J Musculoskel Neuron Interact 2004; 4(4):381-382

Hylonome

Perspective Article

Summary - Bone Metastasis

Session Chair: H.J. Donahue

Penn State University, M.S. Hershey Medical Center, Hershey, PA, USA

The most lethal attribute of a cancer cell is its ability to metastasize. In patients with bone metastasis complications may be manifested in osteolysis, spinal cord compression, hypercalcemia, increased fracture incidence and unrelenting pain[1]. Furthermore, patients with bone metastasis are seldom cured. Clearly, the prevention of metastasis should, at the very least, improve the quality of life for the patient and may increase survival rates. Unfortunately, an incomplete understanding of cancer metastasis to bone has hindered development of effective treatments specifically targeting metastasis[2]. In this session five speakers offered diverse perspectives on how best to identify novel therapeutic targets for treating bone metastasis.

The first speaker was **Tatiana Byzova, Ph.D**. from the Lerner Research Institute, Cleveland Clinic Foundation. Dr. Byzova discussed the role of bone sialoprotein (BSP) and $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins in prostate cancer cell metastasis to bone. Dr. Byzova presented data suggesting that recognition of BSP is regulated by the activation state of $\alpha_v\beta_3$ integrin. Stimulation of lymphocytes or osteoblasts with model agonists markedly enhances $\alpha_v\beta_3$–dependent cell adhesion to BSP. Dr. Byzova has also demonstrated that tumor cell migration to SPARC is mediated by $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins and is controlled by an autocrine loop in which VEGF engages VEGFR-2. Her group found that SPARC is a key protein that attracts prostate cancer cells to bone. SPARC-deficient bone extracts support minimal migration of prostate cancer cells and addition of purified SPARC restores the rate of cell migration. Additionally, both bone matrix proteins, BSP and SPARC, are able to influence VEGF signaling in tumor cells. BSP, purified SPARC and recombinant SPARC increased VEGFR2 expression. Adhesion of prostate cancer cells to SPARC also induced VEGF production, and this increase was completely inhibited by anti-$\alpha_v\beta_3$ and $\alpha_v\beta_5$ blocking peptide cRGDfv. Importantly, the upregulation of VEGF production by SPARC via $\alpha_v\beta_3$ and $\alpha_v\beta_5$ is a prostate cancer specific phenomenon, which provides prostate cancer cells with significant growth advantage in bone tissue. It was concluded that the integrin-SPARC-VEGF axis might have potential as a therapeutic target for bone metastasis.

Paul J. Kostenuik, Ph.D. from Amgen, Inc. next revisited the seed and soil theory of bone metastasis promulgated by Stephen Paget. Dr. Kostenuik discussed findings from other laboratories suggesting that very high concentrations of anti-resorptive agents such as bisphosphonates can reduce tumor burden in animal models. The inhibition may result from decreased osteoclastic bone resorption and decreased release of extracellular matrix sequestered growth factors that may contribute to tumor growth. There is also evidence that bisphosphonates can have direct cytotoxic effects on tumor cells. However, Dr. Kostenuik contends that it is unlikely that tumor cells within the bone of cancer patients are exposed to concentrations high enough to be cytotoxic. Thus, it is difficult to disassociate the therapeutic benefit of the anti-resorptive properties of osteoclasts from their potential cytotoxic effects. Osteoprotegerin (OPG) is anti-resorptive, by neutralizing RANKL, but is not cytotoxic. This allows one to directly assess the role of an anti-resorptive agents on metastasis. Dr. Kostenuic described data suggesting that OPG treatment reduces osteoclast numbers and bone resorption in nude mice that were injected (intracardiac) with human MDA-MB-231 breast cancer cells. This anti-resorptive effect was associated with complete suppression of tumor-associated osteolysis and a significant reduction in skeletal tumor burden[3]. In head-to-head studies using the MDA-MB-231 model, OPG suppresses skeletal tumor burden to an extent that was similar to that observed with a very high dose of zoledronic acid (5 mg/kg). OPG also prevents osteolysis and suppresses skeletal tumor burden in normal mice injected intracardiac with C-26 murine colon adenocarcinoma cells[3]. OPG treatment has no influence on the growth of C-26 cells but causes suppression of skeletal tumor burden within bone[4]. This further suggests that the localized reduction of skeletal tumor burden in OPG-treated animals is an indirect consequence of bone resorption suppression.

Sarah Mosely, Ph.D. of Genentech discussed the role of TGF-β and TGF-β antibodies in different mouse models of metastasis. The central role of TGF-β in bone development and turnover strongly suggests it would be a good therapeutic target for bone metastasis. TGF-β has been demonstrated to directly

The author has consultancies with OrthoLogic and Merck.

Corresponding author: Henry J. Donahue, Ph.D., Penn State University, M.S. Hershey Med. Center, 500 University Drive, P.O. Box 850, Hershey, PA 17033, USA
E-mail: hdonahue@psu.edu

Accepted 4 August 2004

modulate tumor cell growth, differentiation and migration. The group at Genentech has explored the use of antibodies to TGF-β on the development of lung and bone tumors. Dr. Moseley and her colleagues tested anti-TGFβ antibodies in syngeneic, immunocompetent mice using cell lines derived from spontaneous tumors or oncogene-driven tumors. Quantitative endpoints were used to measure tumor number and size as well as destruction of soft and hard tissues. Using these animal models, the Genentech group found that inhibition of TGF-β has distinct effects on primary tumors and secondary lung and bone tumors in a manner related to the nature of the animal model. Thus, this research group's models have allowed them to distinguish between the various tumor-associated biological activities of TGF-β.

Dr. Moseley's presentation was followed by that of **Yibin Kang, Ph.D**. from Memorial Sloan-Kettering Cancer Center. Dr. Kang described work wherein metastatic MDA-MB-231 cells are selected *in vivo* for their metastatic potential to bone. Dr. Kang's group has isolated several subpopulations of MDA-MB-231 cells with enhanced metastatic ability to bone. Transcriptomic profiling was utilized to identify gene sets whose expression pattern is associated with, and promotes the formation of metastasis to bone but not other tissues. Cells with the bone metastasis gene profile are present in the parental population and become selected *in vivo* as highly metastatic entities. Many genes in this group encode secretory or cell surface proteins implicated in cell homing to bone, angiogenesis, invasion and osteoclast recruitment, thus influencing the tumor microenvironment in favor of metastasis. When overexpressed, these genes promote osteolytic bone metastasis by acting cooperatively. Two of these genes, interleukin-11 and CTGF, encode osteolytic and angiogenic factors whose expression is further increased by the bone-derived prometastatic cytokine TGF-β. Inhibition of TGF-β signaling by stable siRNA-mediated repression of Smad4 blocked the positive feedback of bone matrix to tumor cells and inhibited formation of bone metastasis. Dr. Kang's findings provide a conceptual framework and an experimental system for the identification of genes mediating metastasis to different organs[5].

The final presentation was by **Henry J. Donahue, Ph.D**. of Pennsylvania State University. Dr. Donahue discussed research focusing on the role of gap junctional intercellular communication (GJIC) in breast cancer metastasis to bone. Dr. Donahue's group has demonstrated that the metastatic breast cancer cell line MDA-MB-435 (435) expresses a different repertoire of gap junction proteins, connexins (Cx), than do non-metastatic breast epithelial cells or 435 cells expressing the metastasis-suppressing gene BRMS1. 435 are Cx32 positive and Cx43 negative whereas non-metastatic breast epithelial cells and metastasis suppressed cells are Cx32 negative and Cx43 positive[6]. Additionally, metastatic cells display greater heterotypic GJIC with bone cells than homotypic communication with themselves. On the other hand, metastasis suppressed breast cancer cells display abundant homotypic GJIC with themselves but little heterotypic communication with bone cells[7]. Interestingly, when Cx43 negative 435 cells are genetically engineered to express Cx43, Cx32 expression decreases as does that of osteopontin, an extracellular matrix protein associated with increased cancer cell metastasis to bone. Importantly, Cx43 expressing 435 cells are less metastatic to lung than are vector controls. Taken together, these data strongly suggest GJIC contributes to breast cancer metastatic potential.

In summary, the five presentations during this session emphasize the progress being made in understudying bone metastasis. However, what also emerged is that this area of cancer biology is relatively understudied and would benefit from an increased effort focused on cancer cell behavior in, and interaction with, the bone microenvironment. Important areas to be addressed include:

- The role of inter and extracellular matrix proteins in attracting cancer cells to the bone microenvironment.
- The relative contribution of anti-resorptive agents, including bisphosphonates and OPG agonists, to reducing tumor burden.
- The role of TGF-β and other growth factors sequestered in the bone extracellular matrix in bone metastasis.
- The use of genomics, proteomics and metabolomics in identifying genes, proteins and pathways, which contribute to bone metastasis.
- The nature and consequences of heterotypic interactions between cancer cells and bone cells.

References

1. Mundy GR. Mechanisms of bone metastasis. Cancer 1997; 80:1546-1556.
2. Woodhouse EC, Chuaqui RF, Liotta LA. General mechanisms of metastasis. Cancer 1997; 80:1529-1537.
3. Morony S, Capparelli C, Sarosi I, Lacey DL, Dunstan CR, Kostenuik PJ. Osteoprotegerin inhibits osteolysis and decreases skeletal tumor burden in syngeneic and nude mouse models of experimental bone metastasis. Cancer Res 2001; 61:4432-4436.
4. Capparelli C, Kostenuik PJ, Morony S, Starnes C, Weimann B, Van G, Scully S, Qi M, Lacey DL, Dunstan CR. Osteoprotegerin prevents and reverses hypercalcemia in a murine model of humoral hypercalcemia of malignancy. Cancer Res 2000; 60:783-787.
5. Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordon-Cardo C, Guise TA, Massague J. A multigenic program mediating breast cancer metastasis to bone. Cancer Cell 2003; 3:537-549.
6. Saunders MM, Seraj MJ, Li Z, Zhou Z, Winter CR, Welch DR, Donahue HJ. Breast cancer metastatic potential correlates with a breakdown in homospecific and heterospecific gap junctional intercellular communication. Cancer Res 2001; 61:1765-1767.
7. Kapoor P, Saunders MM, Li Z, Zhou Z, Sheaffer N, Junze EL, Samant RS, Welch DR, Donahue HJ. Breast cancer metastatic potential: correlation with increased heterotypic gap junctional intercellular communication between breast cancer cells and osteoblastic cells. Int J Cancer 2004; 111:693-697.