Chapter 16
Therapeutic Targeting of the Bone Pre-metastatic Niche

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Abstract Most of relevant strategies designed for therapeutic targeting the highly lethal, bone-metastasizing and AR-resistant prostate cancers, have been reported and discussed elsewhere in this book. In this chapter, we aim to provide an overview of the rationale underlying the proposal of most of promising new therapeutic alternatives, most of which are still the early phase of evaluation.

Once more, we strongly wish to outline that the deeper understanding of the intricate crosstalks between the manifold molecular pathways responsible for the gain of invasive and metastasizing abilities of tumor cells, is giving rise to a previously unthinkable picture of the complex prostate cancer biology. A new, fascinating therapeutic era is opening up for the treatment of advanced prostate cancers.

16.1 Mechanisms of Bone Metastasis Onset

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Bone metastases are the most frequent event in patients with advanced prostate cancer. They confer a high level of morbidity to patients, with a 5-year survival rate of 25 % and median survival of approximately 40 months. The molecular basis for the development of resistance to treatment is linked to some critical changes in the bone microenvironment that can confer on an advantage on cancer cell

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survival and proliferation (Bussard et al. 2008). These microenvironment-linked resistance mechanisms have just led us to consider new strategies to therapeutic bone- targeting.

The concept of "seed and soil", proposed by Stephen Paget in 1889, hypothesized the existence of an interplay between the metastatic properties of cancer cells (seed) and the favourable properties of the stromal/bone microenvironment (soil), conditioning the selective homing and growth of cancer cells. The "soil" must provide favourable conditions for cancers cells to successfully survive, clonally expand, and establish a nourishing vasculature (Bussard et al. 2008).

Further, the extension of this concept by David Lynch and colleagues, led to the formulation of the metastatic niche model. According to this hypothesis, the formation of a favourable microenvironment, (so-called "premetastatic niche"), before the tumor cells reach the metastatic destination, is critical for their engraftment. These niches facilitate then the formation of micrometastasis, and their subsequent transition into macrometastasis (Bussard et al. 2008).

Bone is an extremely metabolically active tissue. In order to maintain skeletal integrity, it undergoes continuous dynamic remodelling by sequential phases of bone resorption, mediated by osteoclasts, followed by osteoblast-mediated bone formation. The functions of osteoclasts and osteoblasts are tightly regulated in physiological conditions, to secure a perfect final balance between bone formation and degradation. To maintain skeletal homeostasis, multi-directional cross-talks among osteoblasts, osteoclasts, and hematopoietic cells are the rule. They take place through the mediation of systemic hormones and local bone-derived growth factors, including parathyroid hormone (PTH), 1,25-dihy-droxyvitaminD3, thyroxine, prostaglandins, bone morphogenic proteins (BMPs), TGFβ, IGF, and IL-1 and IL-6, in response to mechanical stresses and hormonal changes (Thudi et al. 2011; Kaplan et al. 2006).

Osteoblasts produce several local and systemic factors that are important for bone regulation, including receptors for PTH, prostaglandins, estrogen, vitamin D3, and PDGF, FGF and TG. Importantly, osteoblasts are intimately involved also in osteoclast differentiation, which involves the RANK/RANKL pathway.

RANKL production is influenced by osteotropic factors such as parathyroid hormone, 1,25-dihydroxyvitamin D, and prostaglandins. Crosstalks with the Wnt pathway allow a higher-layer control of osteoblast functions and bone formation. Current data indicate that the activation of Wnt/βbeta-catenin signaling is a major responsible for the increased bone mass. In particular, the overexpression of Wnt10b over-expression in animal models increases bone mass, while the over-expression of Wnt7B and beta-catenin in osteoblastic precursor cells induces their differentiation into mature osteoblasts (Thudi et al. 2011).

Vascular endothelial growth factor (VEGF) and its receptors (VEGFR-1, VEGFR-2 and VEGFR-3) provide for new vessel formation and their maintenance. VEGF also plays a crucial role within bone and bone marrow, with autocrine and paracrine mechanism. In the early metastatic bone modifications, hematopoietic progenitor cells (HPC) seem to have a main role. HPC and osteoprogenitor cells both express VEGFR-1, while endothelial progenitor cells (EPC) express

VEGFR-2. Pre-metastatic niches are particularly rich in tumor-derived VEGFA, placental growth factor (PlGF), and TGFβ, in response to which tumor-associated immune cells, such as HPC and macrophages cluster, prepare the "soil" for the imminent arrival adhesion and invasion of the tumor cell in the future metastatic sites (Schroten et al. 2012).

Accumulation of clusters of myeloid cells, fibronectin, growth factors and matrix remodelling proteins accelerate the micrometastatic process. The bone microenvironment is per se an important source of growth factors like TGFβ, IGF- 1, FGF, PDGF, BMPs, cytokines, chemokines, calcium ions, and cell adhesion molecules that contribute to make it a fertile soil conducive for the growth and proliferation of metastatic cancer cells. Moreover, the marrow stromal cells act also be in concert with the tumor cells in the homing, differentiation and proliferation processes, via the production of vascular cellular adhesion molecule-1 (VCAM-1), cadherin (**11**) and fibronectin. Thus, bones become a highly favourable microenvironments for prostate cancer cells, promoting their cell growth and proliferation (Schroten et al. 2012; Thudi et al. 2011).

Physical factors such as hypoxia, acidic pH, and high extracellular calcium concentrations also contribute to create this permissive environment for tumor growth. In order to continue this symbiotic relationship, cytokines and growth factors produced by cancer cells directly or indirectly impact osteoclastic bone resorption. This bidirectional interaction between the cancer cells and bone microenvironments results in the creation of a "vicious loop" that increases bone destruction to ultimately facilitate the establishment of cancer metastases in the bone.

The humanized RANKL monoclonal Denosumab, approved by FDA on November 2010 for the treatment of solid tumors with metastatic bone disease (MBD), or the tyrosine kinase SRC/BCR-ABL inhibitor dasatinib, already introduced in clinical trials, have been discussed elsewhere in this book.

16.2 Molecular Therapies for Metastasizing Disease

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An impressive number of other new possible drugs and/or targets for alternative molecular therapies for metastasizing disease is actively in progress (Thudi et al. 2011).

Recently, the results of the ZEUS study indicated that we should reserve the use of potent osteoclast inhibition—with either zoledronic acid or denosumab—for men with bone metastatic prostate cancer (Smith et al. 2012).

Rather than preventing bone metastasis, zoledronic acid and denosumab have both been shown to significantly reduce the incidence of skeletal events, such as pathologic fractures and spinal cord compression, while the metastasis-free survival resulted only modestly prolonged by about 4 months in these patients.

A great deal of research has recently concerned the role of hypoxia-related factors HIF-1α and HIF-2α in prostate cancer progression (Mimeault and Batra 2013). Experimental evidences have revealed that they are key regulators of the adaptation of prostate bone-metastasis-initiating cells and corresponding differentiated progenies to oxygen and nutrient deprivation. HIFs are strongly induced by the niche-overexpressed (EGFR), insulin-like growth factor-1 receptor (IGF-1R), stem cell factor (SCF) receptor KIT, transforming growth factor-β receptors (TGF-βRs) and Notch, as well as by their downstream signalling elements such as phosphatidylinositol 3′-kinase (PI3K)/Akt/molecular target of rapamycin (mTOR).

Activated HIFs, in turn, induce and sustain the expression of induced pluripotency-associated transcription factors (Oct-3/4, Nanog and Sox-2), glycolysis- and epithelial-mesenchymal transition (EMT) programme-associated molecules, including CXC chemokine receptor 4 (CXCR4), snail and twist, microRNAs and, once more, VEGF.

This extraordinary melting of gene products create a powerful substrate which sustain self-renewal ability, survival, and treatment resistance of metastasizing prostate cancer cells.

On multivariate analysis, HIF1α recently emerged as an independent risk factor for progression to metastatic PC and development of CRPC in patients on androgen-deprivation therapy. No one prostate cancer not expressing HIF1α give rise to metastasis or developed CRPC (Ranasinghe et al. 2013).

Targeting of HIF signalling network represents then a very promising strategy to eradicate not only the bulk of prostate cancer, but also to directly hit bone-metastatic cancer cells, to prevent disease relapse and to increase the responsiveness of CRPCs to chemotherapy. Moreover, expression of HIF1α is a strong candidate for future new molecular screenings for the assessment of the risk to develop CRPC.

Among the possible tools for new screening tests aimed to the identification of the metastatic prostate cancer compartment, it has been proposed also the “old” member of the intermediate filament family of proteins, vimentin (Satelli and Li 2011).

Vimentin, is physiologically expressed in normal mesenchymal cells, where it maintain cellular integrity and provide resistance against stress. As well, this protein is overexpressed in prostate epithelial cancer, in which it correlates with high tumor growth, invasion, poor prognosis, and has been recognized also as a marker for epithelial-mesenchymal transition (EMT).

For these reasons, vimentin seem to be attractive for prostate cancer therapy, and this is particularly interesting, considering the recent discovery of a vimentin-binding mini-peptide of potential use for therapy. Further researches on this topic are in progress.

An atypical isoform of trypsin, PRSS3/mesotrypsin, represents another promising target for therapy of bone-metastasizing cancer cells. Its over-expression has been found associated with breast, lung, pancreatic cancers. In primary prostate tumors, it has shown prognostic significance, indicating systemic progression following prostatectomy. Mouse orthotopic model with bioluminescent imaging has confirmed that PRSS3/mesotrypsin is critical for prostate cancer metastasis.

To further support this idea, silencing of PRSS3 inhibits anchorage-independent growth of prostate cancer cells in soft agar assays, and suppresses invasiveness in Matrigel transwell assays and three-dimensional (3D) cell culture models. By converse, the treatment with recombinant mesotrypsin directly promotes an invasive cellular phenotype in prostate cancer cells.

This has fueled the search for new inhibitors of mesotrypsin activity to be used to suppress prostate cancer cell invasion (Hockla et al. 2012).

Maspin (mammary serine protease inhibitor) expression, has been correlated instead with a better prognosis in prostate, as well as in most of malignant solid tumors, as bladder, lung, gastric, colorectal, head and neck, thyroid and melanoma. In all these tumors, however, maspin is frequently down-regulated.

Maspin is a member of the serine protease superfamily, and a selectively increased adhesion by the presence of maspin may contribute to the inhibition of tumor metastasis. Possible therapeutic approaches could be to re-activate the system that inhibits the expression of maspin, identifying activating substances or possibly introducing maspin in cancer cell, up-regulating maspin to reduce the risk of metastasis.

However, the finding that maspin is usually over-expressed in pancreatic, gallbladder, colorectal, and thyroid cancers indicates that it may play different roles in human cancers, this deserving further studies to better define all the possible therapeutic implication of targeting maspin (Berardi et al. 2013).

Besides multiple reports indicating the association between the aberrant expression of EGF receptors with hormone-refractory and metastatic prostate cancer, to date the molecular mechanism linking EGF signaling to prostate cancer metastasis remains unclarified. Experimental models of PCa metastasis showed that EGF could induce epithelial-mesenchymal transition (EMT) and increase invasiveness, also through the extracellular signal-regulated kinase 1/2 (ERK1/2)-dependent phosphorylation, ubiquitination, and degradation of the epithelial protein lost in neoplasm (EPLIN), a putative suppressor of EMT and tumor metastasis. Pharmacological inhibition of the ERK1/2 pathway effectively antagonized EGF-induced EPLIN degradation. This indicates that blockade of EGF signaling could be useful to prevent and/or retard prostate cancer metastasis (Zhang et al. 2012).

As well, there was found a tendency for upregulation of the EGFR family members HER2, and EGFR and downregulation of HER3 in the prostate cancer lymph node metastases in comparison to the primary tumors. This indicate the existence of a rationale supporting possible combined strategies for EGFR- and HER2-targeted therapy of metastasizing prostate cancers, and further studies concerning this eveniences are in progress (Carlsson et al. 2013).

Of a particular interest, a role in predicting metastasis has been emerged also for non-cancerous prostate cancer, from several recent studies in vitro and on prostatectomy tumor tissue (Bijnsdorp et al. 2012).

The cell-communication protein connexin-26 (Cx26) has been suggested as a marker to predict the development of metastasis, when expressed in the adjacent noncancerous tissues (rather than cancer tissues) of prostatectomy sections. It appears then promising for select patients who may benefit from adjuvant therapy to decrease the risk of metastasis.

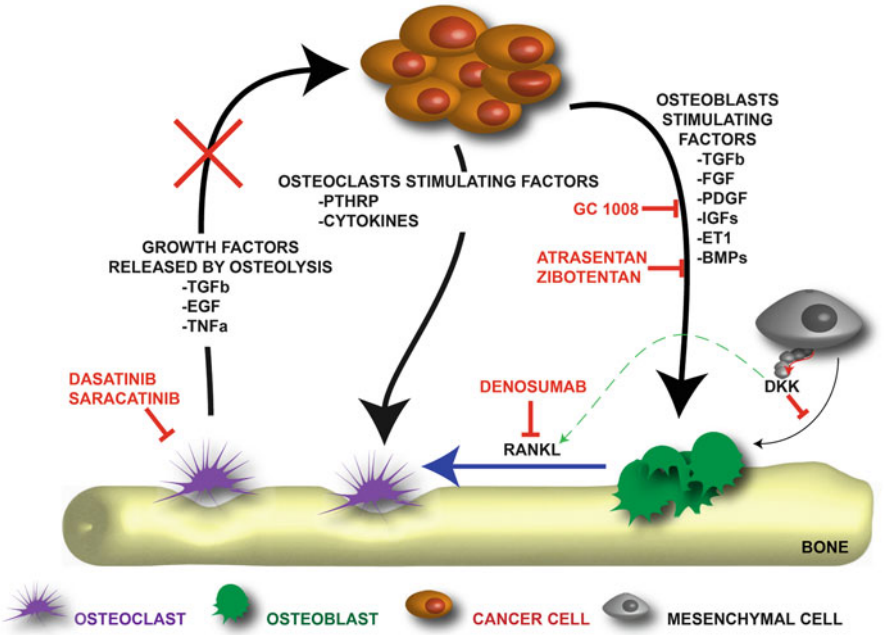

Fig. 16.1 Therapeutic targeting of the bone pre-metastatic niche. In the bone, the formation of a conducive microenvironment, called "premetastatic niche", before the tumor cells arrive at the metastatic destination, is critical for engraftment of the disseminated tumor cells and facilitate formation of micrometastasis, and subsequent transition into macrometastasis. To maintain skeletal homeostasis, cross-talk among osteoblasts and osteoclasts is necessary and it is possible through systemic hormones and local bone-derived growth factors, in response to mechanical stresses and hormonal changes. There is a bidirectional interaction between the cancer cells and bone microenvironment, mediated by production and release of growth factors like TGFβ, IGF-1, FGF, PDGF, BMPs, cytokines, chemokines, calcium ions, and cell adhesion molecules resulting in the creation of a "vicious cycle" that increases bone destruction, ultimately resulting in establishment of cancer metastases in the bone. Genetic ablation of Src results in osteopetrosis, with a decrease in osteoclast-mediated bone resorption and increased osteoblast differentiation and bone formation. TGF-β1 controls bone homeostasis coordinating the bone formation to sites where old bone degradation is occurring. Endhotelin-1 (ET-1) is a potent vasoconstrictor, that binds to its receptors A or B; it stimulates osteoblasts, causing the secretion of growth factors, with increase in tumor cells proliferation and ET-1 production, thus maintaining disease progression. Wnt signaling has a fundamental role in normal osteogenesis; the overexpression of Wnt or a deficiency in Wnt antagonist Dickkopf-related protein 1 (DKK1) result in an increase in bone formation. DKK-1 has been reported to be downregulated in prostate cancer patients in advanced stage, providing a strong basis for the further exploration of the Wnt signaling pathway as a future target in the treatment of bone metastasis in prostate cancer. In this figure, are briefly represented mechanism of action of some drugs, affecting the osteogenic pathway; in detail, denosumab is a RANKL monoclonal antibody; dasatinib and saracatinib inhibit SRC pathway; GC1008 is a monoclonal antibody directed against all three isoforms of TGF-β; Atrasentan and zibotentan are oral ET-A receptor antagonist

Moreover, circulating bone marrow-derived CD90, CD73, and CD105-expressing Mesenchymal Stem Cells (BM-MSCs) have been found to show an innate tropism for tumor tissue in response to the inflammatory microenvironment of prostate cancer tissue. MSCs represent 0.01–1.1 % of the total cells present in core biopsies from primary human prostatectomies. They not only may contribute to prostate carcinogenesis, but may also potentially be used to deliver cytotoxic or imaging agents for therapeutic and/or diagnostic purposes (Brennen et al. 2013).

The intriguing role of miRNAs in prostate cancer progression has been discussed elsewhere in this book. Nevertheless, we would further outline that they looks very promising as "multifunctional" tools for prostate cancer.

They act, in fact, either in suppressing or promoting prostate cancer growth, metastasis, and in maintaining the pluripotency of prostate cancer stem cells.

The low expression of miR-335 was significantly associated with high Gleason Score ($P = 0.04$), advanced clinical stage ($P = 0.04$), and positive metastasis ($P = 0.02$), but not with prognosis in PCa patients. By converse, overwhelming evidence establishes the role of microRNAs as essential actors in the metastasis generation of prostate cancers. Specific microRNAs then appear particularly attractive to be manipulated, either by mimicking or inhibition, to hit metastasizing prostate cancer cells. However, a lot of work is needed, to better understand their role in prostate physiology and cancer, before they may enter the clinics (Fang and Gao 2013; Xiong et al. 2013; Fenderico et al. 2013).

Overall, a great workload is still necessary to reach definitive data about new molecular therapeutic strategies toward metastatic prostate cancer, however, the works are actively on, and the endlessly emerging data are extremely exciting (Fig. 16.1).

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