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Metastases in Prostate Cancer

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Prostate cancer (PCa) prognosis and clinical outcome is directly dependent on metastatic occurrence. The bone microenvironment is a favorable metastatic niche. Different biological processes have been suggested to contribute to the osteotropism of PCa such as hemodynamics, bone-specific signaling interactions, and the "seed and soil" hypothesis. However, prevalence of disseminating tumor cells in the bone is not proportional to the actual occurrence of metastases, as not all patients will develop bone metastases. The fate and tumor-reforming ability of a metastatic cell is greatly influenced by the microenvironment. In this review, the molecular mechanisms of bone and soft-tissue metastasis in PCa are discussed. Specific attention is dedicated to the residual disease, novel approaches, and animal models used in oncological translational research are illustrated.

Prostate cancer (PCa) is the most common cancer and the second-leading cause of cancer-associated death in men (Siegel et al. 2014). Because of the progress made in the treatment of the primary tumor, mortality in cancer patients is now increasingly linked to the metastatic disease (Fig. 1) (Sleeman and Steeg 2010). PCa metastasizes to different organs with a propensity to bone (Gandaglia et al. 2013). More than 50 years ago >20% of patients presented with bone metastasis at diagnosis (Murphy et al. 1982). Data from older studies report median overall survival times of 30–36 months and a median overall survival of ∼18 months in the castration-resistant setting, which in recent years has improved to a median overall survival of 42 months and 2-year overall survival of 72% (95% confidence interval [CI], 68–76) (James et al. 2015). Survival times were influenced by performance status, age, Gleason score, and metastasis distribution. Visceral involvement alone or with bone metastasis is a negative prognostic factor and should be considered a sign of a more aggressive disease in patients presenting with metastatic disease (Gandaglia et al. 2015).

The spine, pelvis, and ribs are the most frequently observed sites of bone metastasis (Kakhki et al. 2013). This distribution is often multifocal, and the more frequent involvement of the axial skeleton suggests an affinity to the hematopoietic active red bone marrow. This is substantiated by the clinical observation that

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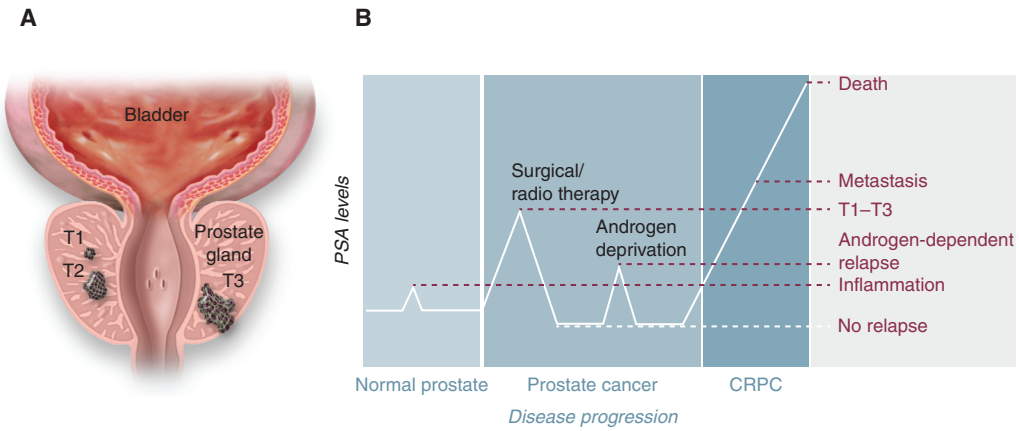

Figure 1. Overview of prostate cancer (PCa) progression and therapeutic options. (*A*) Schematic drawing of different primary PCa stages, as defined by T category of the TNM staging system: T1, confined, not palpable tumor; T2, confined, palpable tumor; T3, palpable tumor, grown through the prostate capsule and spreading to the neighboring tissues. (*B*) Diagram of prostate serum antigen (PSA) blood levels over cancer progression. PSA serum level is used as a diagnostic marker to monitor both the progression of the disease and the effectiveness of the treatments received by the patient. CRPC, castrate-resistant prostate cancer.

with extensive metastases to the axial skeleton, secondary (embryological) sites of hematopoiesis may be activated and may become secondary sites of metastasis. Historically anatomical factors, such as the venous Batson's plexus along the spine, were thought to support this process (Batson 1940). Blood flow in the bone marrow of the adult human is about 2.5 L of blood per minute. Unlike other organs, the arterial supply of the bone marrow ends directly in large vessels (sinusoids). Sinusoids are characterized by an endothelium allowing dynamic opening of pores within the endothelial cells themselves. Blood flow within the sinusoids is slow and nearly stagnant in some areas. These traits not only allow an easy egress of hematopoietic stem cells (HSCs) into the circulation but also facilitate cancer cell extravasation and lodging in the bone marrow. Interesting to note is that sinusoids, which are also part of the spleen, are not prone to be a metastatic site, and this questions the exclusive role of the architecture of the bone marrow sinusoids in the osteotropism of PCa (Hensel and Thalmann 2016).

Despite early detection of the primary tumor, bone metastases are detected in up to 10% of patients already at initial diagnosis of PCa. Additionally, 20%–30% of the patients subjected to radical prostatectomy (RP) for organ-confined (stage T1–T3) PCa will relapse and fatally progress to advanced disease, in which 70%–80% of those patients will harbor bone metastases. Most likely, the majority of recurrences are caused by disseminated tumor cells (DTCs or occult "micrometastases") that had already colonized the target tissue before the time of diagnosis and treatment of the primary tumor. This strongly suggests that in a significant proportion of early-diagnosed PCa the primary tumor already harbors cancer cells with stem-like cell properties (cancer stem-like cell, CSC-like), which are also able to colonize distant organs (metastasis initiating cells, MICs) (Valastyan and Weinberg 2011). Importantly, the microenvironment that harbors the metastatic site must be favorable to this colonization and potentially characterized by biological and molecular features that support the homing of malignant cells and their growth.

Metastasis is a highly inefficient process, in fact, only 0.001%–0.02% of DTCs eventually colonize to distant organs resulting in tumor growth (Luzzi et al. 1998; Schneider et al. 2005). The metastatic process can be described as a multistep process that is initiated in the primary tumor and results in distant tumor growth. The first step is the acquisition of

characteristics from a sessile/epithelial to a mesenchymal/invasive phenotype, described as epithelial-to-mesenchymal transition (EMT), which allows the cells to disseminate. This step of "dedifferentiation" is crucial for the acquisition of invasive characteristics and the dissemination of cancer cells from the primary tumor to the neighboring and distant tissues. To form distant metastasis, the DTCs have to leave the primary site, survive in circulation, attach in the vasculature, migrate and colonize, go into dormancy, and reactivate at the distant site. All this is facilitated by the permissive microenvironment. Much effort has been put into the understanding of these processes aiming to identify the best therapeutical window to target either the cancer cells, the microenvironment, or both (Sterling et al. 2011; Weilbaecher et al. 2011).

CIRCULATING AND DISSEMINATING TUMOR CELLS AND THE METASTATIC MICROENVIRONMENT

The Seed and the Soil Hypothesis

Stephen Paget, more than 100 years ago, observed in women autopsies that, "in cancer of breast the bones suffer in a special way" (Paget 1889). This landmark observation has led to the "seed and soil" hypothesis that postulates the reciprocal need of the seed (cancer cell) and the soil (microenvironment) so that metastasis can occur in distant individual organs. The uniqueness of the microenvironment in the individual organs (liver, lung, bone, etc.) supports or opposes the colonization events that lead to the secondary tumor growth.

The tumor cells that leave the primary site and enter circulation are defined as circulating tumor cells (CTCs); only a fraction of these cells has the capability to extravasate at a distant site and persist/survive as DTCs. Of these DTCs, an even smaller fraction is capable of forming metastasis (Pantel and Speicher 2015; Yang and Weinberg 2008).

Two Main Models of Metastasis

The first model hypothesizes that metastasis-initiating cells need to undergo deep molecular rearrangements to proceed through the various steps of the metastatic cascade, and is often referred to as the "phenotypic plasticity model." To leave the primary tumor site, cancer cells must undergo EMT. This process enables them to become more invasive and motile, allowing migration toward gradients of oxygen and nutrients brought by the vasculature associated to the tumor, often leaky, unorganized, and incompletely formed. However, recent literature has pointed out that EMT might be a dispensable process for the occurrence of metastasis but fundamental for the acquisition of chemoresistance (Zheng et al. 2015).

The concept of "epithelial plasticity" being a process that requires somatic mutations and (epi)genetic changes exclusively in the cancer cells is too simplistic. A fundamental contribution to the maintenance and progression of the primary tumor from a confined to an invasive state is provided by different cell types and extracellular matrix (ECM) components, which constitute the stroma (van der Pluijm 2011). It has been documented that cancer cells can "activate" different cellular components of the stroma, such as fibroblasts, and can recruit inflammatory, endothelial, and mesenchymal cells. These cellular components can, in turn, support cancer cell proliferation and invasion (Mueller and Fusenig 2004; Bhowmick and Moses 2005). This combination of environmental factors and molecular properties on the tumor side and stromal side, respectively, allows cancer cells to enter the bloodstream as CTCs (Carmeliet and Jain 2011). The controversy with this might be due to the notion of irreversibility of EMT (Thiery 2002) and the need of cancer cells, once they reached their metastatic site, to undergo mesenchymal-to-epithelial transition (MET) to regain their proliferative and metastatic behavior. This would imply proteome and transcriptome changes that do not seem to be found in the DTCs of several cancers, which appear epithelial (Braun et al. 2000; Schardt et al. 2005; Dasgupta et al. 2017). Cancer cells with metastatic potential would be required to show a high degree of epithelial–mesenchymal plasticity to progress throughout the different stages of the metastatic spread. However,

EMT-associated transcription factors have been shown by a number of groups to be associated with both positive and negative metastatic effects (Yang and Weinberg 2008; Ocaña et al. 2012; Tsai et al. 2012; Vanharanta and Massagué 2013), leaving this a yet open question.

The second model hypothesizes the selection of subpopulations of cancer cells within the tumor that are genetically predisposed for metastasis; this model is frequently referred to as the "genetic" or "clonal" model (Nowell 1976; Ruiz et al. 2011). This model addressed the clinical observation that some metastases do not display a differentiated phenotype. According to this model, clones or subpopulations of tumor-initiating cells bear a set of genetic alterations that cause a permanent activation of EMT features, which render them fit for the metastatic process. These genetic alterations could be either an intrinsic feature of these cancer subpopulations (driver mutations), developed during tumorigenesis (for instance, when the tumorigenic alterations hit a cell early in its process of differentiation, like a tissue stem cell), or an acquired trait (passenger mutations) developed in response to environmental factors—like the selective pressure imposed by treatment with chemotherapeutic agents. Another interesting hypothesis comes from the observation that the CTCs can also be found in clusters. In this scenario, the cancer cells may facilitate their capability to dock and proliferate by taking with them their own "cancer soil" as passenger soil, as described for lung cancer (Fig. 2) (Duda et al. 2010).

In the process of initial seeding, cell–cell interactions and cell adhesion to the ECM play a critical role. The ECM of the growing cancer undergoes numerous alterations in terms of both biochemical and physical properties (i.e., stiffness, elasticity, and tension) (Miles and Sikes 2014). Integrins play a pivotal role in tumor progression, as they can couple ECM-derived mechanical cues with intracellular signaling pathways (Friedland et al. 2009).

Metastatic PCas show higher levels of active β1 integrin, which confers both an enhanced capacity to colonize distant organs, through the adhesion to ECM molecules like fibronectin and collagen type I, and a survival advantage, through an increase in the resistance to anoikis—the programmed cell death induced by insufficient adhesion to the growth substrate (Lee et al. 2013; Jin et al. 2014).

Cancer cells also express other integrins, like αv and β3, that promote their adherence to a broader variety of proteins of the ECM of other organs, like osteopontin, thrombospondin, vitronectin, fibronectin, intracellular adhesion molecule (ICAM-1), and vascular adhesion molecule (VCAM-1) (Thalmann et al. 1999; Zhao et al. 2007; Lu et al. 2011; Schneider et al. 2011), which has led to the concept of "osteomimicry" by PCa cells (Koeneman et al. 1999). PCa cells also take advantage of the chemokine-(C-X-C-motif) axis CXCL12/CXCR4 as homing mechanism to the bone, resulting in an enhanced capacity to contact the bone marrow niche and establish long-term dormancy. Among other compounds, PCa cells secrete the chemokine CXCL16, which boosts the recruitment of bone marrow stromal cells (MSCs). This signaling axis, in turn, promotes the conversion of MSC into cancer-associated fibroblasts (CAFs), which secrete high levels of CXCL12, which potentiate the EMT conversion of cancer cells and up-regulate their expression of the cognate receptor CXCR4 (Jung et al. 2013). Cancer cells also up-regulate the expression of matrix metalloproteases that facilitate extravasation/migration of the cancer cells (Sun et al. 2004). This process has been described for several cancer types and has therefore become an attractive target for therapeutical treatment of solid tumors (Schneider et al. 2011).

RESIDUAL DISEASE AND TUMOR DORMANCY

Once disseminated, cancer cells can survive as DTCs at the metastatic site for decades. The dynamics of metastatic outgrowth varies considerably between cells, cancer types, and individual patients. This survival can be defined either as a quiescent (dormant or senescent) state or as an equilibrium, in which, although the disseminated metastatic foci will not grow to overt, clinically relevant metastases, they will

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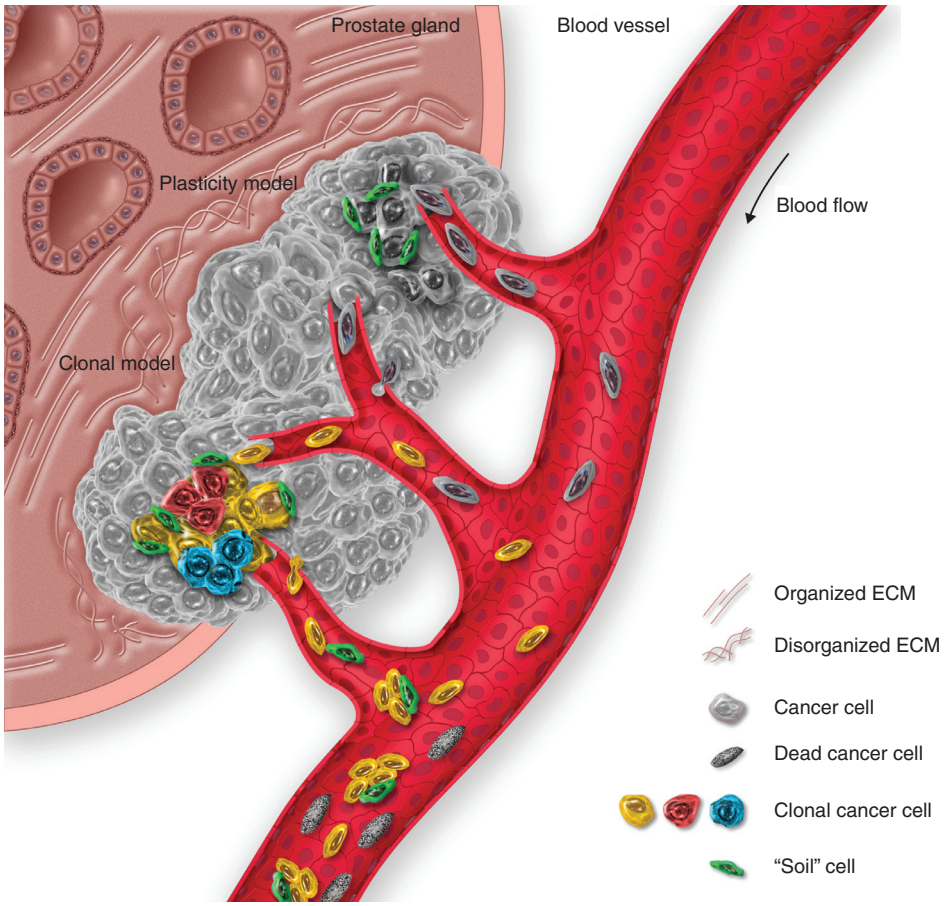

Figure 2. The metastatic spread of prostate cancer (PCa) from the primary site. The primary lesion growth is characterized by a dysregulation of the prostate architecture, inducing changes in the extracellular matrix (ECM) composition and architecture, together with the induction of an inflammatory state that activates stromal cells and favors the recruitment of blood vessels to the lesion. As the tumor recruits new blood vessels, the systemic dissemination of cancer cells (in the form of circulating tumor cells, or CTCs) can take place. Two are the main models that explain the metastatic spread: "plasticity model" and "clonal model." According to the plasticity model, as the cancer cells progress through malignancy, they may collect hits that make them fit for the metastatic process. The clonal model, on the other hand, theorizes that within the heterogeneous cancer cell subpopulations, clones with different fitness are generated, including some with the characteristics required for the metastatic spread. In addition, clusters of cells may form between spreading cancer and stromal cells from the primary lesion, the latter forming the "soil" cells that can facilitate the spreading and the survival of CTC. However, most of the CTCs will not survive in the bloodstream and will circulate as dead CTCs until clearance.

still release cancerous cells into circulation (Ghajar 2015). Dormancy can, in this regard, be induced at a cellular level, in which case the cells undergo a G_0-G_1 arrest (Aguirre-Ghiso 2007; Ghajar 2015), or it can be induced at the population level as a cell-depleting event (e.g., apoptosis) (Sherwood et al. 1971; Gimbrone 1972; Aguirre-Ghiso 2007; Rakhra et al. 2010).

This latter hypothesis is supported by the fact that CTCs can still be detected, for instance, in the circulation of breast cancer patients whose primary tumor had been removed and who do not present signs of relapse (Meng 2004; Ghajar 2015). CTCs have also been detected in the circulation of PCa patients after RP, although correlation with patient clinical outcome is still

controversial (Adsan et al. 2002; Morgan and Dearnaley 2014; Thalgott et al. 2015). Persistence of DTCs in the bone marrow of PCa patients after RP, instead, is a predictor of recurrence (Morgan et al. 2009).

One key element that characterizes DTC growth dynamic and confers capacity to disseminate is the interaction with the microenvironment of the target organ and, in particular, with the local perivascular niches.

Given that cancer cells leave the primary site via the lymphatic or vascular site, it is no surprise that DTCs are found in close proximity of the vascular basement membrane (Chambers et al. 2002; Kienast et al. 2010; Ghajar et al. 2013; Price et al. 2016). This particular microenvironment is defined as the perivascular niche and has a central role in normal tissue development and differentiation by homing stem cells and maintaining them in a stem-like state through the balancing of pro- and antiangiogenic factors (Christov et al. 2007; Butler et al. 2010; Goldman and Chen 2011; Xiao et al. 2013).

In PCa, the main site of metastasis is the bone marrow (Bubendorf et al. 2000). Bone marrow is the main site of hematopoiesis in adults, a process in which the long-term, lifelong persistence of the HSCs is temporally associated with the high proliferative rates required to maintain hematological homeostasis (Mendelson and Frenette 2014; Calvi and Link 2015). Therefore, mechanisms must be present in the bone marrow to support both processes. Moreover, the bone marrow is also a site of intense cellular trafficking (Méndez-Ferrer et al. 2008; Mazo et al. 2011; Casanova-Acebes et al. 2013), and its peculiar vasculature structure has the characteristics that best allow this function (Nombela-Arrieta et al. 2013).

It is then conceivable to hypothesize a correlation among the normal stem cell maintenance, the dormancy of the DTCs, and the role of the perivascular niche. The "endosteal" (or "vascular") and the "perivascular" niche have both been described as the two major HSC niches. In the bone marrow, long-term repopulating HSCs have been found both in the endosteal and perivascular spaces, two stem cell niches where HSCs may reside in a state of quiescence, which allows their long-term repopulating abilities and ensures protection against genotoxicity (Cheshier et al. 1999; Cheng 2000; Arai et al. 2004; Nombela-Arrieta et al. 2013). Interestingly, bone marrow stromal cells have been shown to support and promote their fate by protecting them from oxidative stress and limiting their entry into the cell cycle (Ludin et al. 2012).

PCa cells compete with HSCs for the occupancy of the limited niches in the bone marrow (Braun et al. 2005; Zhang et al. 2003), and reducing the niche size hampers dissemination (Shiozawa et al. 2011). However, once the DTCs have occupied ("hijacked") the vascular niche, they acquire a stem cell phenotype (Shiozawa et al. 2016). The acquired stem-like phenotype, together with the protective microenvironment in which PCa DTCs reside, confers DTC a high degree of resistance to therapy (Kobayashi et al. 2011; Chéry et al. 2014). Of particular interest is the active role of a stable microvasculature on the initiation of the metastatic growth (Ghajar 2015).

Once DTCs are awakened from their dormant state, they may start to reform macrometastases of osteoblastic or osteolytic nature, depending on the origin of primary tumor; PCa leads to mainly osteoblastic lesions, whereas breast cancer leads to osteolytic lesions (Fig. 3) (Logothetis and Lin 2005).

Tumor cells influence and are being influenced by the bone microenvironment, evading from the immune system and acquiring bone-related properties (osteomimicry, as previously mentioned) (Özdemir et al. 2014). The "preference" of tumor cells to colonize the bone has been attributed to a specific bone-related gene expression signature that tumor cells have before the metastasis, as it has been shown by studies in breast cancer (Kang et al. 2003). Additionally, cancer cells recruit bone marrow stromal cells to the primary site where they become CAFs, which contribute to the metastatic potential of malignant cells (Jung et al. 2013).

BONE METASTASIS: THE VICIOUS CYCLE

Osteoclast and osteoblasts mediate constantly the dynamic remodeling of the bone tissue.

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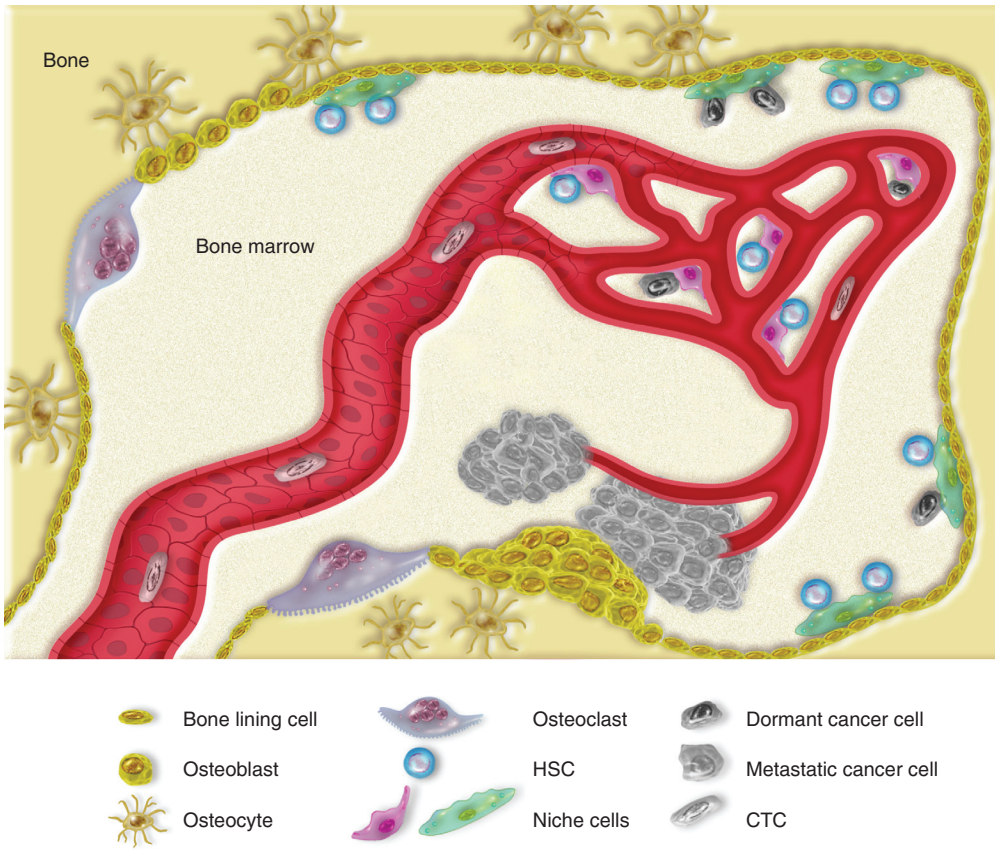

Figure 3. Dissemination of prostate cancer (PCa) cells in the bone marrow: from DTCs to overt metastasis. The hematopoietic bone tissue consists of two main parts: the bone tissue and the bone marrow. Bone is a highly regulated tissue that undergoes constant remodeling to keep its architecture and mechanical properties; as osteoclasts resorb weak bone, skeletal stem cells undergo local expansion, followed by differentiation into osteoblasts. Depending on the local microenvironment and the tissue architecture, osteoblasts will further differentiate either into bone-producing osteocytes or bone-lining cells—namely, the layer of cells that is in contact with the marrow cavities where hematopoiesis occurs. To ensure the lifelong production of blood, hematopoietic stem cells (HSCs) reside in specific areas, or niches, supported by specialized stromal cells (or niche cells) and can be found both at the endosteal and perivascular sides of the bone marrow. Within these niches, HSCs are induced in a state of quiescence, protected from cellular stresses, and prevented from further proliferation. Prostate CTCs may disseminate to the bone marrow and compete with the HSCs for the space in the niches. Within the niches, DTCs could remain dormant for an indefinite amount of time. Eventually, DTCs may exit their dormant state and start proliferating, bending the coupled processes of bone resorption and bone formation to support their growth. Most frequently, this vicious cycle produces hyperplastic bone tissue, eventually forming clinically relevant osteosclerotic metastasis.

Tumor cells produce cytokines (e.g., tumor necrosis factor α [TNF-α], IL-11, VCAM-1, MMP1, Jagged1, parathyroid hormone-related protein [PTHrP]), which stimulate the osteoclast maturation or, indirectly, promote osteoclast differentiation by stimulating bone marrow osteoblast to produce IL-6 and receptor sctivator of nuclear factor κB (RANKL). Bone matrix resorption then releases transforming growth factor β (TGF-β) and insulin-like growth factor-1 (IGF1), which promote proliferation and survival of the cancer cells, closing the circle to what is known as the "vicious cycle," which effectively promotes osteolytic metastasis

(Weilbaecher et al. 2011; Ell et al. 2013). However, the use of antiresorptive agents, like bisphosphonates, as strategy to inhibit bone resorption and interfere with bone metastasis revealed no effect on cancer cell proliferation in animal studies (Sasaki et al. 1995; van der Pluijm et al. 2005; Yuen et al. 2006). This suggests that other mechanisms, such as the coupling of angiogenesis and osteogenesis previously mentioned, support tumor cell growth in the bone. Interestingly, microRNA (miRNA) have recently also been reported to play a fundamental role in osteoclastogenesis. *Dicer1*, *Dgcr8*, and *Ago2* block osteoclast differentiation (Sugatani and Hruska 2009; Mizoguchi et al. 2010), whereas ectopic expression of miR-155 (Mann et al. 2010; Mizoguchi et al. 2010; Zhang et al. 2012) and repression of miR-21(Sugatani et al. 2011) inhibit osteoclast differentiation. Recently, it has been shown that osteoclastogenesis is inhibited by that miR-133a, -141, and -219, whereas miR-190 might inhibit osteoclasts differentiation (Ell et al. 2013). Although bone lesions in PCa are typically osteoblastic (Mundy 2002; Logothetis and Lin 2005), the coexistence of osteobastic and osteolytic responses have been documented (Theriault 2012). Among the variety of factors that orchestrate the balance between osteoclastic- and osteoblastic-activity, TGF-β and Wnt signaling are two fundamental networks that regulate the maintenance and expansion of osteoprogenitor cells and their differentiation toward osteoblasts.

The canonical Wnt signaling pathway seems to play a role in the onset of castration resistance in PCa (Wang et al. 2008). Moreover, alterations of canonical Wnt signaling, such as modulation of the *dickkopf* (DKK) genes or mutations of sclerostin (SOST), which inhibits LRP5, contribute to disrupt bone formation, a process whereby Wnt signaling exerts a crucial role (Semenov et al. 2005). Wnt signaling also induces the expression of osteoprotegerin (OPG), which prevents the binding of RANKL to RANK, thereby inhibiting osteoclast function and leading to a "bone active effect" (Rentsch et al. 2009).

The TGF-β superfamily also exerts a major role in the context of the bone microenvironment and its remodeling in PCa. TGF-β supports the development of bone metastasis from PCa in animal models (Fournier et al. 2015). In particular, bone morphogenetic proteins (BMPs) and Noggin, which antagonizes BMP signaling, are functionally involved in skeletal and joints morphogenesis, bone remodeling, and in different cellular processes, including osteogenesis (Chen et al. 2004). One of the main BMPs involved in the recruitment of osteoblasts is represented by BMP6 (Dai et al. 2005). On the other hand, the recruitment of osteoclasts and stimulation of their activity in PCa is mediated by MMP-7, which cleaves RANKL (thereby stimulating osteoclastogenesis) (Lynch et al. 2005), and Noggin, which antagonizes BMPs and impairs bone formation (Schwaninger et al. 2007; Secondini et al. 2011).

SOFT-TISSUE METASTASIS

The common paradigm of tumor progression is that tumor cells from an advanced tumor unidirectionally migrate to lymphatic sites and then toward distant organs to form secondary metastases (Halsted 1894). This view has been challenged by recent studies showing that tumor cells can, at any point of tumor formation, multidirectionally seed to distant organs, whereas secondary and tertiary metastases can form independently from the primary clone (Haffner et al. 2013; Beltran et al. 2016). Presence of lymph node (LN) metastases is a frequent consequence of PCa associated with high risk, poorer outcome, and limited therapeutic possibilities, such as surgical resection of pelvic LN, radiotherapy, and androgen deprivation therapy (ADT). Surgical resection may not be effective in removing all LN metastases because of imaging and detection limitations, whereas multiple surgeries are not an option because of postoperative scarring and other complications (Sankineni et al. 2015). Disease-free survival is directly dependent on LN staging and the number of metastases. The frequency of mitochondrial mutations, affecting the metabolism of tumor cells, is lower in LN, liver, and lung metastases compared with bone metastases (Arnold et al. 2015). Visceral metastases in men with metastat-

ic, castration-resistant prostate cancer (CRPC) constitute a high prevalence and are linked to poor outcomes (Halabi et al. 2014). B-cell lymphoma 2 (BCL-2), BCL-XL, myeloid cell leukemia 1 (MCL-1) and survivin expression has been measured in primary PCas and small cohorts of lymph node and bone metastases (Krajewska et al. 1996, 2003; Zellweger et al. 2005). Their expression has been associated with transgression of the prostate capsule, risk of relapse, and metastatic progression (Scherr et al. 1999). Interestingly, soft-tissue metastasis transcriptional and protein profile differs from that of bone metastasis. In particular, nuclear survivin was observed in soft-tissue metastasis, whereas bone metastases show relative overexpression of cytoplasmatic survivin, suggesting that cancer cell apoptosis-inducing drugs may exert various effects and may show very different efficacies, depending on the site of the metastasis. Additionally, in liver and LN tissues, the angiogenic expression profile was different (Morrissey et al. 2008), suggesting that factors involved in tumor vascular recruitment and maintenance may also be affected by the microenvironment.

PCa adenocarcinomas show osteotropism, resulting in osteoblastic and osteolytic lesions, however, neuroendocrine (NE) tumors metastasize prevalently to visceral sites (Marcus et al. 2012). NE cases, both the spontaneous ones and those arising following ADT therapy, are associated with low prostate serum antigen (PSA) values, visceral metastases, and poor survival (Palmgren et al. 2007).

The mechanisms of different organotropic properties of various PCa types remain to be elucidated.

EXPERIMENTAL MODELS OF BONE METASTASIS

With the life expectancy of men increasing, PCa has become a major medical problem. Once the tumor has metastasized, outcome is dismal. Research on PCa bone metastases has been hampered by the limited number of experimental models available.

The reasons for this are multiple: poor growth potential of human prostate tumor tissue in nude mice, slow development of immune deficient mice strains, limited cell lines, and lack of spontaneous PCa in animals with the exception of ACI/Seg and Lobund-Wistar rats. With the advent of mutant nude mice with a deficient cell-mediated immune response and only slightly impaired humoral antibody formation, xenografts of cell lines and human tumor tissue became possible and opened a new era of research (van Weerden and Romijn 2000), albeit still impeded by an increased natural killer (NK) cell activity in these animals. A number of preclinical models using state-of-the-art molecular imaging for cell tracking and drug response have been developed (Buijs et al. 2007; Eaton et al. 2010; van den Hoogen et al. 2010).

Cancer cell tracking and drug response can be studied by intracardiac delivery of human PCa cells that stably express either bioluminescence (e.g., luciferase) or fluorescence (e.g., near infrared fluorescent [NIRF] proteins or green fluorescent protein [GFP]) reporters as a model of bone metastasis. Orthotopic and intraosseous cell delivery models are used for the study of primary PCa and metastatic PCa and, in particular, the interactions between cancer cells and bone microenvironment (Dai et al. 2016).

Several efforts to obtain xenografts from patient samples (patient-derived xenografts, PDX) have been attempted. The model PC-82 was the first androgen-dependent PCa xenograft established, achieving a success rate of about 5% over many years (Hoehn et al. 1980; van Weerden and Romijn 2000). In vivo growth rate was improved by the introduction of Matrigel, in which cells were suspended and mixed with Matrigel, allowing the propagation of the CWR (Case Western Reserve) xenograft tumor series, such as the CWR-22 xenograft (Pretlow et al. 1991, 1993). Seven additional xenograft models were established in BALB/c mice using intact tumor piece implantation and testosterone administration from primary and metastatic PCa (LN and skin) (van Weerden et al. 1996). Bone and LN metastasis-derived PCa xenografts were developed by coinjection of tumor cells with Matrigel in severe combined immunodeficient (SCID) mice (Klein et al. 1997). The bone me-

tastasis-derived LAPC-9 model is dependent on androgens for growth, secretes PSA, and shows spontaneous tumor reinitiation after prolonged androgen deprivation (Craft et al. 1999). Another model (Garcia et al. 2014) that has been used to study the transition from androgen-dependent to -resistant tumor growth is the LN metastasis-derived LAPC-4 model, established by the same group (Craft et al. 1999).

The BM18 PCa xenograft model, developed from a bone metastasis biopsy, retains androgen-dependent growth and survival properties, whereas it recapitulates the luminal phenotype observed in human PCa with stem cell characteristics similar to those of castration-resistant NKX3.1 cells (CARNs) described in the mouse (Wang et al. 2009; Germann et al. 2012). Similar results were also achieved in the LuCaP model (Ellis et al. 1996) and MDA model (Navone et al. 1997).

Taking into account the importance of stromal–epithelial interactions in tumor development and progression, a cell-cell recombination model was generated by the coinoculation of nontumorigenic LNCaP cells (Horoszewicz et al. 1983) with organ-specific fibroblasts from the bone into athymic nude mice, showing the ability to form solid tumors (Gleave et al. 1991). By altering the stromal and hormonal environment in vivo, an androgen-independent, tumorigenic LNCaP subline, C4-2, capable of growing tumors in the castrated host was derived. C4-2 cells secrete PSA autonomously and metastasize to the LN and bone with an incidence of 11%–50%, while showing a higher incidence of axial skeleton metastases in castrated hosts. From C4-2 osseous metastases, several cell lines were isolated and denoted as B2, B3, B4, and B5 (Thalmann et al. 2000).

In recent years, several genetically engineered mouse models (GEMMs) of PCa have been established that recapitulate the stages of PCa development, from PIN lesions to localized and invasive adenocarcinoma, to LN metastasis. Telomerase reactivation in the prostate-specific probasin (PB) promoter-driven Smad4 conditional knockout, TP53/PTEN double null model (PB-TP53/PTEN) leads to prostate tumors that progress to bone metastases (Ding et al. 2012). However, there are limited GEMMs that progress to bone metastases (Grabowska et al. 2014), which can provide insight on the mechanisms of human PCa metastatic cues.

BONE METASTASES IN THE CLINIC

Metastatic PCa remains an important clinical problem given the growing number of men with advanced disease, its impact on the quality of life, and, ultimately, as a cause of mortality. Osteoblastic bone lesions to the axial skeleton are the most common metastasis in men with advanced PCa. Palliative treatment is a priority, with the goals of relieving pain, improving mobility, and preventing complications such as pathologic fractures or epidural cord compression.

ADT remains the treatment for metastatic PCa, and whereas this reduces the symptoms and tumor growth, recurrence of CRPC is almost certain. Histopathology of end-stage bone metastases acquired at autopsy or a result of surgical resections for spinal cord compressions or pathological fractures (Maitland and Collins 2008; Collins et al. 2005) has shown that, even within an individual patient, bone metastases are heterogeneous. It is important to note that, although nuclear androgen receptor (AR) staining is generally prominent in most cells, non-NE, AR-negative tumor cells are clearly observed in both CRPC and treatment-naïve metastasis (Colombel et al. 2012). These findings indicate that AR-independent cell survival in the bone microenvironment does occur, and any mechanisms that contribute to such survival are of great interest. Heterogeneity of metastatic disease suggests that second generation, AR-directed therapies such as abiraterone and enzalutamide will most likely need to be conjoined by therapies directed against non-AR pathways and bone-targeting therapies.

ADT increases bone resorption, reduces mineral density, and increases risk of fracture, thus, indirectly leading to occurrence of bone metastasis in CRPC patients (Ottewell et al. 2014). Bisphosphonates have been shown to prevent bone loss associated with ADT, however, a positive effect on fracture prevention is lacking. Bisphosphonates aside current treatments

of bone metastases include surgery, bone-targeted radiopharmaceuticals, and denosumab.

Denosumab is a Food and Drug Administration (FDA)-approved humanized monoclonal antibody that binds to RANKL, a key factor in the pathway for osteoclast formation and activation, as previously discussed. ADT patients treated with Denosumab showed increased bone mineral density and reduction in vertebral fractures (Smith et al. 2009).

Currently, the only FDA-approved bone-targeting radioisotopes for patients with symptomatic metastatic CRPC are Strontium-89 chloride (Sr-89) and Samarium-153 lexidronam (Sm-153). Multiple clinical trials have shown both to be effective agents for bone pain palliation. Radium-223 chloride (Ra-223) is the first radiopharmaceutical drug to show a prolongation of overall survival in these patients and palliative benefits (Goyal and Antonarakis 2012).

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