

# Skeletal implications of prostate cancer

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## Abstract

This manuscript reviews the theories behind the propensity of prostate cancer to cause bone metastases and skeletal implications of the prostate cancer biology and treatment modalities. The escape of tumor cells from the primary tumor in the prostate to secondary tumor sites in the axial skeleton probably occurs before the primary tumor is detected. Several theories offer explanations for the observed proclivity of prostate tumors to selectively colonize the axial skeleton. The interaction between the tumor cells and cells that populate bone marrow, in particular osteoblasts and osteoclasts, is important for creating a “fertile” environment where tumor cells can establish and grow. Prostate cancer cells are capable of producing growth factors that can affect both osteoblasts, resulting in osteoblastic bone formation, and osteoclasts, resulting in excessive bone resorption. In addition to the capability to progress from testosterone-dependent to testosterone-independent phenotype, the hallmark of metastatic prostate cancer is osteosclerosis similar to one induced experimentally in nude rats using CWR22 human prostate cancer cell line. Metastatic bone disease caused by excessive bone formation and bone resorption is the major cause of morbidity in patients with prostate cancer. The most common symptoms include pain, pathological fractures, spinal cord compression, cranial nerve palsies, bone marrow suppression and hypercalcemia. The introduction of prostate-specific antigen in clinical practice created a shift to where more prostate cancer patients with early disease receive androgen ablation treatment, which in return causes more bone loss and cancer-associated osteoporosis. Introduction of third generation bisphosphonates to treat skeletal consequences of malignancy further stressed the important interaction between the bone marrow stroma and cancer cells. Nevertheless, animal models and human prostate tumor cell lines that mimic all aspects of skeletal conditions in prostate cancer patients including osteoblastic bone response are needed to develop and screen for novel therapeutic and diagnostic modalities.

**Keywords:** Cancer, Prostate, Bone Metastases, Bone Formation, Bone Resorption, Prostate-Specific Antigen, Cancer-Associated Osteoporosis

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## Introduction

Prostate cancer is the second most common cancer in American men. The American Cancer Society estimates that approximately 180,000 new prostate cancer patients will be diagnosed every year in the US. The lifetime risk of developing prostate cancer is about 10 percent, and the risk that men will die of prostate cancer is less than 5 percent<sup>1</sup>. Morbidity and mortality are consequences of bone metastases that occur in approximately half of patients diagnosed

with prostate cancer. During the next millennium, as the aged population increases, it is expected that the incidence and mortality of prostate carcinoma will continue to increase.

To metastasize successfully, cancer cells have to detach from the primary tumor, invade blood or lymphatic vessels, travel in the circulation to a distant site and establish a new cellular colony. The growth of the prostate cancer cells is initially androgen-dependent, and therefore androgen ablation therapy has been the most effective treatment for patients with metastatic prostate cancer. However, androgen ablation appears to be effective for only a limited duration of time due to the progression of prostate cancer tumor cells from an androgen-dependent to an androgen-independent state. Tumor progression, in addition to tumor ability to cause osteoblastic bone metastases, is the hallmark of disseminated prostate cancer.

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## Why bones?

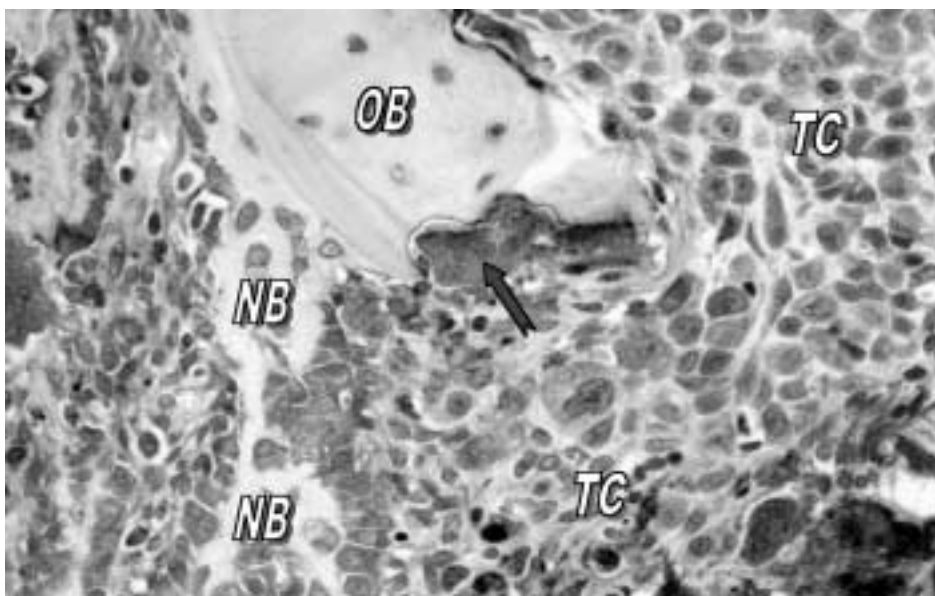
The skeleton has long been recognized to be the most common target organ of prostate cancer metastasis, and the appearance of osseous metastasis signals the final, incurable stage of the disease. The bone metastases are predominantly situated in red bone marrow, most commonly in the spine, pelvis and ribs, although lesions in the proximal femora and humeri are not uncommon, while metastases below the knees and elbows are extremely rare. The reasons underlying the proclivity of prostate cancer to metastasize to bone still remain unclear, though at present several mechanistic theories should be considered.

The “mechanical” hypothesis proposed by Ewing<sup>2</sup> is composed of 2 parts, the first dealing with local tumor spread and the second regarding tumor formation by trapping or sieving of tumor emboli. It was proposed that tumors spread locally along the lines of least resistance, similar to the way that plant roots move through the soil. Therefore, those areas in direct line with the path of least resistance will become the target for local metastasis. This mechanism may involve copious secretion of enzymes by the tumor cells into the adjacent area or it could be due to physical pressure exerted by the expanding neoplasm<sup>3</sup>. The second mechanism involves the filtering (sieving) of tumor emboli from blood or lymph. The ability of tumor cells to form emboli-cell clumps, is well documented<sup>4</sup>. The embolus is called homotypic if it consists of tumor cells only, or heterotypic if it consists of blood components such as platelets or lymphocytes in addition to tumor cells. Trapping of the emboli usually occurs in the first capillary bed encountered. Indeed, in most cancers where multi-cellular tumor emboli are released in circulation or lymph, they do not easily traverse small vessels or nodes, resulting in limited distribution of tumor emboli due solely to circulatory patterns and mechanical loading<sup>4,6</sup>. The fact that tumor cells shed from the primary neoplasm, often entering the venous circulation, may account for the relatively large number of liver and lung metastases observed. The trapping of emboli is not specific and therefore, an organ with extensive capillary or sieving type structure such as lungs, liver or lymph nodes can stop emboli, thereby producing apparent organ specificity. Many tumor cells, however, are able to escape this sieving as a result of their deformability<sup>7-9</sup>. The predominant distribution of bone metastases in the axial skeleton, in which most of the red marrow resides, suggests that sluggish blood flow at these sites and vascular sinusoids lined by endothelial cells that lack a basement membrane and display 60Å fenestra might help the escape of tumor cells from blood vessels and facilitate homing of cancer cells to bones<sup>10</sup>. Even though the proportion of the blood supply in the skeleton is estimated to be approximately 10% of the cardiac output, bone is the most common site of prostate cancer metastases<sup>11</sup>. There are differences in hemodynamics between organs that are affected with metastases. For example, liver and lung are the two most common sites of metastases but the spleen with blood

volume comparable to the blood flow in the liver is almost never affected with metastases. Although hemodynamic and trapping theories alone cannot satisfactorily explain the frequency and location of observed bone metastases, the fact is that slow blood flow and nutritional and functional vascularization facilitates access of circulating cancer cells and/or cancer emboli to the axial skeleton. In any case, blood circulation explains much about why various tumors spread preferentially to certain tissues.

The high incidence of bone metastases from cancer of the prostate without corresponding lesions in the liver or lung makes it unlikely that malignant cells spreading to bones pass through the hepatic and pulmonary circulation. Even if liver and lung tissues are not receptive as the sites for the establishment of metastatic disease from a particular cancer, tumor cells are still unlikely to pass through its narrow capillaries, particularly when aggregated as tumor emboli. A satisfactory explanation for skeletal predilection of metastatic disease from breast and prostate cancers has been provided through studies in animals and human cadavers demonstrating the existence of low pressure, high volume plexus of deep vertebral veins<sup>7,12,13</sup>. Venous blood from both the pelvis and the breast flows not only to venae cavae, but also directly into the vertebral-venous plexus in particular when intrathoracic and intra-abdominal pressure is elevated, which *per se* may help detachment of the cancer cells from the primary tumor site. Thus, in some instances, the axial skeleton may be the site of metastatic tumor growth because it could be the first organ encountered by cells leaving a primary tumor site<sup>14-16</sup>.

Some of the apparent organ-specific metastases may be explained by mechanical hypotheses, physical extension of the tumor, trapping emboli in capillary beds, or favorable venous drainage. Presumably once the tumor cells or embolus reaches the skeleton, it still needs to establish and invade the bones. Paget’s hypothesis<sup>17</sup> predicts the growth of tumor foci to be the direct result of the microenvironment provided by the specific organ. When one considers the rigors of the blood circulatory and lymphatic systems, host defense mechanisms, and the need to lodge in the appropriate “soil”, it might appear that cells shed from a primary tumor (“seed”) have little chance of surviving and, in fact, this is what occurs. Only 1% of the tumor cells injected in the circulation survive the first 24 hours, and only 0.1% remain viable at the site of metastases two weeks later<sup>18</sup>. Paget’s theory, at the time when it was proposed, relied mostly on fragmentary data, although analogy with normal growth factors makes the theory very plausible. The multi-cellular environment of bone marrow is highly metabolic and consists of hematopoietic stem cells that give rise to all blood cell elements and osteoclasts, and of mesenchymal stem cells that differentiate into osteoblasts, chondrocytes, adipocytes and stromal cells. In reality cancer cells that invade bone are in contact with all cell types that exist in the bone marrow, but osteoblasts and osteoclasts are probably the most important because these two cell lines are the ultimate effectors of



**Figure 1.** Nude rats were injected with intra-tibial injections using human, testosterone-dependent, prostate cancer cell line CWR22. Figure 1 depicts changes in the proximal tibial metaphysis seven weeks after inoculation of tumor cells. Active osteoblasts are readily seen along the newly formed bone matrix (NB). Multicellular, TRAP positive osteoclast resorbing old bone matrix (OB) is indicated by the red arrow. Prostate tumor cells (TC) occupy most of the bone marrow space. Paraffin; TRAP immunostain; Magnification x10.

bone response to cancer invasion. It was the independent work of several laboratories providing compelling evidence regarding the influence of host-organ microenvironment on growth of metastatic tumor cells that revitalized Paget's original "seed and soil" theory<sup>19,23</sup>. The development of novel and disease relevant animal models has made it possible to show that the outcome of metastasis depends on the interaction of metastatic cells with host factors<sup>19,24</sup>. The work of Stephenson et al.<sup>25</sup> and Pettaway et al.<sup>26,27</sup> clearly demonstrated that human prostate cancer cells implanted into the prostate of athymic nude mice were more tumorigenic and metastatic compared to the same cells implanted subcutaneously. Bone provides extremely "fertile soil" for cancer cells since a variety of growth factors that are stored in bone matrix are readily released in their active form into the bone microenvironment during the process of physiologic bone remodeling<sup>28,29</sup>.

### Bones have it all!

Obviously, bone tissue has characteristics that can facilitate metastatic prostate tumor cell growth including a favorable vascular network in the axial skeleton which features considerable blood supply, slow circulation, thin sinusoidal walls, and a direct venous system from prostate to bone. The skeletal sites affected with metastatic tumor growth are also known for their metabolism and turnover during which many growth factors and cytokines are locally released providing an excess of nutrients that can help survival, growth and

spread of the cancer cells<sup>28-30</sup>.

Even though in the majority of cancer of the prostate patients (CaP) with bone metastases, serum markers of bone resorption and bone formation are elevated, the primary feature of prostate carcinoma is osteosclerotic bone metastases. The classic radiological appearance of a bone metastasis in prostate carcinoma patients is an osteosclerotic lesion secondary to osteoblastic bone formation<sup>31-33</sup>. Bone sites with prostate carcinoma metastases often display a distinctive osteoblastic reaction, characterized by high bone turnover rates with increased osteoid surface, osteoid volume, and mineralization rates<sup>34,35</sup>. Hypotheses explaining the predominantly osteosclerotic nature of CaP describe many osteoblast stimulating factors produced by cancer cells including peptides with selective mitogenic activity for osteoblasts including urokinase-type plasminogen activator (uPA), transforming growth factor beta (TGF- $\beta$ ), insulin-like growth factor I (IGF-I), fibroblast growth factor (FGF), bone morphogenetic protein 6 (BMP-6), and endothelin 1 (ET-1)<sup>36-38</sup>. In addition, it has been proposed that the process of bone resorption releases growth factors that allow proliferation and differentiation of cells of osteoblastic lineage<sup>39-41</sup>. We hypothesize that increased bone resorption observed only at the surfaces of an "old" bone also provides an additional source of the calcium required for rapid and extensive mineralization of the newly formed bone<sup>42</sup> (Figure 1). Some of the candidate molecules produced by cancer cells that could activate osteoclastic bone resorption include parathyroid hormone related protein (PTHrP), interleukins 1, 6 and 11 (IL-1, -6, -11), tumor necrosis factor alpha and beta

(TNF- $\alpha$ , TNF- $\beta$ ), and transforming growth factor alpha and beta (TGF- $\alpha$ , TGF- $\beta$ ). Parathyroid hormone-related protein (PTHrP) was originally discovered as a product of tumors that produce hypercalcemia and PTHrP has subsequently been demonstrated to be a product of many normal and malignant tissues, including prostate carcinoma<sup>43</sup>. PTHrP production by breast carcinoma is very common, occurring in 50-60% of cases with an even higher incidence rate when the patient is hypercalcemic<sup>44</sup>. In addition to a well-established role in breast cancer, recent studies also suggested that PTHrP could have a similar role in the development of bone metastases in patients with prostate cancer<sup>45-48</sup>. Unfortunately, there is very little experimental data available to elucidate the mechanisms of osteoblastic bone metastases. One reason is the lack of a dependable animal model that can reproducibly develop osteosclerotic lesions following inoculation with human prostate cancer cell lines like the one presented in Figure 1 depicting histological appearance of bone changes following intra-osseous administration of human prostate cell line CWR22 in nude rats<sup>42</sup>. Although naturally occurring prostate carcinoma has been reported in some canine<sup>49</sup> and rodent<sup>50-52</sup> species, these species do not demonstrate the bone metastasis seen as a primary feature in the human disease and therefore the use of these models remained limited. Therefore, predictive animal models that model disease conditions in humans are needed for developing and screening drugs, antibodies and tumor biomarkers as agents for treatment, and detection and monitoring of patients with metastatic prostate carcinoma.

### Skeletal consequences of anticancer therapy

Prostate-specific antigen (PSA) monitoring has created a huge shift in the population of patients in whom androgen ablation is initiated. PSA is expressed in more than 99% of all prostate cancers, and is considered a very reliable marker for monitoring progress of the disease and effectiveness of therapy<sup>53</sup>. Diagnosis of recurring disease following local therapy is made on the basis of an increase in PSA levels. Patients diagnosed prior to metastasis of the tumor based on PSA levels have a median life expectancy of 10-15 years, which is in sharp contrast with patients who present with metastatic bone disease (3-5 years). It is widely believed that all prostate cancers in humans are androgen-dependent in their early stage, with acquired independence resulting in therapy resistance. Patients are treated with androgen ablation when PSA levels begin to rise, even though there is substantial uncertainty with regard to the benefit of initiating treatment this early. The impact of long-term androgen ablation on quality of life is very high and includes impotence, hot flashes, depression, mood changes, anemia, obesity, muscular atrophy and osteoporosis. In one study at 9 years follow-up, 50% of the men who had undergone androgen ablation experienced an osteoporotic fracture compared to only 10% of the group that did not receive androgen ablation<sup>54</sup>. Muscular atrophy and inactivity are contributing factors to bone loss and increased inci-

dence of fractures. In nearly all prostate cancer patients who die from the disease, prostate cancer progressed despite initial treatment with androgen ablation therapy. Managing hormone refractory prostate carcinoma remains a difficult challenge for clinicians and patients. In the past, cytotoxic chemotherapy was considered inactive, but recent advances have altered this view and there have been some promising data with secondary hormonal therapies such as casodex, prednisone, ketoconazole and diethylstilbestrol. Some cancer therapies can contribute to bone loss and create so called "cancer osteoporosis"<sup>55</sup>. As discussed earlier, evidence exists that increased bone resorption may be a facilitating factor in spreading prostate carcinoma to bones, and it is not clear at this time whether or not androgen ablation and subsequent increase in bone resorption further facilitate survival and progression of the cancer cells. The novel nitrogen-containing bisphosphonates have shown some promising results in curbing lytic bone metastases in breast cancer<sup>56,57</sup>, myeloma<sup>58</sup>, and prostate cancer patients<sup>59,60</sup> and could have additional anti-tumor effect due to inhibition of prenylation<sup>61</sup>. Future novel therapeutic approaches targeting the prostate carcinoma-bone stroma interaction could include interference with growth factors/growth factor receptor, ligand-dependent as well as ligand-independent androgen receptor, and extracellular matrix-integrin signaling pathways and their downstream effector molecules. Predictive animal models that model disease conditions in humans are highly desirable tools for developing and screening drugs, antibodies and tumor biomarkers as agents for treatment, detection and monitoring of patients with metastatic prostate carcinoma.

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