1 Biology and Pathophysiology of Bone Metastasis in Prostate Cancer

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1.1 Distribution and Preferential Site of Bone Metastasis in Prostate Cancer Patients

Several studies have attempted to correlate the extent of skeletal metastatic involvement, the number of bone metastases (BMTs) identified by bone scintigraphy or the distribution of BMTs (axial vs appendicular) with survival in patients with advanced prostate cancer (PC) [1, 2]. The number of BMTs has recently been evaluated as a prognostic predictor [3]. Patients with metastatic castration-resistant PC with a higher number of BMTs had a shorter progression-free survival (PFS) and overall survival (OS; hazard ratio 2.0; 95 % confidence interval 1.7–2.4). Patients with 1–4 BMTs have much better PFS and OS than those with 5–20 BMTs [4]. It should, however, be taken into account that among the predictors of prognosis, coexisting non-osseous metastatic disease is an important determinant of prognosis in patients with BMTs [5, 6].

It is well known that a BMT most commonly affects the axial skeleton and that patients with BMT confined to the vertebrae have a better prognosis. Several studies have shown that the distribution and sites of predilection were similar in PC and breast cancer, with the ribs, spine and ilium reported to be those for BMT. However, recent data have shown that in early stages of breast cancer and PC the distribution in the thoracic skeleton is higher for the former than for the latter. In PC the distribution is 80 % in the spine and pelvis. In the advanced stages and in cases of extensive BMT, it seems that there are no differences in skeletal distribution between breast and PC, with a high frequency of BMT to the ribs and sternum in patients with PC as well [7, 8]. Interestingly, BMT is rarely observed in the mid-distal bones of the extremities, unlike that reported in a few other studies [9].

1.2 Pathology of Bone Metastasis from Prostate Cancer

Prostate cancer BMT is usually defined as "osteoblastic" by conventional radiographs. However, recent studies have shown a high heterogeneity of lesions, with synchronous osteolysis in BMT of PC, even when the overall character seems to be blastic [10]. Histomorphometric studies have shown that blastic lesions are mixed in nature, with increased activity of both osteoblasts and osteoclasts [11]. In bone biopsies of prostate BMT, an increase in the osteoid surface and osteoid volume and an elevation in the mineral

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apposition rate, demonstrating an accelerated state of bone formation, have been demonstrated. It was interesting to note that the new bone was formed in the marrow spaces and not adjacent to the bone surface; that is, the bone may form de novo in the marrow without the requirement of pre-existing bone resorption. Spindle-shaped cells or flat cells were seen lining woven osteoid and entrapped as osteocytes in the woven bone [12]. Surprisingly, well-differentiated osteoblasts, defined as cuboidal cells with basophilic cytoplasm lining the osteoid, were rarely observed on the woven bone, but they were observed in areas of bone repair secondary to bone necrosis. The osteoid is not fully mineralised and woven bone is formed, which has a low level of mineral density and a poorly organised lamellar bone. Furthermore, trabecular bone in metastatic lesions showed an increase in connectivity and surface irregularity, suggesting that strong effects of bone resorption and bone formation might occur in osteoblastic BMT [13]. In "osteoblastic" metastases osteoclasts were observed in the usual focal pattern on the surface of woven or lamellar bone or osteoid, and on the eroded surface area the number of osteoclasts was found to have greater than normal values [12]. Despite the osteoblastic nature of BMT, approximately half of 101 biopsies of BMT in bisphosphonate-naive PC patients were osteopaenic and half were osteodense, and this pattern was also reproduced in individual patients [12]. The undermineralised woven bone and the osteopaenic/osteolytic component of BMT may contribute to the histological frailty observed in the skeleton in PC patients, even in dense metastatic lesions (Fig. 1.1).

Bone metastases in castration-resistant PC patients were characterised according to expression levels of steroidogenic enzyme and androgen receptor splice variants. It was found that increased tumour expression of steroidogenic enzymes in individual patients is associated with advanced tumour stage. Interestingly, there are distinct subgroups of CRPC patients with BMTs expressing high levels of AKR1C3 (that convert circulating dehydroepiandrosterone and androstenedione (synthesised in the adrenals) into 5-androstenediol and testosterone) or expressing high levels of ligand binding domain (LBD)-truncated, constitutively active androgen receptor splice variants (AR-Vs). The possible clinical relevance of this is that patients with high AKR1C3 expression and low AR-V expression may show a good response to treatment with abiraterone acetate (Cyp17 inhibition) and/or would benefit from drugs targeting AKR1C3, whereas patients with a high expression of constitutively active AR-Vs will probably not respond to abiraterone acetate or to any therapy targeting androgen synthesis or the LBD of the AR [14].

1.3 Pathophysiology of Bone Metastasis

1.3.1 Pathophysiological Heterogeneity

The osteoblastic lesion is a very complex multistep process that is not fully understood in detail. It is the result of releasing osteoblast-promoting factors such as bone morphogenetic protein (BMP), Wnt family ligand, endothelin-1 and PDGF from PC cells and of a closed interaction with bone matrix, stroma cells and bone cells. Another characteristic of BMT from PC is the biological and pathophysiological heterogeneity. The high level of heterogeneity of the BMTs in PC from the pathological point of view reflects the great complexity of the biology and molecular regulation that underlie their pathophysiology. Lytic and blastic metastases share many molecular mechanisms that give an account of similar therapeutic outcome treating them with bone-modifying agents such as zoledronic acid and denosumab. The complexity of the bone response in PC invasion is underscored by the variety of soluble factors, signalling pathways and transcriptional regulators involved. The abnormal bone response is further promoted by the potential for osteomimicry of the tumour cells signalling in a paracrine fashion within the bone environment and an autocrine signalling cascade of the bone cells themselves. These interactions between the PC cells and bone cells often yield a predominantly osteoblastic response. However,

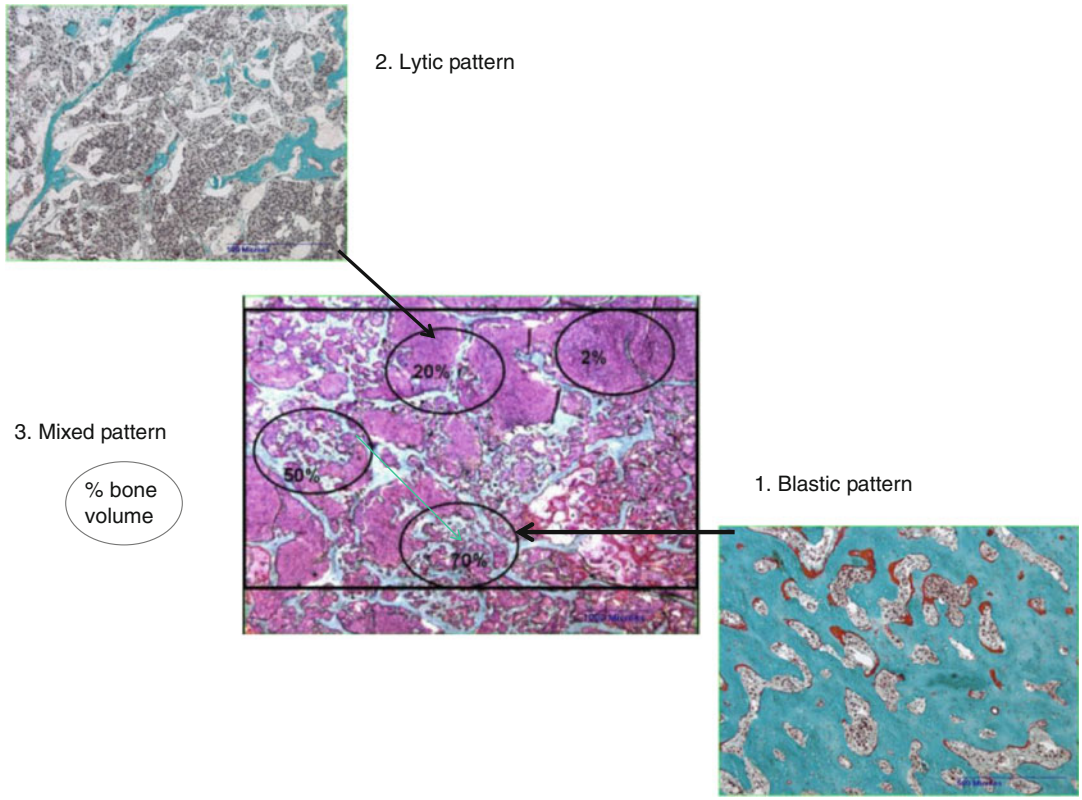

Fig. 1.1 Histopathology of bone metastasis (BMT) from prostate cancer (PC). In the same patient, BMTs are heterogeneous, with predominantly blastic (*1*) and predominantly lytic metastases (*2*). Furthermore, as shown in the histopathological sections in the same specimen of a single metastasis, there is an alternation of predominantly lytic and blastic area (mixed pattern) (*3*): 2–20 % Bone volume: predominantly lytic area; 50–70 % bone volume: predominantly blastic area (Modified from Roudier et al. [12]). *Green* bone, *red* osteoid, *grey/pink* tumour stroma

the formation of osteoblastic bone is also often associated with a significant osteolytic component, leading to a mixed, woven bone response in the same patient at different metastatic sites.

Bone remodelling proteins and transcripts in human specimens of PC BMTs were analysed in detail [15]. The main bone remodelling proteins that were recognised were assessed in lytic and blastic BMTs: BMP-2, BMP-7, dickkopf-related protein 1 (DKK-1), receptor tyrosine-protein kinase erbB-3 (ErbB3), endothelin-1 (ET-1), NEL-like protein 1 (NELL-1), tumour necrosis factor receptor superfamily 11B (OPG), phosphoglycerate kinase 1 (PGK1), sclerostin, substance P, a putative osteoblastic factor EMI domain-containing protein 1 (Emu1) and two putative osteolytic factors, matrix metalloproteinase-12 (MMP-12) and secreted frizzled-related protein 1 (SFRP1). Interestingly, many of these proteins and transcripts were equally expressed in lytic and blastic BMTs, such as BMP-2, BMP-7, DKK-1 and sclerostin. Instead, expression of some of these, such as OPG, Emu1, PGK1 and substance P, was higher in prevalent blastic lesions than in lytic lesions, but not the transcripts. OPG, PGK1 and substance P have been proven to inhibit osteoclastogenesis and induce osteoblastic differentiation. Emu1 has been shown to be prevalent in the epithelium during embryonic development and it has been hypothesised that Emu1 in PC aids adhesion The single proteins are probably not the unique drivers for conditioning the evolution towards a blastic phenotype of the metastasis, and a possible explanation for the characteristic "predominantly osteoblastic phenotype" is that PC expresses a

disproportionate number of pro-osteoblastic and pro-osteolytic factors and the relative prevalence of the former will determine the pathological aspect of the lesion [15, 16].

1.3.2 The Role of Osteoclasts in Blastic and Mixed Bone Metastases

Independently from the phenotype of the lesion, osteoclasts, mainly in the first phases of BMT development, are principally responsible for the initiation, development and clinical consequences such as pain, fracture and hypercalcemia of the evident bone lesion (Fig. 1.2).

Osteoclasts have two pivotal functions in the development of bone lesions: they reabsorb the bone, creating the necessary space for the penetration and development of metastasis into the bone, and they enrich, as a direct consequence of the bone matrix breakdown, the bone micro-environment of a plethora of growth factors and tumour-seeking factors that sustain the proliferation of the cancer cells, which is essential during the first phases of metastasis. These mechanisms are the basis of the "seed and soil" concept, where the bone micro-environment factors represent the fertile ground (the soil) and the "seed" represents cancer cell growth.

Physiologically, bone resorption and bone formation in skeletal remodelling are almost always tightly coupled. The bone resorption by osteoclasts is regulated by the RANK/RANKL/OPG axis, where osteoblasts expressing RANKL induce recruitment, differentiation and activation of osteoclasts, binding and activating of RANK, and conversely expressing OPG, the RANKL decoy receptor, and osteoblasts inhibit the excess osteoclastogenesis. The ratio RANKL/OPG in bone micro-environment drives the equilibrium between bone formation and resorption.

Expression of RANKL by stromal cells/osteoblasts and osteocytes is regulated by cytokines and paracrine hormones that stimulate bone resorption [17] such as interleukin-1 (IL-1), IL-6, IL-11, IL-17, prostaglandin E2 (PGE2), parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHrP), which stimulate osteoblasts or their progenitors to express RANKL and/or to downregulate the expression of OPG [18]. Recently, the role of osteocytes through the Wnt/DKK-1 and sclerostin pathway has been elucidated (Fig. 1.2) [19].

The tumour cells co-opting the normal process that regulates bone resorption interfere with the balance of the RANKL/RANK/OPG axis. The tumour/bone interface is replete with factors that stimulate bone resorption directly produced by tumour cells themselves, by macrophages and T cells associated with metastasis or by stromal cells influenced by metastasising cells. PTHrP, IL-8 and PGE2 have been shown to increase expression of RANKL and downregulate OPG expression either in vitro in the osteoblast/tumour cell coculture or in vivo using the BMT model [17–20].

Parathyroid hormone-related peptide is not physiologically present in the circulation, but it has been found to be widely distributed in most fetal and adult tissues [21], suggesting that it might act in an autocrine/paracrine manner. This peptide plays an important role in regulating many tissues including cancer tissue [22]. PTHrP is expressed by many types of cancer cells, such as breast cancer and PC, and has been proposed as an antigen for cancer immunotherapy [23–26]. PTHrP, as PTH in physiology, stimulates osteoblasts expressing PTHR1 receptor to express RANKL, which activates osteoclasts [27]. Interestingly, it has been found that T cells also express PTHR1 and are activated by PTH and PTHrP [28, 29], contributing to osteoclast activation via RANKL. It has been demonstrated that in mice bone resorption may be prevented by the immunosuppressor abatacept, a CTLA4-Ig preventing T-cell activation [30].

In addition to PTHrP, IL-8 plays an important role in the activation of osteoclasts. IL-8 is the human homologue to murine MIP-2 belonging to the family of chemokine CXC and is constitutively produced by osteoblasts [31]. IL-8 is overexpressed in the breast cancer cell line [32], and it is believed that it acts before PTHrP in the early stages of

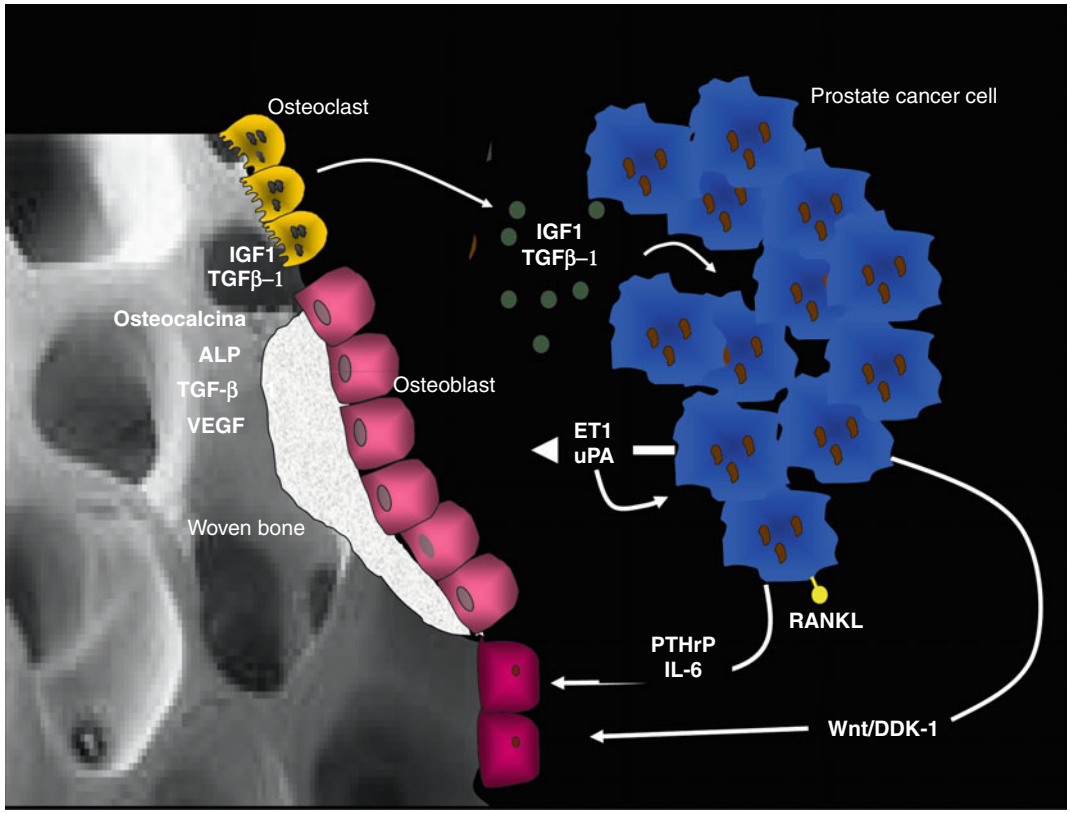

Fig. 1.2 Physiopathology of blastic bone metastasis (BMT). Osteoclasts (*yellow cells*) reabsorbing bone facilitate the expansion of PC metastasis and make available in the bone microenvironment factors promoting penetration and growth of metastasis (TGF beta, osteopontin, FGF, PDGF, VEGF, IGF-1 and IGF-1 are described in detail in the text). In turn, PC cells express cytokines (RANKL, DKK-1 and hormone such as PTHrP) that maintain osteoclast activity and cytokine and factors such as uPA and ET-1, inducing osteoblast bone formation. In the micro-environment of a BMT site, the high bone turnover is characterised by the alternation of osteoclast (lytic) areas and osteoblastic (woven bone) areas, resulting in a disorganised and frail bone structure

breast cancer metastasis stimulating osteoclasts via RANKL [32, 33] and then initiating the vicious cycle that maintains osteolysis in cancer metastasis. It has been suggested that IL-8 might also directly stimulate osteoclasts [33], increase angiogenesis and suppress osteoblast activity [34, 35].

Cancer cells in BMT produce many factors that activate T cells, as discussed above. T cells of patients with breast, prostate and lung cancer support osteoclastogenesis by secreting TNF alpha and expressing RANKL. In addition, T cells suppress the osteoprotegerin action secreting TRAIL (TNF-related apoptosis-inducing ligand), therefore inhibiting the anti-osteoclastogenic effect of osteoprotegerin [36]. In turn, cancer cells produce many factors such as PTHrP, IL-7 and IL-,8 which could recruit or activate T cells with the consequence of further stimulating osteoclastic bone resorption. These mechanisms contribute to the imbalance towards the osteolytic phenotype of the bone lesion.

Studies using RANKL inhibitors have shown the almost complete dependence of tumour-mediated osteoclastogenesis on RANKL. Treatment of mice with OPG-Fc prevented the progression of osteolysis induced by the breast cancer cell line MDA-MB-231 [37]. RANKL inhibition has been shown to prevent the implantation and development of osteolytic lesions in the PC3 cell line in animals [38, 39].

The efficacy of RANKL inhibition was also demonstrated in mixed BMTs in animals, where OPG-Fc blocked the establishment and progression of bone lesions [40, 41]. Recent data indicate that cathepsin G activity at the tumour–bone interface plays an important role in tumour-induced osteolysis and suggest that cathepsin G might be a potentially novel therapeutic target in the treatment of BMT. In a mouse model that mimics osteolytic changes associated with breast cancer-induced BMTs, it has recently been demonstrated that cathepsin G, cooperating with MMP9 and MMP13, is able to cut the extracellular domain of RANKL, generating active soluble RANKL, which is critical for widespread differentiation and activation of osteoclast precursors [42].

Furthermore, some RANKL-independent ways for osteoclast activation in BMT have been found. Some cancer cells, such as PC and breast cancer, may express RANKL and directly activate osteoclasts [43, 44]. Breast cancer cells, myeloma cells and other cancer cells could directly activate osteoclasts in the early stages of BMT via IL-8 production and via MIP-1, a member of the CXC chemokine family that is naturally secreted by osteoblasts and is primarily associated with cell adhesion and migration. It is chemotactic for monocytes and monocyte-like cells, including osteoclast precursors. It directly stimulates osteoclast formation and differentiation in a dose-dependent manner, through the receptors CCR1 and CCR5 expressed by osteoclasts. Moreover, neutralising antibody against MIP-1 blocks MIP-1-induced osteoclast activation [45, 46].

1.3.3 The Role of Osteoblasts in Blastic and Mixed Bone Metastases

In blastic metastases the number and activity of osteoblasts are amplified. Osteoblast differentiates from bone marrow mesenchymal stem cells. A variety of factors contribute to osteoblast formation, including insulin-like growth factor, endothelin-1, BMPs and sclerostin and Wnt proteins (Fig. 1.2) [47, 48].

1.3.4 Endothelin-1

Production of endothelin-1 (ET-1) from PC cells has proven to induce a blastic metastasis promoting osteoblast differentiation and activity. ET-1 is a small vasoconstrictive peptide that plays a key role in vascular homeostasis. ET-1 promotes osteoblast function by binding to ET receptor subtype A (ET_A). The activation of receptor ET_A stimulates phosphate transport and is important for the initiation of bone matrix calcification. ET-1 also increases osteoblast proliferation and inhibits osteoclast formation and motility, and recently it has been suggested that these actions might be indirect and mediated through the Wnt/DKK-1 pathway, inhibiting DKK-1 [49–51]. ET-1 can also enhance the mitogenic effect of other growth factors, such as insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) [52]. Furthermore, ET-1 has been found to be elevated in androgen-resistant advanced PC. However, there are some doubts with regard to the pivotal role of ET-1 in osteoblastic lesions from PC, because a clinical trial with atrasentan, a selective ET receptor antagonist, produced a modest effect on metastatic PC [53].

1.3.5 Bone Morphogenetic Proteins

The expression of several BMPs has been detected in BMTs from PC. BMPs seem to have a crucial role in contributing to osteoblastic phenotype of BMT in PC. BMPs are members of TGF-beta family and are known to be involved in cancer cell migration. In PC tissues, the expression of BMP-7 was higher in metastatic bone than in normal tissue.

The BMPs are not only expressed by osteoblasts and stored in the bone matrix, but are also actively expressed from PC cells. The osteoblastic effects of BMPs are confirmed by the expression of noggin (an antagonist of BMPs) in PC cell lines. A recent study suggests that BMP-4 signalling inducing apoptosis and Smad-mediated gene expression can be repressed by IGF-1 by activating mTOR signalling in prostate epithelial cells

(NRP-152), suggesting a crosstalk between BMP and IGF signalling. It has been recently demonstrated that BMP-7 secreted from bone stromal cells induces reversible senescence in prostate cancer stem-like cells (CSCs) by activating p38 mitogen-activated protein kinase and increasing expression of the cell cycle inhibitor, p21, and the metastasis suppressor gene, NDRG1 (N-myc downstream-regulated gene 1). This effect of BMP-7 depended on BMPR2 (BMP receptor 2), and BMPR2 expression correlated inversely with recurrence and BMT in PC patients. Importantly, this effect was reversible upon withdrawal of BMP-7 [54]. Recently, it has been shown that using CaP/bone stromal cell line coculture models, one possible mechanism underlying the castration resistance induced by BMTs involves BMP-6 induction by bone stroma-derived WNT5A. BMP-6, in turn, permits CaP cells to proliferate in the absence of androgens [55].

1.3.6 Wnt/DKK-1 Pathway

Canonical Wnt proteins bind at the cell surface at a co-receptor consisting of frizzled (FZD) and low-density lipoprotein receptor-related protein 5/6 (LRP5/LRP6). The activation of the canonical pathway signal results in the stabilisation and accumulation of beta-catenin, which upon translocation into the nucleus serves as co-factor for the T-cell factor family of transcription factors [56]. Canonical Wnt signalling directly controls multiple steps of osteoblast development, regulating the fate of mesenchymal precursors by determining the commitment to a chondroblastic or osteoblastic lineage [48, 57]. Furthermore, the Wnt, indirectly dependent on the activation of beta-catenin, suppresses osteoclast recruitment and activity via osteoprotegerin (OPG). In fact, OPG is a Wnt-responsive target gene and was found to be reduced in beta-catenin knock-out osteoblasts and upregulated in cells with hyperactive Wnt signalling [48, 57]. Interestingly, a reciprocal regulation of RANKL by Wnt was observed in osteoblasts where enhanced Wnt signalling led to increased RANKL expression and vice versa [58].

The canonical Wnt pathway is regulated by a large number of antagonists, including the DKK family and secreted frizzled-related proteins (SFRPs). DKK-1 is present in mature osteoblast/osteocytes, suggesting that the Wnt/DKK-1 balance might regulate bone homeostasis [59]. DDK-1 binds the Wnt co-receptors LRP5 and LRP6 and blocks canonical Wnt signalling [60]. In the presence of DKK-1, osteoblast differentiation is impaired and Wnt-mediated suppression of osteoclast differentiation via osteoprotegerin is blocked, resulting in a dysregulation of RANKL/osteoprotegerin expression with increased osteoclast activity [61].

Direct evidence that canonical Wnt signalling participates with Wnt antagonists in adult bone biology modulating bone remodelling is also of great interest in understanding bone metastasis development and the phenotype of the single metastasis The Wnt signal has recently been found to be expressed in PC and in multiple myeloma [62, 63]. Interestingly, in early stage of PC BMT, it has been supposed that an "osteolytic phase" driven by an overexpression of DKK-1 favours tumour establishment within the bone [47] and a molecular switch with suppression of DKK-1 signal mediates the transition to an osteoblastic phase of BMT [47]. Overexpression of DKK-1 in prostate C4-2B cells changes a mixed osteolytic–osteoblastic phenotype to an osteolytic phenotype. The equilibrium between Wnt and DKK-1 expression could dictate the phenotype of BMTs and may speculatively explain the heterogeneity of histological aspects of BMTs found in individual patients or the shift from osteoblastic to osteolytic aspects in the single metastasis. Other studies suggest that non-canonical Wnt signalling also stimulates osteoblast differentiation, through BMP-dependent and BMP-independent signalling pathways [64].

1.3.7 VEGF

Vascular endothelial growth factor, as in breast cancer BMT, has been shown to be upregulated in PC and is associated with clinical stage, Gleason score, progression and survival [65, 66]. It has

been recently demonstrated that osteocytes are also critical mediators in the bone metastatic niche, not only through soluble factors and cell contact but also via tumour-generated pressure [67].

1.3.8 Role of Mineralised Bone Matrix Resorption in the Vicious Cycle of Lytic Metastasis

The mineralised bone tissue contributes actively to the development and overgrowth of the metastases themselves. Bone breakdown by osteoclasts releases a variety of growth factors previously stored in proactive form by osteoblasts during the bone formation phase and physiologically destined for bone remodelling modulation and bone response to bone inflammation or trauma healing [68]. It is well known that the bone matrix represents a mine of growth factors (such as procytokines); chemotactic and adhesive factors for bone cells and cancer cells, such as TGFβ, PDGF, BMPs, FGFs, IGF-1 and IGF-2; and bone matrix proteins such as osteopontin, osteocalcin, osteonectin and bone sialoprotein [69]. Interestingly, many of these factors may also be expressed actively in breast cancer and PC.

The concentration of these molecules in the micro-environment of the bone remodelling site is a critical regulator of cellular proliferation, differentiation, extracellular matrix deposition and mineralisation, is responsible for the coupling between bone resorption and bone formation and serves as survival and growth factors for cancer cells. Furthermore, physical factor such as tumour-generated pressure acting on osteocytes and factors generated during osteoclast activity, such as low oxygen content, acid pH and high extracellular calcium concentration, are combined to sustain the favourable vicious cycle of tumour growth [67, 70].

1.3.9 TGFβ

Of the growth factors stored in the bone matrix, TGFβ is not the most abundant, but has been well-studied, particularly in cancer bone disease. TGFβ binds to a heterodimeric receptor and can activate either the canonical Smad signalling pathway or Smad-independent pathways [71]. TGFβ, of all the factors delivered from bone matrix, is the major stimulator of cancer cells to express PTHrP, which is expressed in many osteolytic cancer cell lines, and its expression is higher in BMTs than in non-skeletal metastases. As a consequence of the increased PTHrP expression via TGFβ, more osteoclasts reabsorb more of the bone matrix, expanding the lytic bone lesion and increasing locally the concentration of TGFβ and other growth factors. TGFβ, as discussed above, stimulates COX-2 expression in osteoblasts, in bone marrow cells and in breast cancer cells. COX-2 expression in breast cancer cells correlates with the secretion of IL-8 and IL-11, which may induce osteoclastogenesis either via RANKL or independently of RANKL respectively. TGFβ is also reported to act on the tumour cells to induce the production of VEGF and connective tissue growth factors (CTGF) [72]. Runx2 gene expression, regulating the expression of osteopontin and metalloproteases MMP-9 and MMP-13, which are involved in bone resorption and osteoclast recruitment, may be modulated by TGFβ both in cancer cells and in osteoblasts.

1.3.10 IGF-1

The insulin-like growth factors 1 and 2 are among the most abundant non-collagen proteins in mineralised bone. Both IGFs act in cancer and in metastases promoting angiogenesis and inducing cell proliferation and cancer invasion. IGF-1 released from bone by osteoclast bone resorption binds to the type I IGF receptor (IGF-IR) on cancer cell membrane and induces the transcription factor NF-kB, which in turn stimulates target gene transcription, stimulating cancer cell proliferation and chemotaxis and inhibiting apoptosis, leading to BMTs. IGFs promote osteoblasts to increase bone matrix apposition and decrease collagen degradation [73]. IGF-1 is upregulated in PC metastases to the bone and contributes to cancer cell proliferation and chemotaxis. In clinical studies, levels of IGF also correlate with

cancer progression, as high levels of IGF-1 are associated with a Gleason score 7. The protein level of IGFs and IGF-binding proteins (IGFBPs), which serve as carrier proteins for IGFs, could be mediated by proteolysis of IGFBPs. Indeed, hydrolysing IGFBPs by urokinase-type plasminogen activator (uPA) increases IGF levels and stimulates osteoblast proliferation. The cleavage of IGFBP-3 by PSA also increases IGF-1 expression, rendering IGF-1 available to bind to its receptor and stimulate osteoblast proliferation [74].

Over expression of uPA has been shown in PC cells, and uPA seems to increase metastasis to the bone. uPA is associated with an aggressive disease phenotype, progression and metastasis to the bone and can be used as a factor in the prognosis and progression of PC [75]. The cleavage of IGFBP-3 by PSA also increases IGF-1 expression, rendering IGF-1 available to bind to its receptor and stimulate osteoblast proliferation. In PC biopsies of BMTs, IGF-IR is increased. Neutralising antibodies against human IGF-1 or mouse or human IGF-2 decreases the development of bone lesions in a prostate xenograft model. Currently, taking all data together, the complex role of IGFs in BMTs phatophysiology has not yet fully elucidated [76].

Finally, it is relevant that many bone matrix-derived factors, including TGFβ, PDGF and BMPs, have the ability to induce the epithelial–mesenchymal transition of cancer cells, which greatly enhances their malignant phenotype, and therefore implicates them in the activation of dormant tumour cells [77].

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