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PROSPECTS

Skeletal Metastases: Decreasing Tumor Burden by Targeting the Bone Microenvironment

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Abstract Several common cancers often metastasize to the skeleton in advanced disease. Bone metastases are incurable and cause protracted, severe symptoms. Growth of tumor in bone is driven by a vicious cycle: tumor-secreted factors stimulate bone cells, which in turn release growth factors and cytokines. The bone-derived factors fuel the vicious cycle by acting back on the tumor cells. The vicious cycle offers novel targets for the treatment of advanced cancers. Treatments can inhibit bone cells (osteoclasts and osteoblasts) that are stimulated by tumor-secreted factors. Drugs can also inhibit tumor responses to factors enriched in the bone microenvironment, such as transforming growth factor-β. Animal models show that these approaches, especially combination treatments, can reduce tumor burden. The results suggest a novel paradigm in which tumor growth can be effectively inhibited by drugs that target cells in the bone microenvironment and not the tumor cells themselves. J. Cell. Biochem. 102: 1333–1342, 2007.

Key words: bone metastases; osteoblastic metastases; osteolytic metastases; transforming growth factor beta; endothelin-1; Wnt signaling; bisphosphonates; hypoxia

Breast and prostate cancers are the leading causes of cancer death among women and men—second only to lung cancer. Early detection and treatment of these cancers has increased 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 drops to 26% for patients initially diagnosed with distant metastases, while prostate cancer survival rate drops to 33% with distant metastases [Jemal et al., 2007]. The skeleton is a preferred site for breast and prostate cancer metastasis. Many other common cancers, including lung and renal tumors, melanoma, and multiple myeloma also attack the skeleton. Skeleton metastases are radiographically classified as osteoblastic or osteolytic, resulting from imbalances between osteoblast-mediated bone formation and osteoclast-mediated bone resorption. Osteoblastic lesions, characteristic of prostate cancer, are caused by an excess of osteoblast activity leading to abnormal bone formation. In breast cancer, osteolytic lesions are found in 80% of patients with stage IV metastatic disease [Kozlow and Guise, 2005], and are characterized by increased osteoclast activity and net bone destruction [Kakonen and Mundy, 2003]. Breast cancer bone lesions span a spectrum; most are osteolytic, but up to 15% are osteoblastic or mixed. Regardless of diagnosis, most patients have an evidence of both abnormal bone resorption and formation. Autopsy of bone metastases shows phenotypic heterogeneity both within a particular lesion and between lesions from a single prostate cancer patient [Roudier et al., 2003]. Both osteoblastic and osteolytic bone metastases cause skeletal-related events (SREs), complications that include bone pain, hypercalcemia, pathologic fractures, and spinal cord and nerve compression syndromes [Coleman, 1997]. SREs increase morbidity and diminish the quality of life.

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The vicious cycle of bone metastasis: Metastasis to bone occurs late in tumor progression through multiple steps. Cancer cells must detach from the primary tumor and invade blood vessels. Cancer cells in the bloodstream are attracted to preferred target tissues [Kakonen and Mundy, 2003]. Tumor cells within the skeleton adhere to the endosteal surface and colonize bone. The bone microenvironment is comprised of osteoblasts, osteoclasts, mineralized bone matrix, and other cell types. Crosstalk between tumor cells and the microenvironment fuels a vicious cycle of tumor growth and bone remodeling [Kozlow and Guise, 2005; Yoneda and Hiraga, 2005], illustrated in Figure 1. Tumor cells secrete factors that stimulate osteoclast-mediated bone destruction, which releases factors immobilized within the bone matrix. Tumor cells also stimulate osteoblast proliferation and maturation, resulting in additional production of growth factors into the local microenvironment. The locally enriched factors surrounding the tumor cells encourage their growth and appear to alter their phenotype to make them resistant to available anti-tumor treatments.

Signaling pathways: Signals from bone activate numerous tumor pathways that drive the vicious cycle. Wnt proteins released by metastatic prostate cancers stimulate osteoblasts and have autocrine effects on tumor proliferation [Hall et al., 2006]. An inhibitor of Wnt signaling, DKK1, can regulate metastatic progression by opposing osteogenic Wnts early in metastasis and 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 [Koeneman et al., 1999]. Metastatic breast cancer cells express bone sialoprotein [Barnes et al., 2003] 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 and matrix metalloproteinases (MMP) and upregulates adhesion proteins that allow tumor and bone cells to bind [Pratap et al., 2005]. Runx2 expression by cancer cells may

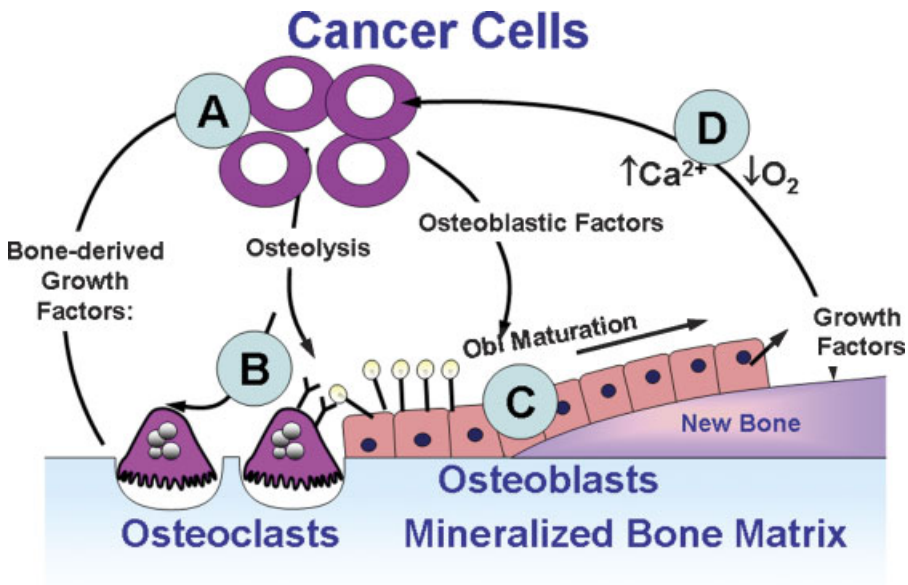

Fig. 1. Vicious cycle of bone metastases. Factors (such as MMPs, CXCR4, and VEGF) attract metastatic tumor cells to bone and facilitate survival within the bone microenvironment. Physical factors within the bone microenvironment, including hypoxia, acid pH, and extracellular Ca^{2+}, and bone-derived growth factors, such as TGFβ and IGFs, activate tumor expression of osteoblast-stimulatory factors, including VEGF and ET-1. Osteoclast-stimulatory factors, including PTHrP, IL-8, and IL-11 are also increased. PTHrP and IL-11 act on early osteoblasts to increase expression of RANKL (lollipops), which stimulate bone resorption via the RANK receptor on osteoclasts (Y's). The factors stimulate bone cells, which in turn release factors that promote further tumor growth in bone, driving a vicious cycle. The vicious cycle offers targets for therapeutic intervention in addition to the tumor cells (**A**) themselves; (**B**) Osteoclastic bone resorption; (**C**) Osteoblastic proliferation and maturation; (**D**) Physical milieu: O_2, Ca^{2+}. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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 by double-feeding the vicious cycle.

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 TGFβ and IGFs I and 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 [Vessella and Corey, 2006; Morrissey and Vessella, 2007; Mitsiades et al., 2007]. Hypoxia, acidosis, and high calcium, plus growth factors such as TGFβ and IGFs, combine to drive the vicious cycle of bone metastasis [Kingsley et al., 2007].

TREATMENT STRATEGIES

Therapeutic paradigm: There are four major targets for therapeutic intervention against bone metastases: (A) the tumor cells themselves, but also (B) osteoclastic bone resorption; (C) the activity of osteoblasts; and (D) the specific bone microenvironment surrounding the tumor cells themselves, as outlined in Figure 1. Tumor cell proliferation is the conventional target for cytotoxic chemotherapy and adjuvant therapies aimed at sex steroid and growth factor receptors and heat shock protein (HSP) 90, for examples. These are not specific to bone metastases. However, specific factors in the microenvironment such as TGFβ activate receptors that can be inhibited with drugs entering clinical trials. Such factors are enriched in the bone microenvironment due to their synthesis by osteoblasts, their storage in bone matrix, and their release at locally high concentrations by osteoclastic bone resorption. Thus, the targets we propose for therapeutic intervention have overlapping functions to drive the vicious cycle, suggesting that combined inhibition of different steps may be more effective than single inhibitors. The large amounts of bone matrix (the soil in the seed and soil model of Steven Paget [Fidler et al., 2007]) at metastatic sites provide a source of tumor-stimulating factors unique to bone among mesenchymally derived tissues such as fat and muscle and probably contributes to the high clinical incidence of skeletal metastases.

Targeting osteoclasts forms the basis for approved clinical treatments of all tumor types that attack the skeleton. Bisphosphonate drugs have been developed for osteoporosis through several generations of compounds; all have high affinity for the hydroxyapatite mineral phase of bone matrix. They are cellular poisons of low specificity that are metabolized to non-hydrolyzable ATP analogues or, in the case of the nitrogen-containing bisphosphonates, by inhibiting farnesyl pyrophosphate synthase and subsequent Rho protein prenylation steps [Russell, 2007]. Two other classes of anti-resorptive drugs are presently in clinical trials [Shoback, 2007]. A human monoclonal antibody, denosumab, neutralizes RANK ligand to prevent osteoclast activity and maturation from hematopoietic precursors. Several small molecule inhibitors of cathepsin K are being tested. This secreted protease is selectively expressed by osteoclasts and is necessary for efficient bone resorption. The various classes of osteoclast inhibitors act at different points in the cell lineage. Denosumab should eliminate mature osteoclasts entirely. Bisphosphonates poison osteoclasts that have eaten bone. Cathepsin K inhibitors should act indirectly to prevent the resorptive action of otherwise healthy osteoclasts. All of these types of inhibitors should decrease osteolytic bone destruction but may have different secondary actions. Osteoclasts are secretory cells that communicate with other cells in the bone milieu. The secretory functions are poorly understood but may be preserved in cells treated with cathepsin K inhibitors. Possible differential effects on metastases are unknown, but bisphosphonate and RANK ligand inhibition have similar and non-additive effects to decrease bone metastases in a standard mouse model using the MDA-MB-231 human breast cancer cell line [Zheng et al., 2007]. The potential for these agents to inhibit tumor cells directly remains controversial since it is unclear whether the drugs will achieve sufficient concentrations in tumor cells in vivo.

Targeting osteoblasts is a much less well-developed therapeutic strategy than anti-resorptive treatments. Markers of activity of both osteoblasts and osteoclasts are high in cancer metastases. Osteoblast activity is

depressed in multiple myeloma. In prostate cancer the majority of bone metastases are osteoblastic, presumably due to tumor secretion of osteoblast-stimulatory factors. Many such factors have been identified, such as the IGFs, platelet-derived growth factor B, bone morphogenetic proteins, and the small peptide vasoconstrictor endothelin-1 (ET-1), which can stimulate bone formation by suppressing DKK1, an inhibitor of Wnt signaling [Clines et al., 2007]. Inhibitors of the G protein-coupled endothelin A receptor are in Phase III clinical trials in men with advanced prostate cancer [Carducci and Jimeno, 2006]. One anabolic agent for the treatment of osteoporosis has been approved: daily administration of parathyroid hormone (PTH) to stimulate osteoblast function. Its use in cancer patients at risk of bone metastases is contraindicated. Nonetheless, bone loss and osteoporosis are major side effects of most cancer therapies and often require treatment [Guise, 2006]. Endothelin was an unexpected stimulator of bone formation and causal agent in osteoblastic metastasis. It seems more likely that centrally important regulators of the osteoblast lineage and bone formation [Cohen, 2006; Lian et al., 2006] would be subverted by cancer cells metastatic to the skeleton. This predicts that the BMP and Wnt signaling pathways should be the major targets for treatment interventions.

The bone morphogenetic proteins are a family of growth factors that stimulate bone formation and are part of the TGFβ superfamily [Gazzerro and Canalis, 2006]. Breast cancer cells express BMPs and BMP receptors, while the factors have both growth inhibitory and stimulatory effects on cancer cells. Overexpression of a dominant negative type II bone morphogenetic protein receptor in T47D breast cancer cells inhibited proliferation. Different BMPs may have different growth effects on breast cancer cells. Increased expression of the bone morphogenetic protein receptor IB is associated with increased tumor grade, proliferation, cytogenetic instability, and poor prognosis of estrogen receptor-positive breast carcinomas. Overexpression of BMP-2 in MCF-7 breast cancer cells increased the invasive ability of these cells in vitro and in vivo and enhanced estrogen-independent growth of these cells in a xenograft mouse model. Overexpression of the BMP antagonist, noggin, in two prostate cancer cell lines decreased osteolytic and osteoblastic lesions after injection into the tibia of immunodeficient mice [reviewed in Siclari et al., 2007]. Addressing the roles of BMPs and their signaling in bone metastases is complicated by the wide variety of ligands and secreted antagonists in the family, as well as multiple receptors. It is not presently clear that the BMPs offer a viable target for therapeutic development against skeletal metastases. However, very recent data [Buijs et al., 2007] indicate that BMP-7 opposes the epithelial-mesenchymal transition and TGFβ signaling in prostate cancer cells. Daily administration of BMP-7 inhibited growth of prostate cancer cells implanted as xenografts in bone but not orthotopically.

The Wnt signaling pathway has a known role in both oncogenesis and osteogenesis. Wnt signaling activates osteoblasts and Wnt signaling inhibitors like dickkopf-1 (DKK1) inhibit this activation. Activation of the Wnt signaling pathway also promotes mammary carcinogenesis. Downregulation of inhibitors of Wnt signaling, secreted frizzled-related protein 1 (sFRP1) and the transcription factor TCF-4, was identified in a subset of breast cancers. Deletion of the chromosomal region containing sFRP1 is often detected in breast cancer. Aberrant hypermethylation (gene-silencing) of sFRP1 was also associated with an unfavorable prognosis for breast cancer. Changes in methylation of the DKK1 promoter may be responsible for changes in expression over time in prostate cancer bone metastases [Hall et al., 2005, 2006]. Increasing Wnt activity by knocking down DKK1 expression with DKK1 short hairpin RNA caused osteolytic PC3 prostate cancer cells to induce osteoblast activity, and decreasing Wnt activity by overexpressing DKK1 converts prostate cancer cells with a mixed osteolytic-osteoblastic phenotype to an osteoblastic one [Hall et al., 2005]. Wnt signaling contributes to prostate cancer osteoblastic bone metastasis formation and may in the same way contribute to breast cancer bone metastasis. Suppression of the Wnt signaling pathway may reduce osteoblastic bone metastasis. A green tea compound epigallocatechin 3-gallate (EGCG) inhibits Wnt signaling and reduces breast cancer cell proliferation and invasiveness. Oral administration of EGCG reduced breast cancer tumor progression in animal models. However, Wnt signaling inhibition has been suggested to be one of the mechanisms that multiple myeloma induces bone destruction by inhibiting bone formation [Mitsiades et al., 2007]. Multiple myeloma cells and multiple myeloma patients with advanced osteolytic lesions secreted

the Wnt inhibitor, secreted frizzled-related protein-2 (sFRP-2), which inhibits bone formation. Further research is needed to test the role of the Wnt signaling inhibitors in cancer bone metastasis [Hall et al., 2006]. As is the case with BMP signaling, the Wnt pathway is of huge complexity—with a very large number of ligands and antagonists that act through complex receptors and signaling pathways—in both bone cells and cancer cells [Bodine and Komm, 2006].

Targeting the physical microenvironment can be approached in two ways. One is to use unique properties of bone to deliver drugs, which is the basis of the specificity for bone of the bisphosphonates. The idea of using conjugation to a bisphosphonate to target drugs to bone is not new. Src inhibitor-bisphosphonate conjugates have been developed and tested [Boyce et al., 2006], validating the approach. Bisphosphonates can target classical small molecule agents selectively into the bone microenvironment using a variety of coupling chemistries and linkers [Zhang et al., 2007]. However, it is not clear that subsequent release from bone provides good pharmacokinetic delivery of active drug to the desired targets such as local tumor cells.

Could the microenvironment itself be changed to suppress metastases? Facets of the microenvironment that are conducive to metastatic colonization and growth probably include hypoxia, low pH, and high calcium [Kingsley et al., 2007]. These physical parameters are not practical to change in vivo. However, the responses of the tumor cells to such parameters are druggable, as discussed next. The idea of normalizing the tumor microenvironment has been reviewed recently [Fukumura and Jain, 2007].

Targeting tumor cells can be tailored for bone specificity, since the bone milieu provides an unusual mix of extracellular conditions that tumor cells elsewhere seldom experience: low pH, low pO_2, high Ca^{2+}, high extracellular nucleotides such as ATP [Hoebertz et al., 2003], and high concentrations of bone-derived protein factors such as IGFs I and II, TGFβ, and lesser amounts of BMPs, PDGFs, and FGFs [Hauschka et al., 1986]. Signaling by the tumor cell in response to many of these factors can be inhibited by small synthetic molecules, natural products, and neutralizing antibodies. This area is obviously too broad to survey in a single article. We focus here on the leading candidates for rapid translation into clinical treatments. In the section "FUTURE PROSPECTS", below, we speculate on additional targets that may warrant more intensive future study.

TGFβ is not the most abundant growth factor in bone, but it has the best-established role in cancer metastases. TGFβ binds to a heterodimeric receptor and can activate the canonical Smad signaling pathway or Smad-independent pathways. TGFβ is deposited in the bone matrix by osteoblasts and released and activated during osteoclastic resorption [Dallas et al., 2002] and can regulate bone development and remodeling. Advanced cancers often lose growth inhibition by TGFβ; the factor then drives metastases by activating epithelial-mesenchymal transition and invasion, increasing angiogenesis and suppressing immune surveillance of tumor cells [Elliott and Blobe, 2005].

TGFβ stimulates bone metastases by inducing pro-osteolytic gene expression in cancer cells, such as parathyroid hormone-related protein (PTHrP), which is expressed by many osteolytic cancer cell lines. Its expression is higher at sites of bone metastases compared to non-osseous metastases. Among factors released from bone during resorption, only TGFβ increases PTHrP production. The consequent increase in bone resorption releases more bone matrix factors to act on cancer cells, sustaining a vicious cycle [Kakonen and Mundy, 2003; Siclari et al., 2006]. PTHrP is not the only factor regulated by TGFβ. The factor increases COX-2 expression in osteoblasts, bone marrow stromal cells, and in breast cancer cells. COX-2 expression in bone-seeking subclones of a breast cancer cell line correlates with increased production of interleukin-8 (IL-8), which induces osteoclast formation and activity independently of RANK ligand and can also induce IL-11, which increases osteoclasts via RANK ligand. IL-11 does not increase bone metastases in the absence of other pro-metastatic factors such as osteopontin, a protein whose expression is regulated by Runx2, which is in turn increased by TGFβ in breast cancer cells. Cancer cells that cause bone metastases often secrete the proteases MMP-9 and MMP-13, which are regulated by Runx2. Such proteases are involved in bone resorption and osteoclast recruitment, while cathepsin K is essential for normal bone turnover. Cancer cells express a number of osteoblasts markers such as osteopontin, bone sialoprotein, and osteocalcin that

are regulated by Runx2 in both osteoblasts and cancer cells [Barnes et al., 2003; Pratap et al., 2005], while TGFβ could regulate gene expression in parallel in cancer cells and bone cells [Kang et al., 2003].

Breast cancer cells expressing a reporter gene under the control of a TGFβ-sensitive promoter were micro-PET imaged in an animal model of metastases. The reporter was activated only in bone and not in adrenal metastases [Kang et al., 2005], demonstrating the bone-specific role of TGFβ signaling. Knockdown of Smad4, engineered expression of inhibitory Smad7 or dominant-negative TGFβ type II receptor dramatically decrease bone metastases in breast and melanoma models. Small-molecule inhibitors of TGFβ type I receptor kinase give similar results in mouse models and such compounds may soon reach the clinic [Biswas et al., 2006]. Consistent with these animal data, human breast cancer bone metastases show nuclear staining for phosphorylated Smad proteins, indicating active TGFβ signaling in tumor cells housed in bone [Kang et al., 2005].

Extracellular calcium occurs at very high concentrations in bone, where it contributes to the vicious cycle of metastasis. Calcium is the primary inorganic component of the bone matrix. Active osteoclastic bone resorption can raise extracellular calcium up to 40 mmol/L. Calcium binds to the G-protein-coupled extracellular calcium-sensing receptor (CaSR) to decrease cAMP and activates phospholipase C. The CaSR is widely expressed in normal tissues and overexpressed by breast and prostate cancers. It suppresses secretion of PTHrP by normal breast epithelium, but breast and prostate cancer cells secrete *increased* amounts of PTHrP in response to CaSR agonists. TGFβ and Ca^{2+}released during osteolysis may act together to activate the CaSR and increase PTHrP release, perpetuating osteolysis and bone matrix destruction. Ca^{2+} increases proliferation of several prostate cancer lines that metastasize to the skeleton. Knockdown of the CaSR by shRNA in a prostate cancer cell line decreased formation of bone metastases in mice. High cytoplasmic expression of CaSR in breast cancer clinical samples positively correlates with metastases to bone rather than viscera. Allosteric regulators of the receptor set-point for extracellular Ca^{2+} have been developed, and these could have future applications to the treatment of bone metastases, although their current clinical use is in the management of hyperparathyroidism via effects on systemic PTH production in response to circulating Ca^{2+} [Brown, 2007].

FUTURE PROSPECTS

What can we expect? Current clinical treatments for established bone metastases are palliative. They effectively reduce SREs and improve patient quality of life, but they do not increase survival [Kozlow and Guise, 2005]. Data on whether the newer antiresorptive treatment directed against RANKL, denosumab, has better efficacy in patients with bone metastases should become available from clinical trials in progress. We now also appreciate that most types of cancer treatment cause bone loss [Guise, 2006]. The vicious cycle model (Fig. 1) predicts that such increased bone turnover could accelerate bone metastases. In many animal models, single treatments increase survival and decrease tumor burden [Yin et al., 2003; Yoneda and Hiraga, 2005]. These successes have yet to be reproduced in patients. A major morbidity in patients with bone metastases is intractable pain. Several factors in the vicious cycle are established nociceptive agents, including low pH and ET-1. Preclinical animal models need to be expanded to include testing the effects of treatments on bone pain, which remains a seriously understudied area, probably due to the complexity of the assays [Halvorson et al., 2006].

Lessons from animal models: It is unrealistic to expect animal models to provide novel insights and breakthroughs. Mice carrying endogenous or xenografted tumors are essentially assays to test specific experimental questions regarding the roles of one or a few factors or pathways in a specific physiological response. Such models have usefully informed clinical trials with endothelin A receptor antagonists, for example [Yin et al., 2003; Titus et al., 2005; Carducci and Jimeno, 2006]. Preclinical model experiments carried out in vivo are much more carefully controlled and homogeneous than a clinical trial, so it is not surprising that the former can generate much cleaner and impressive responses (including reduced tumor burden and increased survival) than the latter. A tougher issue is how metastatic lesions change over time and whether this is an important consideration in designing treatment strategies.

Hall et al., 2005 showed that changes in the phenotype of prostate cancer bone metastases may be due to changes in Wnt signaling in the bone microenvironment and changing expression of the Wnt inhibitor DKK1 [Hall et al., 2006]. Such gene expression changes may underlie the variable phenotype of metastatic lesions seen at autopsy in a single patient, where the tumor cells are probably genomically relatively homogeneous [Roudier et al., 2003]. It is likely that the phenotype of the bone cells is also temporally altered, in particular the osteoblast lineage, which changes over the course of more than a month and employs a large number of signaling pathways [Lian et al., 2006], of which the Wnts are an important part [Bodine and Komm, 2006]. Understanding the temporal co-progression of tumor and bone in vivo will require a sophisticated combination of molecular readouts from the cells as well as externally regulatable expression of genes under study.

Combination treatments: In animal models combined treatments often work better than single ones, such as combined inhibition of bone resorption with bisphosphonates plus attacking one of the other parts of the vicious cycle, but, again, the preclinical animal results, need to pass the sterner tests posed by clinical trials.

Too many factors? A search of the literature will quickly show that there are too many candidate factors released by or acting on tumor cells in the microenvironment [reviewed in Siclari et al., 2006] for practical targeting in the clinic. This dilemma is reinforced by the classic work of Kang et al., 2003, which showed that the bone-selectivity of breast cancer metastasis in an animal model cannot be conferred by transduction of a single pro-metastatic gene but requires a set of four or more genes. Given the high cost of development and clinical testing for a single agent, it is financially impractical to develop four such agents for combination treatment. There are two alternatives: (1) to find and inhibit the most important individual targets, or (2) to identify central, upstream mediators and inhibit them to achieve pleiotropic downstream responses in multiple pathways.

Targeting central upstream pathways: IGFs, hypoxia, HSP90, extracellular nucleotides. We have discussed above the possibility of targeting TGFβ signaling. The focus on this factor originates from its known presence in the bone microenvironment. The most abundant non-structural proteins in mineralized bone matrix are the insulin-like growth factors II and then I [Hauschka et al., 1986]. Both IGFs act through the IGF-IR to maintain cell growth. IGF-I and IGF-II are important in bone development. In cancer and metastases IGF receptor signaling promotes transformation and angiogenesis, induces cell proliferation and invasion and is anti-apoptotic. Both IGFs act through the IGF-IR to maintain cell growth. Their specific contributions to bone metastases are surprisingly understudied. Different bone-seeking subclones of the MDA-MB-231 breast cancer cell line had altered sensitivity to IGF-I in migration and anchorage-independent growth assays. In prostate cancer biopsies of bone metastases, IGF-IR and its substrate IRS-1 are increased. Stable overexpression of IGF-IR increases neuroblastoma growth and osteolysis in the injected tibia of mice. A dominant-negative IGF-IR similarly decreases bone metastases. Neutralizing antibodies against human IGF-I or mouse or human IGF-II, but not against mouse IGF-I, decreased development of bone lesions in a prostate cancer xenograft model. However, engineered overexpression of IGF-I had no effect on two models of prostate cancer bone metastases [Rubin et al., 2006]. Overall, it is quite unclear whether bone-derived IGFs are important contributors to bone metastases. In addition to matrix-derived (and ultimately osteoblast-synthesized) cytokines and growth factors, the bone microenvironment is physically unlike many other organ sites in the body. Other possible major pathways can arise from tumor cell crosstalk with other cells in the microenvironment, such as platelets, which can transfer lysophosphatidic acid to tumor cells. Recent works suggests that the lysophosphatidic acid receptor may be a useful target for therapy against bone metastases [Boucharaba et al., 2006].

Hypoxia: In response to hypoxia tumor metastases secrete proteins that can drive tumor growth. Hypoxia also contributes to resistance to radiation and chemotherapy. Rapidly proliferating solid tumors are susceptible to hypoxia when they outgrow their poorly formed vascular supply, which is unable to meet the increasing metabolic demands of the tumor. Bone is a hypoxic microenvironment that can potentiate 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. Cancer cells can survive at low oxygen levels such as the hypoxic bone microenvironment, where they can enter the vicious cycle of bone metastasis [Kingsley et al., 2007]. Hypoxic signaling is mediated by hypoxia-inducible factor 1 (HIF-1). In 13 human tumor types (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 of the breast [Zhong et al., 1999]. Many proinvasive, prometastatic, and proangiogenic factors are regulated by the hypoxic response pathway, and many of the factors implicated in bone metastases are transcriptionally regulated by both HIF-1 and TGFβ-regulated Smad complexes binding to closely spaced response elements in promoters, such as that for VEGF [Sanchez-Elsner et al., 2001]. Many drugs indirectly inhibit HIF signaling, but there are currently no small molecules that appear to be highly specific for HIF-1α. A role for this pathway can most clearly be tested by stable shRNA knockdown of HIF-1α in tumor cells that are then tested in vivo. Hypoxia is a popular area of cancer research, and improved anti-HIF compounds should become available for testing in the future.

Extracellular purine and pyrimidine nucleotides such as ATP have potent direct effects on osteoblasts and osteoclasts by binding to P2 receptors [Hoebertz et al., 2003]. These nucleotides are found in the bone microenvironment and metabolized by a variety of ectonucleases. They are also produced and act upon tumor cells through P2 receptors [White and Burnstock, 2006]. Inhibitors of these are under development for cardiovascular indications, and the role of these ligands and receptors warrants further study in cancer and metastasis.

HSP90 is a cellular chaperone necessary for the stable expression of proteins in the steroid hormone receptor signaling, HIF-1alpha, AKT/PKB, ERBB2, C-RAF, CDK4, mutant p53, survivin, telomerase, and other pathways that are important in cancer cells [Powers and Workman, 2006; Workman et al., 2007]. As such, HSP90 is a promising novel target for cancer therapy. HSP90 can be effectively inhibited with natural products such as geldanamycin. A less toxic derivative, 17-allylamino-17-demethoxygeldanamycin (17-AAG), effectively decreases growth of prostate and other cancer xenografts and has completed Phase I trials. However, 17-AAG increases bone metastases in a breast cancer model by direct effects of the drug to increase osteoclastic bone resorption [Price et al., 2005]. Such osteoclastic bone resorption is elevated in all types of bone metastases and causes severe insult to the skeleton, resulting in pathological bone loss [Guise, 2006]. In addition, osteoclastic resorption releases growth factors from bone to fuel a vicious cycle of bone metastasis, predicting that undesirable side-effects of 17-AAG will include increased clinical morbidity in the form of SREs and accelerated bone metastases. These side-effects of this otherwise very promising new class of drugs should be preventable by addition of anti-resorptive therapy to the treatment protocols. This cautionary tale emphasizes the importance of preclinical testing in animal models.

Will microenvironment-targeted agents increase efficacy of conventional cytotoxic antitumor treatments? Early diagnosis remains the best defense against cancer, since metastatic disease is seldom curable, suggesting that the microenvironment protects metastatic cells from killing by standard cytotoxic agents. If the microenvironment is fundamentally changed by the tumor cells, then normalizing it [Fukumura and Jain, 2007], may restore tumor chemosensitivity or radiosensitivity. These possibilities need further testing in animal models. For example, treatment of animals with an anti-resorptive bisphosphonate increased the sensitivity of prostate cancer bone metastases to killing by a cytotoxic microtubule agent and by a tyrosine kinase inhibitor [Kim et al., 2005].

Will lessons from bone metastases inform other organotropic metastatic models? Despite the difficulties of studying bone, the field of bone metastases has made substantial progress. Bone provides advantages in being imageable in vivo by standard X-rays, while its physical integrity permits accurate quantitative analysis post mortem for the assessment of tumor burden and bone responses. In this review, we advocate a paradigm in which drugs (such as anti-resorptives and endothelin A receptor antagonists) that target bone cell responses to tumor can decrease tumor burden without directly inhibiting the tumor cells themselves. Other organ microenvironments must similarly respond to tumor factors, which could then be targeted to reduce metastases. We found that endothelin was increased (due to loss of the

metastasis suppressor Rho GDI2) in bladder cancer cells that metastasize to lung, an organ responsive to ET-1. The same endothelin A receptor antagonist that effectively reduced breast cancer osteoblastic metastases also reduced lung metastases in a bladder cancer model [Titus et al., 2005].

CONCLUSIONS

Complex crosstalk between tumor cells and the bone microenvironment promotes a vicious cycle of bone metastasis through multiple extracellular factors and signaling pathways. The metastatic milieu contains physical factors, such as hypoxia, acidosis, and extracellular calcium, plus proteins such as TGFβ and Wnt gene family ligands that contribute to the vicious cycle. These factors activate signaling pathways in cancer cells, causing a more aggressive tumor phenotype. The ongoing interactions between tumor and bone cells in the skeleton may alter the phenotypes of the participating cells as metastatic lesions progress. Understanding the interactions between tumor and bone will identify potential targets for chemotherapeutic intervention and microenvironment-selective agents to halt tumor growth and bone metastasis and reduce the morbidity of SREs.

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