2 Metastatic Disease to the Musculoskeletal System

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1. INTRODUCTION

Bone is a dynamic tissue that undergoes continuous remodeling. It goes through a balanced process that entails repeated cycles of bone resorption coupled with synthesis of new bone matrix (Fig. 1). These remodeling cycles are influenced by an individual's age, endocrine and nutritional status, and level of physical activity. This ongoing tissue turnover is important for meeting the often conflicting need of the skeleton to maintain structural support for the body while also providing a source of ions for mineral homeostasis. The maintenance of skeletal mass in the face of continuous bone remodeling requires the coordinated activities of osteoblasts and osteoclasts, the two cell types responsible for skeletal matrix formation and resorption *(1)* (Fig. 1). Advances in our understanding of the precise mechanisms that control the cellular interactions and coupled activities of these two cell types have provided new insight into a number of diseases affecting the skeleton. These disorders are characterized by an imbalance of remodeling with subsequent increase in bone resorption, decreased bone mass, and loss of skeletal stability and integrity. This is particularly true for neoplastic diseases, in which a number of common human malignancies have a propensity to spread to the skeleton, resulting in significant morbidity and mortality from bone destruction *(2)*.

1.1. METASTATIC DISEASE TO THE SKELETON

The strength and integrity of bone is dependent on the maintenance of this delicate balance between resorption and formation *(3)*. Complex regulatory interactions exist between a metastases and the host bone that disrupt this balance, facilitating dissemination and progression of certain types of tumors within the skeleton. Increasingly, evidence suggests that in order for tumors to successfully establish and grow in skeletal tissues, tumor cells must be able to interfere with normal bone cell function and indirectly tip the balance in favor of bone resorption *(4)*. Thus, it has become clear that in order for tumor cells to form a metastatic deposit and grow in the skeleton, bone resorption by osteoclasts must occur *(5)*. Recent research has provided new insights into osteoclast biology and the regulatory control of bone remodeling. This new knowledge has led to an increase in our understanding of the interactions between tumor cells and the bone microenvironment.

Tumor metastasis is the leading cause of death for patients with cancer, and the skeletal system is one of the most common sites to be affected by metastatic disease. However, not all tumors share the same likelihood of dissemination to the skeleton. Of the cancers that spread to bone, carcinomas of the breast and the prostate possess a special affinity, accounting for more than 80% of all cases of metastatic skeletal disease *(2)*. Other tumors that frequently spread to the skeleton include carcinomas of the lung, kidney, and thyroid *(2)*. This special osteotrophism or affinity to metastasize to bone involves characteristics of these tumors that allow them to establish and grow in bone, as well as unique features of the bone microenvironment, which makes the skeleton a particularly congenial place for these cells *(6)*. More than 100 yr ago, Stephen Paget referred to this as the "seed and soil" hypothesis, to explain the special affinity of breast cancer for the "fertile soil" of the bone microenvironment *(7)*.

1.2. CARCINOMA OF THE BREAST

Breast cancer is one of the most common malignancies in women. Up to one-third of women with early stage breast cancer will eventually succumb to their disease and many of them will have developed bone metastases during the course of their
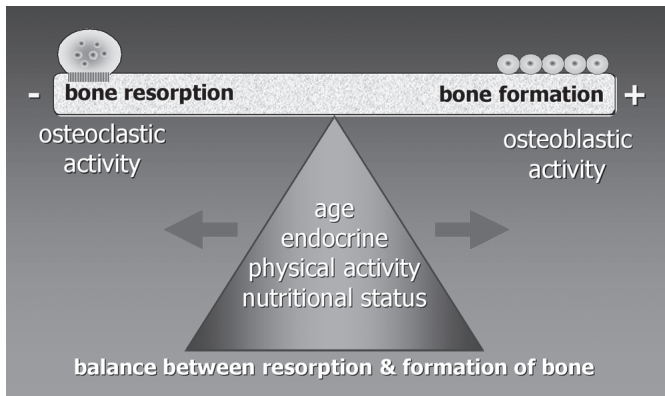
Fig. 1. Bone is a dynamic, metabolically active tissue. In order to maintain structural support for the body while providing a source of ions for mineral homeostasis, the skeleton must undergo continuous remodeling. This is a balanced process that entails repeated cycles of bone resorption by osteoclasts coupled with synthesis of new bone matrix by osteoblasts. An individual's age, endocrine and nutritional status, and level of physical activity influence these remodeling cycles. The maintenance of bone mass in the face of continuous bone remodeling requires the coordinated balanced activities of osteoblasts and osteoclasts in order to sustain the skeleton.
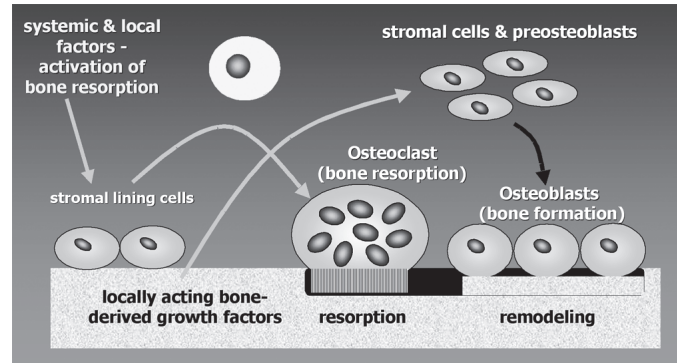

Fig. 2. The activities of the principal bone cells are highly regulated and link to maintain skeletal homeostasis. The temporal sequence in bone remodeling is initiated by osteoclastic bone resorption. The systemic (hormonal) or local (growth factor and cytokine) signals that activate bone resorption target the osteoblast/stromal cells, which regulate the activity of osteoclasts in a paracrine fashion. Osteoclasts are recruited from their hematopoietic/macrophage progenitors, to differentiate, attach to sites of bone resorption and develop a specialized ruffled border that facilitates transport of protons and proteases to degrade bone matrix. The microenvironment of the bone contains a rich supply of mitogenic growth factors synthesized by osteoblasts as part of the bone matrix, which are released by osteoclastic resorption. These osteoblast-derived growth factors funtion to regulate the proliferation and differentiation of osteoprogenitor into active osteoblasts, which then synthesize new matrix to replace the bone lost through resorption.

illness *(8)*. A significant percentage (50–70%) of patients with metastatic breast cancer will have skeletal involvement, contributing significantly to their morbidity *(9)*. In approx 50% of these patients, bone will be the predominant site of metastatic spread and in 20–25% of these patients the skeleton will be the only site of metastasis *(9)*. Approximately 80% of patients with bone-limited disease at the time of diagnosis developed skeletal complications (bone pain, fracture, and hypercalcemia), as will 60% of those with bone and visceral disease and 21% of those with no bone disease *(10)*.

1.3. CARCINOMA OF THE PROSTATE

Likewise, metastatic disease with bone loss and skeletal complications is common in patients with carcinoma of the prostate. Although relatively few patients will manifest bone metastases at initial diagnosis, a significant portion of these men will develop skeletal complications over the course of their disease *(11)*. One-third of patients will experience some adverse skeletal manifestation, including vertebral collapse requiring spinal orthosis, spinal cord compression, and pathological bone fracture *(12)*. Patients with high-grade tumors and those with progressive disease have the highest risk for bone metastases *(11)*. The tumor will have spread to the skeleton in 85–100% of patients who die of their disease *(13)*.

To help explain the interactions between tumor cells that metastasize to bone and the skeletal microenvironment, this chapter first reviews the biology of normal bone remodeling and some of the biological principles of metastasis. Some intriguing animal model studies that have added immensely to the understanding of this complex process are described. Finally, some of the current strategies used to treat this devastating complication of malignancy are briefly discussed.

2. THE BIOLOGY OF BONE REMODELING

Bone is a dynamic, metabolically active tissue throughout life. After skeletal growth is complete, remodeling of both cortical and trabecular bone is ongoing, and results in an annual turnover of approx 10% of the adult skeleton *(14)*. These bone-remodeling cycles are both temporally and spatially "coupled" and involve regulatory mechanisms that closely link the activities of these two cell types (Fig. 2). Bone resorption is, for the most part, a unique function of the osteoclast *(15)*, a specialized multinucleated polykaryon, which is derived from the hematopoietic monocyte/macrophage lineage *(16)*. The initial steps in this temporal sequence involve the proliferation of immature osteoclast precursors, differentiation into osteoclasts, matrix adherence, formation of a specialized ruffled border between the cell and the bone surface, and subsequent resorption *(1)*. The recognition and attachment of the osteoclast to bone matrix is controlled by specific integrin binding ($\alpha v\beta 3$) *(17)*. Integrin binding to the bone matrix signals the osteoclast to organize the cytoskeleton leading to polarization of the cytoplasm and the development of a specialized ruffled border that permits the establishment of an isolated space adjacent to the underlying bone surface *(18)*. The osteoclast then resorbs bone by the production of proteolytic enzymes and hydrogen ions, which are exported into the localized environment under the ruffled border of the cell *(19)*. A proton pump, similar to the vacuolar ATPase in the intercalated cells of the kidney, pumps hydrogen ions across the membrane of the cell, and lysosomal enzymes are also released creating the optimal conditions for the degradation of the matrix *(19)*. The conclusion of bone resorption is
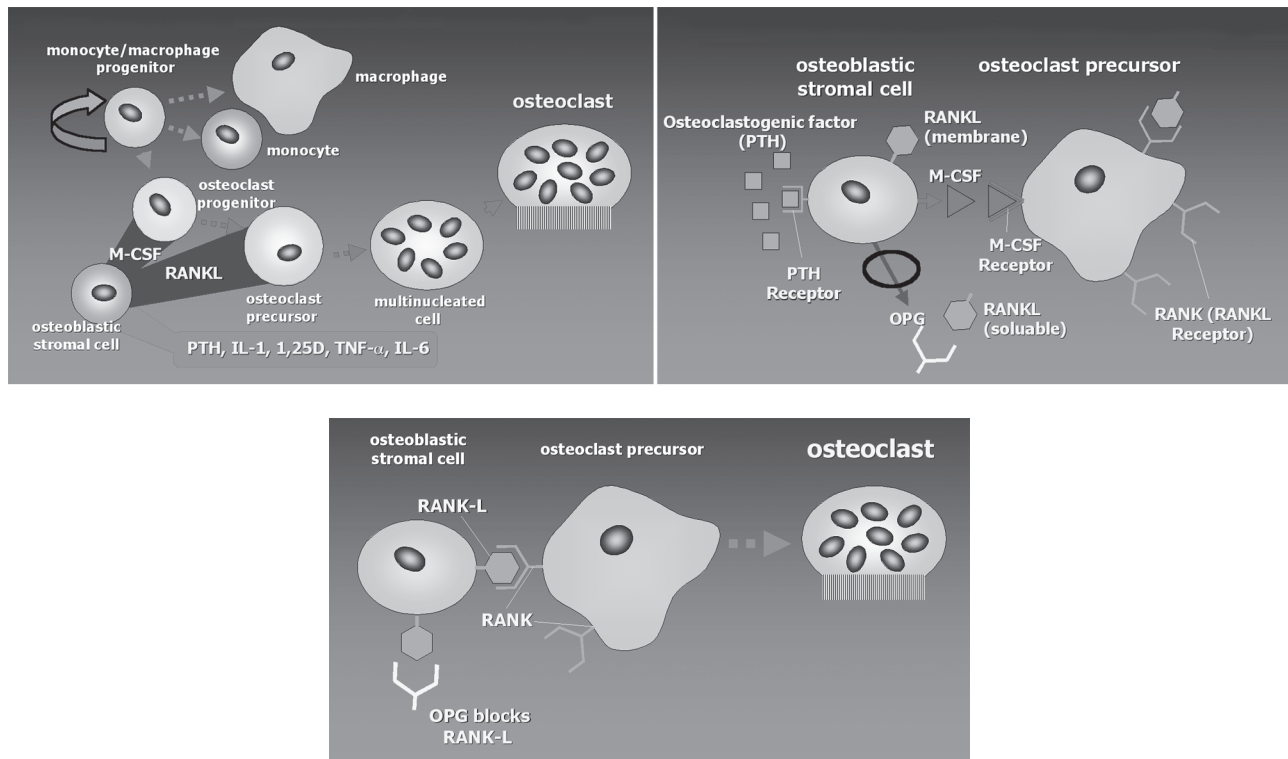
Fig. 3. Osteoclast commitment and differentiation are regulated by the expression of three critical molecules, macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor (NF)-κB ligand (RANKL), and osteoprotegerin (OPG). Cells of the osteoblastic lineage play a paracrine role in the regulation of osteoclast formation and function. **(A)** The factors, which stimulate osteolytic bone resorption (e.g., parathyroid hormone [PTH], parathyroid hormone-related protein promoter [PTHrP], vitamin D3, interleukin [IL]-1, IL-6, tumor necrosis factor [TNF], and prostaglandins), interact with receptors on osteoblast/stromal cells stimulating the expression of M-CSF and RANKL. **(B)** M-CSF is a secreted protein, which interacts with its receptor on monocyte/macrophage progenitors causing these cells to become committed to the osteoclast lineage, creating a pool of osteoclastic precursors. RANKL is expressed on the cell membranes of osteoblasts/stromal cells. **(C)** When osteoclast precursors, which express the receptor RANK, are exposed to RANKL through cell-to-cell interaction with osteoblasts/ stromal cells, they will differentiate into mature activated osteoclasts. RANKL can also bind with OPG, which is a soluble receptor for RANKL, and acts as a decoy in the RANK–RANKL signaling system to inhibit osteoclastogenesis. M-CSF, RANKL, and OPG appear to be the molecular mediators of osteoclastogenesis, and provide a common pathway mediating the activation of bone resorption and controlling physiological bone turnover. The ratio of RANKL:OPG is an important determinant of osteoclast formation and activity and directly determines the rate of both physiological and pathological osteoclastic bone resorption.

most likely mediated by osteoclast apoptosis, however, the signals are still poorly understood. Drugs that inhibit bone resorption, such as bisphosphonates, induce osteoclast apoptosis, therefore, the cessation of osteoclast activity may be as important as their formation in the regulation of bone remodeling *(20)*.

A large number of hormones, growth factors, inflammatory mediators, and cytokines are all known to stimulate osteolytic bone resorption through stimulation of osteoclast formation and function *(21)*. How such a diverse group of factors (e.g., parathyroid hormone [PTH], parathyroid hormone-related protein promoter [PTHrP], vitamin D3, interleukin [IL]-1, IL-6, tumor necrosis factor [TNF], and prostaglandins) could all mediate the same important biological process has remained a mystery until recently, but this fact suggests some common pathway *(22–24)*. It has long been known that cells of the osteoblastic lineage played an important paracrine role in the regulation of osteoclast formation and function *(25)*. In cell culture studies, osteoclast formation from bone marrow requires the addition of 1,25(OH)2 vitamin D3, and the presence of stromal cells in the osteoblastic lineage that produce macrophage

colony-stimulating factor (M-CSF) as well as some other biological activity that has been recently identified *(25)*. This activity has now been characterized with the discovery of three new family members of the TNF ligand and receptor signaling system, which have been shown to play a critical role in the control and regulation of bone turnover *(26–30)*. These include the receptor activator of nuclear factor (NF)-κB ligand (RANKL) *(29,30)*, its receptor, (RANK) *(27,31)*, and its decoy receptor osteoprotegerin (OPG) *(28,32)*. These three molecules appear to be the molecular mediators of osteoclastogenesis and provide a common pathway mediating the activation of bone resorption and controlling physiological bone turnover (Fig. 3).

Most of the previously mentioned factors, which stimulate osteoclasts, do so by upregulating the expression of RANKL mRNA in osteoblasts/stromal cells, which will then express RANKL on their cell membranes *(25,27)*. Osteoclast precursors from the monocyte/macrophage lineage express the receptor RANK, and will differentiate into mature activated osteoclasts, when they are exposed to RANKL through cell-to-cell interaction with osteoblasts/stromal cells in the presence of

M-CSF *(27,28)*. RANKL can also bind with OPG, which is a soluble receptor for RANKL and acts as a decoy in the RANK–RANKL signaling system to inhibit osteoclastogenesis *(32)*. The ratio of RANKL:OPG is an important determinant of osteoclast formation and activity in vivo and directly determines the rate of bone turnover *(28)*. The process of the recruitment and differentiation of osteoclasts is shown schematically in Fig. 3.

During the process of resorption of bone, mitogenic growth factors stored within the matrix are released into the local microenvironments *(22–24)*. These osteoblast-derived growth factors, synthesized as a part of the extracellular matrix, function to regulate the proliferation of osteoprogenitor cells, causing them to differentiate into mature functional osteoblasts. These osteoblasts synthesize new bone matrix, replacing the bone that was lost through resorption, assuring a balance in skeletal remodeling (Fig. 2 *[33]*).

3. THE BIOLOGY OF METASTATIC DISEASE

In order for a tumor to metastasize, the cells must have the capacity to escape the primary site, travel via the circulatory system, and establish disease at a new distant site. To accomplish this formidable feat, a number of important molecular steps must take place, and this process is remarkably similar for the vast majority of different tumor types with the capacity for metastasis *(34)*.

The pattern of spread of metastasis is dependent both on the regional venous drainage of the primary organ, as well as selective characteristics of the target tissue resulting in homing of tumor cells to these preferential sites *(35)*. The propensity of tumors arising in the breast, prostate, and lung for bony metastasis suggests that there is selective homing of these tumor cells to the skeletal microenvironment. However, a comparison of prostate, breast, and lung tumors shows differences in the distribution of bony metastases, which are most likely explained by different patterns of regional venous drainage *(36,37)*. The high incidence of the spread of prostate cancer to the axial skeleton is partially explained by the drainage of Batson's plexus, where connections between the vertebral venous plexus and the marrow spaces allow metastases from prostate cancer to spread preferentially to the lower vertebrae *(36–38)*. This suggests that specific biological characteristics of the metastatic site and patterns of blood flow from the primary organ play a role in distant spread of disease. Additional evidence supporting this concept comes from animal model studies where the route of administration of tumor cells influences the occurance of bone metastases *(39)*. Intracardiac injection of tumor cells has been shown to consistently produce skeletal metastases in a number of animal models, whereas intravenous or subcutaneous injection does not produce bony lesions *(39–41)*. Other important biological factors for the dissemination of a malignancy involve angiogenesis, cell adhesion, invasion, and growth factors produced by tumor and host cells, as well as the local environment of the metastatic site *(34)*.

3.1. ANGIOGENESIS

A strong correlation has been observed between tumor aggressiveness and the degree of vascularization of a number of different types of cancers, including breast and prostate *(42–45)*. This data suggests that the capacity of a malignancy to generate new blood vessels (tumor angiogenesis) is important both in progressive growth of the primary tumor and its ability to form metastases *(46)*. A rich vascular bed not only increases the supply of nutrients to the primary tumor, but also increases the likelihood for dissemination. These newly formed vessels are, in all probability, more permeable to tumor cells facilitating entrance into the circulation *(47)*.

The balance between stimulatory and inhibitory growth factors regulates tumor angiogenesis, and a number of studies have demonstrated that metastatic potential directly correlates with tumor cell expression of several gene products, which function as pro-angiogenic molecules *(48)*. These factors include vascular endothelial growth factor (VEGF), basic fibroblast growth factor, IL-8, type IV collagenase (matrix metalloproteinases [MMP]2 and MMP9), and others *(34,47)*. The production of these growth factors leads to tumor growth and causes a concomitant increase in vascularization through stimulation of endothelial cell proliferation and migration, as well as a break down of extracellular matrix *(34)*. The proteolytic activity of type IV collagenase facilitates the migration of endothelial cells through the altered extracellular matrix toward the source of the angiogenic stimulus *(34,47,48)*. The expression of VEGF in Dunning prostatic adenocarcinoma has been shown to correlate with microvessel density and metastatic potential, where the highest mRNA and protein levels for VEGF were expressed by the most highly metastatic cell lines *(49)*. Recent studies have demonstrated that the pleiotropic transcription factor NF-kB regulates the expression of multiple genes including *IL-8* and *MMP-9*, and is constitutively actived in prostate cancer cells *(48)*. The blockade of NF-kB in the highly metastatic PC-3M human prostate cancer cell line resulted in significant inhibition of *VEGF*, *IL-8*, and *MMP-9* with subsequent inhibition of angiogenesis, invasion, and metastasis, in both cell culture and in animal models *(48)*. Additionally, angiogenesis in a metastatic focus probably plays a role in the establishment of tumor cells at sites of secondary disease. In an animal model of breast cancer, bone metastases contained large numbers of newly formed blood vessels at the periphery and within tumor tissue *(50)*. In cell culture studies, breast tumor cells stimulated proliferation, migration, and differentiation of bone marrow-derived endothelial cells *(50)*. Cytokine-stimulated endothelial cells may also participate in the establishment of a metastasis and help mediate bone destruction by targeting osteoclast precursors to sites of active bone resorption *(51)*.

3.2. CELL ADHESION

The establishment and subsequent growth of metastatic tumor cells in bone is also dependent on attachment to specific extracellular matrix components and to other cells (endothelial and stromal) in the skeletal microenvironment. Cell adhesion molecules (CAM) mediate several important cell-to-cell and cell-to-extracellular matrix interactions *(52,53)*. These attachments, through specific matrix binding, may signal tumor cell localization, migration, and proliferation and may also induce local expression of cytokines that stimulate bone resorption *(24,53)*.

A category of CAMs, the integrins, has been seen to play an important role in the metastasis of tumor cells to bone *(34)*. Integrins are a family of transmembrane receptors that bind to

a variety of extracellular matrix proteins, are involved with cellular signal transduction and may be critical for the attachment of tumor cells to extracellular matrix (53,54). The αvβ3 integrin, which mediates osteoclastic recognition and attachment to bone matrix, is also highly expressed in bone-residing breast carcinoma cells (55). Integrins interact with matrix through the Arg-Gly-Asp (RGD) peptide sequences present in extracellular matrix proteins (34). The addition of RGD peptides that compete with matrix constituents for integrin binding has been shown to inhibit metastasis of melanoma cells (56). Tumor cell attachment to vascular endothelium and to matrix constituents, such as laminin and fibronectin, are integrin-mediated (52). These proteins underlie endothelial cells and this binding may be an important initial step in tumor cell colonization of a metastatic site (53). Synthetic antagonists to laminin inhibit osteolytic bone metastasis formation by A375 cells in nude mice (57), supporting a role for matrix interactions in the establishment of tumor cells in the skeleton. The integrin α4β1 mediates cell–cell and cell–matrix interactions through adhesion to vascular cell adhesion molecule (VCAM)-1 and fibronectin (58). Transfection of Chinese hamster ovary cells with α4β1 resulted in bone and pulmonary metastases, whereas α4β1 negative cells yielded only pulmonary metastases (58). Antibodies against α4 or VCAM-1 inhibited bone metastasis, suggesting that α4β1 expression, can influence tumor cell trafficking and retention in skeletal tissues (58).

In addition to mediating the retention of tumor cells in bone, matrix interactions may also alter the cells' biological behavior, favoring proliferation and growth at the metastatic site (59). Bone extracts promote increases in chemotaxis and invasive ability of bone metastasizing prostate and breast cancer cells, but not that of non-bone metastasizing tumor cells (60). Exposure of certain types of tumor to growth factors that are found in the bone microenvironment might enhance their ability to adhere to bone matrix. Treatment of osteotropic PC-3 human prostatic carcinoma cells with transforming growth factor (TGF)-β (which is abundant in bone matrix and released in active form by osteoclastic resorption), causes an increase in synthesis of α2β1 integrin and promotes the adhesion and spreading of PC-3 cells on bone-derived collagen (24,61).

3.3. INVASION

The ability of tumor cells to invade tissues, with transversal of the extracellular matrix as well as angio-lymphatic channels, are critical early steps in the development of metastatic disease, and requires local proteolysis of matrix proteins and cell migration (62). The proteolytic breakdown of constituents of the extracellular matrix facilitates invasion and requires expression of specific proteases. The production of proteolytic enzymes aid tumor cells with detachment from the primary site, invasion of adjacent stroma, entrance and exodus from the circulation, and the establishment at a distant focus. The MMPs are a large family of proteolytic enzymes that are involved with the cleavage and turnover of many different components of the extracellular matrix and play an important role in physiological matrix remodeling (63). A large number of soluble MMPs have been characterized, which can be divided into three groups, including collagenases, stromelysins, and gelatinases, based on their in vitro substrate specificity (63). The production of

MMPs by many different tumor types has been demonstrated, and their expression levels have been shown to correlate with invasion, metastasis, and poor prognosis in several human cancers (34,64). Transfection of nonmetastatic cells with specific MMPs will produce a metastatic phenotype, and pharmacological agents, which act as specific MMP inhibitors, have been shown to inhibit metastasis in a number of animal models (64–67). In addition to playing a role in tumor invasion by facilitating extracellular matrix degradation, MMPs, through their proteolytic activity, may also help to maintain a microenvironment, which promotes tumor growth (63).

TNF-α is a key regulatory molecule in matrix catabolism, including the stimulation of osteoclastic bone resorption through the RANK–RANK-ligand signaling pathway (68). A number of different types of tumors have been shown to produce TNF-α, and its secretion by tumor cells is dependent on MMP activity (69). The inhibition of MMPs prevents activation and release of TNF-α from the plasma membrane of cells and results in a concomitant decrease in TNF-transcription and translation (70). Because TNF-α has been shown to increase the expression levels of MMPs (71), a vicious cycle could be set up where TNF-α stimulates MMP expression resulting in further TNF activity. This would simultaneously enhance tumor invasion and bone resorption, thus aiding in the establishment metastatic disease in the skeleton.

Tissue inhibitors of metalloproteinases (TIMPs) are produced by nearly all known cells that produce MMPs, bind with MMPs forming inactive complexes, and thus participate in the regulation of proteolysis and matrix turnover (72,73). These inhibitors, in addition to their physiological roles in the balance of matrix degradative activity, appear to be important as regulators of metastases (34). Transfection of metastatic cells with TIMPs or treatment with exogenously added TIMP has been shown to inhibit metastatic disease, including the development of osteolytic bone lesions (64,74,75).

Tumor invasion may involve the direct production of MMPs by tumor cells or, alternatively, induction of proteolytic enzyme expression by the host (52). Host fibroblasts and stromal cells associated with some invasive breast cancers express a gene that encodes stromelysin-3 (76). Stromelysis-3 RNA was found in 95% of invasive breast cancers, however, stromelysin protein and RNA were detected in the fibroblastic cells immediately surrounding the tumor, but not in the carcinoma cells or in stroma at a distance from the lesion (77).

3.4. THE ROLE OF GROWTH FACTORS IN TUMOR ESTABLISHMENT AND PROLIFERATION IN METASTATIC SITES

The establishment of metastatic disease requires tumor cell proliferation at the new site. Tumor cell products can impact the local environment of a metastasis in a reciprocal fashion, leading to a growth advantage in selective tissues. Such mechanisms appear to play a role in the case of metastatic disease to the skeleton. The microenvironment of the bone contains a rich supply of mitogenic growth factors (fibroblast growth factors 1 and 2, insulin-like growth factors (IGF)-1 and IGF-2, numerous bone morphogenetic proteins, TGF-βs, and others). These factors are stored within bone matrix and released by osteoclastic resorption (22–24) (Fig. 2). These osteoblast-derived growth

factors function normally to regulate the differentiation and proliferation of indigenous bone cells (playing a physiological role in bone remodeling as previously described). However, these factors have also been shown to stimulate the growth of established cancer cell lines *(24)*. Demineralized extracts of bone matrix and the conditioned media from resorbing bone cultures both contain growth stimulatory activity for several tumor cell lines with metastatic potential for the skeleton, and the extent of bone resorption correlates with this mitogenic effect *(78)*. IGF-1 and IGF-2 have been shown to affect the growth of breast *(79)* and prostate *(80)* cancer cell lines. As a result, tumor cells with the capacity to stimulate osteoclastic bone resorption will enrich their local environment with the release of mitogenic factors, which can in turn, stimulate tumor proliferation and progression of disease.

3.5. THE INTERACTION OF METASTATIC TUMOR CELLS WITH OSTEOCLAST

Tumor cells utilize a number of different strategies to stimulate osteoclastic resorption, tipping the balance in normal bone remodeling in favor of bone destruction. By far, the most important of these mechanisms involves tumor cell production of factors that stimulate osteoclastic differentiation and activation. A number of different cytokines and growth factors capable of stimulating bone resorption by osteoclasts are expressed by metastatic as well as primary tumors of the skeleton. The list of factors includes most importantly, PTHrP *(81,82)*, prostaglandin E *(83)*, IL-1, IL-6, IL-11 *(84–87)*, and TNF-α and -β *(85,86,88)*. The activated osteoclast may participate in its own regulation in an autocrine/paracrine fashion by constitutively expressing pro-resorptive cytokines and, therefore, pathological bone lesions with large numbers of active osteoclasts may be, to a degree, self-perpetuating *(85,86)*.

3.6. THE ROLE OF PTHRP

PTHrP is an autocrine/paracrine growth factor and a tumor product, which is homologous with the first 13 amino acid of PTH *(89)*. This molecule shares a common receptor with PTH, was first identified for its role in hypercalcemia of malignancy, and, like PTH, is a potent activator of osteoclastic activity *(89–91)*. PTHrP stimulates osteoclastic bone resorption by increasing osteoblast production of RANK-ligand and decreasing osteoblast production of OPG, *(6)*, thereby tipping the balance of bone remodeling to favor bone breakdown.

3.6.1. PTHrP and Breast Cancer

Clinically, PTHrP has long been suspected to play a causal role in breast cancer-mediated osteolysis. In vivo studies have shown that breast cancer cell lines expressing PTHrP frequently metastasize to bone in nude mice *(82)*. PTHrP is expressed in 50 to 60% of cases of human primary adenocarcinoma of the breast, and these patients are more likely to develop bone metastases *(90,92)*. Of particular interest is the fact that PTHrP expression in bone metastases from breast cancer patients is higher than in the primary tumor, suggesting that the bone microenvironment has somehow enhanced tumor cell production of this factor *(92–95)*. In an elegant series of experiments using an animal model of breast cancer metastasis to bone, it was shown that TGF-β released from bone by osteoclast resorption may feedback, and in a paracrine fashion upregulate PTHrP expression by the metastatic lesions in the
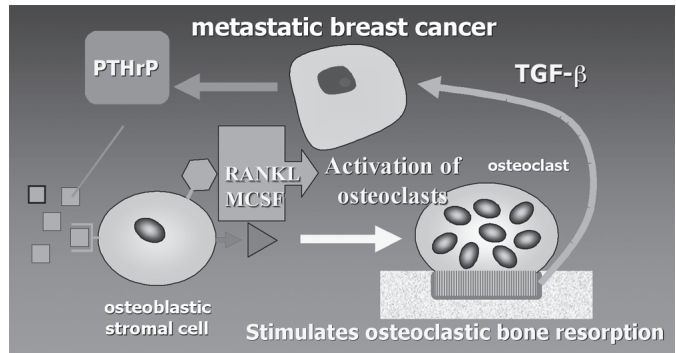

Fig. 4. The initial steps in the establishment of metastatic cancer in bone is the stimulation of osteoclastic resorption, tipping the balance in normal bone remodeling in favor of bone destruction. The secretion of tumor cell products, such as parathyroid hormone-related protein promoter (PTHrP), which stimulate osteoclastic differentiation and activation, mediates this process. Active transforming growth factor (TGF)-β released from bone matrix by osteoclast resorption will then feedback, and in a paracrine fashion upregulate PTHrP expression by the metastatic breast cancer cells. This positive feedback loop sets up a vicious cycle with the resultant osteolysis associated with metastatic breast carcinoma. PTHrP stimulates osteoclastic bone resorption by increasing osteoblast production of RANK-ligand and decreasing osteoblast production of OPG, thereby tipping the balance of bone remodeling to favor bone destruction.

skeleton (Fig. 4) *(96)*. In vitro studies demonstrated that TGF-β significantly increased PTHrP production by human MDA-MB231 breast carcinoma cells *(96)*. TGF-β signaling blockade using a dominant-negative mutant of the TGF-β type II receptor, rendered the cells unresponsive to this TGF-β effect in vitro, and likewise, the signaling blockade also cause significantly less bone destruction and formed fewer tumors in bone in an in vivo animal model *(6,96)*. This intriguing data suggests that tumor cell stimulation of osteoclastic bone resorption by PTHrP, with subsequent release of TGF-β, can provide positive feedback, stimulating further production of PTHrP by tumor cells, setting up a paracrine loop with the resultant osteolysis associated with metastatic breast carcinoma (Fig. 4).

3.6.2. PTHrP and Prostate Cancer

The role of PTHrP in skeletal metastases from carcinoma of the prostate is less apparent. Although prostate cancer is characterized by metastases that are osteoblastic, histological and biochemical studies indicate an increase of both bone resorption and bone formation in these lesions, suggesting that the interactions between tumor cells and the bone microenvironment are quite multifaceted *(97–100)*. Despite this, it seems clear that the stimulation of osteolysis is an important, and most likely, necessary component for the establishment of metastatic prostate cancer in bone *(39)*. PTHrP is expressed and secreted by both normal and neoplastic prostatic epithelial cells, and a number of studies have provided evidence suggesting a role for PTHrP in the development of bone metastases *(101–104)*. However, this association is complex and appears to be different from the observed role of PTHrP in breast cancer dissemination to the skeleton. PTHrP expression has been demonstrated in a number of prostatic carcinoma cell lines *(105)*. However, transfection of a PTHrP expression vector into the rat

prostate carcinoma cell line MATLyLu was not associated with any difference in the incidence of bone metastasis, size of metastatic foci, or tumor cell proliferation in an animal model *(106)*. Likewise, PTHrP protein was found to have a lower expression in the bone metastases than in the primary prostate tumor in human studies *(107)*, which is in contrast to the observations in breast carcinomas *(92–95)*. In vivo studies have shown that PTHrP expression does have a positive influence on prostate tumor growth and size when these cells were placed in the soft tissues of a rat hind limb, and also protected cells from apoptotic stimuli *(105)*.

3.7. RANK–RANKL SIGNALING PATHWAY: RELATIONSHIP TO PROSTATE AND BREAST

Recent reports have provided new insights into alternative molecular mechanisms whereby prostate carcinoma cells may directly mediate osteolysis. In vitro studies have shown that prostate tumor cells are capable of directly inducing osteoclastogenesis from osteoclast precursors in the absence of underlying bone stroma *(108)*. The malignant prostate cells were shown to produce a soluble form of RANKL, which accounted for the tumor-mediated stimulation of osteoclast formation *(108)*. Additionally, in vivo studies demonstrated that administration of OPG completely prevented the establishment of metastatic lesions in bone, emphasizing the important role that osteoclast activity plays in the establishment of skeletal metastases in cancer of the prostate *(108)*. Studies in human tissues have demonstrated the production of RANKL and OPG mRNA and protein in normal prostate and prostate cancer *(109)*, providing additional data supporting the concept of direct modulation of bone turnover. Of interest is the fact that RANKL and OPG expression was significantly increased in all of the bone metastases from prostate cancer compared with nonosseous metastases or the primary tumors in these studies *(109)*.

The significance of RANKL expression in the prostate gland is unclear at this time, but it seems likely that the RANK–RANKL signaling pathway will undoubtedly be found to play some role in normal prostatic physiology. Of interest in this regard is the fact that transgenic mice, which lack RANKL or RANK, demonstrate a mammary gland defect with the failure to form lobulo-alveolar mammary structures during pregnancy, resulting in the death of newborns *(110)*. RANKL-rescue experiments showed that RANKL acted directly on RANK-expressing mammary epithelial cells *(110)*. These findings suggest that this signaling pathway, which serves such a critical role in the regulation of bone remodeling, is also essential for normal mammary gland development. Further study will be needed to unravel the complex inter-relationships between the breast, prostate, and the skeletal system. However, it seems likely that such investigations will lead to new and novel paradigms in mammary and prostate glandular development and neoplasia, as well as an evolutionary rationale for the complex interactions and inter-relationships between hormonal regulation, gender, and the musculoskeletal system *(110)*.

3.8. ESTROGEN RECEPTOR AND BREAST CANCER METASTATSIS TO BONE

The hormone estrogen is a mitogen for breast tumor cells that express estrogen receptor. A role for estrogen in the dissemination of these carcinomas to the skeleton has been sug-gested, but the mechanism remains unclear *(6)*. For patients with cancer of the breast, bone metastasis is involved in nearly 50% of all distant recurrence events *(111)*. A higher rate of bone metastases is seen in lymph node positive compared with node negative patients, and, suprisingly, estrogen receptor positive tumors demonstrated a higher rate of bone recurrence than estrogen receptor negative carcinomas *(112–115)*. This is despite the fact that estrogen receptor positive patients have a lower overall rate of distant recurrence, and a better prognosis compared with estrogen receptor negative tumors *(115,116)*. Additionally, it seems likely that estrogen receptor signaling plays some role in bone metastasis, given that tamoxifen, an estrogen receptor antagonist, has been shown to help reduce bone recurrences in clinical studies *(112)*. The mechanism of this effect may be mediated at least in part by estrogen regulation of PTHrP expression. Estrogen has been shown to regulate the levels of PTHrP in early gestational tissues, as well as increase PTHrP expression in the estrogen receptor-positive breast carcinoma cell line MCF-7. Whether estrogen plays a role in enhanced PTHrP expression in the bone microenvironment remains unclear, but the clinical importance of these observations merits additional investigation, and it may enhance our understanding of tumor-induced osteolysis.

4. THERAPY FOR PATIENTS WITH METASTATIC BONE DISEASE

The development of enhanced methods for early detection along with better local treatment, has led to an improvement in outcome for many patients diagnosed with cancer. However, the treatment of patients who develop metastatic disease remains limited and, in many cases, palliative, despite the extensive use of radiation and chemotherapeutic agents. New or novel strategies that delay or prevent the development of metastatic disease would afford an opportunity to significantly improve both the quality and length of life for many patients diagnosed with a malignancy.

It seems clear that the resulting bone damage in metastatic disease to the skeletal system is because of osteoclastic bone resorption. Given that the rate-limiting step in bone destruction is the osteoclast, inhibiting the activity of these cells seems to be a reasonable primary therapeutic objective. Thus, the insights that have been gained in our understanding of osteoclast and bone biology have led to the development of new therapeutic approaches in the treatment of metastatic bone disease *(3)*. Effective anti-bone-resorptive agents are currently available, and continue to be developed, for the treatment of these patients.

Osteoclasts are inhibited by a class of drugs known as bisphosphonates, which are analogs of pyrophosphate, with a carbon atom replacing the oxygen and a variety of different side chains *(3)*. By inhibiting the osteoclast, bisphosphonates have been shown to reduce bone resorption regardless of cause. Thus, they have proved to be beneficial in the treatment of a number of conditions characterized by pathological bone loss including metastatic disease, osteoporosis, and inflammatory disorders like rheumatoid arthritis.

A number of clinical studies, as well as investigations in animal models, have documented the efficacy of bisphosphonates for the treatment of skeletal metastases in both breast and pros-

tate cancer *(3)*. Through their inhibition of osteoclastic activity, possibly by inducing osteoclast apoptosis *(20)*, there appears to be a reduction in the skeletal events with bisphosphonate therapy, i.e., pain, fracture, and hypercalcemia, in patients with metastatic cancer. Despite what appears to be a clear benefit with bisphosphonate therapy, better treatments are still needed for patients with metastatic bone disease. Such improvements will most likely come with the development of new pharmacological agents that inhibit osteoclast function.

5. CONCLUSIONS

It is clear that the molecular mechanisms involved in osteolytic metastatic disease are multifaceted and complex involving bidirectional interactions between the metastasizing tumor cells and the bone microenvironment. What has emerged from the study of this process is a central role for the production of factors by specific bone-seeking tumor cells, which facilitate recruitment and activation of osteoclasts, leading to bone resorption, loss of matrix, and bone destruction. The subsequent release of mitogenic growth factors from the matrix would prove to be advantageous by altering tumor cells' behavior, aiding in their retension and colonization of the bone. These reciprical interactions could, in turn, set up a series of vicious paracrine cycles promoting the proliferation, adhesion, and invasion of cancer cells, as well as further bone resorption, supporting the establishment and progression of skeletal metastatic disease. The hope is that with a better understanding of the molecular mechanisms that mediate the loss of bone, more effective treatments will emerge, and ultimately, we will be able to prevent this devastating complication in patients with common malignancies who develop metastatic carcinoma.

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