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MEETING REPORT

Osteoimmunology in Corfu: Meeting report from the 4th International Conference on Osteoimmunology 2012

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Meeting report from the 4th International Conference on Osteoimmunology, Corfu, Greece, 17–22 June 2012

Over the past 40 years it has become clear that bone cells exist in an environment, which is rich in external signals that regulate their function. These signals have multiple effects and originate from the variety of cells in the bone and bone marrow. The organization of bone and bone marrow dictates that multiple different cell types, including those responsible for bone formation and resorption, hematopoiesis, cartilage and fat development, reside in close physical proximity, facilitating their interactions. To fully understand how bone cells function, we need to appreciate all the signals that influence their activity. The study of the interactions of bone, hematopoietic and immune cells in health and disease has come to be known as osteoimmunology. To promote the development of this field the 4th International Conference on Osteoimmunology: Interactions of the Immune and Skeletal Systems was held from 17 to 22 June 2012 in Corfu, Greece. Over 115 participants attended the meeting, which explored a variety of themes. These included the effects of cancer on bone, the role of osteocytes in regulating the bone microenvironment and the interactions of osteoblast and osteoclasts with hematopoietic and immune cells in health and disease. Specific sessions at the meeting were the following:

Cancer and bone

Yibin Kang from Princeton University presented data about genes, which are expressed by breast cancers and influence the ability of cancers to metastasize and grow in bone. His group found that transforming growth factor-β (TGF-β), which is released from bone matrix by osteolysis, signals breast cancer cells to enhance Jagged1 expression. Jagged1, in turn, interacts with Notch on stromal cells to promote tumor growth and osteoclast differentiation. Importantly, inhibition of the TGF-β and Notch signaling significantly reduced the development of bone metastases in animal models. They also demonstrated that vascular cell adhesion molecule-1 (VCAM1) mediates the transition of dormant tumor cells to those capable of metastasizing by promoting osteoclastogenesis via binding to VLA-4 on osteoclast precursors. Finally, they found that VCAM1 was upregulated in the breast tumors of patients who have early relapse of their cancer after therapy.

David Roodman from the University of Indiana discussed the factors in the bone microenvironment that enhance the growth of multiple myeloma cells and lead to myeloma-induced bone disease. Osteoclasts in bone that is affected by myeloma produce a variety of factors that directly or indirectly increases tumor growth and/or angiogenesis. These include annexin II, interleukin 6 (IL-6), macrophage inflammatory peptide-1α and activin A. In addition, myeloma cells produce factors that inhibit osteoblasts. Among these are VLA-4 on myeloma cells and VCAM1 on marrow stromal cells. Additional myeloma-produced factors suppress osteoblasts and inhibit bone growth. These include hepatocyte growth factor, IL-3, dickkopf1 (DKK1), soluble frizzled-related protein-2, IL-7, tumor necrosis factor-α (TNF-α) and activin A.

Colin Dunstan from the University of Sydney examined the role of IL-6 in the development of breast and prostate cancer metastases to bone. His group found in animal models that there was a direct correlation between production by tumors of IL-6 and the ability of the tumors (breast and prostate cancer) to grow in bone. Most recently they demonstrated *in vitro* that treatment of human prostate and breast cancer cell lines with RANKL increased the expression of IL-6 and, in turn, treatment of the tumor cells with IL-6 increased the expression of RANK, the receptor for RANKL. *In vivo* studies found that tumor cell lines, which had either IL-6 or RANK expression inhibited, had significantly reduced growth in bone but not in soft tissue. These data imply that there is a 'feed-forward' loop for tumor cell growth in bone, which enhances bone metastases

and involves RANK and IL-6 expression by tumors and RANKL expression by bone cells.

Basic concepts into osteoimmunology: osteoblasts–osteocytes anabolic pathways

Matthew Warman from Harvard Medical School discussed how identifying the mutations that were responsible for the development of four human genetic diseases led to the discovery of new strategies for improving bone mass and bone strength. The diseases were: osteoporosis-pseudoglioma syndrome, autosomal dominant high bone mass, van Buchem disease and sclerosteosis In all of these patients produce too little or too much bone. A common feature of these diseases is that they are caused by mutations in the cell surface receptor LRP5 or the secreted protein sclerostin, which are involved in canonical Wnt signaling pathways. This discovery led to the recognition that osteocytes, the major source of sclerostin, regulate bone mass and respond to mechanical load. As sclerostin is a negative regulator of osteoblast development, this pathway provides targets that can be manipulated to improve bone strength. Currently, inhibitors of sclerostin are in clinical trials.

Dr Thomas Clemens from the Johns Hopkins School of Medicine presented data about the actions of insulin on the skeleton. Insulin and insulin-like growth factors (IGFs) evolved from a common precursor. This system consists of a single receptor and multiple ligands, which function in lower animals to enable a broad range of physiological processes. In mammals, the insulin and IGF system mediate more specific functions: insulin primarily regulates fuel accumulation, storage and energy expenditure whereas IGFs are central to cell proliferation, survival and growth. Recent studies suggest that the direct effects of insulin on bone cells, in turn, regulate energy homeostasis. To examine the role of the IGF/insulin system in bone, Dr Clemens studied mice that lacked IGF1 or insulin receptors in osteoblasts. His group found that insulin suppressed the Runx2 inhibitor, Twist2, which promotes osteoblast differentiation. Treatment with insulin induced a striking increase in the production of osteocalcin, which has been reported to affect glucose utilization and energy expenditure. Furthermore, osteoblast-specific insulin receptor-deficient mice had all their fat depots enlarged including those in marrow. These data suggest that insulin signaling in the osteoblast controls bone metabolism and bone development.

Kyoji Ikeda from the National Center for Geriatrics and Gerontology, Aichi, Japan reported on signaling between osteoclasts and osteoblasts. Cells in the osteoblastic lineage and marrow stromal cells support the formation of osteoclasts by multiple pathways including presenting RANKL to hematopoietic cells. Dr Ikeda has found a factor (ctcl) that is produced by mature osteoclasts and stimulates the recruitment and differentiation of osteoblasts via marrow stromal cells. Osteoclast differentiation by RANKL is not sufficient for full expression of ctcl, but its expression is dependent on the type of substrate used to culture the cells. The production was increased when the cells were cultured on dentin as compared with conventional plastic tissue culture plates. Stromal cells appear to be at least one of the target cells affected by ctcl. Hence, this factor has many characteristics of a new coupling factor.

Brendan Lee of the Baylor College of Medicine spoke about microRNA regulation of Notch signaling during skeletal development. Notch signaling is an evolutionarily conserved mechanism for specifying cell fate differentiation and patterning. Human mutations affecting this pathway cause defects in somatic segmentation in the form of spondylocostal dysostosis. This pathway is also important for specifying osteoblastic and osteoclastic differentiation in both normal and certain pathologic conditions. Alterations in Notch signaling and interactions of Notch signaling with p53 pathways may result in the development of osteosarcomas. Integral to the modulation and integration of these pathways is their regulation by microRNAs. One example of this is the expression of miR34c during osteoblastic differentiation. By regulating Notch signaling pathway components, miR34c modifies the coupling of osteoblastic differentiation to osteoblastic function. Notch activity is dependent on the stage of osteoblast differentiation as early expression leads to a decrease in Runx2, whereas later expression causes an increase in differentiation.

Basic concepts in osteoimmunology: cross-talk

Georg Schett of the University of Erlangen-Nuremberg demonstrated that purified human anti-mutated citrullinated vimentin antibodies isolated from the synovial fluid of rheumatoid arthritis (RA) patents directly increased osteoclast formation and activity, both *in vitro* and *in vivo*. His group also found that in RA patients there was a direct relationship between autoantibody formation and bone destruction.

Louis C Gerstenfeld of Boston University discussed how heightened TNF-α levels in diabetic mice cause delayed fracture healing. His group has found that TNF-mediated upregulation of FOXO1, a pro-apoptotic genes, resulted in chondrocyte apoptosis and delayed fracture healing in diabetic mice.

Nicole J Horwood of the University of Oxford reported that direct contact of monocyte with mesenchymal stem cells (MSC) promoted osteogenic differentiation. Her group also found that the production of oncostatin M (OSM) by monocytes led to the activation of STAT3 signaling in MSCs. This, in turn, upregulated osteoblast-associated genes and the expression of the receptors for OSM and leukemia inhibitory factor. Finally, upregulation of these receptors amplified the anabolic effects of OSM on osteoblasts.

Lionel B Ivashkiv from Weill Cornell Medical College demonstrated that the Notch pathway transcription factor RBP-J is essential for feedback inhibition of TNF-α-mediated osteoclastogenesis. His group has found that RBP-J inhibits the activation of NFATc1 and c-Fos (a positive regulator of NFATc1). In addition, RBP-J also prevents degradation of the transcriptional repressor IRF-8 (a negative regulator of NFATc1). These findings identify RBP-J as a key negative regulator of osteoclastogenesis, which prevents excessive bone resorption during inflammation.

Basic concepts in osteoimmunology osteoclasts and more

Steven Teitelbaum from Washington University described how integrin 'inside out' signaling regulated the osteoclast cytoskeleton. He showed that talin, a ubiquitous integrin-interacting scaffold protein that is typically found at cell–matrix attachment sites, activates β-3 integrins in osteoclasts. This interaction is analogous to the role of talin in other adherent cells, where

it connects integrins to the actin–myosin machinery. Conditional deletion of talin in the osteoclasts of mice, using the cathepsin K promoter to drive Cre expression, produced a phenotype of markedly increased bone mass. In addition, these mice had decreased osteoclast erosive surfaces in a serum transfer arthritis model. Conditional deletion of Rap1, which promotes talin/β-integrin recognition, in mice (using the cathepsin K promoter to drive deletion) produced a similar osteopetrotic phenotype. Curiously, the phenotype of the conditionally deleted Rap1 mice was much more dramatic than that of integrin β-3-deficient mice. This result suggested that talin interacts with multiple integrins in osteoclasts and may be an important target to regulate the osteoclast cytoskeleton and function.

Natalie Sims of the University of Melbourne spoke about the multiple roles of OSM in the bone. OSM is a product of activated macrophages and stimulates RANKL production in osteoblasts. It is also produced by osteoblasts and osteocytes and, interestingly, stimulates IL-6, RANKL, IL-1 receptor and OSM receptor expression in synovial fibroblasts. Thus, OSM may amplify the inflammation and bone erosion of inflammatory arthritis. Furthermore, OSM can stimulate bone formation when injected into the calvarial of mice, is involved in the anabolic action of PTH and inhibits sclerostin production by osteocytes.

Sakae Tanaka of the University of Tokyo reviewed two novel areas. The first was studies of the epigenetic regulation of osteoclast differentiation, particularly the role of histone demethylation in the induction of NFAT. The second was studies examining the mechanisms of how risedronate was anti-resorptive *in vivo*. His group has examined the effects that risedronate had in bim-deficient mice (which show decreased osteoclast apoptosis). They found that risedronate induced an increase in bone mass and a decrease in serum carboxy-terminal collagen crosslinks in these mice, suggesting that the anti-resorptive effect of risedronate is not primarily due to induction of osteoclast apoptosis.

Mary Beth Humphrey of the University of Oklahoma has found that immunoreceptor tyrosine-based activation motif (ITAM) adapter signaling has dual actions in osteoclasts. It has a positive role in regulating osteoclast function and cytoskeletal rearrangement, but it can also negatively regulate osteoclast differentiation and function by recruiting SHIP-1 and DAP12 (an ITAM protein in osteoclasts). Her group found that the adapter molecule Dok3 is involved in the actions of the DAP12/SHIP-1 complex. They demonstrated that Dok3-deficient mice are osteopenic and have preosteoclasts, which are hypersensitive to RANKL stimulation.

New 'up and coming' ideas

Janet Rubin of the University of North Carolina reported on how mechanical signals are translated into biochemical signals and, particularly, on how they influenced the differentiation of mesenchymal cell into osteoblasts. She showed that exercise stimulates the activation of β-catenin and blocks mesenchymal cell differentiation into adipocytes while fostering osteoblastogenesis. This process requires the activation of the intracellular kinase Akt by mTORC2, which inhibits the degradation of β-catenin and shifts precursor differentiation fates from adipogenesis to osteoblastogenesis. This process appears to be strongly dependent on mTORC2, which guides the assembly of stress fibers to form focal adhesion sites. Cytoskeletal reorganization elicited by mTORC2 appears to be a crucial step in the translation of mechanical signals into biochemical processes.

Richard Flavell of Yale University reported on studies of the effects that hydroxyapatite crystals had on bone. Hydroxyapatite crystal deposition causes a very severe inflammatory disease, known as the Milwaukee shoulder, which is associated with rapid and severe bone destruction. His group showed that hydroxyapatite crystals function as 'danger-associated molecular patterns' and lead to a profound activation of the inflammasome pathways through activation of NALP3. The inflammasome is an effective cytokine production machinery in cells, which synthesizes large amounts of the cytokine IL-1.

Hiroshi Takayanagi of the University of Tokyo demonstrated new mechanisms regulating the spatial interaction between osteoblast and osteoclasts. Usually, these major bone cell phenotypes are not colocalized but spatially separated to form distinct areas of bone formation and bone resorption. His group has found that spatial separation of osteoclast from osteoblasts is guided by semaphorin 4D, which previously was identified as an axon guidance molecule. His group has found that semaphorin 4D is expressed by osteoclasts, repels osteoblasts from bone resorption sites and, through this mechanism, inhibits bone formation. As a result, semaphoring-4D-deficient mice and mice that are treated with semaphorin-4D-neutralizing antibodies have increased bone mass. Hence, strategies to inhibit the function of semaphorin 4D are an interesting target for the development of drugs to treat diseases like osteoporosis.

In addition to the talks from invited speakers, the program contained 10 short (15 min) presentations that were chosen from the submitted abstracts. These included studies of the role of TGF-β in RANKL-mediated osteoclastogenesis, the role of the parathyroid hormone receptor in hematopoiesis, how RANKL expression by T-lymphocytes influences experimental autoimmune encephalomyelitis, the role of the microbiota in regulating bone mass, the influence of T-lymphocytes on the effects of estrogens on bone, the role of IL-34 in osteoclastogenesis, the role of the nuclear receptor NR4a1 in osteoblast function and a description of how Wif-1 deficiency uncouples cartilage and bone destruction in TNFα-induced arthritis. There were also 60 additional posters presented in two poster sessions at the meeting.

Summary

It is clear that osteoimmunology is an evolving field. Most rewarding for the organizers was the presence of a large number of young investigators at the meeting. These are the future of the field and it appears from their enthusiasm and excellent oral and poster presentations that the future of the field is bright.

Conflict of Interest

The authors declare no conflict of interest.