Review

Molecular Biology of Bone Metastasis

Lauren A. Kingsley, Pierrick G.J. Fournier, John M. Chirgwin, and Theresa A. Guise

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

Abstract

Metastasis is a final stage of tumor progression. Breast and prostate cancer cells preferentially metastasize to bone, wherein they cause incurable osteolytic and osteoblastic lesions. The bone matrix is rich in factors, such as transforming growth factor-β and insulin-like growth factors, which are released into the tumor microenvironment by osteolysis. These factors stimulate the growth of tumor cells and alter their phenotype, thus promoting a vicious cycle of metastasis and bone pathology. Physical factors within the bone microenvironment, including low oxygen levels, acidic pH, and high extracellular calcium concentrations, may also enhance tumor growth. These elements of the microenvironment are potential targets for chemotherapeutic intervention to halt tumor growth and suppress bone metastasis. [Mol Cancer Ther 2007;6(10):2609–17]

Introduction

Breast and prostate cancer are a leading cause of cancer death among women and men — second only to lung cancer. Mammography and prostate-specific antigen testing have improved early detection and treatment of these cancers, slowing their increase in incidence over the past decade and increasing the 5-year survival rate to 98% for breast cancer and 100% for prostate cancer when detected at the earliest stages. However, the breast cancer survival rate drops dramatically to 83% for patients initially diagnosed with regional spread and to 26% for those with distant metastases. Prostate cancer survival rate drops to 33% with distant metastases (1).

The skeleton is a preferred site for breast and prostate cancer metastasis. Within the skeleton, metastases present as two types of lesions: osteoblastic or osteolytic. These lesions result from an imbalance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption. Osteoblastic lesions, characteristic of prostate cancer, are caused by an excess of osteoblast activity relative to resorption by osteoclasts, leading to abnormal bone formation. In breast cancer, osteolytic lesions are found in 80% of patients with stage IV metastatic disease (2). The lesions are characterized by increased osteoclast activity and net bone destruction (3).

Breast cancer bone lesions span a spectrum in which the majority are osteolytic, but up to 15% are osteoblastic or mixed (2). Although bone metastases are classified by their radiographic appearance, most patients have evidence of abnormal bone resorption and formation. For example, autopsy examination of prostate cancer bone metastases found marked phenotypic heterogeneity both within a particular lesion and between lesions from a single patient (4). Both osteoblastic and osteolytic bone metastases lead to numerous skeletal complications, including bone pain, hypercalcemia, pathologic fractures, and spinal cord and nerve compression syndromes (5). Such complications increase morbidity and diminish quality of life in these patients.

Metastasis to bone occurs in the late stages of tumor progression and is a multistep process. Cancer cells first detach from the primary tumor and migrate locally to invade blood vessels. Once in the bloodstream, cancer cells are attracted to preferred sites of metastasis through site-specific interactions between tumor cells and cells in the target tissue (3). Tumor cells that metastasize to the skeleton adhere to the endosteal surface and colonize bone. The bone microenvironment is composed of osteoblasts, osteoclasts, and the mineralized bone matrix, plus many other cell types. It is highly favorable for tumor invasion and growth. Crosstalk between tumor cells and the microenvironment promotes a vicious cycle of tumor growth and bone destruction (2, 6). This vicious cycle is shown in Fig. 1. Tumor cells secrete factors which stimulate osteoclast-mediated bone destruction and the consequent release of numerous factors immobilized within the bony matrix that act on cancer cells, promoting a more aggressive tumor phenotype and potentiating cancer spread and bone destruction.

Crosstalk between tumor and bone activates numerous signaling pathways which drive the vicious cycle. In prostate cancer bone metastasis, for example, Wnt proteins released by tumor cells stimulate osteoblasts and have autocrine effects on tumor proliferation (7). An inhibitor of Wnt signaling, Dkk-1, can regulate metastatic progression by opposing osteogenic Wnts early in metastasis and

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Note: The bone microenvironment changes the phenotype of tumor metastases to the skeleton.

Requests for reprints: Theresa A. Guise, P.O. Box 801420, Charlottesville, VA 22903. E-mail: tag4n@virginia.edu

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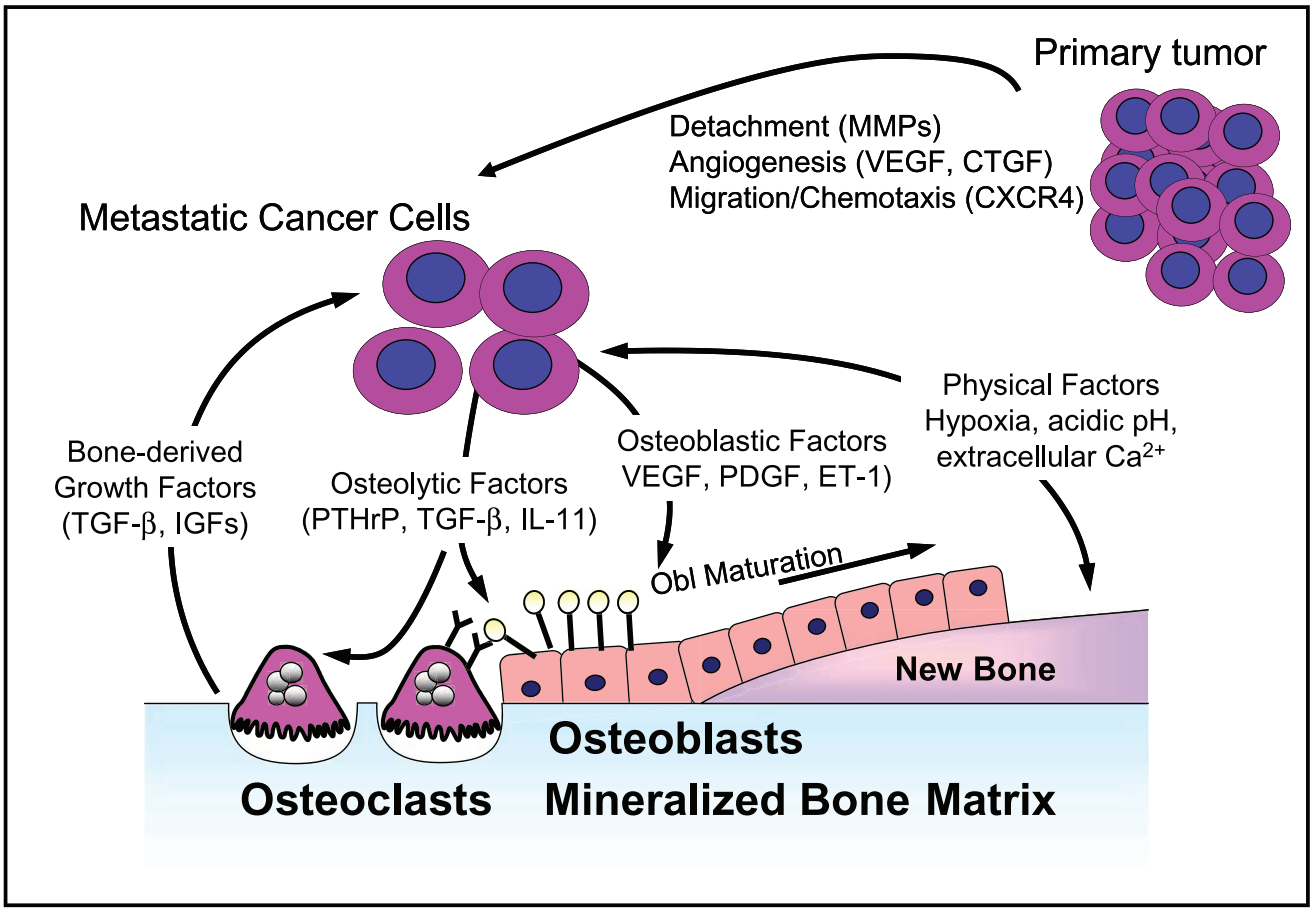

Figure 1. The vicious cycle of bone metastases. Factors, such as MMPs, chemokine receptor 4 (*CXCR4*), vascular endothelial growth factor (*VEGF*), and connective tissue growth factor (*CTGF*), target metastatic tumor cells to bone and facilitate survival within the bone microenvironment. Physical factors within the bone microenvironment, including hypoxia, acidic pH, and extracellular Ca^{2+}, and bone-derived growth factors, such as TGF-β and IGFs, activate tumor expression of osteoblast-stimulatory factors, including vascular endothelial growth factor, platelet-derived growth factor (*PDGF*), and ET-1. Osteoclast-stimulatory factors, including PTHrP, TGF-β, and IL-11, can also be increased. These factors stimulate bone cells, which in turn release factors that promote tumor growth in bone.

controlling the phenotypic switch from osteolytic to osteoblastic lesions later in metastasis.

Tumor cells and bone cells may rely on the same signaling pathways and transcription factors to facilitate their cooperative interactions at sites of metastases. This phenomenon has been suggested to represent ''osteomimicry'' on the part of the tumor cells (8). For example, metastatic breast cancer cells express bone sialoprotein (9) under control of Runx2 and MSX2 transcription factors, which are also important regulators of osteoblast functions. Runx2 activity in both cancer cells and osteoblasts stimulates the production and release of angiogenic factors andmatrix metalloproteinase (MMP) into the microenvironment and up-regulates adhesion proteins, which allow tumor and bone cells to bind (10). Runx2 expression by cancer cells may also support tumor-induced osteoclastogenesis. Expression of similar surface proteins and secreted factors allows for coexistence of these two cell types and promotes the growth of metastatic lesions.

We believe that the bone microenvironment plays a critical role in the vicious cycle by altering the phenotype of tumor cells to give highly aggressive metastatic lesions. The bone matrix is rich in growth factors, such as transforming growth factor-β (TGF-β), insulin-like growth factor-I (IGF-I), and IGF-II, which are released by osteolysis and can stimulate bone and tumor cell proliferation. Physical properties of the bone matrix, including low oxygen content, acidic pH, and high extracellular calcium concentration, create an environment favorable for tumor growth. Hypoxia, acidosis, and high calcium, plus growth factors, such as TGF-β and IGFs, combine to drive the vicious cycle of bone metastasis (Fig. 2).

Growth Factors as Mediators of the Bone Microenvironment

The destruction of bone by osteoclasts releases calcium and growth factors from the matrix. Ninety percent of the protein

released consists of collagen; among the remaining 10% are IGFs, TGF-β, fibroblast growth factor, platelet-derived growth factor, and bone morphogenetic proteins (11). All of these factors can act on metastatic cells. Although termed ''growth'' factors, they need not increase tumor burden by direct stimulation of cancer cell proliferation. They can also act indirectly to promote angiogenesis and increase tumor production of osteolytic and osteoblastic factors, which remodel the skeleton to accommodate tumor growth.

TGF-β is not the most abundant growth factor in bone, but it has the best established role in osteolytic metastases. TGF-β binds to a heterodimeric receptor and can activate the canonical Smad signaling pathway or Smad-independent pathways through extracellular signal-regulated kinase 1/2, p38 mitogen-activated protein kinase, and c-Jun-NH_2 kinase (12). TGF-β is deposited in the bone matrix by osteoblasts and released and activated during osteoclastic resorption (13). It regulates bone development and remodeling (for review, ref. 14). Advanced cancers often escape growth inhibition by TGF-β, and this factor mediates metastases by activating epithelial-mesenchymal transition and tumor cell invasion, increasing angiogenesis and suppressing immune surveillance of tumor cells (15).

In 75% of patients with biopsied bone metastases, tumor cells stained positive for phosphorylated Smad2 localized to the nucleus (16). When MDA-MB-231 cells transduced with a retroviral vector expressing a reporter gene under the control of a TGF-β–sensitive promoter, micro–positron emission tomography imaging showed reporter activation only in bone and not in adrenal metastases (16), demonstrating that Smad signaling was activated when the tumor cells were in bone. Knockdown of Smad4 (16), engineered expression of the inhibitory Smad7 (17), or introduction of a dominant-negative TGF-β type II receptor (TβRII Δcyt; ref. 18) dramatically decreased bone metastases in breast or melanoma models. Small-molecule inhibitors of TGF-β type I receptor kinase give similar results in mouse models (19–21).

TGF-β may stimulate bone metastases by inducing proosteolytic gene expression in cancer cells, with parathyroid hormone–related protein (PTHrP) having a central role. PTHrP is expressed by osteolytic breast and prostate cancer cell lines, such as MDA-MB-231, MDA-MB-435, and PC-3 (18, 22). Its expression is higher at sites of bone metastases compared with nonosseous metastases (23). Among factors released from bone during resorption, only

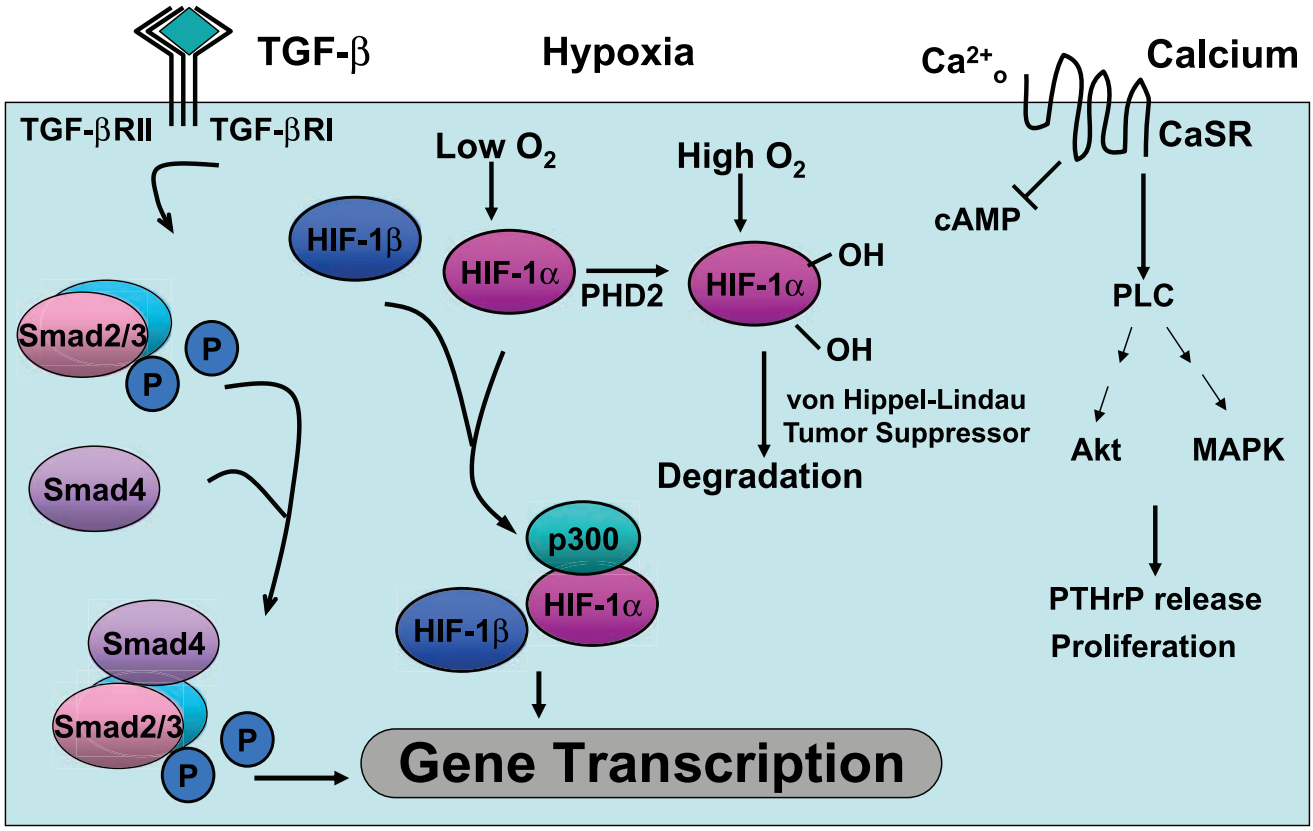

Figure 2. Signaling pathways in bone metastases. The bone microenvironment up-regulates signaling pathways within tumor cells, including the TGF-β, hypoxia, and calcium signaling pathways, enabling survival and tumor growth in bone. TGF-β binding to its receptor activates the Smad signaling pathway to mediate gene transcription. In the hypoxic bone microenvironment, HIF-1α is stabilized and mediates the transcription of hypoxia-responsive genes. Extracellular calcium stimulates the CaSR to stimulate tumor-cell proliferation and result in PTHrP release.

TGF-β increased PTHrP production (18), which occurred via Smad-dependent and Smad-independent pathways (24). This induction was prevented by the expression of TβRII Δcyt in MDA-MB-231 cells (18, 24). These cells gave decreased bone metastases in mice, which could be reversed by overexpression of PTHrP or a constitutively active type I receptor subunit (24). Neutralizing antibodies against PTHrP (22) or inhibitors of its gene transcription (25) decreased osteolytic metastases and tumor burden in cancer models. TGF-β–induced PTHrP increases osteoblastic production of RANK ligand, which stimulates osteoclast formation and activity and promotes bone metastases (26–28). The consequent increase in bone resorption releases more bone matrix factors to act on cancer cells, sustaining a vicious cycle.

PTHrP is not the only factor regulated by TGF-β. Cyclooxygenase-2 is expressed in 87% of the bone metastases from patients (29). Its expression by MDA-MB-231 cells is higher in bone metastases than in cells growing orthotopically. TGF-β increases cyclooxygenase-2 expression in osteoblasts, bone marrow stromal cells, and breast cancer cells, whereas, as an inhibitor of bone resorption, the bisphosphonate risedronate reduced cyclooxygenase-2 immunostaining in bone (29). Media conditioned by TGF-β–treated MDA-MB-231 cells support osteoclast formation, a response blocked by the cyclooxygenase-2 inhibitor NS-398. The inhibitors NS-398, nimesulide, and MF-tricyclic decreased the number of osteoclasts at the tumor-bone interface, as well as skeletal tumor burden in mice inoculated with MDA-MB-231 cells (29, 30). Cyclooxygenase-2 expression in bone-seeking subclones of MDA-MB-231 cells correlates with increased production of interleukin-8 (IL-8; ref. 30). IL-8 induces osteoclast formation and activity independent of the RANK ligand pathway (31) and can also induce IL-11 (32). IL-11 can act on osteoclasts via RANK ligand (33) and by regulation of granulocyte macrophage colony-stimulating factor (34). However, overexpression of IL-11 does not increase bone metastases in the absence of other prometastatic factors, such as osteopontin, connective tissue growth factor, or chemokine receptor 4 (35). IL-11, connective tissue growth factor, chemokine receptor 4, and MMP-1 are all up-regulated in the gene signature of breast cancer cells capable of forming osteolytic bone metastases (35). Osteopontin is a protein secreted by osteoblasts and involved in bone matrix mineralization (36). Its expression is regulated by Runx2 (37), which is increased by TGF-β in breast cancer cells (38). Cancer cells that cause bone metastases often secrete the proteases MMP-9 and MMP-13, which are regulated by Runx2 (10, 38), and cathepsin K (39). These proteases are involved in bone resorption and osteoclast recruitment (40), and cathepsin K is essential for normal bone turnover. Cancer cells express a number of osteoblasts markers, such as osteopontin, bone sialoprotein, and osteocalcin (8), which are regulated by Runx2 in both osteoblasts and cancer cells (37, 41, 42).

IGF-I and IGF-II are the most abundant proteins in bone and important in bone development (for review, ref. 43). IGF signaling is also important in cancer and metastases; it promotes transformation and angiogenesis, induces cell proliferation and invasion, and is antiapoptotic (44). Both IGFs act through the IGF-IR to maintain cell growth. Their specific contributions to bone metastases are surprisingly untested. Different bone-seeking subclones of MDA-MB-231 cells had altered sensitivity to IGF-I in migration and anchorage-independent growth assays, perhaps due to increased expression of IGF-IR compared with parental cells (45, 46). In biopsies from prostate cancer patients with bone metastases, IGF-IR was frequently increased, as was the receptor substrate IRS-1 (47). Stable overexpression of IGF-IR in neuroblastoma cells increased tumor growth and osteolysis when the cells were directly injected in the tibia of mice (48). Similar results were obtained using MDA-MB-231 cells expressing of a dominant-negative IGF-IR, which decreased bone metastases (49). When MDA-PCA-2b prostate cancer cells were injected into human bone grafts in NOD/SCID mice, neutralizing antibodies against human IGF-I or mouse or human IGF-II, but not against mouse IGF-I, decreased development of bone lesions (50). However, engineered overexpression of IGF-I had no effect on two models of prostate cancer bone metastases (51). The development of skeletal metastases depends on the reactions of the cancer cells to the bone microenvironment, whose milieu consists of more than growth factors. It is also characterized by low pO_2, low pH, and high Ca^{2+}.

Physical Properties of the Bone Microenvironment

Hypoxia

Hypoxia is a major contributor to tumor metastasis, regulating secreted products that drive tumor-cell proliferation and spread. Hypoxia also contributes to resistance to radiation and chemotherapy in primary tumors. Solid tumors are particularly susceptible to hypoxia because they proliferate rapidly, outgrowing the malformed tumor vasculature, which is unable to meet the increasing metabolic demands of the expanding tumor.

Bone is a hypoxic microenvironment capable of potentiating tumor metastasis and growth. Hypoxia regulates normal marrow hematopoiesis and chondrocyte differentiation. The medullary cavity oxygen pressure in humans is estimated to be 5% O_2 (52). Cancer cells capable of surviving at low oxygen levels can thrive in the hypoxic bone microenvironment and participate in the vicious cycle of bone metastasis.

Hypoxic signaling is mediated by hypoxia-inducible factor-1 (HIF-1; ref. 53). This transcription factor is a heterodimer of HIF-1α and HIF-1β. HIF-1α expression is regulated in response to oxygen levels, whereas HIF-1β is constitutively expressed. Under normoxic conditions, oxygen-dependent prolyl hydroxylases modify HIF-1α at specific residues within the oxygen-dependent degradation domain. Hydroxylated HIF-1α is recognized and targeted for proteosomal degradation by the von Hippel-Lindau tumor suppressor, which is a component of an E3

ubiquitin-protein ligase (54). When oxygen levels are low, HIF-1α is no longer targeted for degradation by prolyl hydroxylases and instead, heterodimerizes with HIF-1β. The HIF-1 heterodimer enters the nucleus where it binds to hypoxia-response elements in DNA and mediates the transcription of numerous hypoxia-response genes.

Hypoxic signaling is increased in cancer cells exposed to low oxygen levels in the primary tumor. Hypoxia-response genes regulated by HIF-1 include glycolytic enzymes, glucose transporters, and vascular endothelial growth factor, which is important for angiogenesis. Other genes are expressed in a cell-type specific manner, including ones involved in tissue remodeling/migration/invasion, apoptosis, stress responses, proliferation/differentiation, and growth factor/cytokine function (55). Many are also prometastatic, suggesting a role for hypoxia signaling in the vicious cycle of bone metastasis.

In 13 different human cancers, including lung, breast, prostate, and colon, HIF-1α was overexpressed in two thirds of all the regional lymph node and bone metastases examined, including 69% of metastases versus 29% of primary tumors among the breast cancers (56). HIF-1α overexpression was correlated with advanced tumor stage (57), suggesting that increased HIF-1α is associated with a more aggressive and metastatic tumor phenotype.

In vitro, HIF-1α overexpression correlated with increased invasive potential of human prostate cancer cells, as well as enhanced expression of vimentin, cathepsin D, and MMP-2, which are important for cell migration and invasion, and decreased levels of E-cadherin, which is responsible for maintenance of cell-cell contacts and adhesion (58). Vimentin and E-cadherin are involved in epithelial-mesenchymal transition early in metastatic progression. Through upregulation of these proteins, HIF-1 alters the phenotype of tumor cells to increase their metastatic capability.

HIF-1α increases the transcription of factors that could accelerate the vicious cycle of skeletal metastases. MET, a receptor tyrosine kinase that binds hepatocyte growth factor, is overexpressed in advanced breast cancer and is associated with invasion and metastasis. MET expression is mediated by HIF-1α under hypoxic conditions. HIF-1α and MET cooverexpression in primary tumor samples from breast cancer patients who had undergone modified radical mastectomy was independently correlated with metastasis and decreased 10-year disease-free survival (59). HIF-1 also regulates the expression of other factors, including adrenomedullin, chemokine receptor 4, and connective tissue growth factor, with known roles in carcinogenesis and tumor metastasis (35, 55, 60, 61).

Under normoxic conditions, HIF-1α stabilization is regulated by numerous growth factors and cytokines through the phosphatidylinositol-3-kinase/protein kinase B (Akt) and the mitogen-activated protein kinase pathways (62). Growth factors, such as IGFs, fibroblast growth factor, epidermal growth factor (EGF), and tumor necrosis factor-α, have been shown to stabilize HIF-1α. Expression of these factors by tumor cells is associated with enhanced proliferation and tumor spread. Hypoxia and growth factor signaling pathways may synergistically promote the vicious cycle of skeletal metastasis.

Several studies have shown crosstalk between hypoxia and growth factor signaling pathways. In normoxic conditions, the EGF receptor (EGFR) signaling pathway activates HIF-1α–mediated transcription of survivin, a protein which increases apoptotic resistance of human breast cancer cells, thus contributing to a more aggressive cancer phenotype (63). Crosstalk also occurs between the HIF-1α and TGF-β signaling pathways: TGF-β increases hypoxic signaling by selectively inhibiting prolyl hydroxylase 2 and decreasing HIF-1α degradation (64). As discussed previously, TGF-β is important in osteolytic bone metastases, and these results show that TGF-β potentiates HIF-1 signaling within the hypoxic bone microenvironment.

As a regulator of tumor progression and metastasis, the hypoxia signaling pathway is an important chemotherapeutic target. Inhibiting this pathway may prevent the development of HIF-mediated resistance to chemotherapy and radiation therapy. A number of small molecule inhibitors of hypoxia signaling are under development. One such inhibitor is 2-methoxyestradiol, a poorly estrogenic estrogen metabolite and microtubule-depolymerizing agent with antiangiogenic and antitumorigenic properties (65). 2-Methoxyestradiol decreases HIF-1α levels and vascular endothelial growth factor mRNA expression *in vitro* and induces apoptosis of tumor cells (66, 67). 2-Methoxyestradiol is currently being evaluated in phases I and II clinical trials for the treatment of multiple types of cancer, and more potent analogues with improved antiangiogenic and antitumor effects are being developed (68). Other small molecule antihypoxic agents include inhibitors of topoisomerase I and II, such as camptothecin and GL331, and inhibitors of phosphatidylinositol-3-kinase, such as LY294002 — all of which have been shown to inhibit HIF-mediated gene transcription (62). Because HIF-1 crosstalks with multiple signaling pathways, inhibiting hypoxia signaling alone may be inadequate to halt tumor growth and spread (69). However, small molecule inhibitors could be useful in combination with other therapies to halt the vicious cycle of metastasis.

Acidic pH

Acidosis of the bone microenvironment also potentiates the vicious cycle of bone metastasis. Extracellular pH is tightly regulated within bone and has significant effects on osteoblast and osteoclast function. Extracellular acidification results in increased osteoclast resorption pit formation, with osteoclasts being maximally stimulated at pH levels of <6.9 (70). Osteoblast mineralization and bone formation is significantly impaired by acid (71). The combined effect on osteoclasts and osteoblasts is the release of alkaline bone mineral from the skeleton, compensating for systemic acidosis.

Tumor metastasis leads to localized regions of acidosis within the skeleton (70). Increased glycolysis and lactic acid production by proliferating cancer cells and decreased buffering capacity of the interstitial fluid contribute to the

acidic microenvironment within primary tumors (72). The acid-mediated tumor invasion hypothesis states that altered glucose metabolism in cancer cells stimulates cancer cell proliferation and results in a more invasive tumor phenotype (73). Acidosis alters cellular dynamics at the interface between the tumor and normal tissue, promoting apoptosis in adjacent normal cells and facilitating extracellular matrix degradation through the release of proteolytic enzymes. Unlike normal cells, cancer cells have compensatory mechanisms to allow proliferation and metastasis even at low extracellular pH and thus are not susceptible to acid-induced apoptosis.

Hypoxia further promotes acidosis within tumor cells through HIF-mediated overexpression of glycolytic enzymes and increased lactic acid production (74). Together, hypoxia and pH regulatory mechanisms control survival and proliferation of tumor cells. Apoptosis of E1a/Ras-transformed mouse embryo fibroblasts is mediated by hypoxia-induced acidosis rather than as a direct effect of hypoxia exposure (75).

Tumor acidosis promotes the release and activation of proteins, such as cathepsins B, D, and L and MMPs, which degrade the extracellular matrix and facilitate metastasis (73). Cathepsin B is a cysteine proteases expressed by tumor cells, which is activated in an acidic microenvironment and could participate in the vicious cycle of bone metastasis (76). It is expressed at low levels in primary prostate tumors; however, bone metastatic lesions express high levels of activated cathepsin B, suggesting that protease activity is modulated by interactions between tumor cells and the bone microenvironment (77).

Hypoxia-mediated acidosis also activates numerous stress signaling cascades within tumor cells, including the nuclear factor-κB and activator protein-1 pathways, which in turn regulate the transcription of prometastatic factors, such as IL-8, a cytokine important for cell motility, proliferation, and angiogenesis (78). IL-8 expression is induced by prolonged hypoxia and decreased intracellular pH in pancreatic and prostate cancer cells (79). Its overexpression correlates with increasing tumor grade and metastasis in many cancers, including breast and prostate.

Both hypoxia and acidosis have been implicated in resistance of cancer cells to radiation and chemotherapy. Extracellular acidity contributes to chemotherapeutic resistance via a pH gradient that prevents the intracellular accumulation of weakly basic drugs, such as Adriamycin (74). Tumor acidosis is a direct consequence of hypoxia exposure. Thus, therapeutic approaches, which target hypoxia signaling may exert their beneficial effects by correcting pH in cancer cells, making them more susceptible to conventional radiation and chemotherapy.

Extracellular Calcium

Calcium released from the mineralized bone matrix contributes to the vicious cycle of metastasis by several mechanisms. Calcium is the primary inorganic component of the bone matrix and, in the bone microenvironment, levels are maintained within a narrow physiologic range (~1.1-1.3 mmol/L; ref. 80). Active osteoclastic bone resorption causes extracellular calcium (Ca^{2+}_o) levels to rise up to 8 to 40 mmol/L (81).

Calcium effects are mediated through the extracellular calcium-sensing receptor (CaSR), a G protein–coupled receptor, which, in the presence of high Ca^{2+}_o, inhibits cyclic AMP and activates phospholipase C (82). The CaSR is expressed in normal tissues and is overexpressed in several types of cancer, including breast and prostate cancer (83, 84). The CaSR regulates secretion of PTHrP, whose role in osteolytic bone metastases is discussed previously (83). In normal mammary epithelium, the CaSr responds to low Ca^{2+}_o by increasing PTHrP, which activates bone resorption and release of bone matrix calcium. PTHrP production from these cells is decreased by high Ca^{2+}_o or CaSR agonists (85). Unlike normal mammary epithelial cells, breast cancer cells secrete increased levels of PTHrP in response to known agonists of the CaSR: high Ca^{2+}_o, spermine, and neomycin (83). Similar effects were observed in prostate cancer cells (84). Expression of a dominant-negative form of the CaSR in prostate cancer cells prevented Ca^{2+}_o-stimulated PTHrP release, whereas TGF-β pretreatment increased basal and Ca^{2+}_o-stimulated PTHrP (84). Thus, the vicious cycle of bone metastasis includes contributions by the CaSR: TGF-β and Ca^{2+}_o released during osteolysis activate the CaSR to increase PTHrP release, perpetuating osteolysis and bone matrix destruction.

Ca^{2+}_o has also been shown to specifically induce proliferation of PC-3 and C4-2B prostate cancer cells known to metastasize to the skeleton at concentrations of 2.5 mmol/L but does not affect LNCaP prostate epithelial cells, which do not form bone metastases (86). This effect is likely mediated by the CaSR, as knockdown of the CaSR by shRNA decreased PC-3 cell proliferation *in vitro* and inhibited the formation of bone metastases in mice. Clinically, overexpression of cytoplasmic CaSR in breast cancer tumor samples is positively correlated with the bone metastases rather than visceral metastases, suggesting that the CaSR may be a good potential marker for predicting bone metastases (87).

The CaSR activates Akt signaling to promote PC-3 cell attachment *in vitro*. Similarly, bone matrix calcium may act through this receptor to help cancer cells localize to and attach to bone during metastasis. The CaSR also signals in part through the mitogen-activated protein kinase signaling pathway to stimulate PTHrP release. Inhibitors of mitogen-activated protein/extracellular signal-regulated kinase kinase, p38 mitogen-activated protein kinase, protein kinase C, and c-Jun-NH_2 kinase prevented CaSR-stimulated PTHrP release by HEK293 and H-500 Leydig cancer cells in response to high Ca^{2+}_o (88, 89). Increased phosphorylation of EFK1/2, p38 mitogen-activated protein kinase, and SEK1 (upstream of c-Jun-NH_2 kinase) was observed in response to Ca^{2+}_o activation of the CaSR (88, 89).

G protein–coupled receptors, of which the CaSR is one, transactivate tyrosine kinase receptors and activate mitogen-activated protein kinase signaling cascades (90). The CaSR may interact with the EGFR signaling pathway to

stimulate PTHrP release. High Ca^{2+}_o resulted in delayed phosphorylation of extracellular signal-regulated kinase in PC-3 cells (91). An inhibitor of the EGFR kinase or an EGFR-neutralizing antibody prevented extracellular signal-regulated kinase phosphorylation and reduced PTHrP secretion, supporting a mechanism whereby the CaSR transactivates EGFR, resulting in extracellular signal-regulated kinase phosphorylation and increased PTHrP release. Such a mechanism may explain the finding that EGF induced PTHrP in prostatic epithelial cells (92). Inhibitors of the EGFR, such as gefitinib or PKI166, reduced osteoclastogenesis (93) and malignant osteolysis, as well as the growth of cancer cells in bone (94, 95), suggesting that the EGFR may be an important target in the vicious cycle of bone metastasis.

Two classes of therapeutic agents targeting the CaSR have been developed. Calcimimetics, including cinacalcet, increase the affinity of the CaSR for Ca^{2+}_o, which in turn inhibits release of PTH or PTHrP and leads to lower serum calcium levels. Calcimimetics have been approved for the treatment of hyperparathyroidism in end-stage renal disease and for parathyroid cancer (96). A second class of drugs which targets the CaSR is the calcilytics. Calcilytic agents have been proposed as an anabolic therapy for osteoporosis and act similarly to injectable PTH, though these drugs have not yet been approved for clinical use (96). By preventing calcium-stimulated activation of the CaSR and release of PTHrP by tumor cells, calcimimetics and calcilytics may interrupt the vicious cycle and are potentially useful for the prevention and treatment of bone metastases.

Conclusion

Crosstalk between tumor cells and the bone microenvironment promotes a vicious cycle of bone metastasis. This crosstalk occurs via multiple factors and signaling pathways. The bone microenvironment contains numerous physical factors, such as hypoxia, acidosis, and extracellular calcium, and growth factors, like TGF-β, which have been implicated in this vicious cycle. These factors activate signaling pathways in cancer cells, promoting a more aggressive tumor phenotype. Whereas much is understood about the effects of these factors in cancer cells at the primary tumor site, continued research is necessary to further elucidate their role in skeletal metastasis. Understanding the interactions between tumor and bone may help to identify potential targets for chemotherapeutic intervention to halt tumor growth and bone metastasis.

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