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MEETING REPORT

13th International Conference on Cancer-Induced Bone Disease

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Meeting Report from the 13th International Conference on Cancer-Induced Bone Disease, Miami, FL, USA, 6–9 November 2013.

Introduction

The 13th International Conference on Cancer-Induced Bone Disease was held in Miami and organized by the International Bone and Mineral Society (IBMS) and the Cancer and Bone Society (CABS). The Co-Chairs of the conference were Professors Catherine Van Poznack and Russell Tachman from the University of Michigan (Ann Arbor, MI, USA). This meeting is a big favorite for the community of cancer and bone as its format and its size allow a lot of interactions between the attendees. Thanks to the friendly environment surrounded by outstanding scientific sessions, this conference is the right soil for the growth of collaborations and for the expansion of cancer and bone research. The CIBD conference brings together basic and clinical scientists to bridge the gap between basic research and clinical practice. This year clinical vignettes were included at the beginning of each session bringing a clinical view to the basic researchers. The conference also hosted a clinical debate to discuss the inclusion of bone-specific therapy as an integral part of the management of all patients with bone metastasis. Furthermore, the CABS and the IBMS are committed to the education and the mentoring of young investigators, and for the first time organized a young investigators carnival. This special session preceding the meeting brought together young investigators and experienced professors in the field to discuss topics of interest such as mentoring, transition from trainee to faculty, grant proposal and manuscript writing, or translational science. The scientific program focused on the discussion of the recent advances in the understanding of the tumor microenvironment, dormancy and stem cells, plasticity and chemoresistance. All of them are key factors in the establishment and progression of cancer-induced bone diseases.

Session Highlights

Tumor microenvironment

The first session of the meeting focused on the tumor microenvironment as a model of ecosystem failure. Kenneth Pienta (Johns Hopkins School of Medicine, Baltimore, MD, USA) provided an overview of the 'cancer diaspora' during the Greg Mundy Memorial Lecture. Tumors resemble ecosystems in which cancer cells interact with the different cell types and the extracellular matrix of the native host that contribute to cancer progression and metastasis.[1] To examine the role of the stroma during tumor initiation and progression, Marc Lippman (University of Miami, Miami, FL, USA) used dual-species microarray analysis with Illumina beadchips to characterize gene expression profiles of human metastatic tumors and the murine stroma in NSG mice inoculated with MDA-MB-231 cells or primary breast tumors to cause metastases. In these xenograft models, stromal gene expression analysis of all the cancer-bearing tissue showed a differential expression of a group of genes consistent with myeloid cell infiltration.[2] In a different approach, Frank Marini (Wake Forest Institute of Regenerative Medicine, Winston-Salem, NC, USA) studied the contribution of mesenchymal stromal/stem cells (MSCs) to the tumor stroma using a novel multispectral detection system based on the use of multiple antibodies conjugated to different fluorescent dyes.[3] He showed that MSCs contribute to the stroma, in part, as cancer-associated fibroblasts (CAFs), and the phenotypes of CAFs rely on MSC expression of the glycoprotein CD44. Deficiency of CD44 in MSCs decreased the migration of cancer cells and angiogenesis, and reduced the expression of activation markers on fibroblasts, demonstrating that CD44 is a potential target to inhibit stroma formation in tumors. In addition, Raghu Kalluri (MD Anderson Cancer Center, Houston, TX, USA) proposed a link between fibrosis and tumor stroma. He discussed that the fibrotic microenvironment in cardiac disease and kidney fibrosis precedes the emergence of cancer and contributes to the development and progression of disease. This process is mediated by endothelial-to-mesenchymal transition (EndMT), an important source of CAFs that regulates tumor angiogenesis and metastasis by secreting cytokines such as TGF-β. His data suggested that targeting EndMT would be a novel therapeutic strategy to treat cancer patients.[4] Theresa Guise (Indiana University, Indianapolis, IN, USA) discussed how the microenvironment of bone metastases affects the musculoskeletal system, decreasing muscle mass and strength. Skeletal muscle dysfunction was evaluated in mice and it revealed that muscle weakness was caused by bone metastases. The decrease of muscle strength was associated with the oxidation of the ryanodine receptor 1 (RyR1) and a decreased interaction with its stabilizing unit, calstabin,

resulting in intracellular Ca^{2+} leak. A treatment with Rycal, a molecule that stabilizes the interaction of calstabin and RyR1, completely prevented bone metastasis-associated muscle weakness.[5] The animal data indicated that there is a strong correlation between osteolytic area and muscle weakness in mice with bone metastases, whereas tumors in the mammary fat pad had no effect on muscle strength. When mice with bone metastasis were treated with an inhibitor of Tgfbr1 kinase domain, SD208, or with a pan-TGF-β neutralizing antibody, 1D11, the muscle function was improved, suggesting that TGF-β released during bone resorption can act systemically to cause muscle weakness.[6]

Dormancy and stem cells

The next sessions reviewed the last advances in dormancy, focusing on what makes the 'monster' sleep or wake up, new technologies to detect CTC/DTC and the role of the stem cells in dormancy and metastasis. Filippo Giancotti (Memorial Sloan-Kettering Cancer Center, New York, NY, USA) revealed a new role for neogenin,a neuronal receptor for netrins, as a regulator of dormancy in prostate cancer. Analysis of samples from patients with hormone-refractory metastasis or small-cell neuroendocrine prostate tumors showed reduced levels of neogenin, and the loss of neogenin promoted prostate tumor progression to castration resistance and bone metastasis in mice.[7]

A critical point in the study of CTC/DTC would be to examine individual cells and characterize their molecular profile at different times throughout the disease and identify new therapeutic targets. With this purpose, Colm Morrissey (University of Washington, Seattle, WA, USA) tested the currently commercially available technologies and showed that it is possible to reliably analyze the transcriptomic profile of DTC isolated from the bone marrow of patients with prostate cancer (PCa).[8]

In many cancers, the patients never develop bone metastasis even if DTCs are detected in the bone marrow, whereas other organs such as lungs present growing metastasis. To resolve this paradigm, Julio Aguirre-Guiso (Mount Sinai School of Medicine, New York, NY, USA) proposed that the target organ microenvironment could be characterized as permissive (lung) or restrictive when it induces DTC dormancy (bone marrow) under the control of TGF-β2 and Tgfbr3. In a head and neck squamous cell carcinoma model, tumor cell dormancy in the bone marrow is associated with higher levels of TGF-β2, but not of TGF-β1, which activates MAPK p38α/β in the presence of Tgfbr3. In contrast, in lungs (a metastasis permissive site) TGF-β2 levels were low and DTC dormancy was short and was quickly followed by metastatic growth.[9]

Russell Taichman (University of Michigan) provided an overview of the current knowledge on how hematopoietic stem cells (HSCs) regulate metastasis and cancer stem-like cell (CSC) phenotype of solid tumors. Metastatic prostate cancer cells compete to occupy the endosteal niche with HSCs. Once there, the cancer cells can become dormant using the same molecular signals that regulate HSCs dormancy. Dormancy allows cancer cells to escape the immune surveillance mechanism by resembling HSCs in the niche and to resist the conventional radiation and/or chemotherapy. HSC quiescence is regulated by growth arrest-specific 6 (GAS6) protein that binds to tyrosine kinase receptors Axl, Tyro3 and Mer. A balance between these receptors determines whether HSCs remain quiescent or proliferate. When PCa cells bind to osteoblasts in the HSC niche, it increases the expression of Axl and activates GAS6 signaling, inhibiting the proliferation of PCa cells, suggesting that, once DTCs enter the HSC niche, interactions between GAS6 and its receptors may regulate PCa dormancy.[10] Gabriela Dontu (King's College London School of Medicine, London, UK) described a novel experimental *in vitro* model of dormancy that mimics the bone microenvironment and the breast cancer metastatic niche. This system is based on 3D cocultures of breast cancer cells with cell types from the bone marrow, and in which dormant or proliferate conditions can be manipulated.[11]

Plasticity and chemoresistance

The plasticity session focused on the recent molecular advances in the understanding of the contribution of the epithelial–mesenchymal transition (EMT) to cancer progression and metastatic disease. Yibing Kang (Princeton University, Princeton, NJ, USA) showed that the transcription factor Elf5—a regulator of alveologenesis in the mammary gland—suppresses EMT in both mammary gland development and metastasis by directly repressing the transcription of Snail2/Slug, a regulator of mammary stem cells. Besides, dNp63, a regulator of mammary gland stem cell, was frequently overexpressed in basal-like breast cancer and control stem cell activities in normal mammary gland and in cancer stem cells in malignant tissues. His findings suggest that cell lineage regulators during normal mammary gland development can serve as regulators of cellular plasticity and metastasis in breast cancer.[12] Philippe Clezardin (INSERM, Lyon, France) discussed the role of the helix–loop–helix transcription factor (Twist1) in bone metastasis formation. Increased Twist1 expression in mammary tumors is associated with poor survival, as Twist facilitates intravasation of the tumor cells in the circulation and promotes the EMT of CTCs and DTCs. Twist1 overexpression increased osteolytic lesions in mice, whereas repression of Twist1 expression prevented bone metastasis formation.[13]

During the session on chemoresistance, Naoto Ueno (MD Anderson Cancer Center) summarized the last advances in chemoresistance of triple-negative breast cancer (TNBC). TNBC has a heterogeneous response to chemotherapy suggesting that different subtypes of primary TNBC are associated with high or low rate of pathological complete response (pCR), defined as no residual-invasive tumor or *in situ* carcinoma present, and no residual lymph node metastasis. Using molecular profiling, he determined the pCR rates after neoadjuvant chemotherapy on the basis of TNBC subtypes in 146 patients. He found a correlation between TNBC subtypes and pCR status. The basal-like 1 subtype had the highest pCR rate, whereas the basal-like 2 and luminal androgen receptor had the lowest.[14] His findings can help to develop personalized medicine strategies for patients with TNBC. Alexandra Naba (Koch Institute for Integrative Cancer Reseach, Cambridge, MA, USA, MIT) reviewed the role of the extracellular matrix (ECM) in tumor progression and its role as a barrier to drug delivery, enabling chemoresistance. She developed a proteomic method to characterize the ECM composition of normal and tumor tissues using enrichment of ECM components and analysis by mass spectrometry. The analysis of more than 100 ECM proteins in murine lung and colon tissues permitted the identification of characteristic signatures. The analysis of

human tumor xenografts and their stroma demonstrated that the composition of the ECM differs according to their metastatic ability.[15] David Roodman (Indiana University) reviewed the contribution of the bone marrow stromal cells (BMSCs) to chemoresistance in multiple myeloma (MM). Particularly, he discussed the role of a spliced form of X-box-binding protein-1 (XBP1) in the chemoresistance of myeloma cells. XBP1 is induced in the BMSCs of the MM microenvironment. Over-expression of XBP1 in BMSCs increased their expression of VCAM-1, IL-6 and RANKL, and increased the growth myeloma cells, whereas knockdown of XBP1 in BMSCs had the opposite effect. Moreover, he described the role of the ZZ domain of p62, a scaffold protein involved in multiple signaling pathways including RANK-RANKL signaling in myeloma. An antagonist of p62-ZZ was able to induce the apoptosis of myeloma cells, decrease their proliferation as well as osteoclast formation. In addition, the p62-ZZ antagonist induced new bone formation in mice with MM and could then be used for the treatment of MM and bone complications of MM.[16]

Short Talk Highlights

Last but not least, I would like to highlight briefly some short talks that in my opinion constitute exciting new findings in the field of cancer and bone. Michaela Reagan described a 3D scaffold model system to investigate bone and cancer interactions using mesenchymal stem cells from MM patients, and identified miR-199a-3p, miR-15a-5p and miR-16-5p as novel bone anabolic agents.[17] Michelle McDonald using a MM model demonstrated that two-photon and intra-vital microscopy could be used to visualize individual dormant cancer cells and their activation in the skeleton of live mice.[18] Yusuke Shiozawa developed a method for the detection and isolation of human DTCs in mouse bone marrow using flow cytometry, PCR of human Alu sequences and by immunohistochemistry using anti-HLA antibody.[19] Jonathan Page described a crosstalk between β3-integrin and TGF-β receptor, required for cancer cells to sense the rigidity of bone matrix suggesting that integrin inhibition could be a valuable target for osteolytic tumor metastasis.[20] Majd Zayzafoon developed a novel preclinical xenograft mouse model to examine the recurrence and metastasis of human osteosarcoma (OS) using bioluminescent imaging. In this model, OS cells are injected into the mouse tibia, followed 2 weeks later by the amputation of this hindlimb. Mice are then examined using bioluminescence for the recurrence of OS in distant organs. Using this model, he determined that VEGF induced by α-CaMKII controls the levels of OS tumor-initiating stem cells.[21] Caroline Wilson studied the antitumor effects of zoledronic acid (ZOL) and found that ZOL decreased follistatin secretion from ER-negative breast cancer cells, but not from ER-positive cells *in vitro*. Similarly ZOL decreased serum levels of follistatin in patients with ER-negative breast cancer, suggesting that ZOL effect on follistatin is influenced by ER status.[22] Rob Coleman presented the final results of the AZURE trial evaluating the use of ZOL in addition to standard adjuvant therapy in patients with stage II/III breast cancer. This study was performed in 3360 patients randomized to receive standard adjuvant systemic therapy alone or with ZOL over 5 years. ZOL reduced disease recurrence in bone and had a favorable effect on both invasive disease-free survival and overall survival in postmenopausal women. These data suggest that adjuvant bisphosphonates can be a part of the routine adjuvant treatment strategy for postmenopausal women.[23]

Conflict of Interest

The author declares no conflict of interest.

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