The Osteoblastic and Osteoclastic Interactions in Spinal Metastases Secondary to Prostate Cancer

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ABSTRACT: Prostate cancer (PC) is one of the most common cancers arising in men and has a high propensity for bone metastasis, particularly to the spine. At this stage, it often causes severe morbidity due to pathological fracture and/or metastatic epidural spinal cord compression which, if untreated, inevitably leads to intractable pain, neurological deficit, and paralysis. Unfortunately, the underlying molecular mechanisms driving growth of secondary PC in the bony vertebral column remain largely unknown. Further investigation is warranted in order to identify therapeutic targets in the future. This review summarizes the current understanding of PC bone metastasis in the spine, highlighting interactions between key tumor and bone-derived factors which influence tumor progression, especially the functional roles of osteoblasts and osteoclasts in the bone microenvironment through their interactions with metastatic PC cells and the critical pathway RANK/RANKL/OPG in bone destruction.

KEY WORDS: prostate cancer, spine, metastasis, osteoblasts, osteoclasts, RANK, RANKL, OPG bone microenvironment

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Introduction
Prostate cancer (PC) is the fifth most commonly diagnosed cancer type globally and the second most common disease causing mortality in males.\(^1\) Five-year survival rates for localized PC are high (almost 100%), however this drops to less than 33% in patients with metastatic disease.\(^2\) Over 90% of patients with advanced PC develop bone metastases, with the most common site of establishment being the spine.\(^3,4\) These patients are at risk of suffering due to severe symptoms such as bone pain and instability, pathological fracture, neurological deficit, and metastatic epidural spinal cord compression which may ultimately cause paralysis.\(^5,6\) Despite advances in operative and non-operative management of symptomatic bone and spinal metastases from PC, treatment options are mainly palliative and are limited in efficacy in cases of severe bone loss and destruction, pathological fracture, and bony instability. There is currently no effective therapy for advanced metastatic PC in terms of substantially improving morbidity and prolonging survival.\(^7\) This review summarizes the interactions of the tumor with the bone microenvironment and the role of the bone remodeling cells, osteoblasts and osteoclasts, in the spread of metastatic PC to the bony skeleton and spine.

Spinal Metastasis in PC
In the early stage of PC, localized PC cells are confined to the lobes of the prostate and, depending on the Gleason score (a histopathological method of grading PC), active surveillance, radical prostatectomy or radiotherapy are the current first line treatment options.\(^6\) When the disease progresses, tumor cells develop a more aggressive phenotype which enables them to grow outside their initial localized surrounding, precipitated by genetic mutations as well as interactions
and stimulation from the local environment. Paget proposed the “seed and soil” theory in 1889, whereby neoplastic tumor cells metastasize only to specific environments based on suitability for growth. Indeed, it has been suggested that the highly vascularized nature of the axial skeleton provides such an environment and the spine is the commonest site of skeletal metastasis secondary to PC.

The emerging importance of stem cells in PC associated bone metastasis. PC was originally thought to be due to abnormal proliferating epithelial cells. However more recently has been shown to involve complex interactions between PC epithelial cells and the surrounding stromal tissue. Multiple signaling pathways provide cross-talk between these compartments via androgen receptors, tyrosine kinase receptor signaling, and immune surveillance. The cancer stem cell (CSC) hypothesis proposes that there is a small subpopulation of cancer cells that are more resistant to chemotherapy and toxicity. These cells are able to drive tumor growth and metastasis, thus leading to relapse following treatment. CSCs are more resistant to stress induced by chemotherapy, with an increased expression of anti-apoptotic molecules and a reduced expression of pro-apoptotic genes. Furthermore, it is suggested that these cells exhibit preferential activation of DNA damage checkpoint response and increased capacity for repair, thus chemotherapy may enrich the CSC population within tumors by eliminating sensitive cells. Multipotent mesenchymal stem cells (MSCs) found within the bone marrow have recently been identified as playing an important role in supporting PC growth and survival in bone. These cells possess properties of self-renewal and repair, migrating towards active tumorigenesis and integrating into the niche contributing to the development of cancer-associated fibroblasts. Consequently, because of their tumor-tropic migratory response, MSCs are emerging as promising anti-cancer agents. PC cells require an invasive capacity which is stimulated through type I collagen from bone marrow MSCs, inducing the secretions of proteases from PC cells. In addition, stromal derived factor 1α (SDF1α) receptor and CXC chemokine receptor-4 (CXCR4) are essential for metastatic spread, allowing tumor cells to chemotact towards bone and easily access bone marrow cellular niches, ultimately promoting survival and growth. Furthermore, SDF1α promotes tumor angiogenesis by attracting endothelial cells to the tumor. Therefore, CSCs constitute a repository niche of androgen-insensitive and chemotherapy-resistant cells responsible for secondary metastatic progression. As such, they are an integral target for the cessation of PC progression.

The role of epithelial to mesenchymal transition in metastatic progression. PC is of epithelial origin and the process of epithelial to mesenchymal transition (EMT) is a critical step in the conversion of early stage cancer to an invasive and metastatic cancer in advanced disease progression. EMT is a process whereby polarized epithelial cells convert into motile mesenchymal cells by undergoing phenotypic transitions during malignant progression. Consequently, these cells acquire stem cell-like properties which permit resistance to treatment, adhesive and invasive capacity, changes in morphology and cellular architecture, migratory potential, and the ability to undergo metastatic progression. These cancer cells are thought to hijack core biological processes through key initiating events such as genetic mutation and activation of important oncogenic pathways involved in regulating cell proliferation and migration, cross-talk, angiogenesis, and epithelial-mesenchymal signaling.

Pathological EMT in tumor cells occurs as a result of transcriptional reprogramming of abnormal survival signals via receptors such as fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), transforming growth factor-β receptor (TGF-βR), and insulin-like growth factor-1 receptor (IGF-1R), as well as regulatory kinases such as phosphoinositide 3-kinase (PI3K), protein kinase B (AKT), and mammalian target of rapamycin (mTOR). These factors contribute to bone metastasis through molecular cross talk which is mediated by cytokines and other paracrine factors including vimentin, N-cadherin, platelet-derived growth factor (PDGF), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and Notch-1. These factors are expressed by the circulating tumor cells and are also able to acquire bone-like properties through transcriptional reprogramming of abnormal survival signals through independent activation of signaling pathways.

Xu et al. reported that PC cells undergo EMT through cellular interactions with the host microenvironment, increasing the metastatic potential to bone. A hallmark of EMT is the functional loss of epithelial cell markers and transmembrane adhesion glycoproteins E-Cadherin, cytokeratins, and β-catenin; factors which are proposed to suppress cancer progression. In this process, mesenchymal cell markers, namely N-Cadherin, fibronectin and Vimentin, are produced. These factors are found at the invasive front between the tumor and interacting microenvironment, signaling pathways that facilitate migration and survival. Zhan et al demonstrated the plasticity of cancer cells which, upon induction via these soluble factors, developed the ability to express markers found in osteoblasts; a phenomenon known as osteomimicry. These factors include osteocalcin (OC), bone sialoprotein (BSP), osteopontin (OPN), non-collagenous bone matrix proteins, and receptor activator of nuclear factor kappa-B ligand (RANKL), which together allow cancer cells to survive and thrive within the bone microenvironment.

Matrix metalloproteinases (MMPs), particularly membrane type 1 MMP (MT1-MMP), have been reported in PC EMT through both paracrine and autocrine pathways and are capable of cleaving E-cadherin, thus disrupting its function by interfering with cell adhesion and polarity of the cells. Furthermore, secreted MMPs (MMP-2, -3, -9, 28) have been implicated in PC cell EMT. MMP proteolysis serves a path-clearing mechanism in facilitating the movement of
cancer cells or groups of cells through the extracellular matrix (ECM). Cleavage of certain ECM components leads to the unmasking of sites, generating fragments with new biological activities and modulating migration, growth and angiogenesis.

Bone enriched growth factors, cytokines, proteases, and components of the ECM in bone marrow, provide a hospitable environment for harboring circulating PC cells. The process of metastasis is dependent on both the intrinsic properties of tumor cells as well as the response of the host bone microenvironment. Studies using bone xenograft models have demonstrated that injection of PC cells adjacent to bone leads to cancer cell migration towards bone. Thus, it appears that a bidirectional interaction between bone cells within the bone microenvironment and the PC cells exists, which facilitates tumor growth in the spine. During PC progression to metastatic disease, cancer cells acquire characteristics that promote interactions with the local microenvironment. The tumor releases vascular endothelial growth factor (VEGF), which initiates angiogenesis, the process of new blood vessel formation leading to an enhanced blood supply to the tumor. The surrounding stromal cells produce growth factors and cytokines such as transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), fibroblast growth factor (FGF), PDGF, and bone morphogenetic proteins (BMPs) that can act directly on cancer cells as well as induce angiogenesis, and stimulating growth and spread of the tumor. Disruptions to intracellular adhesion lead to detachment and dissemination of the tumor cells from the primary site, followed by local invasion into the blood vessels and lymphatic system, extravasation, migration, and invasion of the bone marrow. The tumor cells proliferate and colonize bone through migration and adherence, followed by degradation of the ECM. Once PC cells enter the bone compartment, close interactions with factors of the surrounding bone cells, matrix, and the environment occur in a reciprocal fashion via cytokine mediators, which allow osteoblastic, osteoclastic, and mixed lesions to form. The mechanism by which PC metastasizes to bone within the spine is illustrated in Figure 1.

A rich venous plexus known as Batson’s plexus, surrounds the prostate and connects to the venous drainage of the spine. This collection of veins provide a migratory route for tumor cells to localize in the lumbosacral region spinal metastases, which is a common occurrence in advanced PC.

Normal Bone Physiology and Remodeling

Bone physiology is a balanced and dynamic state consisting of continuous and coordinated cycles of bone formation by osteoblasts and bone resorption by osteoclasts, critical for maintenance of the structural integrity of bone. Osteoblasts are derived from MSCs of bone marrow stroma and synthesize and secrete proteins that form the ECM of bone, facilitating the deposition and mineralization of bone while maintaining the structural integrity of the skeleton. Formation of bone via osteoblasts involves the production of type 1 procollagen. This undergoes processing, modification, and secretion forming cross-linked arrangements whereby osteoblasts become

![Figure 1. Mechanisms involved in bone metastasis. At the primary site within the prostate, the tumor secretes factors which promote growth and angiogenesis. It also secretes factors that allow detachment from primary site and migration into blood vessels. Cancer cells then migrate through the blood circulation and are attracted towards bone. Subsequent extravasation and growth within and invasion of bone occurs involving complex interactions between the tumor and the local bone microenvironment. This ultimately leads to osteoclast-induced bone destruction and pathological fracture, and the expanding tumor mass impinges on the adjacent neural structures and spinal cord, causing neurological deficit and paralysis.](image-url)
osteocytes once embedded in the bone. The newly formed bone matrix induced by osteoblasts is subsequently mineralized with the deposition of hydroxyapatite crystals to increase the resistance to compression. Osteoblasts are also responsible for the regulation of osteoclast bone resorption via the secretion of factors that induce osteoclast differentiation, activation, and survival. The differentiation and growth of osteoblasts is regulated by complex signaling pathways including BMPs, IGFs, TGF-β, and Wnt. Differentiated osteoblasts secrete growth factors that become embedded in the bone matrix during formation. These factors are released into the microenvironment during the bone resorption process. Runx-related transcription factor 2 (Runx2) is the master transcription regulator of osteoblast differentiation and is over-expressed in PC metastasizing to bone. It has the ability to increase oncogenic potential through the regulation of genes involved in metastasis and invasion, which facilitate interactions between the tumor and bone microenvironment causing severe osteoblastic lesions. Runx2 functions in many regulatory processes such as the suppression of cell growth, epigenetic control of genes in mitosis, and bone turnover, which become unregulated in PC.

Osteoclasts are derived from hematopoietic cells of the monocytic-macrophage myeloid lineage. These cells form through the cytoplasmic fusion of their mononuclear precursor resulting in multinucleated bone resorbing cells. The differentiation and maturation of osteoclasts is regulated through cytokines released from osteoblasts. The cytokine macrophage colony stimulating factor (M-CSF) controls proliferation and promotes survival of osteoclast precursors. Furthermore, it stimulates activity in the mature resorbing cells such as proliferation, motility, and cytoskeletal organization. Osteoclasts are involved in the process of bone resorption; the degradation of mineral matrices such as calcified cartilage and bone during growth and development, skeletal homeostasis, repair, and remodeling. They tightly adhere and bind to the bone surface through polarization via αvβ3, αvβ5, and α2β1 integrins, using actin-rich podosomes to form sealed cytoplasmic extensions (transcytotic vesicle or vacuole) with the underlying bone matrix. Within this zone, they form a ruffled border membrane, which increases surface area and enables secretion of acid proteases, lysosomal enzymes, protons, and Cathepsin K onto the extracellular compartment of the bone surface. These secretions are essential for bone matrix solubilization and acid protease induced digestion of the organic matrix. Tartrate resistant acid phosphatase 5b (TRAP5b) is integral to the bone matrix digestion process. TRAP5b mediates two functions: phosphatase activity, which facilitates enzymatic degradation of bone, and the generation of a reactive oxygen species, which degrades collagen. Osteoclastic bone destruction results in disintegration of the collagen from within the matrix as well as the release of calcium, growth factors, and cytokines. Osteoclastic action is highly up regulated in PC leading to excessive bone destruction.

Pathological Bone Turnover in PC Bone Metastasis

The bidirectional interactions of bone cells (osteoblasts and osteoclasts) with PC cells not only suggest that tumor-derived growth factors affect bone cells, but also that cells in the bone microenvironment stimulate metastatic tumor growth and progression. The pathological condition of bone is caused by dysfunctional signaling mechanisms for bone remodeling due to the presence and influence of the invading tumor, thus resulting in the destruction of bone. Within the bone matrix there are several factors, such as TGF-β, IGF, FGF, and PDGF, which are stored during bone formation and released during resorption. In PC, close cross-talk between the bone microenvironment and the tumor results in the release of these growth factors into the microenvironment, up-regulating differentiation and activity of osteoblasts, the bone forming cells, and subsequently osteoclasts (bone resorbing cells). Increased production and release of these growth factors from dissolved bone during uncontrolled turnover and remodeling results in co-stimulation of bone and tumor cells. Consequently, enhanced tumor survival, growth, proliferation, as well as osteoclastogenesis and accelerated bone destruction, occurs. Osteoblastic, osteoclastic, and mixed lesions can occur in PC skeletal metastases, due to an imbalance between osteoblastic mediated bone formation and osteoclast mediated bone resorption. In particular, PC is known to often cause osteoblastic lesions caused by over-expression of numerous pro-osteoblastic factors, such as RANKL, parathyroid hormone-related protein (PTHRP), BMPs, TGF-β, IGFs, FGFs, interleukins (ILs), PDGF, and VEGF, leading to un-regulated osteoblastic function via the activation of proliferation and differentiation signaling pathways such as Wnt. Furthermore, conditions within the bone environment, such as increased calcium release, hypoxia, and acidity, allow the tumor to thrive. The newly formed bone contains ECM proteins such as Type I collagen, osteonectin, and BSP, which act as chemoattractants to PC cells.
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IGF-1, basic FGF, and PDGF which further stimulate tumor growth.

Figure 3 illustrates the cycle involving bone and tumor cell interactions in the bone microenvironment and the various factors involved.

The RANK/RANKL/OPG Signaling Mechanism Involved in Bone Destruction

Local bone destruction is a significant aspect of PC bone metastasis in the spine and is mediated through osteoclasts. As such, the RANK-RANKL-OPG axis has been well established as a key regulatory pathway of bone activity in physiological conditions and is highly up-regulated in pathological conditions. Receptor activator of the nuclear factor kappa-B receptor (RANK), RANKL, and osteoprotegerin (OPG) are members of the tumor necrosis factor (TNF) family. RANK is a membrane-bound TNF receptor expressed by osteoclast precursors and mature osteoclasts and controls the development and apoptotic processes in bone. RANKL is a type II transmembrane protein and is expressed on the surface of bone marrow stromal cells and by mature osteoblasts. RANKL is essential for differentiation, activation, and survival of osteoclasts. The release of RANKL from tumor cells into the bone microenvironment has been suggested to stimulate migrating to bone. The various factors which are implicated in PC bone metastasis are summarized in Table 1.

Bone destruction from osteoclast activity leads to the release of growth factors from the matrices which possess the ability to act on metastatic cancer cells. These factors indirectly promote angiogenesis and stimulate tumor-derived osteolytic and osteoblastic factors, subsequently remodeling the skeleton and accommodating tumor growth. TGF-β is the most abundant growth factor in the PC bone microenvironment and regulates bone development and remodeling via the promotion of osteoprogenitor differentiation. Osteoblasts deposit TGF-β into the bone matrix where it is then released and activated through osteoclastic resorption.

In PC, TGF-β mediates metastasis though the activation of EMT, tumor cell invasion, and suppression of immune surveillance by increasing angiogenesis. It stimulates bone metastasis via the induction of proteolytic gene expression through PTHrP within tumor cells. TGF-β-induced PTHrP acts to increase osteoblastic production of RANKL, consequently stimulating osteoclast formation and activity thus promoting bone metastasis. Additionally, there is an irregular control of the resorption pathway, leading to bone destruction and resulting in the release of several bone matrix factors such as IGF-1, basic FGF, and PDGF which further stimulate tumor growth. Figure 3 illustrates the cycle involving bone and tumor cell interactions in the bone microenvironment and the various factors involved.
endothelin-1 is a small vasoconstricting peptide produced by the vascular endothelium, and has a key role in vascular homeostasis. ET-1 production is stimulated by cytokines (ILs), growth factors (TNFα, TGF-β, PDGF), leading to growth and proliferation of tumours. Activation of the ET A receptor by ET-1 promotes tumour cell growth and survival, mediating processes involved in tumour invasion and metastasis, angiogenesis and the inhibition of apoptosis. ET-1 may also increase osteoblast proliferation and bone formation by crosstalk with Wnt signaling, leading to the suppression of the inhibitor of Wnt signaling, Dickkopf-1 (Dkk1).15

Insulin-like growth factors

IGFs are survival factors—potent mitogens and anti-apoptotic factors involved in cell proliferation and differentiation. IGF-I and II are abundant in the bone matrix and are released during bone resorption. IGF-1R signaling plays a role in malignant cell transformation, cancer progression, and metastatic spread of different types of tumours. IGFs promote survival and angiogenesis in PC.16

Integrins

Integrins are cell surface receptors for extracellular matrix proteins and play a key role in cell survival, proliferation, migration and gene expression, apoptosis, cell adhesion, angiogenesis and proteinase expression. Integrin signaling has been shown to be de-regulated in PC. Integrins expressed in PC play an important role in colonization of prostate tumour cells in the bone. PC cells express the same integrins; αvβ3 and α2β1 which are express by osteoclast. These factors are able to facilitate tumour spreading in the bone, by aiding in attachment and migration to the bone matrix, facilitating extracellular matrix remodelling and cancer cell growth.17

Cadmherins

Cadmherin-11 mediates homophillic cell adhesion in a calcium dependent manner. Its expression is associated with osteoblast differentiation and may function in cell sorting, migration, and alignment during the maturation of osteoblast.18 E-cadherin, is prominently associated with intracellular adhesion mechanisms for tumour invasiveness, metastatic dissemination.19

Matrix metalloproteinases

MMPs are proteolytic extracellular matrix-degrading enzymes, which are produced by both the cancer cells and the stromal cells. They degrade ECM components including; collagens, laminins, fibronectin, and vitronectin, which can clear a path to facilitate cell migration and invasion. The expression of MMPs is regulated by many growth factors, such as TGFβ which up-regulates MMPs expression and activity such as migration, invasion, angiogenesis activation and growth.20

RANK

RANK is the receptor expressed on the surface of osteoclasts precursors and mature osteoclasts. The receptor becomes activated upon binding of RANKL, activating several downstream signalling pathways for differentiation, maturation and survival of osteoclasts in the process of bone resorption.21

RANKL

RANKL is expressed by osteoblasts and bone marrow stromal cells. RANKL binding to RANK leads to differentiation of osteoclast precursors as well as activation and survival of mature osteoclasts. RANKL is produced in two forms; a cell surface and a soluble molecule. Furthermore, it has been established that PC cells secrete soluble RANKL, which can act to directly activate osteoclasts.22

Osteoprotegerin

OPG is a soluble decoy receptor for RANKL (ligand) and acts by binding RANKL, thus, preventing it from binding and activating RANK receptor on osteoclasts. Thus, it inhibits osteoclastogenesis; osteoclast maturation, activation and survival and bone resorption.23

Parathyroid hormone related peptide

PTHrP is expressed by PC cells. This induces RANKL expression through osteoblasts differentiation, which further stimulates osteoclast activation and formation, leading to bone destruction.24 This activity protects PC cells and osteoblasts from apoptosis.25

Chemokines

Chemokines are primarily known in the regulation of the motility (chemotaxis) of hematopoietic cells (immune system cells) and their ability to stimulate directional migration of nearly all classes of leukocytes during inflammation through the activation of a group of cell surface receptors. Chemokines are believed to mediate cell adhesion, migration, invasion and angiogenesis during the metastasis of tumour cells to bone. Bone contains chemotactic factors that attract PC cells towards the microenvironment. CXCR4 (expressed on PC cells) and stromal-derived factor-1 (SDF-1), are elevated in metastatic PC cell lines and in bone metastasis.26,27

Transforming growth factor

TGFβ is a widely expressed and abundant growth factor involved in the regulation of cellular proliferation, chemotaxis, differentiation, immune response, and angiogenesis. Production of TGFβ by PC-associated stroma has been shown to increase the growth and invasiveness of prostate epithelial cells. Bone is one of the largest reservoirs of TGFβ1, which is released from the bone matrix during bone resorption following PC cell metastasis.28

Table 1. The various mediators implicated in PC bone metastasis; their function and mechanism of action.

| FACTOR                          | FUNCTION                                                                 |
|---------------------------------|--------------------------------------------------------------------------|
| Bone morphogenetic proteins    | BMPs play an integral role in endochondral ossification, osteogenesis and bone repair through the initiation of osteoblast differentiation (through Runx2 induction). In PC, BMPs are involved in proliferation, migration and invasion of in cancer cells in bone metastasis. Furthermore, BMPs promote VEGF expression, leading to angiogenesis and endothelial cell migration in PC cells. The expression levels of BMPs increase while those of the cognate receptors decrease with progression of disease. As PC cells produce BMPs, and BMPs generally decrease the proliferation of PC cells, however in advanced progression, loss of BMP receptor expression permits PC cells escape from the physiologic constraint on cellular proliferation imposed by BMPs, leading to a more aggressive phenotype by altering the tumour microenvironment. |
| Endothelin-1                    | Endothelin-1 is a small vasoconstricting peptide produced by the vascular endothelium, and has a key role in vascular homeostasis. ET-1 production is stimulated by cytokines (ILs), growth factors (TNFα, TGF-β, PDGF), leading to growth and proliferation of tumours. Activation of the ET A receptor by ET-1 promotes tumour cell growth and survival, mediating processes involved in tumour invasion and metastasis, angiogenesis and the inhibition of apoptosis. ET-1 may also increase osteoblast proliferation and bone formation by crosstalk with Wnt signaling, leading to the suppression of the inhibitor of Wnt signaling, Dickkopf-1 (Dkk1). |
| Insulin-like growth factors     | IGFs are survival factors—potent mitogens and anti-apoptotic factors involved in cell proliferation and differentiation. IGF-I and II are abundant in the bone matrix and are released during bone resorption. IGF-1R signaling plays a role in malignant cell transformation, cancer progression, and metastatic spread of different types of tumours. IGFs promote survival and angiogenesis in PC. |
| Interleukiins                   | Interleukiins are pro-inflammatory cytokines and a key regulator of immunosuppression in advanced cancer. It is involved in the regulation of proliferation, apoptosis, migration, invasion, and angiogenesis. IL-6 has been implicated in PC bone metastasis progression—stimulates RANKL production, chemotherapeutic resistance, and androgen independence (androgen receptor dysfunction). IL-6 induces AR expression, which leads to TGFβ activation and consequently MMP-9, resulting in invasion of PC cells. |
| Integrins                      | Integrins are cell surface receptors for extracellular matrix proteins and play a key role in cell survival, proliferation, migration and gene expression, apoptosis, cell adhesion, angiogenesis and proteinase expression. Integrin signalling has been shown to be de-regulated in PC. Integrins expressed in PC play an important role in colonization of prostate tumour cells in the bone. PC cells express the same integrins; αvβ3 and α2β1 which are express by osteoclast. These factors are able to facilitate tumour spreading in the bone, by aiding in attachment and migration to the bone matrix, facilitating extracellular matrix remodelling and cancer cell growth. |
| Cadherins                       | Cadherin-11 mediates homophillic cell adhesion in a calcium dependent manner. Its expression is associated with osteoblast differentiation and may function in cell sorting, migration, and alignment during the maturation of osteoblast. E-cadherin, is prominently associated with intracellular adhesion mechanisms for tumour invasiveness, metastatic dissemination. |
| Matrix metalloproteinases       | MMPs are proteolytic extracellular matrix-degrading enzymes, which are produced by both the cancer cells and the stromal cells. They degrade ECM components including; collagens, laminins, fibronectin, and vitronectin, which can clear a path to facilitate cell migration and invasion. The expression of MMPs is regulated by many growth factors, such as TGFβ which up-regulates MMPs expression and activity such as migration, invasion, angiogenesis activation and growth. |
| RANK                            | RANK is the receptor expressed on the surface of osteoclasts precursors and mature osteoclasts. The receptor becomes activated upon binding of RANKL, activating several downstream signalling pathways for differentiation, maturation and survival of osteoclasts in the process of bone resorption. |
| RANKL                           | RANKL is expressed by osteoblasts and bone marrow stromal cells. RANKL binding to RANK leads to differentiation of osteoclast precursors as well as to activation and survival of mature osteoclasts. RANKL is produced in two forms; a cell surface and a soluble molecule. Furthermore, it has been established that PC cells secrete soluble RANKL, which can act to directly activate osteoclasts. |
| Osteoprotegerin                 | OPG is a soluble decoy receptor for RANKL (ligand) and acts by binding RANKL, thus, preventing it from binding and activating RANK receptor on osteoclasts. Thus, it inhibits osteoclastogenesis; osteoclast maturation, activation and survival and bone resorption. |
| Parathyroid hormone related peptide | PTHrP is expressed by PC cells. This induces RANKL expression through osteoblasts differentiation, which further stimulates osteoclast activation and formation, leading to bone destruction. This activity protects PC cells and osteoblasts from apoptosis. |
| Chemokines                      | Chemokines are primarily known in the regulation of the motility (chemotaxis) of hematopoietic cells (immune system cells) and their ability to stimulate directional migration of nearly all classes of leukocytes during inflammation through the activation of a group of cell surface receptors. Chemokines are believed to mediate cell adhesion, migration, invasion and angiogenesis during the metastasis of tumour cells to bone. Bone contains chemotactic factors that attract PC cells towards the microenvironment. CXCR4 (expressed on PC cells) and stromal-derived factor-1 (SDF-1), are elevated in metastatic PC cell lines and in bone metastasis. |
| Transforming growth factor      | TGFβ is a widely expressed and abundant growth factor involved in the regulation of cellular proliferation, chemotaxis, differentiation, immune response, and angiogenesis. Production of TGFβ by PC-associated stroma has been shown to increase the growth and invasiveness of prostate epithelial cells. Bone is one of the largest reservoirs of TGFβ1, which is released from the bone matrix during bone resorption following PC cell metastasis. |
Table 1. (Continued)

| FACTOR                        | FUNCTION                                                                 |
|-------------------------------|--------------------------------------------------------------------------|
| **Platelet-derived**          | PDGFs are potent stimulator of cell proliferation, migration, survival,  |
| **growth factor**             | chemotaxis, angiogenesis and transformation. PDGF is known to play a    |
|                               | major role in cell-cell communication for normal development and also    |
|                               | during pathogenesis. PDGF from tumour cells regulates commitment of      |
|                               | stromal mesenchymal cells to differentiate into osteoprogenitor cells   |
|                               | and induces proliferation and migration of osteoblast cells, suggesting  |
|                               | a role for PDGF in bone formation. PDGF also stimulates bone resorption  |
|                               | by increasing the number of osteoclasts and up-regulating matrix degrading |
|                               | enzyme expression.                                                      |
| **Epidermal growth factor**   | EGF is over-activated in PC and cross-talk with the androgen pathway     |
|                               | is linked to progression from androgen-responsive disease to castrate    |
|                               | resistant phenotypes. EGFR signalling activates androgen receptor        |
|                               | pathway even in the circumstances of androgen deprivation. Furthermore,  |
|                               | EGFR itself may be under the regulation of androgen signalling pathway.  |
|                               | EGF stored within the bone matrix, is released in the bone resorption.   |
|                               | EGF thus, plays an important role in regulating cellular growth,         |
|                               | proliferation, survival and function of PC cells.                      |
| **Fibroblast growth factor**  | FGFs have a broad range of biologic activities that play an important    |
|                               | role in tumorigenesis including promotion of proliferation, motility,    |
|                               | and angiogenesis and inhibition of cell death. FGFs play a key role in   |
|                               | the cellular growth and maintenance of normal prostatic epithelium and   |
|                               | are expressed in normal prostatic stroma. FGFs are expressed as          |
|                               | autocrine growth factors by prostate cancer cells and can also be        |
|                               | expressed in the tumour microenvironment as paracrine growth factors    |
|                               | FGFs released from the bone matrix as a result of PC induced bone       |
|                               | resorption, thus leading to up-regulation of signalling activity.        |
| **Vascular endothelial**      | VEGF is a critical mediator of angiogenesis—formation of new capillaries  |
| **growth factor**             | from existing blood vessels and vasculature. It plays an important role  |
|                               | for tumour growth and metastasis by providing oxygen and nutrients to   |
|                               | move and migrate through the blood vessel circulation. VEGF signalling   |
|                               | is important for osteoblast and osteoclast activity and survival.       |
|                               | Osteoblasts and osteoclasts also express VEGF receptors (VEGFRs), which  |
|                               | are up-regulated and over-activated in PC, leading to dys-regulated bone |
|                               | destruction.                                                          |

Figure 3. The cycle involving bone and tumor cell interactions in the bone microenvironment. Matrix metalloproteinases (MMPs), chemokines (CXCL12/CXCR4), and vascular endothelial growth factor (VEGF) are involved in tumor cell attraction and targeting of bone as well as facilitating survival and metastasis within the bone microenvironment. Physical factors including hypoxia, acid pH, and high extracellular calcium levels influence this process. The tumor secretes several osteoblastic and osteoclastic factors in response to the bone microenvironment. Tumor derived osteoclast-stimulatory factors include PTHrP and IL-1, IL-6, IL-8, TNF, M-CSF, and RANKL. Bone-derived pro-osteoblastic growth factors released from the resorption process include TGF-β, IGFs, EGFs, and FGFs, which in turn upregulate BMPs, VEGF, and RANKL. Chemokines, MMPs and cathepsin are secreted by the tumor, which in turn stimulates bone cells to release factors which promote further tumor growth in bone. OPG is downregulated due to the over-stimulation of RANKL. The increased RANKL-to-OPG ratio leads to increased osteoclast formation, survival, and activity, which increases the rate of bone remodeling and turnover.
osteoblast-derived activation of osteoclasts, leading to bone destruction in skeletal metastasis in PC.

OPG is a naturally occurring protein that shares its homology with members of the TNF family. Osteoblasts and stromal cells secrete OPG, which acts as a soluble decoy receptor and exerts its effects through the binding and subsequent sequestering of RANKL. This prevents it from binding to RANK and activating downstream signaling events leading to osteoclast gene expression, differentiation, and survival, thus inhibiting bone destruction. OPG and RANKL, therefore, share a synergistic relationship to maintain homeostasis. While OPG has been shown to block the pathological increase in osteoclast activity and number, in cancer, RANKL expression is upregulated by stimulatory osteoclastic factors that are released from the tumor. This leads to down-regulation of osteoblastic factors in the environment resulting in decreased OPG expression which causes unregulated and abnormal bone formation that ultimately leads to bone destruction.

The RANK/RANKL/OPG system plays a critical role in osteoclastogenesis and bone destruction in PC. The binding of osteoblasts causes RANKL to bind to the RANK receptor expressed on the cellular surface of osteoclast precursors leading to the commitment of the monocytic precursor to the osteoclastic lineage and the subsequent differentiation and activation of osteoclasts. Following the activation of the receptor (RANK), several downstream signaling cascades are initiated. These cascades control processes such as osteoclastogenic differentiation, cell survival and growth as well as actin reorganization, motility, and osteoclastic resorptive activity. The NF-kB pathway initiates the transcription of a wide variety of genes encoding angiogenic factors, cytokines, cell adhesion molecules, and anti-apoptotic factors which are involved in tumor cell invasion, metastasis, survival, and chemo-resistance. Due to the critical role of this pathway in PC, it provides an important target for therapeutic developments. The pathways in osteoclastogenesis through the RANK-RANKL-OPG triad system are illustrated in Figure 4.

**The Role of VEGF in Promoting Angiogenesis in PC Bone Metastasis**

It is well established that tumors require a blood supply to establish, grow, and metastasize. Angiogenesis is the formation of new blood vessels from the existing vasculature within the growing tumor. It involves neovascularization of cancer cells through the division and migration of endothelial cells. This promotes the expansion, sustainable growth, progression, and metastasis of tumors to distant sites. As tumor cells proliferate, the tumor mass expands beyond a capacity that can be supported by the existing vasculature. As a result, nutrient and oxygen levels fall, leading to an accumulation of metabolic waste. The tumor cells respond to this condition by secreting...
Recent studies have shown that suppression of PI3K/Akt/tumor cell apoptosis, as well as inhibit MMP-9 production, tumorogenicity and metastasis by inducing endothelial and antibody has been shown to inhibit angiogenesis, reduce signal mediator and a target for RTKIs. Sorafenib and sunitinib. Multi-receptor tyrosine kinase inhibitors (RTKIs) such as, angiogenic drugs have been introduced into the clinical setting VEGF and VEGF receptors (VEGFRs).

As such, the up-regulated VEGF production in osteoblasts and studies have shown that increased VEGF in osteoblasts is strongly correlated with osteogenesis (bone formation), stimulating chemotactic migration, and osteoblast differentiation. Osteoblastogenic BMPs regulate VEGF expression and VEGF promotes osteoblastic activity. VEGF has a direct role in osteoclastogenesis through promoting the activation and survival of osteoclasts with mature osteoclasts increasing expression of VEGF receptors. VEGF also possesses the ability to up-regulate RANK on endothelial cells thereby increasing its sensitivity to RANKL and promoting angiogenesis. As such, the up-regulated VEGF production in tumor cells and local bone cells may be responsible for close bidirectional attraction and interaction leading to excessive bone formation and active resorption, resulting in osteoblastic lesions and osteolytic destruction in PC.

Angiogenesis inhibition via blocking the VEGF pathway has become an important therapeutic strategy. Tumor cells as well as tumor cell lines have been reported to express both VEGF and VEGF receptors (VEGFRs). Several antiangiogenic drugs have been introduced into the clinical setting including anti-VEGFA antibody, known as bevacizumab and multi-receptor tyrosine kinase inhibitors (RTKIs) such as, sorafenib and sunitinib. VEGF2 is a major endothelial cell signal mediator and a target for RTKIs. The anti-VEGFR2 antibody has been shown to inhibit angiogenesis, reduce tumorigenicity and metastasis by inducing endothelial and tumor cell apoptosis, as well as inhibit MMP-9 production. Recent studies have shown that suppression of PI3K/Akt/mTOR signaling using mTOR inhibitors reinstates sensitivity of phosphatase and tensin homolog (PTEN) deficient cancer cells to tyrosine kinase inhibitor-mediated apoptosis. This may have therapeutic applications in the future. The various growth factors implicated in PC bone metastasis and their current use or potential for therapeutic targets is summarized in Table 2.

The Function of Growth Factors in PC Bone Metastasis

Several growth factors have been implicated in PC bone metastasis. The mineralized bone matrix contains a wide range of growth factors, where IGF-1 is one of the most abundant. IGF-1 is involved in regulating cell differentiation, proliferation, migration, and apoptosis. Mitogenic effects of IGFs are mediated through binding with IGF-1 receptor and activation of the PI-3 kinase/Akt signaling pathway. Once metastasis has been established in the bone, osteoclastic bone resorption is activated. Growth factors such as IGF-1 and TGF-β are released into the bone marrow cavity where they interact with and influence metastatic tumor cells. The IGF-1R is an RTK that, upon activation by IGF-1, shows mitogenic and anti-apoptotic effects. Increased IGF-1R signaling results in activation of growth-promoting intracellular signaling pathways, including the ras-raf-MAPK (mitogen-activated protein kinase) and PI3K cascades. IGFs are proposed to bypass inhibition by stimulation of autocrine androgen synthesis, survival signaling, and enhancement of androgen receptor nuclear localization by stabilizing microtubules. IGF-1 is crucial for osteoblast differentiation and found abundantly in the bone microenvironment.

BMPs are members of the TGF-β superfamily and are potent inducers of osteoblast differentiation. BMPs initiate new bone formation by recruiting progenitor stem cells and initiating their growth and differentiation into bone. They are also involved in stimulating cancer cell migration. In addition, BMP mRNA and protein expression has been found in human PC cell lines from bone metastasis specimens. Furthermore, receptors have been expressed in the cancer cell lines and tissue. In vivo, inhibition of BMP activity by noggin inhibits the development of osteoblastic lesions and reduces the osteoblastic component in mixed lytic/blastic lesions. Osteoblast-derived BMP-2 can activate the Akt, MAPK, and ERK pathways, which in turn induce IKKa/b phosphorylation and NF-kB activation, resulting in the activation of β1 and β3 integrins and contributing to the migration of PC cells. BMP signaling also activates the intracellular receptor type I kinase, followed by phosphorylation of Smad, which translocates to the nucleus and induces the expression of genes important for bone formation. BMPs are implicated in homing, migration and invasion of PC cells to bone that ultimately leads to promotion and formation of osteoblastic lesions.
### Table 2. Growth factors implicated in Prostate Cancer bone metastasis. Role in tumour stimulation and proliferation and current and potential therapeutic targets.

| Growth Factor | Receptor | Experiments | Findings | Drug Availability | Ref |
|---------------|----------|-------------|----------|-------------------|-----|
| Insulin-like growth factor | IGF-1R | PC xenograft models | Involved in anti-apoptosis, cell differentiation and proliferation, transformation and angiogenesis (bone metastasis). Uregulates expression of both RANK/RANKL, stimulates differentiation of osteoclast and represses OPGL. | CIXUTUMUMAB (IMC-A12): IGF-IR inhibitor-fully human monoclonal antibody (Phase II) | 108 |
| IGF-I & IGF-II | | Clinical studies: Phase I/III | Reduces IGF-IR activation by preventing IGF-I and IGF-II interaction with its receptor. Promotes receptor internalization and degradation. | GANITUMAB (AMG 479): IGF-IR inhibitor-fully human antibody (lgG1) | 110 |
| Epidermal growth factor | EGFR | Transwell or Boyden chamber Dunn chamber 3D invasion | Modulation of cell proliferation and survival for tumorigenesis. Inhibition of downstream signalling pathways. | GEFITINIB & ERLOTINIB: Oral anilinoquinazolone compound that blocks EGFR tyrosine kinase activity (Phase I/II with radiation) | 112 |
| | | wound healing assay | Initiation of growth, vascularization, and progression of tumours through proliferation and evasion of cell death. Enhance angiogenesis through paracrine action on endothelial and other stromal cells. | AZ8010: ATP-competitive FGFR tyrosine kinase inhibitor | 113 |
| Fibroblast growth factor | FGFR1-4 | Transwell or Boyden chamber Dunn chamber wound healing assay | bFGF is highly angiogenic and regulates protease expression, e.g. urokinase-type plasminogen activator, collagenase, which may be involved in metastatic cascade. | PEG-interferon alfa-2b BGJ398 E-3810 TSU-68 Dovitinib lactate (TKI258) BIBF 1120 | 114 |
| FGF8 & FGF9 | | Wound healing assay | | | |
| | bFGF | | | | |
| | FGF-2 | | | | |
| Platelet derived growth factor | Cognate α-PDGFβ-PDGF | In vivo studies | Promotes tumorigenesis, supports stromal cell recruitment and malignant transformation. | CEDIRANIB (AZD2171): IMATINIB Tyrosine kinase inhibitor CP-673,451: PDGFR-β Inhibitor Anti-angiogenesis, growth inhibition | 115 |
| (PGDF-D) | | Clinical studies: Phase I & II | Proliferation f connective tissue, monocyte/macrophage and smooth muscle cell chemotaxis, angiogenesis. | | |
| Vascular endothelial growth factor | VEGFR-1 | Transwell or Boyden chamber | Induction of endothelial cell apoptosis and reduction of endothelial cell MMP-9 production | BEVACIZUMAB: Approved SORAFENIB: Approved | 116 |
| (VEGF) | VEGFR-3 | 3D culture Orthotopic PC xenograft | | Anti-angiogenic | |
| | VEGFR-2 | | | Axitinib (AG-013736) Vatalanib (PTK787) | 108 |
| Transforming growth factor | TGF-β1 | Transwell or Boyden chamber Dunn chamber wound healing assay | Sensitivity to TGF-B inhibition is lost with tumour progression. Causes osteoblast and osteoclast recruitment, differentiation, bone matrix production (ECM), cell growth angiogenesis, immunosuppression. | NICOTUZUMAB: Inhibits TGF-B activity leading to tumour regression. | 117 |
| (TGF-β) | TGF-β2 | | | | |
| Bone morphogenetic Proteins | BMP-2 | | | | |
| BMP-6 | BMPR-I | In vitro studies | Proliferation, apoptosis, invasion differentiation and migration of cellular targets. Acts in conjunction with RANKL to form mixed osteolytic/ blastic lesions. | Targets: BMP-7, 9, 15 Tumour suppression through increased Noggin activity which prevents osteoblastic/ osteoclastic activity. | 118 |
| | BMPR-II | In vivo studies | | | |
stroma increases the growth and invasiveness of prostate epithelial cells. TGF-β has been shown to promote osteoblastic bone metastases in experimental models. Bone is an abundant reservoir for TGF-β1, which is released from the bone matrix during bone remodeling following PC cell migration and establishment. TGF-β is activated by cleavage of its precursor by osteoclast-derived or PC secreted proteases. TGF-β signaling occurs through a transmembrane receptor serine–threonine complex which consists of type I and II receptor kinases. The binding of TGF-β1 to the type II receptor leads to the formation of a heterodimeric complex with the type I receptor, resulting in phosphorylation. The receptor-associated Smads are subsequently recruited to the activated receptor complex. The phosphorylated regulatory Smad2/3 interact with the co-Smad, Smad4. This complex then translocates into the nucleus, binding to specific DNA sequences, consequently recruiting co-activators or co-repressors to regulate the transcription of TGF-β target genes. Activated TGF-βR1 also induces activation of SMAD-independent pathways such as PI3K, AKT, and MAPK. Thus, TGF-β is a potential therapeutic target in advanced PC using TGF-β receptor type I (TGF-βR1) kinase inhibitors.

Communication between epithelial and stromal compartments via FGF family signaling pathways have important roles in homeostasis of the normal prostate. Upon binding of FGF to its receptor, FGFR dimerization is induced leading to phosphorylation and activation of various downstream signaling pathways including MAPK-PI3K/AKT, phospholipase-C (PLC), and signal transducer and activator of transcriptions (STATs). FGFs are expressed as autocrine growth factors by PC cells, however they can also be expressed in the tumor microenvironment as paracrine growth factors. They promote cancer progression by increasing proliferation and preventing cell death. FGFRs are well known angiogenic factors stimulating angiogenesis through paracrine actions on endothelial and other stromal cells in the tumor microenvironment. FGF signaling is therefore a promising therapeutic target in aggressive PC. Several FGFR small-molecule inhibitors have entered clinical trials with the action of inhibiting multiple tyrosine kinases.

De-regulation of EGF receptor (EGFR) is often associated with carcinogenesis which can be caused by its overexpression, gene amplification, mutations, or deletions. Increased expressions of EGFR and its ligands (EGF and TGF-β) have been described in prostate tumors, and autocrine activation of EGFR signaling regulates the growth of androgen-independent PC. EGFRs belong to the human epidermal receptor (HER) axis, which binds the EGF, activating downstream signaling events through PI3 kinase and MAPK. This activates the Akt family of kinases and STATs, resulting in downstream events that regulate cellular proliferation, growth, survival, and migration. Normanno and Gullick demonstrated that factors released under the control of EGFR signaling modulate bone remodeling through the induction of RANKL in bone marrow stromal cells, thus activating osteoclasts. EGFR signaling also regulates the ability of MSCs to release growth factors that promote neo-angiogenesis and tumor cell migration. A number of anti-EGFR drugs are currently awaiting Food and Drug Administration approval or are under evaluation in clinical trials.

The Role of PTHrP in PC Bone Metastasis

PTHrP was initially identified as a product associated with humoral hypercalcemia of malignancy resulting from human tumors. It binds to the type 1 PTH receptor (PTHR1) and functions through endocrine, autocrine, paracrine, and intracrine actions to modulate development, growth, and differentiation of normal cells as well as inducing metastasis of malignant cells via cytokine interaction. Studies of the downstream signaling events of PTH-1R have focused mainly on the cAMP-dependent protein kinase A (PKA) and protein kinase C (PKC) pathways. At the cellular level, PKA and MAPK-ERK (extracellular signal-regulated kinase) pathways govern the majority of the effects induced by PTH and PTHrP on osteoblasts. The MAPK cascade is also called the ERK cascade and found to be involved in cell adhesion, proliferation, differentiation, viability, and apoptosis. Activation of the MAPK cascade involves phosphorylation of MAPKKK (Raf-1), MAPKK, and MAPK. Active MAPK subsequently phosphorylates transcription factors, other downstream kinases, and substrates leading to biologic activity. PTHrP regulates MAPK and is critical in the regulation of cellular functions including migration, angiogenesis, survival, and calcium release and transport in osteoclasts.

Recently, PTHrP has been found to be important in androgen dependent resistance of PC tumor cells to apoptosis as well as tumor proliferation and progression. Over-expression of PTHrP is involved in the promotion of metastasis to bone, especially the initial osteolysis and subsequent osteoblastic phase. PTHrP enhances bone remodeling and the release of several biological factors such as VEGF, ILs, and Endothelin-1 as well as various growth factors including IGFs, FGFs, TGF-β and PGDF, providing a fertile environment for tumor growth. PTHrP also induces osteoblast differentiation and protects PC cells and osteoblasts from apoptosis. It participates in the indirect activation of osteoclasts through stimulation of osteoblastic RANKL production and decreasing OPG leading to the activation of osteoclastogenesis. This leads to the process of bone destruction in osteolytic metastasis whereby the bone matrix releases stored immobilizing growth factors, such as TGF-β, which is activated and released as a result of osteoclast-activated resorption activity. The release of growth factors from the matrix further stimulates the cancer cells to up-regulate production of PTHrP.
The Key Role of MMPs and Chemokines in PC Bone Metastasis

Tumor metastasis involves the interactions between invading cancer cells and the surrounding stromal tissue. Such interactions promote degradation of the ECM via specialized proteolytic enzymes, which are produced by both the cancer cells and the stromal cells.\textsuperscript{138} MMPs are potent zinc and calcium dependent proteolytic enzymes that play a vital role in this process of degradation and digestion of structural components of the ECM. This is an essential step for tumor invasion, migration, metastatic progression, and tumorigenesis.\textsuperscript{139–141} Moreover, MMPs are involved in the cleavage and release of growth factors, cell surface receptors, cell adhesion molecules, and chemokines from the matrix as a result of degradation, leading to enhanced tumor growth and establishment of a more aggressive phenotype due to acquired resistance.\textsuperscript{142,143} In particular, MMP-7 is involved in the cleavage of RANKL from the osteoblast cell surface, forming a soluble form of RANKL. Among the other MMPs, MMP-2 (gelatinase A) and MMP-9 (gelatinase B), referred to as type IV collagenases or gelatinases, are specifically associated with PC metastasis.\textsuperscript{138} MMP-2 and MMP-9 induce tumor angiogenesis,\textsuperscript{138} with MMP-9 found to play a vital role in tumor induced osteoclast recruiting.\textsuperscript{142} Dong et al demonstrated that MMP-9 activity was originating from newly recruited osteoclasts during the early colonization of the bone marrow spaces by tumor cells.\textsuperscript{142,144} MT1-MMP is a membrane-anchored protease capable of activating pro-MMP-2 on the cell surface, as well as promoting tumor angiogenesis and growth. Additionally, it degrades several components of the ECM, including type 1 collagen, which is the most abundant protein in bone. It has been proposed that MT1-MMP is able to function as enzymes that shed and release non-ECM substrates, particularly RANKL, from the tumor cells, which activates osteoclastic bone degradation.\textsuperscript{144,145} As such, MMPs are potential therapeutic targets against cancer using endogenous and synthetic tissue inhibitors of metalloproteinase (TIMPs).\textsuperscript{146}

Evidence from bone xenograft models demonstrate that PC cells injected adjacent to the bone migrate toward bone, suggesting that there is chemo-attraction between the tumor and the bone microenvironment. Chemokines interact with cell surface receptors promoting oncogenic and cellular transformation by acting as growth factors that increase proliferation and tumor angiogenesis.\textsuperscript{72} CXCL12 (also known as SDF-1) from bone stromal cell binds to CXCR4 receptor and activates downstream signaling through the PI3K/Akt and MAPK/MEK pathways. CXCR4 is widely expressed in the cellular environment and interacts only with the SDF-1 ligand. It is involved in the immune response, protecting the tumor from the host response as well as activating key survival pathways such as anti-apoptosis, leading to growth and proliferation of tumor cells.\textsuperscript{147} CXCR4 has been implicated in the “homing”-directed dissemination of circulating cancer cells to microenvironments of high chemokine concentration such as lymph nodes and bone marrow.\textsuperscript{148} During this process, the circulating cancer cells mimic hematopoietic and immune cells in terms of localizing to high CXCL12-expressing sites, firm adhesion to endothelial cells, transmigration across the blood vessel wall, and migration towards the chemokine source.\textsuperscript{148} The binding of CXCL12 from the bone microenvironment to CXCR4 expressed on the surface of PC cells activates cell signaling pathways and leads to the expression and secretion of proteases.\textsuperscript{148} SDF-1 is thought to recruit osteoclast precursors expressing CXCR4 to induce MMPs (2,9,14), which promote proteolytic breakdown of the ECM. In addition, it triggers the angiogenic switch through up regulation of VEGF.\textsuperscript{149,150} The SDF1α/CXCR4 signaling pathway plays a major role in migration by promoting adhesion, invasion, proliferation, and cancer growth in bone.\textsuperscript{144,145} As such, receptor antagonists present potential targets to reduce growth and proliferation of tumors.\textsuperscript{151}

A major characteristic of bone-homing PC cells is their capacity to release copious levels of IL-6, which aids in facilitating bone invasion and growth of metastatic lesions.\textsuperscript{152} Additionally, osteoblasts also express IL-6 receptors. As such, IL-6 is a major pleiotropic, pro-inflammatory cytokine playing a role in immune response, cell differentiation, hematopoiesis, wound repair, and bone remodeling.\textsuperscript{35} It has an effect on cell proliferation, apoptosis, and angiogenesis through activation of Janus kinase (Jak), STAT factor 3, phosphatidylinositol-3-kinase MAP/k, and PI3-K-Akt.\textsuperscript{59} IL-6 also stimulates osteoclastic bone resorption by inducing RANKL expression in osteoblastic cells thereby affecting the growth of PC cells in a paracrine and autocrine manner.\textsuperscript{155} IL-6 activates the androgen receptor in a ligand-independent manner and contributes to PC progression in castrated patients. Additionally, IL-6 may protect PC cells from cell death induced by certain chemotherapeutic agents through activation of STAT3 and anti-apoptotic proteins such as bclXL. Elevated IL-6 levels in patients with castration-resistant prostate cancer (CRPC) are independently associated with decreased survival.\textsuperscript{153} Therapies that can counteract the up-regulated IL-6 activity on tumor growth and survival may benefit patients with advanced PC. The various chemokines and cytokines that are currently being investigated in PC bone metastasis for potential therapeutic targets are summarized in Table 3.

Emerging Therapies for PC Spinal Metastasis

Skeletal metastases as a consequence of advanced PC often cause severe and debilitating symptoms for patients that are refractory to current treatments thereby highlighting the need for more effective and novel therapies. Denosumab (XGEVA, Amgen) is a monoclonal human antibody which binds to and neutralizes RANKL, preventing its binding to the RANK receptor. Administered subcutaneously, it inhibits the activation and activity of osteoclasts and has
Table 3. Chemokines and cytokines implicated in Prostate Cancer bone metastasis. Role in tumour stimulation and proliferation as well as current and potential for therapeutic targets.

| CHEMOKINE                          | RECEPTOR | EXPERIMENTS                                      | FINDINGS                                                                 | DRUG AVAILABILITY                                                                 | REF   |
|-----------------------------------|----------|--------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-------|
| Stromal derived factor-1 (SDF-1)  | CXCR-7   | In vitro studies                                 | Involved in migration, MMP expression, survival, invasion and angiogenesis. Enhanced proliferation and recruitment of immune cells that promote tumour growth | PLERIXAFOR (AMD3100); Receptor antagonists, that block binding pocket of CXCR4.    | 154   |
| CXCL-12                           | CXCR-4   | Transwell assay (Boyden chamber)                 | In vitro studies                                                         | Inhibition of CXCR4 sensitises PC to chemotherapy                                | 108   |
| CXCL-11                           | CCr-2     | [Phase II clinical studies]                      | Promote the migration of monocytes and macrophages to sites of inflammation. Involved in migration of cancer cells. Recruitment of Natural Killer cells and other immune cells (T cells). | CARLUMAB (CNTO888); anti–human monoclonal antibody MLN1202; CCR2 blocker MOGAMULIZUMAB KW-0761; Human monoclonal antibody targeting CCR4 | 108   |
| CCL2                              | CCr-2     | [Phase II clinical studies]                      | Mediates adhesion of PC cells.                                            | None                                                                              | 157   |
| CCL22                             | CCr-4     | Transwell assay (Boyden chamber)                 | Promotes proliferation and growth of PC cells through chemotaxis.         | Anti-CXCL8 antibodies (ABX-IL8); inhibit angiogenesis, MMP production and reduces tumour growth | 159   |
| CX3CL1 fractalkine                 | CX3CL1-R  | In vivo studies                                  | Induces MMP expression and tumour growth                                 | None                                                                              | 158   |
| CXCL-1                            | CXCR-1    | Transwell assay (Boyden chamber)                 | Induces proliferation and growth of PC cells through chemotaxis.          | Anti-CXCL8 antibodies (ABX-IL8); inhibit angiogenesis, MMP production and reduces tumour growth | 159   |
| CXCL-6                            | CXCR-2    | In vivo studies                                  | Induces systemic immune responses (TNF and ILs).                          | ARRY-382: csf-1 inhibitor Prevents osteoclast (macrophage) differentiation and inhibit tumour cell proliferation | 160   |
| CXCL-8                            | CSF-1R    | Transwell assay (Boyden chamber) Dunn chamber 3D invasion | Regulates the survival, proliferation, differentiation and function of cells in the monocyte lineage including macrophages and osteoclasts. | PLX3397: Inhibition of PC tumour growth                                           | 161   |
| Macrophage colony stimulating factor | M-CSF     | [Phase II clinical studies]                      | Involved in immune response. Potentiated by hypoxic environments and chemically induced stresses including exposure to chemotherapy agents. Induce tumour cell proliferation and through growth factor stimulation, pro-angiogenic and anti-apoptotic effects. | SILTUXIMAB (CNTO 328); Chimeric Human monoclonal antibody against IL-6 Induces apoptosis, inhibits expression of angiogenic growth factors and down-regulates genes involved in tumourigenesis. | 162   |
| Interleukins                       | IL-1R     | [Phase II clinical studies]                      | Involved in immune response. Potentiated by hypoxic environments and chemically induced stresses including exposure to chemotherapy agents. Induce tumour cell proliferation and through growth factor stimulation, pro-angiogenic and anti-apoptotic effects. | SILTUXIMAB (CNTO 328); Chimeric Human monoclonal antibody against IL-6 Induces apoptosis, inhibits expression of angiogenic growth factors and down-regulates genes involved in tumourigenesis. | 163   |
| IL-6                              | IL-6R     | Xenograft models                                 | Involved in immune response. Potentiated by hypoxic environments and chemically induced stresses including exposure to chemotherapy agents. Induce tumour cell proliferation and through growth factor stimulation, pro-angiogenic and anti-apoptotic effects. | SILTUXIMAB (CNTO 328); Chimeric Human monoclonal antibody against IL-6 Induces apoptosis, inhibits expression of angiogenic growth factors and down-regulates genes involved in tumourigenesis. | 164   |
| IL-8 (CXCL-8)                      | CXCR-1    | [Phase II clinical studies]                      | Involved in immune response. Potentiated by hypoxic environments and chemically induced stresses including exposure to chemotherapy agents. Induce tumour cell proliferation and through growth factor stimulation, pro-angiogenic and anti-apoptotic effects. | SILTUXIMAB (CNTO 328); Chimeric Human monoclonal antibody against IL-6 Induces apoptosis, inhibits expression of angiogenic growth factors and down-regulates genes involved in tumourigenesis. | 162   |
| IL-11                              | IL-11R    | [Phase II clinical studies]                      | Involved in immune response. Potentiated by hypoxic environments and chemically induced stresses including exposure to chemotherapy agents. Induce tumour cell proliferation and through growth factor stimulation, pro-angiogenic and anti-apoptotic effects. | SILTUXIMAB (CNTO 328); Chimeric Human monoclonal antibody against IL-6 Induces apoptosis, inhibits expression of angiogenic growth factors and down-regulates genes involved in tumourigenesis. | 164   |
been found to be clinically effective in inhibiting subsequent bone resorption.\cite{165,166} Zoledronic acid (Zometa, Novartis) is a widely used bisphosphonate for bone metastases, acting to decrease fracture risk via inhibition of the catalytic action of farnesyl pyrophosphate synthase. This leads to a reduction in osteoclastic activity and induction of apoptosis, inhibiting bone resorption as well as inhibiting tumor cell adhesion to bone.\cite{147,167} The structural similarity of bisphosphonates to inorganic phosphate assists them in incorporating into bone and binding to hydroxyapatite. Once inside the bone, they act to decrease bone resorption by decreasing the availability of hydroxyapatite crystals and osteoclast-mediated resorption,\cite{70} and inhibiting recruitment, differentiation, attachment and survival of osteoclasts.\cite{70} Furthermore, they act on osteoblasts to indirectly inhibit osteoclast differentiation and activation\cite{168} and inhibit RANKL expression in PC cells, diminishing osteoclast activity even more.\cite{70}

An international phase III double-blind study comparison conducted between denosumab and zoledronic acid in PC patients reported that denosumab was superior in delaying metastatic skeletal complications by an average of more than 3 months.\cite{34} Additionally, bone turnover markers were highly suppressed with denosumab treatment. However, the results of this study concluded that there were no differences in overall survival time between patient treatments.\cite{169} Another study tested the effectiveness of denosumab in prolonging bone metastasis-free survival in men with advanced CRPC. These results revealed a risk reduction of 15% and an extension of metastasis-free survival of 4.2 months. In addition, risk of symptomatic bone metastases was 33% lower in the denosumab group.\cite{87} Several systemic agents that possess selectivity for anti-neoplastic effects on bone metastases are currently being tested for palliative efficacy and effect on prolonging survival in patients with advanced PC. These therapies include endothelin receptor antagonists (atrasentan and zibotentan), proto-oncogene Src (c-Src tyrosine kinase) inhibitors, and radiopharmaceuticals (strontium-89 and samarium-153).\cite{87} More recently, alpharadin (radium-223), an alpha radiation-emitting agent, has been of interest due to its ability to target bone metastasis, which is attributable to its similar chemical and physical properties to calcium.\cite{170} The various therapeutic targets currently employed for the treatment of PC bone metastasis are illustrated in Figure 5.

Endothelins and their receptors are emerging as potential targets for PC bone metastasis. The unregulated endothelin pathway in PC plays a vital role in the proliferation, growth, and invasion via up-regulation of tumor proteases

Figure 5. Therapeutic targets for the various mediators involved in PC bone metastasis. Potential targets for PC skeletal metastases include the cancer cells themselves, the tumoral blood supply, cancer-associated fibroblasts, osteoclasts and osteoblasts. Important PC pathways may be blocked by neutralizing antibodies, receptor antagonists and inhibitors.
(MMPs) as well as urokinase-type plasminogen activators, inhibition of apoptosis, and promotion of VEGF-induced angiogenesis in tumors.\textsuperscript{56} Furthermore, endotoxins promote abnormal osteogenesis in the bone microenvironment, proliferation of osteoblasts, bone remodeling, and release of growth factors that stimulate survival of cancer cells.\textsuperscript{56,171,172} Endothelin-1 (ET-1) is a potent vasoconstrictor. Activation of the endothelin receptor A (ET\textsubscript{A}) can lead to induction of a survival pathway, whereas activation of the endothelin receptor B (ET\textsubscript{B}) results in clearance of circulating ET-1 as well as stimulation of apoptosis.\textsuperscript{173} In PC, ET-1 activates the ET\textsubscript{A} receptor, mediating the signaling cascade which promotes tumor survival, growth, angiogenesis, and invasion, thus inhibiting apoptosis.\textsuperscript{173} Once ET-1 binds to and activates ET\textsubscript{A}, interactions and activation of a G-protein triggers the parallel activation of several signal-transduction pathways. This includes phospholipase C activity, which causes an increase in intracellular Ca\textsuperscript{2+} levels, proliferation of ET\textsubscript{B}, and ras/raf/MAPK pathways.\textsuperscript{56} This cascade of events ultimately induces nuclear transcription of several proto-oncogenes, including c-myc, c-fos, and c-jun, which possess the capability of influencing cell growth and proliferation.\textsuperscript{56} A selective ET\textsubscript{A} antagonist known as Atrasentan has been the focus of pre-clinical trials where it has been shown to prevent osteoblastic lesions in mouse models.\textsuperscript{56} Zibotentan is an oral ET\textsubscript{A} specific antagonist shown to inhibit cell invasion, proliferation, and metastasis.\textsuperscript{57} A phase II trial of patients with CRPC bone metastasis showed improvements in overall survival.\textsuperscript{57} The current and potential therapeutic targets for PC bone metastasis are discussed in Table 4.

Better understanding of the underlying molecular mechanisms contributing to and which drive metastasis of PC cells to secondary skeletal sites, particularly the spine, is critical in order to prevent or improve the devastating complications related to pathological fracture and metastatic epidural spinal cord compression. Targeting critical pathways involved in pathological bone turnover may inhibit these skeletal complications and thus improve pain, function, and quality of life in patients with advanced metastatic PC.

| INTERVENTION | MECHANISM OF ACTION | EVIDENCE |
|--------------|---------------------|----------|
| Bisphosphonates | Bisphosphonates inhibit osteoclast activity in PC bone metastasis. The suggested MOA is inhibition of tumour cell adhesion and invasion of the extracellular bone matrix and/or antiangiogenic effects.\textsuperscript{174} Bisphosphonates are pyrophosphates which have two phosphate groups, and bind with high affinity to calcium which is abundantly found in the bone. Once ingested by osteoclasts, bisphosphonates induce apoptosis in these cells and thus prevent further bone loss.\textsuperscript{70,168} ZA contains nitrogen in its structure, which is effective against a variety of cancers in both osteolytic and osteoblastic lesions.\textsuperscript{175} | In a Phase III trial, ZA significantly reduced the incidence of SRE by 36% and delayed the first SRE by more than 5 months compared with the placebo.\textsuperscript{174} |
| Denosumab (PROLIA, amgen inc. Xgeva, amgen inc.) | Denosumab is a monoclonal antibody that binds to RANKL, a protein involved in the formation, function, and survival of osteoclasts, causing inhibition of osteoclastic bone resorption. | Showed a superior effect compared to zoledronic acid in the prevention of SREs (e.g., fracture, spinal cord compression and radiation or surgery to bone) in a large Phase III study.\textsuperscript{176,177} |
| Radionuclides | Radium 223 is an alpha-emitter that releases a large helium nucleus, causing more biologic damage but over a much shorter path length and with the possibility of killing tumour cells and reducing tumour burden.\textsuperscript{178} Radionuclides residing in family IIA of the periodic table carry the same divalent charge as elemental calcium and are incorporated into bone matrix directly. Others are chelated to organic phosphates, which are incorporated into the matrix.\textsuperscript{175} Other approved radionuclides: Strontium-89 Samarium-153 Phosphorus-32 | A Phase II clinical trial of patients with symptomatic, hormone-refractory PC showed an improvement in survival, PSA levels, and alkaline phosphatase levels compared with placebo. A median overall survival of 14 vs. 11.2 months was reported. The time to a SRE was also significantly longer for patients (13.6 vs. 8.4 months; P = .00046). The time to disease progression based on PSA and alkaline phosphatase levels was significantly superior.\textsuperscript{178} |

(continued)
**Table 4. (Continued)**

| INTERVENTION                  | MECHANISM OF ACTION                                                                 | EVIDENCE                                                                                                                                 |
|-------------------------------|--------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| **SRC kinase inhibition**     | Srec is active or overexpressed during prostate tumor growth and metastasis. SRC signaling is required during osteoclast maturation and activation and plays a role in the formation and maintenance of PC bone metastases. I ntracellular tyrosine kinase inhibitors transduce signals from a range of upstream proteins, including receptors for EGF, PDGF, VEGF that promote several mechanism involved in metastasis, like cell proliferation and survival, cell adhesion, migration, invasion, and dissemination to distant organs. Datasatinib is one of the several inhibitors It has been shown to suppress markers of bone turnover—decrease proliferation of immature osteoblasts while enhancing their differentiation. |
| Dasatinib (SPRycEL, Bristol-Myers Squibb) | In chemotherapy-naive patients with metastatic CRPC, 20/41 had a 35% decrease in uNTX compared with baseline. In addition, 21/42 had a decrease in BAP. Furthermore, among patients who underwent bone scans, 11/22 were stable at 12 weeks and 3/9 were stable at 24 weeks. Prolonged PSA doubling time in 32/39 was observed. |
| Saracatinib (AZD0530)          | 107 patients had stabilized bone disease with metastatic CaRc. 56% of 140 patients in the trial demonstrated decreases in serum Psa of 50% or more. 61 out of 75% of patients and a partial radiological response in 23 of 39 (59%) of patients with metastatic CRPC who had disease progression after docetaxel-based chemotherapy. the median overall survival was 14.8 months in the ArmX plus prednisone group vs. 10.9 months in the control. |
| Bosutinib (Ski-606, Wyeth)    | 180 months in the ArmX plus docetaxel and estramustine chemotherapy resulted in a 50% PSA decline in 75% of patients and a partial radiologival response in 14.8 months in the ArmX plus prednisone group vs. 10.9 months in the control. |
| **Angiogenesis inhibitors**   | Bevacizumab is a humanized monoclonal antibody that neutralizes VEGF-A activity, leading to the suppression of cell proliferation, angiogenesis and invasions in the bone. Bevacizumab monotherapy did not show significant activity in CRPC, however the combination of bevacizumab plus docetaxel and estramustine chemotherapy resulted in a 50% PSA decline in 75% of patients and a partial radiological response in 23 of 39 (59%) of patients with metastable disease in a phase II study. Phase II studies investigating sunitinib monotherapy showed evidence of radiological responses in patients with mCRPC in the absence of PSA decline. Several phase II studies investigating sorafenib monotherapy in mCRPC have demonstrated modest activity with some discordance between PSA and radiological responses. |
| (Avastin, Roche/Genentech)     | 107 patients had stabilized bone disease with metastatic CaRc. 56% of 140 patients in the trial demonstrated decreases in serum Psa of 50% or more. 61 out of 75% of patients and a partial radiological response in 23 of 39 (59%) of patients with metastatic CRPC who had disease progression after docetaxel-based chemotherapy. the median overall survival was 14.8 months in the ArmX plus prednisone group vs. 10.9 months in the control. |
| Sunitinib (Sutent, Pfizer)     | 107 patients had stabilized bone disease with metastatic CaRc. 56% of 140 patients in the trial demonstrated decreases in serum Psa of 50% or more. 61 out of 75% of patients and a partial radiological response in 23 of 39 (59%) of patients with metastatic CRPC who had disease progression after docetaxel-based chemotherapy. the median overall survival was 14.8 months in the ArmX plus prednisone group vs. 10.9 months in the control. |
| Sorafenib (Nexavar, Bayer)     | 107 patients had stabilized bone disease with metastatic CaRc. 56% of 140 patients in the trial demonstrated decreases in serum Psa of 50% or more. 61 out of 75% of patients and a partial radiological response in 23 of 39 (59%) of patients with metastatic CRPC who had disease progression after docetaxel-based chemotherapy. the median overall survival was 14.8 months in the ArmX plus prednisone group vs. 10.9 months in the control. |
| Cabozantinib (XL184, Exelisis) | 107 patients had stabilized bone disease with metastatic CaRc. 56% of 140 patients in the trial demonstrated decreases in serum Psa of 50% or more. 61 out of 75% of patients and a partial radiological response in 23 of 39 (59%) of patients with metastatic CRPC who had disease progression after docetaxel-based chemotherapy. the median overall survival was 14.8 months in the ArmX plus prednisone group vs. 10.9 months in the control. |
| AVE0005, Sanofi-Aventis         | 107 patients had stabilized bone disease with metastatic CaRc. 56% of 140 patients in the trial demonstrated decreases in serum Psa of 50% or more. 61 out of 75% of patients and a partial radiological response in 23 of 39 (59%) of patients with metastatic CRPC who had disease progression after docetaxel-based chemotherapy. the median overall survival was 14.8 months in the ArmX plus prednisone group vs. 10.9 months in the control. |
| Tasquinimod (Active Bech)      | Afibercept, also known as VEGF-Trap, is a protein composed of the extracellular domains of VEGFR-1 and -2 fused with the constant region (Fc) of the human IgG1 antibody. Afibercept acts as a decoy receptor, preventing VEGF from binding to VEGFRs. Tasquinimod is an oral agent with anti-angiogenic and potential anti-neoplastic activities, and has been shown to decrease blood vessel density. Afibercept also known as VEGF-Trap, is a protein composed of the extracellular domains of VEGFR-1 and -2 fused with the constant region (Fc) of the human IgG1 antibody. Afibercept acts as a decoy receptor, preventing VEGF from binding to VEGFRs. Tasquinimod is an oral agent with anti-angiogenic and potential anti-neoplastic activities, and has been shown to decrease blood vessel density. |
| Abiraterone acetate (Zytiga Tablets, Janssen Biotech, Inc.) | In Phase III studies of 1195 patients, AA plus prednisone (398 patients), compared to placebo plus prednisone (398 patients), prolonged overall survival among patients with metastatic CRPC who had disease progression after docetaxel-based chemotherapy. the median overall survival was 14.8 months in the AA plus prednisone group vs. 10.9 months in the control. |
| FDA approved in 2012, in combination with prednisone for the treatment of patients with metastatic castration-resistant prostate cancer. | In Phase III studies of 1195 patients, AA plus prednisone (398 patients), compared to placebo plus prednisone (398 patients), prolonged overall survival among patients with metastatic CRPC who had disease progression after docetaxel-based chemotherapy. the median overall survival was 14.8 months in the AA plus prednisone group vs. 10.9 months in the control. |
| Androgen receptor antagonist   | In addition, MDV3100 is an oral androgen receptor antagonist It directly inhibits AR by binding the receptor irreversibly. This interaction impairs AR nuclear translocation, DNA binding, and recruitment of co-activators. Preclinical studies have demonstrated that MDV3100 binds to the AR receptor with substantially higher affinity compared to Bicalutamide (a clinical AR modulator), resulting in more complete suppression of the androgen receptor pathway. | In a Phase I/II study, MDV3100 showed anti-tumor activity in patients with metastatic CRPC. 56% of 140 patients in the trial demonstrated decreases in serum PSA of 50% or more. 61 out of 109 patients had stabilized bone disease after treatment. |
Author Contributions
Wrote the first draft of the manuscript: SD, DC, GQ. Contributed to writing of the manuscript: SD, DC, GQ. Agree with manuscript results and conclusions: SD, DC, GQ. Jointly developed the structure and arguments for the paper: SD, DC, GQ. Made critical revisions and approved final version: SD, DC, GQ. All authors reviewed and approved of the final manuscript.

DISCLOSURES AND ETHICS
As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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