Published in final edited form as:

Curr Mol Med. 2013 May 1; 13(4): 626–639.

Understanding and targeting osteoclastic activity in prostate cancer bone metastases

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Abstract

Bone metastasis is a debilitating side effect of advanced prostatic carcinoma impacting nearly all of the men developing this disease. Even though a majority of these lesions are considered osteoblastic, it is believed that there is an underlying osteolytic component. Lytic processes are governed primarily by osteoclasts, the primary bone resorptive cell. Osteolysis has been implicated in tumor cell seeding and nourishment of tumor growth via development of pro-tumorigenic changes in the microenvironment. Herein, we provide a current view of the processes involved in regulating osteolysis in the presence of prostate cancer bone metastases. Several factors have been implicated in the division, differentiation, and activation of osteoclasts, including, but not limited to, interleukin-6, receptor activator of nuclear factor kappa B ligand (RANKL), osteoprotegerin (OPG), and parathyroid hormone-related protein (PTHrP). Effector molecules in bone resorption play a significant role, such as matrix metalloproteinases (MMPs), cathepsins, and acid secretion. The primary method for treating skeletal events associated with prostate cancer bone metastases has been bisphosphonates. However, a new therapeutic, denosumab, a monoclonal antibody that inhibits RANKL in a mechanism similar to that attributed to the endogenous mediator OPG, has received approval for treatment of skeletally associated metastases. Additional novel targets are continuously being developed for bone metastases. In this review, we describe the processes involved in osteolysis of the prostate cancer bone microenvironment, and introduce therapeutics that may play a role in inhibiting tumor growth leading to increased survival and quality of life.

Keywords

IL-6; Denosumab; RANKL; OPG; Bisphosphonates; bone microenvironment; osteoclast; osteoblast; prostate cancer

Introduction

Bone is a common site for metastasis in a number of tumors, including prostate, breast, and lung cancer; however, this review will focus primarily on PCa bone metastases. Even though only 8–14% of men have clinically evident bone metastases at diagnosis, it has been suggested that 85–100% of patients dying from this disease will develop bone metastases, even if subclinical (1). Bone metastases are a significant concern for patients as they are typically painful and increase the risk of catastrophic pathologic fracture and other skeletal-related events (SREs). Patient care is further complicated as there is no cure for these lesions despite significant effort to understand the underlying biology and specifically target them

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Conflict of Interest

The authors have no conflict of interest to report.

(2). The development of bone metastases in older patients with advanced disease is of significant concern as this population presents with decreased bone mineral density (BMD), exacerbating the risks of SREs leading to decreased quality of life and possible morbidity. One of the main line treatments for PCa, androgen deprivation therapy, also leads to decreased BMD, and may be a cofactor in SRE development. Skeletal metastases are typically described as osteolytic, those that lead to bone resorption, or osteoblastic, those leading to enhanced bone formation. Many lesions consist of a mix of osteolytic and osteoblastic components. It is often difficult to determine the nature of a metastasis by radiography alone. Typically bone markers, such as alkaline phosphatase (ALP), osteocalcin (OC), and tartrate-resistant acid phosphatase (TRAP) 5b, reveal mixed messages concerning changes in the bone environment, and may speak to the plasticity associated with bone metastasis (3, 4). Even though PCa bone metastases have an osteoblastic phenotype, they are thought to have an underlying osteolytic component important in tumor establishment and progression as previously reviewed (2, 5). One of the tenets of normal bone remodeling is that there cannot be bone production without prior bone absorption. It is only when these pathways fall out of balance that pathologies occur, and tumors are one mechanism that act to disturb this balance for their own growth promotion. Therefore, therapeutic intervention is required to improve quality of life and enhance survivability.

In normal bone, osteoblastic cells typically regulate bone turnover and osteoclastogenic activity. However, in the PCa microenvironment, many of these functions are co-opted by the tumor through the production of pro-osteolytic effectors. Several factors have been described as playing a role in the tumor induced osteolytic microenvironment. One such factor is receptor activator of nuclear factor κ B ligand (RANKL) and it is supportive of the osteoclastic environment. RANKL expression has been shown to be induced by cancer cells leading to induction of osteoclast activity and osteolysis (6). Osteoprotegerin (OPG) is a soluble decoy receptor for RANKL, and therefore inhibits osteoclastogenesis and diminishes osteolysis in the PCa microenvironment. Parathyroid hormone-related protein (PTHrP) has been implicated as having a growth promoting role on PCa once it metastasizes to the bone. Interleukin-6 (IL-6) may also increase osteoclast numbers by promoting the growth of pre-osteoclastic cells. Matrix metalloproteinases (MMPs) and cathepsins lead to the breakdown of the extracellular matrix allowing for degradation of mineralized bone by osteoclasts, and increased invasion and migration of tumor cells. Together, these factors help describe the role that osteoclasts play in the promoting PCa growth in the bone microenvironment, and provide plausible pathways for the inhibition of tumor growth in the bone.

Osteoclast Biology

Osteoclasts are the primary mediators of bone resorption. In orchestration with osteoblasts, the primary mediators of bone formation, and osteocytes, the conductors of these two cell types, bone homeostasis is typically maintained. Osteoclasts help maintain bone structure and function through continual remodeling of the microenvironment in response to a number of physical and chemical stimuli. Osteoclast growth and differentiation is controlled by a number of well described processes (Figure 1). The colony forming unit granulocyte-macrophage (CFU-GM) is part of the monocyte-macrophage lineage of cells, and the earliest precursor to osteoclasts (7, 8). The CFU-GM undergoes a relatively well described progression of differentiation that has been previously reviewed (8, 9). Briefly, macrophage colony stimulating factor (M-CSF; colony stimulating factor, CSF-1), and interleukins-1, -4, and -6 (IL-1, IL-4, and IL-6) expand the osteoclast precursor pool and promote the formation of bone marrow macrophages (Figure 1) (9, 10). At this point, RANKL and M-CSF lead to promotion of a mature osteoclast through the expression of numerous genes associated with a mature osteoclast phenotype. Activation of the osteolytic phenotype is promoted by the presence of RANKL, PTHrP, and IL-6 (Figure 1). These cytokines lead to

subsequent bone resorption which occurs through a defined process which has been previously reviewed in great depth (9, 11, 12). After activation and bone resorption is completed, osteoclasts undergo apoptosis which is enhanced by the presence of TGF- β and may be exploited by therapeutic intervention (e.g. bisphosphonates) (13). PCa cells exacerbate this system by creating many of the pre-osteoclastic cytokines described above, and will be discussed in greater detail below.

Although a number of factors have been implicated in the differentiation of immature osteoclasts, it is well accepted that RANKL is required for terminal osteoclast differentiation and induction of osteolytic activity. RANKL is balanced by the antagonist OPG, which helps to regulate osteoclast activity, and thus bone resorption (14). Osteolysis begins when a mature osteoclast is activated by RANKL, leading to upregulation of MMP-9 and osteoclast polarization. Osteoclast polarization leads to ruffled membrane formation and the osteoclast sealing itself to the bone. Subsequent release of acid, cathepsins, and MMPs lead to breakdown of inorganic and organic ECM and resorption of bone constituent proteins. PCa has been observed to promote bone resorption through the induction of RANKL producing an environment conducive to PCa growth (Figure 2) (15).

PCa is a predominantly osteoblastic disease. However, bone resorption precedes bone production, and thus it is understood that even osteoblastic processes have an underlying osteolytic component. Formation of a vicious cycle is based upon tumor growth promoting the release of calcium and growth factors stored in the bone and extracellular matrix (ECM) which lead to further promotion of tumor growth (2, 12). Indeed, it has recently been suggested that osteoblastic lesions occur at locations on trabecular bone upon which osteoclast resorption has recently occurred (1, 16, 17). This suggests that osteoclast activation may be a key early mediator preparing the metastatic niche and nurturing early PCa metastases. Akech et al. have shown that an induction of Runx2, a transcription factor typically involved in positively regulating osteoblast activity, may play a role in bone lysis (18–20). They found that increased Runx2 expression in PC3, a lytic PCa tumor, induced mediators of osteoclast activity whereas PCa cells with a mixed phenotype, osteoblastic and osteolytic components, such as LNCaP and its derivative C4-2B had lower levels of Runx2 expression.

One of the primary methods of treatment for PCa is castration leading to loss of androgen and inhibition of tumor growth. However, androgen deprivation therapy has also been associated with an increased risk of SRE and osteoporosis (21). These factors are compounded by the fact that older men already have decreased BMD due to andropause resultant from decreased sex steroid (testosterone) production (22, 23). The combined effects can be so great that they lead to bone loss greater than that of postmenopausal women (23, 24). Furthermore, alterations in calcium metabolism and parathyroid hormone lead to excessive bone resorption (25). Further coalescence of nutritional, genetic, and environmental factors accentuates osteoporosis (26). The combination of these factors makes the risk of SRE in men with advanced PCa a serious clinical dilemma. Current and novel therapeutics for PCa metastases must resolve possible conflicts with bone remodeling in aging individuals, so as not to exacerbate these conditions.

It has long been understood that physiologic bone remodeling begins with osteoclast activation. These processes may be mimicked by metastasizing PCa cells leading to underlying PCa pathology (8, 27–29). Recently, Cackowski et al. modulated fetal mouse osteoclast formation and showed that osteoclasts are necessary for angiogenesis, the growth of blood vessels, through an MMP-9 dependent mechanism (30). They further attributed these effects to the presence of RANKL, PTHrP, and MMP-9, as these factors are critical for regulation of osteolysis, but may be exploited by tumors to promote growth. It is therefore

plausible that osteoclasts may promote angiogenesis in the tumor microenvironment, a critical ingredient to tumor growth (31–33). Similarly, PCa cells have been shown to induce an osteoclastic phenotype in mature peripheral blood mononuclear cells (PBMCs) by regulating CD11b, which may be related to a common hematopoietic precursor cell (34). Metastatic PCa cells in the bone microenvironment compete for locations occupied by mesenchymal stem cells which have been suggested to be occupying locations of recent bone resorption (35, 36). These observations detail the importance of osteoclasts not only in preparation of the pre-metastatic niche for tumor cells, but nurturing continued growth of the tumor once seeding is complete.

Mediators of Bone Resorption

Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases are a family of zinc-dependent proteases whose primary purpose is the breakdown of ECM, and thus they have a large role in bone remodeling. MMPs have been found to have a role in migration, invasion, angiogenesis, and cellular differentiation of numerous cell types including osteoclasts and PCa (31, 37–40). MMPs are important mediators of bone resorption, yet only have the capacity to breakdown proteins in non-mineralized bone. The primary MMPs associated with bone resorption are MMP-1, MMP-9, and MMP-13; all of which are collagenases. MMP-9 is also thought to play a role in osteoclast polarization (8). Type I collagen composes the greatest percentage of bone, and is critical to bones strength, durability, and flexibility as evidenced in diseases such as osteogenesis imperfect in which Type I collagen is mutated (41–43). Associations between collagen and PCa bone metastases have been suggested (41, 42).

MMPs are also important mediators of the metastatic process which has been extensively described (44–46). Levels of MMPs have been related to the metastatic characteristics of a number of tumors, and are important in tumor cell migration and invasion. PCa is no exception as MMP-1, -3, -7, and -9 have been shown to be mediators of PCa metastasis, specifically to bone (47). The presence of mineralized matrix has been shown to induce MMPs from PCa cells, and thus may be a mechanism to which facilitating osteolysis during tumor seeding and cell invasion (48). MMP-9 is specifically important for osteoclast polarization and ruffled border formation (8). Osteoclasts must orient themselves towards bone to reposition mediators of bone resorption and seal their external membranes to bone to promote a resorptive environment. Upregulation of integrin $\beta 3$ has been found to be an important mediator of this process and helps maintain adherence of the osteoclast to bone (8). The importance of MMPs in bone metastasis has been shown *in vitro* and *in vivo* through the blocking of activated MMPs in culture to the use of novel inhibitors in animal models (12). MMPs work in concert with cathepsins and an acidified environment to resorb bone (Figure 2) (49). Interestingly, MMPs are responsible for initiating bone resorption, and also play a role in concluding it through breakdown and uptake of collagen I. Therefore, MMPs are not only important in tumor cell migration out of the prostate, but likely play an important role in metastatic formation and growth.

Acid Secretion

One of the first steps of bone resorption is acidification of the environment between bone and the sealed osteoclast to promote breakdown of the inorganic mineralized matrix. Development of ruffled borders on polarized osteoclasts leads to localization of H^+ -ATPase-dependent pumps that push H^+ ions against a gradient into the resorption pit of osteolytic lesions (Figure 2). Increased acidity leads to demineralization of the bone. Osteoclasts then absorb the calcium ions present by a calmodulin dependent mechanism (50). Mineralized bone fragments and collagen are processed within the osteoclast and released into

circulation. N-telopeptide (NTx), C-telopeptide (CTx), and alkaline phosphatase (ALP) are serum based markers of bone turnover (8, 28, 51). Several hormones have been implicated in the production of acid in osteoclasts, including parathyroid hormone (PTH). Therapeutics, primarily diuretics, have been investigated in the inhibition of acid from osteoclasts, and have shown some inhibition of bone resorption. For example, the carbonic anhydrase II inhibitors acetazolamide and indapamide have been shown to inhibit bone resorption (52–54). Proton pump inhibitors used for the amelioration of indigestion, such as omeprazole, have also been found to inhibit acid secretion, though the long term consequences may be severe (55, 56). These data in conjunction with that of MMPs and cathepsins suggest that inhibition of osteoclast function may be sufficient to the inhibition of bone resorption. However, much investigation has also been performed to identify the cytokines involved in osteoclast growth and differentiation.

Cathepsins

Cathepsins are an important class of proteases that modulate the ECM similarly to MMPs. In fact, cathepsins are important in the cleavage of Type I collagen, osteopontin, and osteonectin; all of which are substantial ECM components of bone (Figure 2). There are 15 cathepsins, and each has a specific ligand for which it is active and cleaves at specific locations yielding a variety of breakdown products (57, 58). Cathepsins have been shown to be important mediators of metastasis across a range of tumors, including PCa (57). The primary cathepsins involved in the bone microenvironment at D, K, and L. It has previously been shown that over expression of cathepsin K can lead to increased bone turnover, whereas inhibition of cathepsin K can lead to significant bone loss (59, 60). The presence of cathepsins K and D in the sera of PCa patients has been associated with tumor aggressiveness and contributing to bone resorption (60–63). Cathepsin K is of specific importance because its expression is regulated by RANKL and is released by osteoclasts after acid degradation of the inorganic mineralized matrix thereby allowing collagen degradation prior to resorption (9, 64). For this reason, cathepsin inhibitors have been investigated to slow bone resorption and inhibit tumor growth in bone, but have found difficulty gaining acceptance due to severe toxicity (60). Novel therapeutics such as 2-deoxyglucose (2-DG) have been shown to inhibit metastasis by altering tumor metabolism in other tumor models concerning bone, and inhibiting cathepsin L through a yet to be described mechanism (65–67). Several clinical trials have opened with a number of cathepsin K inhibitors, and have recently been reviewed (68). However, most of the investigation of these inhibitors have been performed in breast cancer and osteoporosis patients, with novel inhibitors only recently being applied to PCa bone metastases (60, 68). These studies provide evidence that cathepsins are a plausible mechanism by which bone resorption and tumor growth may be inhibited as long as toxicity is sufficiently managed.

RANKL

Even though there are a number of factors that lead to increased osteoclastogenesis, the primary mediator of osteoclast differentiation and promotion of the bone resorptive phenotype is RANKL. RANKL is a member of the tumor necrosis factor family, and is also known as tumor necrosis ligand super family member number 11 (TNFSF11) (69). RANKL has been shown to promote osteoclast differentiation, activation, adherence, and survival, leading to increased bone resorption (16, 70–75). Osteoclast activity under RANKL is important in the mediation of bone formation of cortical and cancellous bone, and has previously been shown to mediate bone volume, density, strength, and rigidity.

RANKL is known to exist in at least 3 active forms (RANKL1-3), two of which (RANKL1/2) are membrane bound (76, 77). The different isoforms have variant effects on the process of osteoclastogenesis (77). RANKL1 and RANKL2 contain transmembrane

domains and are typically expressed on osteoblast membranes and thus require cell-cell interactions to activate osteoclasts (77). However, a soluble form of RANKL can be created by tumor necrosis factor converting enzyme (TACE) or membrane bound MMPs cleaving membranous RANKL (6, 78). Specifically, MMP-7 has been implicated in RANKL promotion in prostate cancer osteolysis (6). Evidence suggests that RANKL3, lacking the transmembrane domain, serves to bind RANKL1/2 and possibly inhibit osteoclastogenesis (76, 79).

Activity of RANKL is dependent upon binding to receptor activator of nuclear factor kappa B (RANK; TNFRSF11A), the primary receptor for RANKL (14). Activation of RANK in osteoclasts leads to direct activation of c-fos allowing it to translocate to the nucleus promoting the transcription of at least 24 identified osteoclastic genes, including NFAT-c1 and c-Src (8, 80–82). Activation of RANK polarizes the osteoclast allowing for bone resorption (8, 70). c-fos is critical for osteoclast formation, but is not required for multinucleation to occur (83, 84). These changes subsequently lead to promotion of osteoclastogenesis and osteolysis as described above. Constitutive activation of RANK through tandem repeat expansion is the primary mechanism driving familial expansile osteolysis (FEO) leading to focal lytic disease that can expand and lead to significant skeletally related events similar to those seen in PCa bone metastases (14). RANKL can also increase migration in PCa cells through mitogen activated protein (MAP) kinase p38 and c-Jun activation (85). RANK activation of TRAF5/6 leads to activation of JNK1, c-Src, MAPK, and NF- κ B which have a number of roles, predominantly concerned with cell growth, migration, and survival (9).

The role that RANKL plays is not isolated to that of osteoclasts, but some preliminary studies suggest that RANKL can also alter PCa phenotype directly (86). RANK has been shown to promote migration of PCa cells (85). PCa can also induce osteoclast indirectly by inducing osteoblasts to express RANKL on their membranes, thereby activating osteoclasts to resorb bone (86). Outside of the effects on cancer cells, RANKL also plays an important role on T-helper cells, and has been implicated in the immune inhibition of breast cancer metastases; it is plausible that a similar mechanism is at work in PCa with direct and indirect effects on osteoclastogenesis, though these mechanisms need to be further elucidated (87–90).

Numerous activators of RANKL promote bone resorption through the effects of RANKL. However, these factors are balanced through the presence of IL-4 and IL-13 which typically lead to the production of OPG, the primary antagonist to RANKL (91).

Osteoprotegerin (OPG)

Osteoprotegerin (TNFRSF11B), also known as osteoclastogenesis inhibitory factor (OCIF), is a 120 kDa homodimeric protein that acts as a decoy receptor for RANKL (14). Upon binding RANKL, OPG inhibits RANKL's ability to activate RANK. This, in turn, diminishes osteoclastogenesis and osteoclast activation. Increasing the ratio of OPG to RANKL has been shown to result in increased bone mass (92). Knock-out of OPG in mice leads to severe osteoporosis due to lack of inhibition of RANKL (93). OPG is produced by maturing osteoblast cells to inhibit the maturation of osteoclasts. In bone, OPG is primarily located on the cellular surface of osteoblasts, but is also present on a variety of other tissues, such as lung, heart, and kidney. A single dose of OPG was found to inhibit more than 90% of osteoclasts within 48 hours in mice, showing the potency of OPG as an inhibitor of osteoclast function, but not necessarily osteoclast survival (71, 94). Inducers of OPG include tumor necrosis factor alpha (TNF- α) and bone morphogenetic protein 2 (BMP-2), both of which also play a role in osteoblast differentiation.

It is well described that induction of OPG leads to decreased bone loss under a number of scenarios (95). For example, Bateman et al. observed that hind-limb suspension, a method to investigate disuse and microgravity, mice could be treated with OPG and bone loss ameliorated (96). Furthermore, the production of OPG has been related to estrogen through competition with the estrogen receptor (97). The presence of the estrogen-response element binding protein is thought to be ubiquitous in bone, yet its role in males and PCa has yet to be defined, if it exists. Administration of OPG has been shown to prevent bone loss in ovariectomized mice, and significantly inhibits bone turnover (16). Due to the relation of Vitamin D3 and OPG, it has been observed that vitamin D deficiency leads to decreased PCa growth in bone of animal models (98). Furthermore, several studies suggest that OPG may be a reliable measurement of metastatic bone disease in PCa patients, possibly more so than PSA (99–101). However, a recent study by Corey et al. observed that C4–2B PCa cells overexpressing OPG protected the cells from apoptosis, yet limited growth in bone possibly due to increased BMD and trabecular bone formation (102). It is likely that decreased bone turnover and lysis physically restrict tumor growth. Furthermore, patients with advanced prostatic tumors with metastatic bone disease have been shown to have increased ratios of RANKL to OPG, suggesting that RANKL expression by the tumor may overwhelm endogenous OPG expression (3). It is well described that OPG is an integral component to bone homeostasis, and in conjunction with RANKL and RANK serves to mediate bone turnover. Even though therapeutics aim to mimic the effects of OPG in the metastatic setting further examination of the role of OPG in these patients is necessary to better understand the underlying biology (16).

Interleukin-6 (I L-6)

IL-6 is a member of the “interleukin 6-type cytokine” family and was first identified as a macrophage differentiation factor (103). Other members of this family include, but are not limited to: leukemia inhibitory factor (LIF), oncostatin M, ciliary neurotrophic factor, interleukin 11, and cardiotrophin 1 (47, 103, 104). Numerous physiologic functions besides osteoclastogenesis are associated with this family of cytokines, such as: antibody production from B-lymphocytes, thrombopoiesis, and hepatic acute phase reaction protein synthesis. IL-6 mediates its activities through the IL-6 receptor (IL-6R) which is composed of an 80 kD transmembrane portion (IL-6Rp80) that specifically binds a 130 kd membrane glycoprotein (gp130). The gp130 subunit is common to all members of the IL-6 family of cytokines and is important in signal transduction (105). Heterodimerization in the presence of IL-6 induces a signaling cascade primarily through, Janus activated protein kinases (JAKs), mitogen activated protein kinases (MAPKs), JNK, E2F/Rb, and STAT3 leading to activation of CREB, oncostatin M, and RANKL in osteoclasts (103, 106–110). In addition to the membrane bound receptor, a soluble form of the receptor (sIL-6R) can be produced by proteolytic cleavage or differential splicing of the 80 kDa subunit. sIL-6R can bind IL-6 and interact with gp130 to induce signal transduction and promote PCa growth (111). IL-6 has been found to be critical for osteoclast formation *in vitro* and *in vivo* through the suppression of NF- κ associated pathways (112, 113). Therefore, the production of IL-6 from PCa cells and its role in the bone microenvironment make it a critical cytokine to examine for bone metastasis.

Numerous studies have demonstrated that IL-6 can be correlated with progressive disease in PCa patients and may act as a growth factor for PCa cells (4, 114–116). It has further been shown that IL-6 correlates with tumor burden and PSA acting as a growth factor for PCa (117, 118). Men with castrate resistant PCa had a significant increase in IL-6 compared to men with localized disease, benign prostatic changes, and normal prostates (114, 115, 119). IL-6 and IL-6R have been shown to be expressed on normal prostate epithelium and in PCa (116, 120). It is hypothesized that expression of IL-6 and its receptor play a role in

increasing proliferation, metastasis, and androgen independence in PCa by utilizing STAT3 as a signaling mediator (117, 120–122). Recently, Kim et al. have shown that inhibition of IL-6 in a metastatic PC-3 model led to inhibition of tumor growth and decreased metastasis (119). These effects appear to be tumor type dependent, as IL-6 can also inhibit the growth of melanoma, breast cancer, and osteosarcoma (103). IL-6 has been shown to be a critical mediator of multiple myeloma (MM). MM cells produce IL-6 to stimulate osteoclast leading to increased bone lysis which in turn allows for increased MM growth and invasion (31). Administration of anti-IL-6 antibodies has been shown to inhibit growth of subcutaneously injected PCa in animal models, and may have potential as a therapeutic for clinical disease (122, 123). These data describe the importance of IL-6 in PCa biology and metastasis.

PCa cells injected into the bones of mice have shown IL-6 upregulation, and it is believed that these changes can have direct effects on the localized bone microenvironment and bone remodeling (117). IL-6 promotes osteoclastogenesis primarily through the induction of osteoclastic precursors, and does not appear to induce RANKL directly (124, 125). Interestingly, these effects appear to be tightly related to the concentration of gp130 present on precursor cells. It has been proposed that the IL-6R α itself is not able to induce signal transduction, but must interact with gp130. Therefore, it is likely that gp130 is the primary mediator of IL-6 activity in osteoclast precursor cells. The effects of IL-6 are believed to be independent of RANKL, but may help potentiate the effects of PTHrP.

Parathyroid Hormone Related Protein (PTHrP)

PTHrP is related to PTH, which is involved in regulating hematopoietic stem cell development and calcium regulation which it partially accomplishes through IL-6 (126, 127). Both molecules bind to the PTH-1 receptor (PTH-1R) due to similarities in their N-terminal amino acid sequences (128). PTH-1R is a G-protein receptor that leads to PKA and PKC pathways activation (129, 130). PTHrP has been observed to be secreted directly from breast, lung, and PCa cells; all of which metastasize to bone. PTHrP was first identified due to its role in mediating hypercalcemia in tumor bearing patients (131). PTHrP typically acts in a localized paracrine or autocrine fashion. PTHrP leads to increased RANKL expression by osteoblasts, thereby indirectly leading to the promotion of osteoclastic activity (Figure 2) (9). It is plausible that PTHrP acts in concert with IL-6 to promote RANKL expression (132). However, PTHrP does not lead to increased 1,25-dihydroxyvitamin D production as does PTH, and thus does not control intestinal calcium resorption although it does play a significant role in renal calcium homeostasis. In fact, PTHrP is suppressed by 1,25-dihydroxyvitamin D₃ by mediating post-transcriptional pathways, decreasing mRNA stability, and inducing protein degradation by PCa (133). It has also been shown that PTHrP can act on osteoblasts to increase IL-6 and LIF to activate osteoclasts and inhibit osteoblast differentiation (103, 134, 135).

In normal bone, PTHrP serves to help regulate bone development, specifically endochondral bone growth. For example, selective depletion of PTHrP from osteoblasts leads to decreased bone mass, inhibition of osteoblast survival, and trabecular osteopenia (136, 137). The ability of PCa to secrete PTHrP and the ability of this molecule to regulate bone absorption and serum calcium makes it an interesting target for PCa therapy. PTHrP has also been found to associate with PCa progression, especially bone metastasis (129, 138). Indeed, PTHrP secretion from the tumor microenvironment can lead to an increase in monocyte chemoattractant protein-1 (MCP-1), inducing osteoclast recruitment and differentiation while promoting a pro-tumorigenic immune infiltrate of monocytes (139–141). However, the role of PTHrP is relatively complex, as it may also act as an inducer of anabolic bone growth dependent on the differentiation state of effector cells (129). A recent paper by Liao et al. described overexpression of PTHrP from PCa cells lead to an increase in bone density

(142). However, an increase in osteoclastogenesis was also observed. These observations along with those of others describe complex interactions dependent on the amount of PTHrP produced, the cells present in the microenvironment, and the differentiation state of all players in the PCa microenvironment (20, 139, 143–147).

Therapy

Denosumab

Denosumab (Xgeva^{TM}) is a fully humanized monoclonal IgG2 antibody developed by Amgen with a high affinity for RANKL. Denosumab is thought to have similar effects as OPG in that it is able to bind free RANKL and inhibit binding to RANK, thereby inhibiting osteoclast differentiation and activation (Figure 2). The development and impact of Denosumab have recently been thoroughly reviewed (16, 148). Denosumab has completed phase I and phase II clinical trials establishing sufficient pharmacokinetics and pharmacodynamic relationships for phase III clinical trials in PCa patient cohorts (16, 106, 148–150). The optimal dosing regimen was determined from a phase II trial investigating 5 distinct dosing regimens in bone metastasis patients not previously treated with bisphosphonates. Fizazi et al. showed that denosumab increased time to first SRE by over 3-months when compared to the bisphosphonate zoledronic acid, helping to clear the way for FDA approval (106). It was determined that the greatest suppression of bone resorption occurred with a 120 mg dose every 4 weeks (16, 106, 151). Several other phase II studies were recently completed and currently under way to confirm these findings and further define dosing for various patient populations (148, 152, 153). The preponderance of positive studies has led to the recent approval of denosumab for prevention of bone metastatic skeletal-related events. Denosumab treatments are typically well tolerated with the most reported side effects being back pain, dizziness, arthralgia, and musculoskeletal pain (16). Concerns of hypocalcemia persist even though this effect is typically transient, and the nadir for serum calcium reported around day 10. Due to the effects of RANKL on T and B cells, denosumab may increase the risk of infection in patients, although a significant difference was not appreciated when compared to placebo administration. A number of phase III clinical trials have originated in a number of tumor types, including PCa (106, 149).

Currently, there are 3 phase III studies ongoing investigating the effects of denosumab for metastatic PCa to bone (www.clinicaltrials.gov). As there is no standard of care for bone loss due to androgen deprivation therapy in prostate cancer, new therapeutics are needed to combat the disease itself and the side effects associated with hormone therapy. Many physicians resort to the use of bisphosphonates due to the growing evidence from the osteoporosis field.

Bisphosphonates

Bisphosphonates are one of the primary mechanisms used to control osteoclast activity (Figure 2). There are two classes of bisphosphonates used clinically, the N-containing, and the non-N-containing; each with its own independent mechanism of action. N-containing bisphosphonates include pamidronate (Aredia^{TM}), residronate (Actonel^{TM}), and zoledronic acid (Zometa^{TM}). These bisphosphonates block the enzyme farnesyl diphosphate synthase, part of the mevalonate pathway, and inhibit protein prenylation. Inhibition of prenylation leads to improper membrane integration, and lack of protein function within the osteoclast. This process can also inhibit the Rho family of proteins, leading to inhibition of osteolysis through improper fusion of the osteoclast to the bone. Non-N-containing bisphosphonates include clodronate and etidronate. These bisphosphonates resemble adenosine triphosphate and lead to an inhibition of normal cellular metabolism prompting cells to undergo apoptosis. However, some studies suggest that osteoclast activity is simply inhibited rather

than cells undergoing apoptosis. It has been suggested that glucocorticoids abrogate the apoptotic effects of bisphosphonates (154). Furthermore, breast cancer cells have been shown to protect osteoclasts from apoptosis through M-CSF production (155). Further research is needed to elucidate the role of bisphosphonates on osteoclasts, especially as it pertains to the metastatic bone microenvironment.

Zoledronic acid has been the focus of a number of clinical trials across a variety of tumors for its ability to inhibit osteolysis in bone metastases from prostate, breast, lung, myeloma, and a number of other tumors. At the time of this review, there are 206 total clinical trials concerning the use of zoledronic acid alone (www.clinicaltrials.gov). More specifically, there are 50 clinical trials investigating the role of zoledronic acid for prostate cancer, many in combination with other therapeutics. Zoledronic acid is believed to be one of the best methods for controlling bone metastases, even though toxicity does include normal bone loss and other musculoskeletal related side effects. Zoledronic acid and residronate have been found to inhibit tumor growth while retaining BMD and overall bone strength (64, 156). However, indications are still early, and denosumab has also shown the ability to retain BMD while inhibiting tumor growth in the AZURE (adjuvant zoledronic acid to reduce recurrence) trial (150).

Combination of zoledronic acid and RANKL-inhibition have been shown to inhibit osteolysis in animal models, and clinical trials are ongoing to determine clinical efficacy but preliminary results are encouraging (157, 158). Baron et al. have suggested that the differential mechanisms of action, denosumab acting in the extracellular milieu and zoledronic acid working internally, lead to differential impacts on osteoclast formation and activity and synergistically inhibiting osteoclast viability (94). It has also been discovered that calcium signaling and regulation may play a key role in metastasis and inhibit PCa apoptosis (145, 159, 160). It has been suggested that blocking calcium release from bone using bisphosphonates and OPG may inhibit metastasis to bone (161). Even though these therapeutics impact different aspects of osteoclast biology, it is plausible that further study will elucidate clinical gains in osteolytic inhibition as it pertains to PCa.

Other therapeutics

There are a number of other pathways being explored to inhibit PCa bone metastases outside of bisphosphonates and denosumab, and many of these have been recently and extensively reviewed (12, 57, 94, 151, 158, 161) (Figure 2). As described above, MMPs are integral in ECM degradation and enhance the mobility of PCa cells. Inhibition of MMPs using SB-3CT, a gelatinase inhibitor, PCK3145, a synthetic peptide, and silibinin, a naturally occurring small molecule inhibitor, have been shown to inhibit osteolysis in animal models (6, 12, 162–166). Even though TGF- β has been shown to be of greater importance in breast cancer metastases to bone, TGF- β does play a role in the regulation of osteoclastogenesis and investigation into the role of TGF- β inhibitors in PCa are progressing (8, 167, 168), though with conflicting results.

The use radiation has been of great importance for the treatment of localized prostatic disease. However, the use of systemic radionuclides has been of enhanced recent interest as specific isotopes such as strontium-89 home directly to bone when injected, and reside in relatively high concentrations in these locations (151). Other radionuclides, such as samarium-153, can also be linked to a bisphosphonate to increase their abundance in bone (151). Typically these therapies have been associated with palliative therapy, but new evidence suggests that there may also be therapeutic gain when radionuclides can be directed directly to bone metastases. One method for enhancing radionuclide efficacy is linking the nuclides to antibodies or particles preferentially taken up by osteoclasts, such as bisphosphonates. This therapy allows for high concentrations of radionuclides to be present

in the metastatic microenvironment and spare normal tissue as much as possible due to the relatively short linear energy transfer of beta or alpha particles emitted (151).

The use of monoclonal antibodies and small molecule inhibitors have been used to some success in other tumors, and ongoing clinical trials are currently underway for PCa. For example, vorinostat, a histone deacetylase inhibitor has been shown to inhibit PCa growth in bone, even though it promotes osteolysis through an induction of PTHrP (169). A novel HSP90 inhibitor, PF-04928473, was shown to promote PCa apoptosis by abrogating RANKL signaling and Src phosphorylation and inhibiting NF- κ B, but also inhibiting osteoclastogenesis by similar mechanisms (170). As discussed above, IL-6 is important for osteoclast formation, but it has also been implicated in androgen responsiveness (171). An anti-IL-6 antibody, CNTO328, has been shown to inhibit subcutaneous tumor growth by promoting PCa apoptosis (123, 172). However, the role this therapy plays in bone has not been fully described although the therapy has shown promise in treating ovarian carcinoma, myeloma, and possibly PCa (173, 174). Gadolinium on the other hand can integrate into bone in exchange for calcium, and has been shown to suppress PCa growth and migration while inhibiting osteoclast differentiation (175). Although not yet identified for PCa, coptisine, a naturally occurring isoquinoline alkaloid, has shown efficacy for the inhibition of osteoclast function in normal bone through RANKL inhibition of osteoclast formation, and may hold promise as an anti-tumor therapeutic (176). There are a number of other therapies in development that have been significantly reviewed previously (60, 158). This is but a short list but shows the promise that many novel therapeutics hold for the treatment of this disease.

Conclusion

Prostate cancer skeletal metastases often have a lytic component underlying their osteoblastic phenotype. Several mechanisms are important in promotion of osteoclast development, and activation of osteolytic pathways leading to bone resorption. IL-6 has been shown to be an important mediator in differentiation of osteoclasts while mediation of the balance between RANKL and OPG is important in regulating bone resorption and osteoclast activity. It is important to continue investigation concerning the mechanisms by which prostate cancer cells illicit bone osteolysis and activation of osteoclasts for therapeutic gain. Much information concerning the interactions between PCa and osteoclasts is required. For example, many of the typical laboratory models fail to replicate the osteoblastic environment, yet many of them have strong osteolytic tendencies when analyzed *in vitro* and *in vivo*. Examination concerning the nature of these cell lines is important, and may yield greater context to the underlying pathobiology of PCa as is reflected in the osteolytic bone microenvironment of metastases. Our understanding of PCa biology and the role that osteoclasts play can help define novel targets. Novel therapeutics aimed at presently known targets, and those now under development should yield important insight into the inhibition of PCa growth. For example, the role that PTHrP plays in regulating RANKL expression in osteoblasts as a subsequent regulator of osteoclasts is relatively difficult and poorly understood. Increased description of these pathways may yield useful therapeutic developments for patients with advanced disease. Current clinical trials seek to define the role that novel therapeutics play in the inhibition of PCa bone lysis to improve patient quality of life and increase survival. Continued investigation into the biology of the metastatic prostate tumor bone microenvironment will enable increased target definition, therapeutic development, and inhibition of the pain associated with these tumors.

Acknowledgments

This work was supported by NIH Grant CA093900.

List of Abbreviations

2-DG	2 Deoxy glucose
ALP	Alkaline Phosphatase
BMD	Bone Mineral Density
BMP	Bone morphogenetic protein
CFU-GM	Colony forming unit granulocyte-macrophage
CSF-1	Colony stimulating factor 1
CTx	C-telopeptide
ECM	Extracellular Matrix
FEO	Familial expansile osteolysis
JAK	Janus activated protein kinase
IL	Interleukin
IL-6R	Interleukin-6 receptor
LIF	Leukemia inhibitory factor
MAP	Mitogen activated protein
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemotactic protein - 1
M-CSF	macrophage colony stimulating factor
MM	Multiple Myeloma
MMP	Matrix Metalloproteinase
NTx	N-telopeptide
OC	Osteocalcin
OCIF	osteoclastogenesis inhibitory factor
OPG	Osteoprotegerin
PBMC	Peripheral Blood Mononuclear Cell
PCa	Prostate Cancer
PTH	Parathyroid Hormone
PTH1R	Parathyroid hormone receptor-1
PTHrP	Parathyroid hormone-related protein
RANK	Receptor activator of nuclear factor kappa B
RANKL	Receptor activator of nuclear factor kappa B ligand
SRE	Skeletally Related Event
TACE	Tumor necrosis factor converting enzyme
TNF-α	Tumor necrosis factor alpha
TRAP5b	Tartrate-resistant acid phosphatase 5b

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Summary

Bone metastasis is a painful and frequent complication of advanced prostate cancer (PCa). Although a majority of bone metastases are considered osteoblastic, many of these tumors have an underlying osteolytic component. Herein, we describe the primary players involved in the bone microenvironment that contribute to osteolysis and the promotion of tumor growth. Examination of osteoclast biology, important cytokines such as receptor activator of nuclear factor kappa B ligand (RANKL), osteoprotegerin (OPG), interleukin-6 (IL-6), and parathyroid hormone-related protein (PTHrP) are defined, and interactions between osteoclasts and prostate cancer cells described. Novel therapeutics have been developed to inhibit osteoclastic processes and applied to the prostate cancer scenario with recent developments associated with these therapies examined. This review seeks to describe the field of osteolysis in advanced prostate cancer bone metastases and explore current thinking to inhibit metastatic growth to enhance quality of life and prolong survival in this cohort of patients.

Figure 1. Osteoclast differentiation

Important cytokines are listed at relevant points of differentiation. Legend: CFU-GM=Colony forming unit-granulocyte monocyte; OC=Osteoclast; RANK=Receptor activator of nuclear factor kappa B; RANKL=Receptor activator of nuclear factor kappa B ligand; M-CSF=macrophage colony stimulating factor; OPG=Osteoprotegerin. CFU-GM are the earliest precursor in the osteoclast lineage. Presence of M-CSF leads to expansion of the CFU-GM pool and initiation of differentiation. The presence of RANKL and M-CSF leads to increased expression of genes characteristic of osteoclast. Continued presence of RANKL leads to continued differentiation into mature osteoclasts and promotes bone resorption by converting cells to an active phenotype. Throughout this process, OPG can mitigate the

effects of RANKL leading to lack of differentiation and/or the bone resorptive phenotype. The presence of TGF- β is associated with osteoclast apoptosis and helps mitigate bone lysis.

Figure 2. Therapeutic targeting the microenvironment of prostatic bone metastases

Novel therapeutics are important for the targeting of osteoclastogenesis through osteoclasts themselves and relevant effectors. A snapshot of the prostatic bone microenvironment is shown with the location and impact of important therapeutics. There is significant crosstalk and overlap concerning the mechanisms by which PCa can alter bone homeostasis and promote not only an osteolytic, but an osteoblastic environment. Legend: CFU-GM=Colony Forming Unit Granulocyte-Macrophage; PCa=Prostate Cancer Cell; PTHrP=Parathyroid Hormone Related Protein; RANKL; Denosumab; Anti-IL-6 Ab=Anti-IL-6 Antibody; Interleukin-1,-4,-6=IL-1, -4, -6; OPG=Osteoprotegerin; OB=Osteoblast; Acid=Acid Secretion; MMP=Matrix Metalloproteinase; MMP-I=MMP inhibitor CAI=Carbonic Anhydrase Inhibitor; Bisphos=Bisphosphonates; OC=Osteoclast.