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MEETING REPORTS

Cancer and Bone: Meeting Report from the 3rd Joint Meeting of the European Calcified Tissue Society and the International Bone and Mineral Society

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Concepts of cancer metastasis are that in order to grow in distant organs, cancers need special properties to suit them to those organs. Derived as it is directly from Stephen Paget's "seed and soil" hypothesis from the end of the 19th century, this modern view is that the environment of bone is remarkably hospitable for certain solid cancers such as those of the breast, prostate and lung, and also for hematological malignancies. At the 3rd Joint Meeting of the European Calcified Tissue Society and the International Bone and Mineral Society in Athens, Greece, this subject received considerable attention. Importantly, also at this major bone meeting there was much reported in basic bone biology that is very relevant to mechanisms of skeletal complications of cancer.

In an opening paper at the meeting, David Scadden (Boston, USA) described experiments seeking to identify the source of cells capable of replicating sufficiently to replenish the osteoblast lineage. In genetically engineered mice, when cells were labelled through the osterix or collagen type 1 2.3 promoters they did not replicate, but labelling through the Mx-1 promoter resulted in abundant replenishment of the osteolineage *in vivo*. This work is important for bone cell biology and crucial for the effort to identify what cells in the osteoblast lineage are those that are most important in favoring normal and malignant hemopoietic stem cell growth and survival. Published studies show that myeloproliferative disease and leukemia can be transferred in mice in a manner that is bone microenvironment-dependent (*e.g.*, Walkley *et al.* (1)). Given the great current focus on the role of the microenvironment in the establishment and growth of solid cancers, *e.g.*, of the breast, prostate, lung, etc., this work on hematological malignancy will have much to offer our understanding of the important factors operating in bone to favor malignancy. The importance of the mesenchymal lineage in hematological malignancy is emphasized by the evidence presented that some stage of that lineage can induce changes in the hemopoietic lineage that can even result in development of frank leukemia. This leads to the hypothesis presented that certain stages of the mesenchymal lineage can serve as an initiating "hit" in the process of oncogenesis.

With that introduction, reviews and new data on multiple myeloma were welcome. Aspects of the "myeloma niche", that microenvironment in bone that favors myeloma cell survival and expansion, were considered by Toshio Matsumoto (Tokushima, Japan). He emphasized the importance in this process of products both of malignant and of bone cells. For example, myeloma cells produce macrophage inflammatory protein-1 (MIP-1) that promotes RANKL production by stromal cells to increase bone resorption, as well as increase production of an integrin, VLA4, that binds to stromal cell VCAM-1. The net effect of this myeloma cell activity is to divert monocytes to osteoclasts at the expense of dendritic cells. Further feedback comes from osteoclasts producing both B cell-activating factor and a proliferation-inducing ligand (both are TNF family cytokine members), which are survival factors for myeloma cells. This new understanding of the biology of multiple myeloma and how it interacts with

hemopoietic cells owes much to discoveries of the last 10 years in bone cell biology. So too does the interaction of myeloma with osteoblasts and bone formation. Secreted frizzled-related protein-2 and Dickkopf-1 are both products of myeloma that inhibit bone formation by inhibiting Wnt signaling. Decreased bone formation is a prominent feature of myeloma bone disease, with a further mechanism proposed by Dr. Matsumoto pointing to the increased active TGF-β locally around myeloma cells, thus providing a further inhibitor of osteoblast differentiation and bone formation. Some promising data were reported showing that TGF-β inhibition using a TGF-β type 1 receptor kinase inhibitor could prevent the inhibited osteoblast activity and suppress myeloma growth.

Two further interesting aspects of myeloma pathogenesis came from the group of Claire Edwards (Oxford, UK and Nashville, USA). Based on the hypothesis that host matrix metalloproteinase-7 (MMP7) might contribute to myeloma progression, these investigators inoculated murine myeloma cells, 5TGM1 cells, into C57Bl mice that had been cross-bred with *Rag 2*(-/-) mice, yielding mice with myeloma bone disease identical to that in the C57Bl/KaLwRy strain. Most surprisingly, MMP7 deficiency resulted in enhanced myeloma growth and burden, suggesting that MMP7 has a protective role rather than an enhancing one. Further novelty came from the result of a microarray study showing increased circulating osteopontin in the MMP7-1-mice. The findings that full-length osteopontin induced myeloma cell growth, and that cleaved osteopontin induced myeloma cell apoptosis, led the group to propose an important role for host-derived MMP7, mediated by osteopontin, in inhibiting myeloma growth *in vivo*. Studies with osteopontin-deficient mice are awaited with great interest.

The same group extended their previous work showing adiponectin levels to be decreased in mice (KaLwRij) that are permissive for myeloma. In *in vivo* experiments they used a stimulator of adiponectin levels in C57Bl/KaLwRij mice (the alipoprotein peptide mimetic L-4F), finding that this resulted in significantly reduced growth of 5TGM1 myeloma cells, as well as reduced osteolytic bone disease and increased bone formation. Repeat of the study in KaLwRij mice deficient also in adiponectin showed no effect of the peptide mimetic. The conclusion that increased adiponectin inhibits myeloma is an important one, with the mechanism of this adiponectin action awaiting clarification, as well as the development of new enhancers of its production.

In the case of solid cancers, Marco Cecchini (Berne, Switzerland), working with prostate cancer and studying cancer stem cells, pointed out how the latter cells can remain dormant for years. During this time in cell cycle arrest and status, they are drug- and radiation-resistant. Treatment of prostate cancer spares these cells, but they can regenerate after the tumor shrinks, so a major question is whether this rare population of cancer stem cells can be targeted. He pointed to the “osteomimicry” of prostate cancer cells, by which they mimic osteoblasts with their osteoblast products, such as bone sialoprotein, osteopontin, cadherin-1, and osteocalcin, in ways that favor their growth in bone. This hypothesis that metastatic cells mimic osteoblasts is consistent with recent data indicating that prostatic cancer cells in bone “hijack” the hemopoietic stem cell niche (2), thus indicating that this niche serves as a target for prostate cancer cells and plays an important role in bone metastasis.

Having shown previously that vitamin D deficiency enhances breast cancer growth in bone as a consequence of increased bone resorption (3), the group of Markus Seibel (Sydney, Australia) reported a surprising effect of vitamin D receptor (VDR) ablation (in MDA-MB231 and MCF7 cells by 85% and 80%, respectively, using siRNA). They found that receptor ablation in each cell line resulted in increased cell growth *in vitro*, and increased tumor growth *in vivo*, both in the mammary fat pad and following intra-tibial injection of nude mice. Such a ligand-independent effect of the VDR would be an important one that needs further exploration.

Some novel insights could come from the work of Aymen Idris and colleagues (Edinburgh, Scotland) in three papers concerning the endocannabinoid system in breast cancer metastases. Having shown that ligands specific for the peripheral cannabinoid type 2 receptor (CB2) partially attenuated ovariectomy-induced bone loss in mice (4), they studied this receptor in breast cancer. With only *in vitro* studies so far, they found that selective agonists of CB2 stimulate RANKL formation and support osteoclastogenesis, and at the same concentrations, increase MDA-MB231 cell mobility in culture. Conclusions from their *in vitro* experiments were that tumor-induced osteolysis and osteoclast formation can be inhibited by genetic or pharmacological blockade of CB2. This interesting possibility will require *in vivo* support and further *in vitro* study.

Nadia Rucci (L'Aquila, Italy) and colleagues used an innovative approach in a search for marker genes that might distinguish breast cancers that metastasize to bone from those that metastasize to soft tissues. They used global gene expression arrays, carried out on bone metastases from patients with tumor deposits only in bone, and on bone metastases of patients with deposits also in other organs. Their bioinformatic analysis revealed a surprisingly small number (*i.e.* 13) of genes comprising a signature associated with bone-only metastases, with gene ontogeny analyses focusing these on processes involved in oxygen transport, fatty acid metabolism and circulation. When a similar array study was carried out in MDA-MB231 human breast cancer cells selected clonally from growth in bone or soft tissue in nude mice, 5 of these 13 genes were found also to separate into bone-only metastases.

These early results are productive enough to be encouraging. It will always be difficult to obtain fresh non-bone metastasis from human subjects, but that aspect could be examined in animal experiments.

The meeting also encouraged interest in primary tumors of bone, with Carl Walkley (Melbourne, Australia) presenting his mouse model of osteosarcoma, generated using an osterix promoter transgene to direct Cre expression to committed osteoblast progenitors. Making use of the known predisposition to osteosarcoma conferred by mutations in either the Rb or p53 genes, they bred the transgene to conditional alleles for both p53 and Rb. Mice developed osteosarcoma with a mean latency of ~ 4.5 months, with complete penetrance, and with more faithful mimicking of the human disease than any other experimental model (5). In an approach to investigating the role of PTHR1 signaling in the pathogenesis of osteosarcoma, this group showed that primary and metastatic tumors expressed PTHrP and functional receptor (PTHR1). Preparatory to *in vivo* experiments they have used shRNA knockdown of PTHR1 to show substantial impairment of *in vitro* invasion capacity of the cells in which PTHR1 receptor levels are reduced by more than 80%.

A different, intriguing perspective on bone sarcoma was provided by the group of Dominique Heymann and Frederique Blanchard (Nantes and Paris, France). One variety of primary bone tumor derived from mesenchymal cells is Ewing's sarcoma. The gp130 cytokine, oncostatin M (OSM), acts in human cells through both the OSM and the leukemia inhibitory factor (LIF) receptors. The group reported that whereas OSM inhibits the proliferation of osteosarcoma and chondrosarcoma, this cytokine induced proliferation of 9 out of 10 human Ewing's cell lines. The only other member of this cytokine family to produce a similar effect was LIF and the investigators noted that Ewing's sarcoma cell lines expressed higher levels of LIF receptor (LIFR) than osteo- or chondrosarcomata. When the LIFR was reduced by genetic means, decreasing the LIFR/OSMR ratio, the OSM effect now became inhibitory of Ewing's cell growth, raising the possibility of the LIFR as a therapeutic target in Ewing's sarcoma.

A feature of cancer research today is the great effort being devoted to understanding how the microenvironments of various tissue types can contribute to the establishment and growth of cancers that are often derived from different organs. This requires an understanding of the homing mechanisms to those tissues, the nature of their connective

tissue backbone, how their vascular supplies develop, what cytokines and growth factors their constituent cells can produce in resting and stimulated states, and their relationship with the immune system. There is a wealth of new information on all these matters nourishing our understanding of bone biology. So much so that we can argue with real justification that bone is an ideal tissue in which to study the interaction with cancer. That is a great advantage provided by meetings such as this one that combine basic and clinical bone biology with the study of skeletal complications of cancer.

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