

MEETING REPORTS

Cancer Stem Cells and Bone, and Cancer Cell Dormancy and Bone Metastasis: Meeting Report from Skeletal Complications of Malignancy V

October 25-27, 2007 in Philadelphia, Pennsylvania, USA

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Cancer Stem Cells and Bone (Session 1)

The recognition of cancer stem cells is changing cancer biology. Leukemia stem cells, for example, arise from hematopoietic stem cells (HSCs) and display all the properties of adult stem cells: they give rise to multiple types of differentiated progeny and are capable of self-renewal, e.g., passage through multiple generations of NOD/SCID mice (1;2). Their biology largely determines the biology of leukemia; for example, stem cells evade therapies directed at rapidly proliferating leukemia cells because they are largely quiescent. Solid tumor stem cells have been harder to identify – and whether they are immutable as HSCs are thought to be or plastic remains controversial (3) – but there is now good evidence for stem cells in breast cancer (4) and brain tumors, and some evidence for stem cells in lung and prostate cancer. The focus of the session was on cancer stem cells and bone metastasis.

The conceptual model for cancer stem cells is the normal adult stem cell, a self-renewing population that gives rise to all the differentiated cell types in a given tissue (5). The simplest mechanism for self-renewal of a stable stem cell population is asymmetric division, in which each mitosis of a stem cell gives rise to one daughter cell and one new stem cell. Asymmetric division can be determined by cell-autonomous properties of the stem cell – there are few examples in mammalian biology but one is basal cells of the epidermis (6) – or by residence of stem cells in a niche that determines stemness such that, in each asymmetric division, one

daughter cell remains in the niche as a stem cell and the other leaves the niche to proliferate and differentiate.

Buck Stewler opened the meeting by proposing the hypothesis that pioneer tumor cells that establish bone metastases from solid tumors are authentic cancer stem cells whose stem cell properties are not cell-autonomous but are determined by residence in a cancer stem cell niche in bone (7). Like HSCs, cancer cells home to bone using selectins to identify the bone microvasculature, extravasate through the sinusoidal endothelium, and are directed by binding of CXCL12 (SDF1) to CXCR4 receptors on the cancer cells (8) to specific niche environments, where they take up residence in a perivascular or an endosteal niche, similar to the location of HSC niches. Pioneer tumor cells can give rise to a variety of differentiated progeny and, like normal adult stem cells, can be quiescent for years.

Max Wicha presented new data on breast cancer stem cells (9). His group has characterized normal mammary stem cells from reduction mammoplasties as capable of forming clusters called mammospheres during anchorage-independent growth; these cells can reconstitute a human breast in a “humanized” mouse in which irradiated human fibroblasts are implanted into the mammary fat pad. The enzyme aldehyde dehydrogenase (aldh) is characteristic of several types of stem cells and can be used in an easy fluorescence assay (Aldefluor®) to identify living stem/progenitor cells. His group has previously identified CD44⁺CD24^{low}Lineage⁻ cells as tumorigenic human

breast cancer cells capable of serial passage in NOD/SCID mice (4) and he showed that Aldefluor identified an overlapping set of stem-like cells from human breast cancers. In tissue arrays $aldh^+$ cells are found at the periphery of tumors and display microinvasion, suggesting that they could be poised for metastasis; the number of $aldh^+$ cells in tumor sections is correlated with survival. Finally, an $aldh^+$ population of MDA-MB453 cells metastasizes to bone after intracardiac injection, suggesting that this established cell line harbors stem-like cells that home to bone.

Laura Calvi related the story of the endosteal HSC niche (10). Mice that express a constitutively active PTH1 receptor in osteoblasts have increased hematopoiesis as well as increased numbers of osteoblasts, and stromal cells treated with PTH are better able to support HSCs *in vitro* than WT cells (11;12). Osteoblastic cells expressing the constitutively active PTH1R produced high levels of the Notch ligand, Jagged1, and supported an increase in the number of HSCs, with evidence of Notch1 activation in stem cells *in vivo*. This is among the strongest evidence that osteoblasts are an essential part of an endosteal HSC niche. More recent data show that PTH increases expression of both Jagged1 and the cytokine CXCL12, consistent with a role for these cytokines in the niche.

Catherine Alix-Panabieres (13) described a new enzyme-linked immunospot assay to screen for expression of tumor antigens and showed that bone marrow taken from the iliac crest of breast cancer patients reveals a fingerprint of specific secreted proteins. Cytokeratin 19 was detectable in 54% of patients without overt metastases and 90% of patients with metastatic disease. Subsets of cells expressed a breast stem cell-like phenotype (mucin-1/cytokeratin-19⁺).

Important questions remain: Do cancer stem cells arise from normal tissue stem cells, in the same way that leukemia stem cells arise by oncogenesis of HSCs, or can they arise by events that confer stemness on other cancer cells? How plastic is this state? Is

there a niche that determines asymmetric division of cancer cells, à la HSCs? Stromal fibroblasts derived from mesenchymal stem cells play an important role in instructing cancer cells to metastasize (14;15); do they constitute part of a cancer stem cell niche? Perhaps stromal cells or osteoblasts in bone serve a similar function.

Can we find stem cells in our favorite cancer cell lines, which are clonal and selected for the property of self-renewal in cell culture? Wicha's results, as well as other data published since the meeting (16) suggest that the answer is yes. An attractive alternative, much needed in the field of bone metastasis, is to develop models of spontaneous metastasis from orthotopic sites. For example, a model of spontaneous lung metastasis to bone and soft tissues was created by removing the Peutz-Jeghers tumor suppressor gene (LKB) from lung carcinoma cells by crossing *Lox-Stop-Lox* *Kras*^{G12D} x *LKB1*^{L/L} mice (17). Inhaled *cre* excises the stop sequence from the *Kras* oncogene in the lung and this leads to development of pulmonary adenocarcinomas that metastasize to bone.

Cancer Cell Dormancy and Bone Metastasis (Session 2)

Klaus Pantel opened the session by reviewing the wealth of data from many laboratories on isolation of cells from micrometastases in bone marrow. His group has shown that the detection of micrometastatic cells is an independent risk factor for relapse, suggesting that the pool of disseminated tumor cells may include metastatic stem cells. Most disseminated tumor cells in early stage breast cancer have a breast stem cell phenotype, *CD44*⁺/*CD24*⁻ (18) or *mucin-1*/*cytokeratin-19*⁺ (19). Metastatic cells in bone marrow are in a nonproliferative or dormant state and escape chemotherapy. Mechanisms controlling dormancy are poorly understood, however. Pantel suggested at least four potential factors that may control the escape from dormancy: amplification of *Her2/neu* (present in 38% of circulating tumor cells); accumulation of *p53* mutations; accumulation of genetic imbalances; and the response to stem cell growth factors (EGF

and FGF) that are available in the bone marrow microenvironment.

Robert Vessella (20) reported his work on circulating and disseminated tumor cells in prostate carcinoma. Patients with no evidence of disease after radical prostatectomy have a 50% incidence of disseminated tumor cells within one year after radical prostatectomy and this confers a 7-fold increased risk of recurrence. Of these cells, 70% have genomic alterations compared with preprostatectomy samples, using the technique of comparative genomic hybridization, indicating the accumulation of genetic changes over time. Gene profiling can be used to distinguish low risk, high risk and advanced patients. In preprostatectomy DTC there is reduced expression of prostate epithelial markers such as PSA, suggesting an epithelial-to-mesenchymal transition. In advanced disease DTC revert to an epithelial phenotype.

Heparan sulfates and heparan sulfate-binding growth factors are important in tumor biology, and Larry Suva (21) described studies of overexpression of heparanase in the MDA-MET strain of highly metastatic breast cancer cells. Heparanase-1 expression improved growth *in vivo*, with increased numbers of osteoclasts and increased excretion of NTX. This may be a combined result of promoting tumor invasion through the extracellular matrix by breakdown of the heparan sulfate barrier and increased osteoclastogenesis by bioactive fragments of heparan sulfate. Similar results have been reported in multiple myeloma. Suva also reviewed his data on IL-8 as a RANKL-independent bone resorbing cytokine that is produced by prostate, breast and lung cancer cells.

Further studies of disseminated breast cancer cells from bone marrow were summarized by Rebecca Aft (22). Bone marrow cells enriched for expression of EpCAM by immunomagnetic beads expressed a set of transcripts including *TWIST1*, a gene associated with metastasis. In an independent set of samples, *TWIST1* gene expression was correlated with clinical outcome.

Conflict of Interest: None reported.

Peer Review: This article has been peer-reviewed.

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