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MEETING REPORT

Highlights on the osteoclast

Roberta Faccio[1] and Deborah V Novack[2]

[1]Department of Orthopedics, Washington University, St Louis, MO, USA. [2]Bone and Mineral Division, Department of Medicine, Washington University, St Louis, MO, USA.

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Increased osteoclast differentiation during postmenopausal osteoporosis, inflammatory arthritis or bone metastases is the leading cause of bone loss. A better understanding of the positive signaling pathways governing osteoclastogenesis, identification of negative regulators that can restrain osteoclast differentiation and novel modulators of resorption were the central topics at this 2011 American Society of Bone and Mineral Research meeting during the osteoclast sessions.

Advances in understanding signals emanated by the receptor activator of nuclear factor-κB (RANK) during osteoclastogenesis were made by Liu *et al.*[1] The requirement for tumor necrosis factor (TNF) receptor-associated factor (TRAF)6 and TRAF6 binding to RANK during osteoclast differentiation is well established. Interestingly, Dr Feng's group has identified a novel IVVY motif in the cytoplasmic tail of RANK that has a TRAF-independent role in osteoclast differentiation and function.[2] Now, the phenotype of mice with a knock-in mutation (IVVY ≥IVAF) that disrupts this motif was presented.[3] Mice homozygous for the AF allele (AF/AF) had increased BV/TV by micro-computed tomograpgy at 8 weeks of age compared with WT controls, whereas heterozygous littermates were intermediate. AF/AF mice had decreased numbers of osteoclasts *in vivo* and *in vitro*, with concomitant decreases in expression of osteoclast markers such as cathepsin K, carbonic anhydrase II, TRAP and NFATc1. Interestingly, phosphorylation of IκB, ERK and c-Jun N-terminal kinases was intact. Thus, the mechanism by which the IVVY motif regulates osteoclast differentiation, independent of TRAF binding and nuclear factor-κB activation, remains to be discovered.

New insights into the effects of transforming growth factor-β on RANK ligand (RANKL)-induced osteoclastogenesis were presented by Yasunori Omata in Sakae Tanaka's group.[4] ChIP-seq on Smad 2/3 target genes was performed to identify activating histone methylation signatures and responses to transforming growth factor-β. Of seven targets identified, Nedd9 was selected for further study. Overexpression of Nedd9 in osteoclast precursors increased differentiation, whereas short hairpin RNA decreased it. Nedd9$^{-/-}$ mice were generated, and showed an increase in bone mineral density (BMD) *in vivo* with decreased numbers of osteoclasts. Further work will be needed to demonstrate a mechanism for Nedd9's pro-osteoclastogenic activity.

Much less is known about negative regulators of osteoclastogenesis. Baohong Zhao in Lionel Ivashkiv's group[5] described several elegant experiments demonstrating a negative regulatory role for Notch/RBP-J during inflammatory osteolysis. Although TNF potently stimulates osteoclastogenesis *in vivo* during inflammatory conditions, when added to the precursor cells *in vitro*, it is very inefficient at inducing osteoclast differentiation. Interestingly, Dr Zhao demonstrated that in the absence of RBP-J, TNF induces robust osteoclastogenesis both *in vivo* and *in vitro*, and in a RANKL-independent manner. RBP-J negatively modulates osteoclast differentiation by decreasing Fos activation and preventing downregulation of IRF8, a transcriptional repressor that blocks osteoclastogenesis. Activation of RBP-J by transgenic overexpression of the Notch intracellular domain was effective in blocking osteolysis in a model of inflammatory arthritis. Thus, the ability of RBP-J to specifically block inflammatory bone erosion makes it an intriguing therapeutic target.

Youridies Vattakuzhi in Nicole Horwood's group[6] discussed DUSP1 as another potential brake on inflammatory bone loss. DUSP1 inactivates the mitogen-activated protein kinase p38. Dusp1$^{-/-}$ osteoclast precursors were particularly sensitive to low doses of RANKL and TNF. When challenged in a collagen-induced arthritis model, Dusp1$^{-/-}$ mice had enhanced inflammation and increased bone loss. Interestingly, Dusp1$^{-/-}$ mice on a mixed C57/129Sv background developed spontaneous inflammation and bone loss in the digits.

Although β-catenin signaling in the osteoblast governs osteoclastogenesis by altering RANKL expression levels,[7] the direct role of β-catenin in osteoclasts is a new area of investigation. Millan *et al.*[8] from the University of Cantabria in Spain showed data indicating that β-catenin also suppresses osteoclastogenesis. Recombinant Wnt3a induced β-catenin, reduced osteoclast differentiation and caused osteoclast apoptosis. Mice depleted of β-catenin in the osteoclast lineage (using LysM-Cre) generated more osteoclasts *in vitro* with macrophage colony-stimulating factor and RANKL, but interestingly, *in vivo* effects were limited to cortical bone. Cortical thickness was decreased in femurs at 28 weeks, but there were no changes in vertebral trabecular bone, and the number of osteoclasts in sections seemed to be specifically increased on endocortical surfaces.

Further studies, possibly using pathological models of bone loss, need to be examined to better understand the importance of β-catenin signaling in osteoclasts.

Reactive oxygen species increase with age and can stimulate osteoclast differentiation and survival.[9] FoxO transcription factors limit generation of reactive oxygen species. Thus, Shoshana Bartell in Stavros Manolagas' group[10] overexpressed FoxO3 in the osteoclast lineage (with LysM-Cre) to ask whether limiting reactive oxygen species in these cells could decrease bone resorption *in vivo*. Micro-computed tomography showed modest but significant increases in BMD and BV/TV in FoxO3/LysM-Cre mice compared with controls, with a small decrease in differentiation and resorption *in vitro*. Depletion of the three main FoxO's[1,3,4] with flox mice and LysM-Cre decreased BV/TV and increased OCs, supporting a role for FoxO's in the control of reactive oxygen species and bone mass.

More light was also shed on the molecular mechanisms regulating the bone resorptive process. Resorption of the organic and inorganic components of the bone matrix is achieved by acidification and secretion of proteases. In particular, cathepsin K targeting has been proven to successfully inhibit resorption in preclinical models and new data from clinical trials in postmenopausal women are also showing promising results.[11–13] However, the mechanisms leading to regulation of cathepsin K secretion are still poorly documented. Zhao and colleagues[14] identified Plekhm1 as a critical regulator of cathepsin K secretion. Mice lacking Plekhm1 specifically in the osteoclast compartment (using the Cathepsin K Cre) showed increased BMD as early as 1 month of age by dual-emission X-ray absorptiometry analysis. Although no changes in osteoclast numbers were observed, the resorptive capacity of osteoclasts lacking Plekhm1 was compromised as revealed by a significant decrease in resorption pit formation *in vitro* and diminished C-telopeptide of type 1 collagen levels *in vivo*. Elegant *in vitro* studies demonstrated that Plekhm1 is a lysosomal adapter protein, linking lysosomes to the microtubule machinery in resorbing osteoclasts. Thus, in the absence of Plekhm1, cathepsin K-containing vesicles are retained in the cytoplasm and cannot be secreted into the resorptive lacunae. Through its N-terminal-localized RUN and PH1 domains, Plekhm1 interacts with LIS1, a critical molecule regulating microtubule dynamics and the function of cytoplasmic dynein, a microtubule-based motor complex.[15] Confirming the importance of this complex, depletion of LIS1 in osteoclasts inhibits cathepsin K release into the resorption lacuna and LIS conditional knock-out mice under the LysM-Cre promoter showed increased BMD and BV/TV, starting at 3 months of age. However, differently from the Plekhm1-deficient osteoclasts, LIS1 depletion also impacted osteoclast differentiation. Thus, future studies are required to better understand whether LIS1 has dual functions modulating both osteoclast differentiation and activation, or if impaired cathepsin K release is simply a consequence of cell immaturity.

Cathepsin K release was also shown to be under control of protein kinase C. Two presentations[16,17] demonstrated that lack of the protein kinase C delta leads to increased bone mass *in vivo* due to defective bone resorption. Despite the presence of normal sealing zones and ruffled borders, absence of protein kinase C delta impaired cathepsin K secretion,[16] leading to its accumulation within the cytoplasm. Interestingly, Rottlerin, an inhibitor protein kinase C delta, was able to block lipopolysaccharide-induced bone resorption *in vivo*. It will be interesting to understand how protein kinase C delta affects the secretory machinery, and if it regulates the Plekm1/Lis1 complex.

Bone resorption requires attachment to the extracellular matrix, an event mediated by the αvβ3 integrin and DAP12.[18] However, whether DAP12 activation requires its ligand TREM2 or other ligands is still under investigation. A novel finding presented by Stuible *et al.*[19] identified Siglec-15 as a new binding partner for DAP12. This complex is expressed in mature osteoclasts but not in their precursors, and Siglec-15 depletion by short hairpin RNA reduced osteoclast differentiation in cells of murine and human origins. Even more importantly, Siglec-15 inhibition, by the antibody 25E9, suppressed osteoclast resorption *in vitro* and led to increased BV/TV *in vivo*.

Dynamins, large guanosine triphosphatases best known for having a critical role in endocytic membrane fusion, also contribute to the regulation of the osteoclast cytoskeleton and bone resorption.[20] Although three dynamin isoforms are expressed in osteoclasts, Bruzzaniti's group[21] from Indiana University showed that deletion of dynamin 1 and 2 is sufficient to reduce the fusion activity of osteoclast precursors by half. Confirming defective cell fusion, dendritic cell-specific transmembrane protein and E-cadherin, which are important for the generation of fusogenic osteoclast precursors, were downregulated in dynamin1/2 null cells. Importantly, dynamin1/2cko:cathepsin K-Cre mice showed significant increases in bone density, thus demonstrating the importance of dynamin regulation in cell fusion as well as resorption.

The importance of guanosine triphosphatases in bone resorption was further documented by a high-throughput screen of 2995 knock-out mouse lines by dual-emission X-ray absorptiometry, which identified LRRK1 as a critical regulator of bone mass.[22] Lrrk1$^{-/-}$ mice had the highest total body BMD value (T score = 7.0). LRRK1 is a serine/threonine kinase with guanosine triphosphatases activity. Its disruption resulted in severe osteopetrosis and protected against OVX-induced bone loss. Future studies are required to analyze the molecular and cellular mechanisms regulated by LRRK1.

What is the take-home message from the osteoclast sessions? Although mechanisms of osteoclast differentiation are still being fully elucidated, there is increasing emphasis on understanding negative regulation of osteoclastogenesis as a possible way to restrain differentiation in a more physiological way, especially in the context of inflammation. Additionally, there is renewed focus on elucidating the mechanisms of protease secretion as a novel and more specific strategy to block bone resorption.

Conflict of Interest

The authors declare no conflict of interest.

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