Prostate Cancer in Bone: Importance of Context for Inhibition of Matrix Metalloproteinases

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Prostate cancer is the second most common cause of cancer death in American men. Once metastasis has occurred, there is no curative treatment, and the search for effective therapies for prostate cancer and, in particular, for metastasis to bone is hampered by a lack of suitable animal models of the disease.

In this issue of the Journal, Nemeth et al. *(1)* address aspects of these two difficult issues. They describe the use of the human fetal bone/severe combined immunodeficient (SCID) mouse model to explore the role of matrix metalloproteinases (MMPs) in metastasis of prostate cancer to bone and the ability of the broad-spectrum MMP inhibitor batimastat to reduce bone disease.

Most research into human prostate cancer uses just three cell lines, PC3, DU-145, and LnCaP. After subcutaneous inoculation into immunocompromised mice, these lines grow as tumors but do not metastasize. Orthotopic inoculation can result in some limited metastasis, but still there is the unresolved issue of human tumor tissue in a murine host. The mouse prostate reconstitution model is a step forward, in that normal prostate epithelial cells are transformed with myc and/or ras, mixed with normal mesenchymal cells, and inoculated into the renal capsule of recipient mice *(2).* These mice develop metastatic lesions in the lung, liver, mesentery, and bone. Elegant as the model is, however, this is an artificial transformation. Like all other models where single or multiple oncogenes are overexpressed, the events do not necessarily mimic the complex processes leading to prostate or other cancers.

Cher and colleagues *(3)* used a different approach. SCID mice were implanted with fragments of fetal human bone before the introduction of human prostate cancer cell lines intravenously or directly into the human bone. Tumors developed in the human bone cavity but not in the mouse skeleton. A similar system involving non-obese diabetic (NOD)/SCID mice implanted with adult human bone fragments has also been described recently *(4).* While these models can be used to explore prostate cancer growth potential in bone, they are not useful for exploring the metastatic events that result in tumor cells establishing in the bone environment. Tumor cells can colonize the human bone fragment after either intravenous injection or direct injection into the bone fragment but not after growth of a primary tumor. The models do, however, avoid the incompatibility issue of human tumor cells in murine stromal tissue. A final point that distinguishes the few existing prostate cancer models from the real disease is the fact that PC3 and DU-145 tumors are osteolytic, whereas those of LnCaP are both osteolytic and osteoblastic *(3).* The metastases of human prostate cancer are predominantly osteoblastic or mixed blastic and lytic.

Nemeth et al. *(1)* demonstrate that batimastat, a broadspectrum MMP inhibitor, can prevent the loss of mineralized bone and can reduce the number of osteoclasts recruited to the bone surface after injection of PC3 cells directly into the human bone marrow. Batimastat treatment was accompanied by a reduction in the proportion of proliferating PC3 cells but this was not obviously due to increased apoptosis or reduced angiogenesis.

In vitro, batimastat did not affect the proliferation rate of PC3 cells. Although the mechanism for this batimastat-induced reduction in tumor growth is unclear, it points toward a growthenhancing interaction between the tumor cells and bone marrow stromal cells and/or release of growth factors from the bone matrix that is blocked by inhibition of MMPs *(1).* It is increasingly clear that the response to chemotherapy is dictated not only by the stage of the disease and the phenotype of the tumor cells but also by the context in which the tumor cells find themselves *(5,6).* The study by Nemeth et al. *(1)* is important because it addresses the treatment of the tumors (albeit the PC3 cell line) in the correct context (the human bone).

MMPs are a family of more than 20 members of zincdependent extracellular or embrane-bound proteases that have roles in tissue remodeling and repair and have been implicated in pathologic conditions including tumor growth, invasion, and metastasis *(7).* MMPs are usually expressed by stromal cells, but their expression is elevated in response to tissue damage or the presence of tumor cells. MMPs are also expressed at high levels in many tumors, potentially providing two sources of activity during local invasion and metastasis of the tumor *(8).* Tumor cells are believed to use the pericellular degrading activity of these enzymes to spread to distant sites *(9),* possibly aided by transient or permanent epithelial-to-mesenchymal transition *(10).* Therefore, inhibition of MMP activity has long been seen as an attractive goal for cancer therapy *(11).*

Cancer cells generally show a selective nonrandom pattern of metastasis *(6,12).* For reasons not yet understood, prostate and breast cancers have a strong predilection for spreading to bone *(13–15).* There are two general theories to explain why metastases are not randomly distributed in all tissues. There is, of course, the classical theory of seed and soil put forth by Paget *(16),* which is supported by the present work. Thus, one can imagine that these tumor cells disseminate widely but can grow avidly in bone because the environment provides a favorable soil. What then is the nature of this favorable soil? It appears from the study by Nemeth et al. that prostate or breast tumor cells interact directly with bone cells to activate specific MMPs *(1,17).* Activated MMPs can be one of the important players of the vicious cycle existing between bone matrix turnover and tumor cell growth *(1,18).* The seed-and-soil hypothesis is further strengthened by the observation that PC3 cells injected intravenously colonize the human bone fragment but not the mouse skeleton *(3).* The concept put forward more recently *(19)* that endothelial cells express tissue-specific adhesion molecules that selectively trap those tumor cells that express the correct ligands is a variation on this theme. The second general idea was presented by Muller et al. *(20),* who showed that lung and bone stromal cells secrete a chemokine, CXCL12, and that many breast tumor cells express high levels of the matching chemokine receptor CXCR4. By neutralizing CXCR4 activity with antibodies, they were able to block metastasis to the lung.

The use of MMP inhibitors in both *ex vivo* and *in vivo* studies has shown promising results and has led to the design of clinical trials. Regrettably, the results of the phase III trials have been disappointing because little or no appreciable clinical efficacy has been demonstrated. These studies, however, do not yet exclude MMP inhibitors as potent cancer therapeutics *(9,21,22).* Indeed, in light of the clinical and preclinical studies, the

future clinical trial designs need to consider the following points: 1) MMP inhibitor use in earlier stages of the disease; 2) selection of patients for clinical trials based on analysis of tumor phenotype, especially MMP expression pattern; and 3) emphasis on inhibitor use in combination with other anticancer compounds (cytotoxic drugs) *(21–23).* Thus, more serious attention needs to be given to therapies that treat both the tumor and its context *(5).* For example, MMP inhibitors could be combined with antibodies neutralizing CXCR4 activity to treat metastasis to bone *(20),* if a similar chemoattraction mechanism is found for prostate cancer. The advantage of selective combination therapies is that severe toxicity to host normal tissues may be minimized. Clearly, much remains to be considered and done. Meanwhile, these new models are a step forward toward mimicking the human disease and, therefore, enabling the search for more effective therapies.

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