# COMMENTARIES

# Awakened From Sleep: Dormancy in Stem Cells and Bone Metastases

**Gordon J. Strewler**

*Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA*

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*If I didn't wake up, I'd still be sleeping. —Yogi Berra*

Metastasis to bone requires a series of steps. Before they take up residence in bone, tumor cells must invade the matrix surrounding the primary tumor, intravasate into the blood, attach to the endothelium of bone marrow capillaries, and extravasate from capillaries (1;2). But, the arrival of tumor cells in bone defines the beginning of the metastatic process, rather than its end. In breast and prostate cancer, tumor types that characteristically spread to bone, tumor cells arrive in bone marrow early in the course of the disease and lie dormant there for many months, or even years, before beginning to grow, invade, and destroy the bone. Yet, the nature of dormancy in bone is very poorly understood. What characterizes the dormant niche? How do cells enter it? How do they finally awake from dormancy to begin dividing and produce a clinically evident metastasis?

Breast cancer cells are often found in bone marrow at the time of primary surgery, and their presence predicts a poor prognosis (3;4). In one study, prostate cancer cells were detected in the bone marrow of 54% of men before prostatectomy and 33% of men soon after prostatectomy (5). Studies using animal models support the conclusion from clinical studies that colonization of bone occurs early and with high efficiency; in contrast, subsequent steps in experimental metastasis are completed inefficiently (2). Even if tumor cells are delivered intravenously or by direct intramedullary injection, the development of bone metastasis is remarkably inefficient.

Dormant tumor cells at peripheral sites may exist as single cells or micrometastases. Using fluorescent markers, single quiescent mammary carcinoma cells can be identified at metastatic sites (*e.g.*, liver and lung), recovered, and shown to be viable (6;7). In one of these studies, a "nonmetastatic" breast cancer clone dispersed large numbers of single dormant cells to lung, but did not form tumors; a "metastatic" clone also dispersed large numbers of solitary dormant cells, but these coexisted with a smaller number of actively growing metastases (7). Dormant tumors can also consist of preangiogenic micrometastases, in a state where proliferation and apoptosis are balanced in the absence of angiogenesis, only to be switched to active proliferation by the ingrowth of tumor vessels (8). Cells that ultimately metastasize are those that can adapt to a dormant niche and acquire the capacity to wake up.

Analogies can be drawn between tumor cells and tissue stem cells; both are capable of quiescence, self-renewal, and proliferation (9). Several recent papers suggest that study of the hematopoietic stem cell (HSC) niche in bone marrow may yield insights into the nature of tumor cell dormancy. Genetic approaches to increasing osteoblast number show that osteoblasts govern the size and location of

the stem cell niche. Using a model of conditional ablation of the *Bmpr1a* gene, Zhang *et al.* (10) showed that osteoblast number determines the size of the HSC niche and that slowly cycling HSCs are characteristically in contact with a population of spindle-shaped osteoblastic cells on the surface of ectopic trabecular bone. Calvi *et al.*, (11) using a model in which the osteoblast population was increased by overexpression of the PTH/PTHrP receptor, showed that increased osteoblast number was correlated with increased HSC number (12) and that the Jagged-Notch signaling pathway, which has been shown to regulate the self-renewal of HSCs (13), was implicated in the effects of the PTH receptor.

Arai *et al.* (14) have now examined the determinants of quiescence within the stem cell niche. They found that HSCs that express the receptor tyrosine kinase Tie2 are quiescent cells adherent to osteoblasts. An interaction between Tie2 and its ligand angiopoietin-1 induced adhesion and flattening of HSCs to form a cobblestone pattern on the osteoblast surface and also enhanced their exit from the cell cycle. Quiescent HSCs were resistant to myeloablation with 5-fluorouracil. If angiopoietin-1 expression was increased by injection of recombinant human angiopoietin-1 or expression of angiopoietin-1 from an adenovirus vector, mice were more resistant to myeloablation.

These studies define an HSC niche in which osteoblasts regulate both self-renewal, by Jagged-Notch signaling, and quiescence, by angiopoietin 1-Tie2 signaling. The HSC niche can be defined either architecturally or in molecular terms. HSC can be converted to a dormant state by a specific receptor-ligand interaction, and dormancy can contribute to resistance to chemotherapy. Can a similar niche be defined for cancer cells in bone?

Experimentally, tumor cells can be marked with green fluorescent protein to identify single dormant cells or micrometastases. Certain human breast cancer cell lines display a long latency to metastasis, even after intracardiac injection delivers them directly to bone, suggesting that they may survive a period of dormancy before initiating growth. The ultimate ability of ZR-75-1 breast cancer cells to form metastases is determined by the endothelin-1 receptor, which is expressed on osteoblasts, but not on tumor cells themselves, suggesting a complex relationship between dormancy, survival, and growth in an osteoblast-associated tumor cell niche (15).

Human prostate cancer probably undergoes two distinct periods of dormancy in bone. Following radical prostatectomy, men are often free of clinical metastasis with undetectable levels of the tumor marker prostate-specific antigen for years before recurrence, consistent with prolonged dormancy. Androgen ablation usually induces remission in recurrent prostatic carcinoma, but the remission invariably ends with the development of androgen-insensitive metastases (16). Thus, prostate cancer cells can enter dormancy and leave it in either an androgen-replete or androgen-deprived environment.

The example of HSC illustrates how a niche can be defined and manipulated. Tools are now available to study the tumor cell niche in bone, both by immunochemistry of patient samples and in animal model systems. Given the efficiency of tumor targeting to bone and the refractory nature of frank metastases, understanding how tumor cells fall asleep and awaken in bone may be a key to treatment of metastatic disease.

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