:4451-4458.
24. Bagi CM. Skeletal implications of prostate cancer. J Musculoskel Neuron Interact 2003; 3:112-117.
25. Nelson JB, Hedican SP, George DJ, Reddi AH, Piantadosi S, Eisenberger MA, Simons JW. Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. Nat Med 1995; 1:944-949.
26. Guise TA, Yin JJ, Mohammad KS. Role of endothelin-1 in osteoblastic bone metastases. Cancer 2003; 97(3 Suppl):779-784.
27. Yin JJ, Mohammad KS, Kakonen SM, Harris S, Wu-Wong JR, Wessale JL, Padley RJ, Garrett IR, Chirgwin JM, Guise TA. A causal role for endothelin-1 in the pathogenesis of osteoblastic bone metastases. Proc Natl Acad Sci USA 2003; 100:10954-10959.
28. Rosenbaum E, Carducci MA. Pharmacotherapy of hormone refractory prostate cancer: new developments and challenges. Expert Opin Pharmacother 2003; 4:875-887.
29. Carducci MA, Padley RJ, Breul J, Vogelzang NJ, Zonnenberg BA, Daliani DD, Schulman CC, Nabulsi AA, Humerickhouse RA, Weinberg MA, Schmitt JL, Nelson JB. Effect of endothelin-A receptor blockade with atrasentan on tumor progression in men with hormone-refractory prostate cancer: a randomized, phase II, placebo-controlled trial. J Clin Oncol 2003; 21:679-689.
30. Nelson JB, Nabulsi AA, Vogelzang NJ, Breul J, Zonnenberg BA, Daliani DD, Schulman CC, Carducci MA. Suppression of prostate cancer induced bone remodeling by the endothelin receptor A antagonist atrasentan. J Urol 2003; 169:1143-1149.
31. Eaton CL, Coleman RE. Pathophysiology of bone metastases from prostate cancer and the role of bisphosphonates in treatment. Cancer Treat Rev 2003; 29:189-198.
32. Nishida T, Nakanishi T, Asano M, Shimo T, Takigawa M. Effects of CTGF/Hcs24, a hypertrophic chondrocyte-specific gene product, on the proliferation and differentiation of osteoblastic cells *in vitro*. J Cell Physiol 2000; 184:197-206.
33. Brigstock DR. The CCN family: a new stimulus package. J Endocrinol 2003; 178:169-175.
34. Maillard M, Cadot B, Ball RY, Sethia K, Edwards DR, Perbal B, Tatoud R. Differential expression of the ccn3 (nov) proto-oncogene in human prostate cell lines and tissues. Mol Pathol 2001; 54:275-280.
35. Tsai MS, Bogart DF, Li P, Mehmi I, Lupu R.

Expression and regulation of Cyr61 in human breast cancer cell lines. Oncogene 2002; 21:964-973.
36. Cornish J, Callon KE, Coy DH, Jiang NY, Xiao L, Cooper GJ, Reid IR. Adrenomedullin is a potent stimulator of osteoblastic activity *in vitro* and *in vivo*. Am J Physiol 1997; 273:E1113-1120.
37. Hamada H, Kitamura K, Chosa E, Eto T, Tajima N. Adrenomedullin stimulates the growth of cultured normal human osteoblasts as an autocrine/paracrine regulator. Peptides 2002; 23:2163-2168.
38. Cornish J, Naot D, Reid IR. Adrenomedullin - a regulator of bone formation. Regul Pept 2003; 112:79-86.
39. Zudaire E, Martinez A, Cuttitta F. Adrenomedullin and cancer. Regul Pept 2003; 112:175-183.
40. Miller MJ, Martinez A, Unsworth EJ, Thiele CJ, Moody TW, Elsasser T, Cuttitta F. Adrenomedullin expression in human tumor cell lines. Its potential role as an autocrine growth factor. J Biol Chem 1996; 271:23345-23351.
41. Rocchi P, Boudouresque F, Zamora AJ, Muracciole X, Lechevallier E, Martin PM, Ouafik L. Expression of adrenomedullin and peptide amidation activity in human prostate cancer and in human prostate cancer cell lines. Cancer Res 2001; 61:1196-1206.
42. Hastings RH, Summers-Torres D, Cheung TC, Ditmer LS, Petrin EM, Burton DW, Spragg RG, Li J, Deftos LJ. Parathyroid hormone-related protein, an autocrine regulatory factor in alveolar epithelial cells. Am J Physiol 1996; 270:L353-361.
43. Abasolo I, Yang L, Haleem R, Xiao W, Pio R, Cuttitta F, Montuenga LM, Kozlowski JM, Calvo A, Wang Z. Overexpression of adrenomedullin gene markedly inhibits proliferation of PC3 prostate cancer cells *in vitro* and *in vivo*. Mol Cell Endocrinol 2003; 199:179-187.
44. Chang AC, Jellinek DA, Reddel RR. Mammalian stanniocalcins and cancer. Endocr Relat Cancer 2003; 10:359-373.
45. Welcsh PL, Lee MK, Gonzalez-Hernandez RM, Black DJ, Mahadevappa M, Swisher EM, Warrington JA, King MC. BRCA1 transcriptionally regulates genes involved in breast tumorigenesis. Proc Natl Acad Sci USA 2002; 99:7560-7565.
46. Filvaroff EH, Guillet S, Zlot C, Bao M, Ingle G, Steinmetz H, Hoeffel J, Bunting S, Ross J, Carano RA, Powell-Braxton L, Wagner GF, Eckert R, Gerritsen ME, French DM. Stanniocalcin 1 alters muscle and bone structure and function in transgenic mice. Endocrinology 2002; 143:3681-3690
47. Yoshiko Y, Maeda N, Aubin JE. Stanniocalcin 1 stimulates osteoblast differentiation in rat calvaria cell cultures. Endocrinology 2003; 144:4134-4143.
48. Coffman JA. Runx transcription factors and the developmental balance between cell proliferation and differentiation. Cell Biol Int 2003; 27:315-324.
49. Geoffroy V, Kneissel M, Fournier B, Boyde A, Matthias P. High bone resorption in adult aging transgenic mice overexpressing cbfa1/runx2 in cells of the osteoblastic lineage. Mol Cell Biol 2002; 22:6222-6233.
50. Cramer SD, Chen Z, Peehl DM. Prostate specific antigen cleaves parathyroid hormone-related protein in the PTH-like domain: inactivation of PTHrP-stimulated cAMP accumulation in mouse osteoblasts. J Urol 1996; 156:526-531.
51. Iwamura M, Hellman J, Cockett AT, Lilja H, Gershagen S. Alteration of the hormonal bioactivity of parathyroid hormone-related protein (PTHrP) as a result of limited proteolysis by prostate-specific antigen. Urology 1996; 48:317-325.
52. Schluter KD, Katzer C, Piper HM. A N-terminal PTHrP peptide fragment void of a PTH/PTHrP-receptor binding domain activates cardiac ET(A) receptors. Br J Pharmacol 2001; 132:427-432.
53. Ruchon AF, Marcinkiewicz M, Ellefsen K, Basak A, Aubin J, Crine P, Boileau G. Cellular localization of neprilysin in mouse bone tissue and putative role in hydrolysis of osteogenic peptides. J Bone Miner Res 2000; 15:1266-1274.
54. Wakefield LM, Roberts AB. TGF-beta signaling: positive and negative effects on tumorigenesis. Curr Opin Genet Dev 2002; 12:22-29.
55. Yin JJ, Selander KS, Chirgwin JM, Dallas M, Grubbs BG, Wieser R, Massague J, Mundy GR, Guise TA. TGFβ signaling blockade inhibits parathyroid hormone-related protein secretion by breast cancer cells and bone metastases development. J Clin Invest 1999; 103:197-206.
56. Kakonen SM, Selander KS, Chirgwin JM, Yin JJ, Burns S, Rankin WA, Grubbs BG, Dallas M, Cui Y, Guise TA. Transforming growth factor-beta stimulates parathyroid hormone-related protein and osteolytic metastases via Smad and mitogen-activated protein kinase signaling pathways. J Biol Chem 2002; 277:24571-24578.
57. Dallas SL, Rosser JL, Mundy GR, Bonewald LF. Proteolysis of latent transforming growth factor-beta (TGF-β)-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF-beta from bone matrix. J Biol Chem 2002; 277:21352-21360.
58. Sasaki A, Boyce BF, Story B, Wright KR, Chapman M, Boyce R, Mundy GR, Yoneda T. Bisphosphonate risedronate reduces metastatic human breast cancer burden in bone in nude mice. Cancer Res 1995; 55:3551-3557.
59. Morony S, Capparelli C, Sarosi I, Lacey DL, Dunstan CR, Kostenuik PJ. Osteoprotegerin inhibits osteolysis and decreases skeletal tumor burden in syngeneic and nude mouse models of experimental bone metastasis. Cancer Res 2001; 61:4432-4436.
60. Orr W, Varani J, Gondex MK, Ward PA, Mundy GR. Chemotactic responses of tumor cells to products of resorbing bone. Science 1978; 203:176-179.
61. Roudier MP, True LD, Higano CS, Vesselle H, Ellis W, Lange P, Vessella RL. Phenotypic heterogeneity of end-stage prostate carcinoma metastatic to bone. Hum Pathol 2003; 34:646-653.

62. Bagnato A, Spinella F. Emerging role of endothelin-1 in tumor angiogenesis. Trends Endocrinol Metab 2003; 14:44-50.
63. Martinez A, Vos M, Guedez L, Kaur G, Chen Z, Garayoa M, Pio R, Moody T, Stetler-Stevenson WG, Kleinman HK, Cuttitta F. The effects of adrenomedullin overexpression in breast tumor cells. J Natl Cancer Inst 2002; 94:1226-1237.
64. Belperio JA, Keane MP, Arenberg DA, Addison CL, Ehlert JE, Burdick MD, Strieter RM. CXC chemokines in angiogenesis. J Leukoc Biol 2000; 68:1-8.
65. Menendez JA, Mehmi I, Griggs DW, Lupu R. The angiogenic factor CYR61 in breast cancer: molecular pathology and therapeutic perspectives. Endocr Relat Cancer 2003; 10:141-152.
66. Zlot C, Ingle G, Hongo J, Yang S, Sheng Z, Schwall R, Paoni N, Wang F, Peale FV Jr, Gerritsen ME. Stanniocalcin 1 is an autocrine modulator of endothelial angiogenic responses to hepatocyte growth factor. J Biol Chem 2003; 278:47654-47659.
67. Bakre MM, Zhu Y, Yin H, Burton DW, Terkeltaub R, Deftos LJ, Varner JA. Parathyroid hormone-related peptide is a naturally occurring, protein kinase A-dependent angiogenesis inhibitor. Nat Med 2002; 8:995-1003.
68. Wykoff CC, Pugh CW, Maxwell PH, Harris AL, Ratcliffe PJ. Identification of novel hypoxia dependent and independent target genes of the von Hippel-Lindau (VHL) tumour suppressor by mRNA differential expression profiling. Oncogene 2000; 19:6297-6305.
69. Garayoa M, Martinez A, Lee S, Pio R, An WG, Neckers L, Trepel J, Montuenga LM, Ryan H, Johnson R, Gassmann M, Cuttitta F. Hypoxia-inducible factor-1 (HIF-1) up-regulates adrenomedullin expression in human tumor cell lines during oxygen deprivation: a possible promotion mechanism of carcinogenesis. Mol Endocrinol 2000; 14:848-862.
70. Kunz M, Moeller S, Koczan D, Lorenz P, Wenger RH, Glocker MO, Thiesen HJ, Gross G, Ibrahim SM. Mechanisms of hypoxic gene regulation of angiogenesis factor Cyr61 in melanoma cells. J Biol Chem 2003; 278:45651-45660.
71. Shimo T, Kubota S, Kondo S, Nakanishi T, Sasaki A, Mese H, Matsumura T, Takigawa M. Connective tissue growth factor as a major angiogenic agent that is induced by hypoxia in a human breast cancer cell line. Cancer Lett 2001; 174:57-64.
72. Lal A, Peters H, St Croix B, Haroon ZA, Dewhirst MW, Strausberg RL, Kaanders JH, van der Kogel AJ, Riggins GJ. Transcriptional response to hypoxia in human tumors. J Natl Cancer Inst 2001; 93:1337-1343.
73. Cooper CR, McLean L, Walsh M, Taylor J, Hayasaka S, Bhatia J, Pienta KJ. Preferential adhesion of prostate cancer cells to bone is mediated by binding to bone marrow endothelial cells as compared to extracellular matrix components *in vitro*. Clin Cancer Res 2000; 6:4839-4847.
74. Di Raimondo F, Azzaro MP, Palumbo G, Bagnato S, Giustolisi G, Floridia P, Sortino G, Giustolisi R. Angiogenic factors in multiple myeloma: higher levels in bone marrow than in peripheral blood. Haematologica 2000; 85:800-805.
75. Bianco P, Riminucci M, Gronthos S, Robey PG. Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells 2001; 19:180-192.
76. Martin TJ, Romas E, Gillespie MT. Interleukins in the control of osteoclast differentiation. Crit Rev Eukaryot Gene Expr 1998; 8:107-123
77. Russell RG, Rogers MJ. Bisphosphonates: from the laboratory to the clinic and back again. Bone 1999; 5:97-106.
78. Green JR. Antitumor effects of bisphosphonates. Cancer 2003; 97(Suppl.3):840-847.
79. Shakespeare W, Yang M, Bohacek R, Cerasoli F, Stebbins K, Sundaramoorthi R, Azimioara M, Vu C, Pradeepan S, Metcalf C III, Haraldson C, Merry T, Dalgarno D, Narula S, Hatada M, Lu X, van Schravendijk MR, Adams S, Violette S, Smith J, Guan W, Bartlett C, Herson J, Iuliucci J, Weigele M, Sawyer T. Structure-based design of an osteoclast-selective, nonpeptide src homology 2 inhibitor with *in vivo* anti-resorptive activity. Proc Natl Acad Sci USA 2000; 97:9373-9378.
80. Mantyh PW, Clohisy DR, Koltzenburg M, Hunt SP. Molecular mechanisms of cancer pain. Nat Rev Cancer 2002; 2:201-209.
81. Gallwitz WE, Guise TA, Mundy GR. Guanosine nucleotides inhibit different syndromes of PTHrP excess caused by human cancers *in vivo*. J Clin Invest 2002; 110:1559-1572.
82. Dumont N, Arteaga CL. Targeting the TGFβ signaling network in human neoplasia. Cancer Cell 2003; 3:531-536.
83. Guise TA, Chirgwin JM. Role of bisphosphonates in prostate cancer bone metastases. Semin Oncol 2003; 30:717-723.
84. Corey E, Brown LG, Quinn JE, Poot M, Roudier MP, Higano CS, Vessella RL. Zoledronic acid exhibits inhibitory effects on osteoblastic and osteolytic metastases of prostate cancer. Clin Cancer Res 2003; 9:295-306.
85. Jackson JG, Zhang X, Yoneda T, Yee D. Regulation of breast cancer cell motility by insulin receptor substrate-2 (IRS-2) in metastatic variants of human breast cancer cell lines. Oncogene 2001; 20:7318-7325.
86. Mohan S, Baylink DJ. IGF-binding proteins are multifunctional and act via IGF-dependent and-independent mechanisms. J Endocrinol 2002; 175:19-31.
87. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev 2002; 23:824-854.
88. LeRoith D, Roberts CT Jr. The insulin-like growth factor system and cancer. Cancer Lett 2003; 195:127-137.
89. Tisdale MJ. Cachexia in cancer patients. Nat Rev Cancer 2002; 2:862-871.
90. Smith MR. Bisphosphonates to prevent skeletal complications in men with metastatic prostate cancer. J Urol 2003; 170:S55-57.

91. Cornish J, Callon KE, Bava U, Coy DH, Mulvey TB, Murray MA, Cooper GJ, Cooper GJ, Reid IR. Systemic administration of adrenomedullin (27-52) increases bone volume and strength in male mice. J Endocrinol 2001; 170:251-257.
92. Miki T, Yano S, Hanibuchi M, Kanematsu T, Muguruma H, Sone S. Parathyroid hormone-related protein (PTHrP) is responsible for production of bone metastasis, but not visceral metastasis, by human small cell lung cancer SBC-5 cells in natural killer cell-depleted SCID mice. Int J Cancer 2004; 108:511-515.
93. Chiao JW, Moonga BS, Yang YM, Kancherla R, Mittelman A, Wu-Wong JR, Ahmed T. Endothelin-1 from prostate cancer cells is enhanced by bone contact which blocks osteoclastic bone resorption. Br J Cancer 2000; 83:360-365.
94. Dall'Era MA, Shih SJ, Yang J, Benik S, Gandour-Edwards R, Evans CP. Differential expression of angiogenic cytokines by cell lines and primary cultures of human prostate cancer. Prostate Cancer Prostatic Dis 2001; 4:106-111.
95. Scott PA, Gleadle JM, Bicknell R, Harris AL. Role of the hypoxia sensing system, acidity and reproductive hormones in the variability of vascular endothelial growth factor induction in human breast carcinoma cell lines. Int J Cancer 1998; 75:706-712.
96. Corey E, Quinn JE, Bladou F, Brown LG, Roudier MP, Brown JM, Buhler KR, Vessella RL. Establishment and characterization of osseous prostate cancer models: intra-tibial injection of human prostate cancer cells. Prostate 2002; 52:20-33
97. Yi B, Williams PJ, Niewolna M, Wang Y, Yoneda T. Tumor-derived platelet-derived growth factor-BB plays a critical role in osteosclerotic bone metastasis in an animal model of human breast cancer. Cancer Res 2002; 62:917-923.