Header for SpringerLink: Osteoporosis and Cancer (Section Editors, Mark Nanes and Matthew Drake)

The role of the microenvironment in prostate cancer-associated bone disease

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iThenticate: ok with references

Keywords

Bone microenvironment; prostate cancer; bone metastasis; therapeutic strategies

Word Count: 3,880

Abstract: 126

Abstract

The bone is a common site for metastasis in patients with advanced prostate carcinoma, and provides a 'fertile' milieu, which stimulates tumour growth and associated bone disease. For years the concept of treatment strategies has remained targeting the tumour itself; however the occurrence of chemoresistance remains a challenge now more than ever. The attraction of targeting the bone microenvironment in order to disrupt tumour localisation and proliferation stems from the idea that stromal cells are superiorly stable at a genetic level, thus decreasing the risk of resistance manifestation. In this review, we will discuss recent findings with regards to the pathogenesis of prostate cancer-induced bone disease and recent therapeutic strategies in an aim to evaluate the ever-increasing role of the microenvironment in disease progression.

Introduction

Prostate cancer is the most common form of cancer amongst men in the UK. Skeletal metastasis is a frequent complication of castration-resistant disease causing considerable morbidity. On average, a patient with metastatic disease will experience a skeletal-related event every 3 to 6 months. However, occurrences of this nature are not regular, with frequency of events increasing with cancer progression. As the disease becomes more extensive, treatment options are reduced and reliability of therapeutics decreased [1]. Cancer invasion and metastasis mark the transformation of a locally growing tumour into a systemic, metastatic, life-threatening disease [2].

Metastatic cancer cells produce factors that modulate normal bone remodelling, giving rise to both osteoblastic and osteolytic lesions. Symptoms of patients that have developed cancer-induced bone disease include: extreme bone pain, hypercalcemia, pathological fractures, and in some cases spinal cord and nerve compression [3]. Bone metastasis is a common complication amongst many progressive solid tumour types. Less than 20% of patients, however, will survive for more than five years after the discovery of cancer-induced bone disease [4].

Understanding the patterns of metastasis has historically been and still remains a challenge. In the early 20th century, two theories were formulated in a bid to explain the specific metastatic patterns of certain tumour types: the “mechanical” and the “seed-and-soil” hypotheses. The mechanical hypothesis predicts metastasis outcome by the spread of the primary tumour into the lymphatic system, subsequently resulting in its spread through the venous system, whereas the seed-and-soil hypothesis describes metastasis as a plant going to seed. The seeds can be carried in all directions, but will only survive if they fall on ‘congenial soil’ [5]. Since then, there have been many valuable contributions to the understanding of cancer pathogenesis, metastasis and the dependency of this process on the crosstalk between the tumour and the cancer-microenvironment. Gundem *et al* recently showed very clearly, using whole genome sequencing, the evolution of metastatic prostate cancer from initial tumorigenesis through to metastasis and castration resistance [6]. This work indicates that subclones within the primary tumour develop metastatic potential from the beginning of the disease, rather than the primary tumour developing this metastatic potential as a whole. The pattern of metastatic spread was also investigated, identifying that tumour cells frequently spread from one metastatic site to another. This study lends support to the “seed and soil” hypothesis in that rare subclones develop metastatic potential within the primary tumour and are able to give rise to disease progression as a result of

environmental changes. The now widely known concept of the 'vicious cycle' first proposed by Mundy *et al*, eloquently explains how cancer cells are able to manipulate their immediate environment to support their survival and growth [7]. This has been followed by numerous studies investigating the interactions between the tumour and bone marrow microenvironment and thus therapeutic strategies aimed to exploit those findings.

Homing to bone and the pre-metastatic niche

The initial stages of metastasis involve the detachment of malignant cells from the primary tumour and migration of these cells into nearby vasculature. In the normal prostate gland, cells have restricted migratory capacity. Cell-to-cell adhesion is maintained by a complex of cell adhesion molecules such as selectins and cadherins. Early in the process of migration, prostate cancer cells exhibit alterations in the expression of different molecules that lead to decreased cellular adhesion. The process of epithelial-to-mesenchymal transition (EMT) is now regarded by many to be critical in the development of more migratory and invasive tumour types [8]. However controversially in 2015 two independent studies challenged the traditional role of EMT in metastasis. Zheng *et al* showed that *in vivo* knockout of either twist1 or snail1, two key transcription factors responsible for EMT, did not alter either progression of pancreatic cancer, or the capacity of local invasion or metastasis [9]. Fischer et al. found that lung metastases were comprised primarily of tumour cells that maintained their epithelial phenotype and had not undergone EMT. Notably, both studies identified a potential role for EMT in chemoresistance [10]. Since prostate cancer bone metastasis is traditionally thought to be dependent upon EMT for the early stages of the metastatic cascade, it is intriguing to speculate that this role for EMT may not be as significant as first thought.

Once cells intravasate, the initial attraction of detached cells to distal sites is largely regulated by a series of integrins and chemokines produced by the bone marrow and stromal cells [11]. Among these, stromal-derived factor-1 (SDF-1), also known as C-X-C chemokine ligand 12 (CXCL12) is thought to play a major role. The receptor for CXCL12, C-X-C chemokine receptor 4 (CXCR4), is present on osteoclast precursors and regulates hematopoietic cells homing to bone [12]. Like hematopoietic stem cell (HSC) precursors, cancer cells also expresses CXCR4 and are thus attracted into the bone microenvironment [13]. At a time when the CXCL12/CXCR4 axis was beginning to be recognised as a modulator of migration and survival in many malignant cell types, Sun *et*

al showed provided data for prostate cancer, supporting the concept that CXCL12 and CXCR4 expression is associated with a progressive cancer type [14]. In addition to producing large amounts of CXCL12, osteoblasts also express anchorage molecules such as angiopoietin (Ang-1) and osteopontin (Opn) that also encourage tumour cells into the bone microenvironment. Opn is a glycophosphoprotein with the ability to stimulate HSC and osteoclast adherence to bone matrix. It has a key role in the trans-marrow migration, retention and negative regulation of HCSs within the osteoblastic niche. Furthermore it has been shown in both breast and prostate cancer that Opn is linked to regulation of metastatic spread and has been found to be highly expressed both within metastatic cells and surrounding stromal tissue[15].

Dormancy and the HSC niche

HSCs also have the ability to engage in a reversible state of cell cycle arrest, termed 'quiescence.' Quiescence allows HSCs to escape damage by cellular toxins and stresses, thus maintaining a viable stem cell reserve. In a similar way, disseminated tumour cells (DTCs) also share mechanisms of 'dormancy'. Dormancy is thought to allow tumour cells to evade cell death from chemotherapeutics. Traditional chemotherapy works by targeting rapidly dividing cells. By engaging reversible cell cycle arrest, however, cancer cells become resistant to these effects. Bone metastatic DTCs target the osteoblastic hematopoietic stem cell niche via the CXCL12/CXCR4 axis and compete for occupancy of the niche. Upon binding to the niche, tumour cells are thought to undergo growth arrest resulting in dormancy [16, 17]. Recent evidence also suggests that quiescent cells are more tumourigenic in a murine model of bone metastasis, when compared with rapidly dividing cells, providing further support for the importance of such quiescent, dormant tumour cells [18]. One molecule thought to be implicated in homing to the HSC niche is annexin II. Annexin II is expressed on the surface of osteoblasts and, in conjunction with its receptor, has the ability to regulate homing to bone in a similar fashion to the CXCL12/CXCR4 interaction [19]. Shiozawa *et al* showed that by blocking annexin II or its receptor in animal models of prostate cancer, short- and long-term localisation of cancer cells could be limited [20]. Thus current evidence suggests that prostate cancer is able to utilise HSC homing mechanisms in order to invade and localise within the bone marrow niche. This raises the possibility that approaches which mobilise stem cells from the HSC niche, such as the CXCR4 inhibitor AMD3100, may also mobilise tumour cells and so render them susceptible to chemotherapy [16, 17].

The tumour-bone microenvironment

The term 'bone microenvironment' broadly describes the complex biological interplay between cells of the haematopoietic and mesenchymal origin, the bone marrow stroma and the bone extracellular matrix. The bone matrix serves as a major source of growth factors, including tumour growth factor- β (TGF- β), insulin growth-like factors (IGFs), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and bone morphogenetic proteins (BMPs). Collective, these embedded growth factors make the bone matrix an attractive site for metastasis, enabling the growth of the metastatic tumour *in situ* and increasing the production and release of cytokines and other bone remodelling factors from the tumour itself [21].

Osteoblasts

Normal bone development is regulated by complex interactions between cells of the bone microenvironment including osteoblasts, which control bone formation and osteoclasts, which control bone resorption. Cancer cells use the same regulatory pathways that are involved in normal bone development and remodelling in order to 'hijack' bone turnover. The dysregulation of bone remodelling seen in cancer-induced bone metastasis is the result of the interactions between the tumour cells and the stromal cells of the bone marrow microenvironment.

Osteoblast activation and maturation in multiple myeloma, breast and prostate cancer has been shown to be stimulated by the wnt pathway [22]. Accumulating evidence suggests that wnt released by metastatic prostate cancer cells can stimulate osteoblasts and enhance tumour proliferation, while the inhibitor of wnt signalling, dickkopf-1 (DKK-1) can promote osteolysis, particularly during the early stages of cancer development. Expression of DKK-1 has been shown to be upregulated in early developing prostate cancer with a decline in DKK-1 levels occurring in advanced bone metastases. This suggests that the initial upregulation of DKK-1 is required for the establishment of the tumour, whereas the DKK-1 decrease during bone metastasis can promote wnt expression and thereby result in an increase in osteoblast activity to give rise to the osteoblastic metastases traditionally associated with prostate cancer [23, 24].

Other paracrine factors secreted by prostate cancer that regulate osteoblast proliferation and/or differentiation include: BMP, TGF- β, IGF, PDGF, vascular endothelial growth factor (VEGF) and endothelin-1 (ET-1) [25]. Numerous members of the TGF-β family have been found to stimulate bone formation. Serum TGF- β

concentrations in prostate cancer patients have been found to be elevated in bone metastatic compared with non-bone metastatic patients [26]. Another study showing the importance of one of these factors is the work by Autzen *et al.* who found that expression of BMP6 mRNA was upregulated in primary bone metastatic prostate cancer samples, suggesting that BMPs and specifically BMP-6 could potentially play a role in the mediation of skeletal metastasis [27].

VEGF has previously been shown to regulate bone formation by controlling vascularity within the developing growth plate and has been shown by Street *et al.* to differentiate primary osteoblasts *in vitro,* thus suggesting that VEGF may enhance bone formation and repair [28].

Osteoclasts

Osteoclasts function primarily as mediators of bone resorption and maintain bone homeostatic balance through continual remodelling of the microenvironment in response to various stimuli [29]. Under normal physiological conditions, osteoblastic cells regulate osteoclast activity by maintaining a fine balance between osteoprotegerin (OPG) and receptor activator of nuclear factor κ-B ligand (RANKL) expression. The binding of RANKL to receptor activator of nuclear factor κ-B (RANK) on osteoclast precursors initiates a signalling cascade resulting in activation and differentiation of osteoclasts. The interaction was shown to be an absolute requirement for signalling as early as 1999 by Dougall *et al*, [30] who demonstrated that both whole body RANKL -/- and RANK -/- mice developed abnormally dense bones due to the absence of osteoclasts. Dysregulation of RANKL through the secretion of parathyroid hormone-related protein (PTHrP) has become a typically described mechanism in osteolytic metastasis in breast cancer. RANKL binds to RANK on osteoclast precursors and stimulates the expression of genes such as integrin α v β 3, cathepsin K, matrix metallopeptidase 9 (MMP-9) and H+-ATPase necessary for osteoclast adhesion to the bone and bone degradation. The degradation of bone then promotes the proliferation of tumour cells through the release of growth factors. These growth factors then stimulate the proliferation of both osteoblasts and tumour cells to create a vicious cycle [22]. The dysregulation of the OPG / RANKL and/or RANK crosstalk has been shown to occur in a number of cancers, with the production of soluble RANKL in prostate cancer suggested as a mechanism by which osteoclastogenesis may be initiated [31].

Bone marrow derived adipocytes

Bone is a major regulator of energy metabolism. Adipocytes and osteoblasts share

common precursors known as mesenchymal stem cells (MSCs). Adipogenic differentiation plays an important role in regulating bone mass and homeostasis, as cells of the MSC lineage can be diverted towards the adipogenic or osteoblastic lineage depending on the presence of adipogenic (e.g., peroxisome proliferator-activated receptor-γ (PPARγ)) or osteogeneic (e.g., runt-related transcription factor 2 (Runx2), core-binding factor alpha 1 (Cbfa1)) factors that may be present in the bone microenvironment. A number of studies have suggested that the behavior of tumour cells can be affected by the presence of adipocytes and adipocyte-associated factors. An increase in adipocyte number can lead to an abundance of lipids, which are critical for signaling, cellular trafficking, and migration. Transformed cells have the ability to utilize and store lipids in order to gain a growth advantage compared to normal epithelial cells. Moreover, adipocytes and associated inflammatory cells secrete adipokines and cytokines, which are known to contribute towards tumour proliferation and survival [32]. A recent study has shown a functional relationship between bone marrow adipocytes and metastatic prostate cancer. Herroon *et al*, found that an increase in invasion was observed in prostate cancer cells exposed to adipocyte-conditioned media. This increased invasion was found to be mediated by fatty acid binding protein 4 (FABP4), suggesting that the presence of adipocyte-related factors can give rise to a more progressive phenotype [33]. Furthermore, marrow adipocytes have been found to secrete C-X-C chemokine ligand 1 (CXCL1) and C-X-C chemokine ligand 2 (CXCL2), chemokines postulated to activate osteoclasts, thereby providing a potential mechanism linking marrow adipocytes to the vicious cycle of cancer-induced bone disease [34].

Bone marrow mesenchymal stem cells and stromal cells

Several studies have shown that bone marrow-mesenchymal stem cells (MSCs) have the potential to promote the progression of various tumour types. Due to the multipotent nature of this lineage, MSCs can give rise to a variety of cell types including: osteoblasts, adipocytes, chondrocytes, and fibroblasts [35]. MSCs are known to be recruited to the primary tumour site to facilitate tumour progression and metastasis. Jung *et al.* provide evidence that the recruitment of MSCs to prostate cancer is dependent on the expression of C-X-C chemokine receptor 6 (CXCR6). CXCR6 signalling was shown to support recruitment, conversion and activation of MSCs into CXCL12-secreting cancer-associated fibroblasts (CAFs) [36]. CAFs are well known to play essential roles in primary tumour growth and metastasis, and their crucial role in tumour growth within the bone environment is beginning to emerge [37]. Li *et al.* showed that expression of TGF-β type II receptor was lost in prostate CAFs, suggesting that the loss of stromal

TGF-β responsiveness in the primary site promoted prostate cancer mixed bone metastasis, which was found to be mediated through an increase in the expression of cytokines such as CXCL1 and C-X-C chemokine ligand 16 (CXCL16) [38].

Therapeutic strategies for targeting the tumour-bone microenvironment

Androgen deprivation therapy combined with surgery remains the first line of treatment for localised prostate cancer. However with 90% of patients with castration-resistant disease developing bone metastasis, the effects of primary treatment become more sinister. Androgen deprivation therapy is known to cause bone loss and in 2014 Ottewell *et al* demonstrated, with the use of *in vivo* models, that mimicking androgen ablation increased growth of bone disseminated tumour through osteoclast-mediated mechanisms [39]. As targeting resistant tumour cells remains a considerable challenge, efforts to target the tumour microenvironment present a unique advantage. Stromal cells are far more genetically stable compared to tumour cells and are therefore less susceptible to the possibility of therapeutic resistance. Further, the diversity of tumour-stroma crosstalk that contributes towards cancer progression within the bone microenvironment provides a wide range of potential therapeutic targets [40].

Bisphosphonates

Bisphosphonates are chemically stable derivatives of inorganic pyrophosphate that inhibit calcification by binding to bone mineral, preventing its breakdown by osteoclasts. Second generation bisphosphonates now have an added effect of inhibiting mevalonate pathway enzymes, which also have direct effects on osteoblasts and tumour cells. These compounds can block apoptosis and promote differentiation of osteoblasts, and also promote apoptosis and inhibit invasion of tumour cells [41]. Long-term clinical trials in patients with metastatic hormone-refractory prostate cancer have shown that treatment with the most commonly used bisphosphonate in prostate cancer, zoledronic acid, reduced overall risk of skeletal complications by 36% [42, 43].

Bisphosphonates are generally well tolerated and have beneficial effects on the management of metastatic bone disease and the prevention of treatment-induced bone loss. However, despite obvious clinical benefits, it is clear that only a proportion of skeletal complications are prevented with bisphosphonates and increases in overall survival in patients with cancers metastatic to the skeleton has yet to be achieved [44].

Targeting the RANKL/RANK/OPG interaction

Signalling of the RANKL/RANK/OPG triad has been shown to have significant involvement in bone metastasis from both breast and prostate cancer. Blocking the RANKL/RANK interaction has shown a unique anti-tumour effect within the bone [45]. Denosumab is a humanised monoclonal antibody that binds to and neutralises RANKL, thereby inhibiting osteoclast function and subsequent bone resorption (Figure 1). Phase 3 studies in men with castration-resistant prostate cancer showed denosumab to significantly increase bone metastasis-free survival and delay onset of bone metastasis. However no increase in overall survival was seen [46]. Phase 3 studies comparing denosumab to zoledronic acid in patients suffering from breast, prostate cancer or multiple myeloma suggested that the effect of denosumab on skeletal-related-events was superior to those of zoledronic acid. Denosumab delayed the onset of skeletal-related-events by 8.21 months compared to zoledronic acid. Overall survival, however. was found to be similar between treatment groups [47].

Targeting VEGF signalling

VEGF has been found in many tumour types to exert a driving role in tumour angiogenesis, growth, invasion and metastasis. VEGF is expressed by osteoblasts and promotes chemotactic migration, proliferation and differentiation effects on osteoblasts, as well as stimulating the formation and survival of osteoclasts. The actions of VEGF are thought to contribute to tumour cell recognition of the bone and establishment of tumour cells within the skeleton. Increased VEGF expression has been associated with a more aggressive phenotype in castration-resistant prostate cancer. With therapies targeting the VEGF pathway showing promising early clinical application, these inhibitors are now being investigated in clinical trials [48].

Bevacizumab was the first agent to provide clinical evidence that the use of VEGF inhibitors to target the microenvironment may provide patient benefits. Bevacizumab was FDA approved in 2004 primarily for the treatment of metastatic colon cancer, but has since been used to target other metastatic cancers in combination with cytotoxic agents.

In phase 2 studies, the combination of bevacizumab and docetaxel in hormone-refractory prostate cancer patients showed promising results. These results, however, did not transpire to a phase 3 study where despite a small improvement in progression free survival, overall survival did not improve [49, 50].

Emerging evidence now suggesting suggests that VEGF inhibitors may increase delivery of chemotherapeutics by increasing blood flow to the tumour itself. In the majority of cancers, tumor-associated blood vessels are often abnormal in both structure and function. Abnormal tumour vessels can impede the function of immune cells as well as the transport of chemotherapy and oxygen. Vascular normalisation is a therapeutic strategy aimed at enhancing treatment delivery through the remodelling of tumour vessels in order to partially overcome physiological barriers that prevent effective chemotherapeutic activity. This approach, unfortunately, appears to be both time and dose dependent, with a narrow window of opportunity to increase blow flow that may well differ between cancer types [51]. Accordingly, the studies of Di Lorenzo and Kelly *et* al., in which combining bevacizumab was combined with docetaxel, demonstrate this reality, showing a small improvement but without achieving a significant effect on overall survival. Given the difficult of implementing this strategy, Wong *et al.* have recently proposed an alternative approach termed "vascular promotion therapy." Using co-administration of low-dose cilengitide, an angiogenesis inhibitor and verapamil, a calcium channel blocker, Wong *et al.* were able to show increased vessel dilation and blood flow in both mouse and human cancer models. This approach was associated with increased treatment delivery of gemcitabine, with a resultant reduction in tumour growth and metastasis, along with minimal side effects and an increase in overall survival. These data demonstrates the potential of VEGF inhibitors in combination with other agents to improve efficacy of chemotherapeutics efficacy [52].

Other VEGF inhibitors currently being evaluated include: small molecule receptor tyrosine kinase inhibitors, sunitinib and sorafenib and receptor-specific antibodies IMC-1121B and anti-VEGFR-2 (Figure 1). Sunitinib and sorafenib have already been approved by the FDA for the treatment of advanced renal cell carcinoma and are currently being investigated as therapies for other progressive cancer types including castration-resistant prostate cancer [53]. These data demonstrate the potential of VEGF inhibitors for targeting the tumour microenvironment, and suggest the possibility of future combination therapies to improve chemotherapeutic efficacy.

Endothelin-1 inhibitors

ET-1 has been implicated as having a central role in the mediation of osteosclerotic metastasis, as ET-1 stimulates bone formation and osteoblast proliferation [54]. One way ET-1 regulates osteoblast function is by inhibition of DKK-1 in marrow stromal cells, thus increasing wnt signalling (Figure 1). Furthermore, preclinical data has provided evidence that by blocking the ET-1 receptor, osteosclerotic lesion occurrence

can be prevented. Serum levels of ET-1 have been shown to be elevated in prostate cancer patients with bone metastases. Despite promising initial findings, however, subsequent phase 3 trials evaluating the potential of endothelin receptor antagonists, such as atrasentan, have failed to provide clinically significant benefits to patients with prostate cancer [55].

Bone targeted radiopharmaceuticals

Radium-223 dichloride (radium-223) is an alpha emitter that selectively binds to areas of increased bone turnover, a characteristic traditionally associated with the osteoblastic metastases of prostate cancer. By inducing double-strand DNA breaks, radium-223 treatment results in a potent and highly localized cytotoxic effect in the target areas (Figure 1). The short path of the alpha particles also means that off-target toxic effects in the surrounding tissue can be reduced. In both phase 1 and phase 2 studies, radium-223 has shown a favourable safety profile, with minimal myelotoxicity. Furthermore, phase 2 studies have also observed a reduction in bone pain and an improvement in disease-related biomarkers (e.g., serum levels of alkaline phosphatase and prostate-specific antigen (PSA)). In the pivotal phase 3 radium-223 in symptomatic prostate cancer patients (ALSYMPCA) study conducted by Parker *et al.* in 2013, radium-223 significantly prolonged overall survival in patients with castration-resistant prostate cancer and bone metastases, with a overall 30% reduction in the risk of death [56]. A number of trials are now beginning to look at combining radium 223 with other agents. A phase 1/2 study of the efficacy of radium-223 with docetaxel (NCT01106352) has recently been completed, and results are pending. In addition, a phase 2 trial investigating the combination of radium-223 with abiraterone or enzalutamide (NCT02034552), and a phase 3 trial combining radium-223 with abiraterone and prednisone (NCT02043678) are ongoing [57].

Conclusion

Therapeutic strategies for bone metastasis are driven by our evolving understanding of the molecular interactions between cancer cells and the bone microenvironment. Although recent clinical studies of targetable agents have in many ways been less then successful, many illustrate the potential for bone-microenvironment manipulations as a treatment option for cancer-induced bone disease. The potential for targeting the tumour-microenvironment in combination with chemotherapeutics remains a largely unexplored area. With recent findings demonstrating that the inhibition of many

processes associated with advanced disease significantly improves the efficacy of treatment, this is bound to become a rapidly expanding area of research. As such, further elucidation of the complex interplay between cancer cells and stroma within the metastatic niche will undoubtedly bring to light new avenues for therapeutic strategies.

Compliance with Ethics Guidelines

Conflict of Interest

Christina J. Turner and Claire M. Edwards declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Acknowledgements

This work was supported by Prostate Cancer UK and Cancer Research UK (CR-UK) grant number C38302/A12278, through the Oxford Cancer Research Centre Development Fund and through the University of Oxford Medical Research Fund.

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