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MEETING REPORT

Meeting Report from the 29th Annual Meeting of the American Society for Bone and Mineral Research

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OSTEOCLASTS

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Integrin signaling plays an essential role in osteoclast (OC) function. The importance of the αvβ3 integrin in osteoclastic bone resorption is well established (1-3), and now new evidence indicates a critical role for the α9β1 integrin receptor in osteoclast formation and function (4). Studies from *α9*(-/-) mice and human OC precursors infected with α9 shRNA revealed decreased numbers of mature osteoclasts with disrupted actin rings. α9 was shown to be the only receptor for ADAM8 (A Disintegrin and Metalloproteinase 8). The importance of the ADAM8/α9β1 interaction was demonstrated by increased OC numbers in WT, but not α9 null cultures treated with soluble ADAM8. Mechanistically, the disintegrin motif of ADAM8 formed a complex with the tyrosine kinase Pyk2 and modulated paxillin phosphorylation. Thus, these data indicate that interaction between the α9β1 integrin and its ligand ADAM8 is critical for OC activity by activating a PYK2-dependent signaling pathway. The importance of PYK2 in bone homeostasis was also unveiled through demonstration that PYK2 deletion led to high bone mass through a positive balance between bone formation and bone erosion in aged mice (5). Furthermore, another recent study underscores an important role of Pyk2 in microtubule-dependent podosome organization, bone resorption, and other osteoclast functions (6).

New insights into activation of OC-mediated bone resorption were elegantly presented (7) in work using Cdc42 gain-of-function and Cdc42 flox/flox mice. While Cdc42 null mice are embryonic lethal, rendering difficult the analysis of their bone phenotype, Cdc42 gain-of-function mice (Cdc42GAP), which lack the GTPase-activating proteins, thereby allowing prolonged activation of Cdc42, die soon after birth. Transplant studies of *Cdc42GAP*(-/-) bone marrow cells into lethally irradiated WT mice induced lower bone mass, increased OC numbers and higher levels of bone resorption. This *in vivo* finding correlated with accelerated M-CSF dependent proliferation and RANKL-induced differentiation *in vitro*. Conversely, bone marrow macrophages (BMMs) from *cdc42*(flox/flox) mice treated *in vitro* with retroviral Cre to delete Cdc42 displayed a decreased response to M-CSF, increased apoptosis and diminished OC differentiation. This is the first report indicating an important role for the Cdc42 GTPase in both OC differentiation and bone resorption.

NFκB activation is critical for osteoclast development and survival. Both RANKL and TNFα can induce NFκB activation and both cytokines activate the canonical (p65 and p50) and non-canonical (p52 and RelB) NFκB pathways. However, their effect on osteoclast development is different. RANKL strongly promotes OC differentiation, while TNFα does so only in the presence of permissive levels of RANKL (8). Intriguing findings were presented suggesting that TNFα, independent of RANKL, can promote OC differentiation, albeit to a lesser extent than the osteoclastogenic cytokine (9). A possible mechanism explaining the differential effect of these two cytokines on NFκB-mediated osteoclast differentiation relies on the capacity of TNFα to induce upregulation of both p52 and its precursor

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protein p100 (NFκB2), while RANKL increased protein levels of p52 by promoting p100 degradation (10). Interestingly, deletion of NFκB2 augmented the capacity of TNFα to promote OC differentiation at similar rates to RANKL. Exit from cell cycle is a required step for OC terminal differentiation and involves NFκB. TNFα promoted cell proliferation via activation of cyclinD1 and similarly to *NFκB2*(-/-) cells, deletion of cyclin D1 stimulated TNF-induced OC differentiation. These data suggest that TNFα may limit OC differentiation through a mechanism involving NFκB2 and cyclinD1.

Interesting results on NFκB-induced osteoclast differentiation were also presented (11). NFκB activity is controlled by 2 upstream kinases, IKKα and IKKβ. In this study, the authors examined the role of IKKβ in *in vivo* and *in vitro* osteoclastogenesis by generating myeloid lineage-specific deletion of IKKβ using the Cre-lox system. Deletion of IKKβ in osteoclast progeny was responsible for developmental and survival defects, since knockout bone marrow macrophages formed less OCs in response to RANKL and apoptosis was more sensitive to RANKL and the pro-inflammatory cytokine, TNFα. Interestingly, the deletion of IKKβ in splenocytes was not sufficient to block their differentiation into osteoclasts, suggesting that the microenvironment in the spleen pre-programs OC precursors to differentiate into mature OCs independently from IKKβ activity.

A recent report indicated that RANKL co-stimulatory signals mediated by ITAM-containing receptors FcRγ and Dap12 are critical for osteoclast development *in vitro* and *in vivo* (12). FcRγ and DAP12 modulate calcium influx from the ER through the PLCγ2 pathway (4;5) and thereby mediate upregulation of the osteoclastogenic gene *NFATc1* (12). Mechanisms mediating the activation of PLCγ are still under investigation. Investigators elegantly demonstrated the need for Tec tyrosine kinases during osteoclast differentiation to modulate PLCγ1 and PLCγ2 phosphorylation (13). Specifically, *Tec*(-/-) *Btk*(-/-) mice exhibited an osteopetrotic phenotype due to severe impairment of OC differentiation. *In vitro* analysis showed that the two Tec family members were recruited to lipid rafts upon RANKL stimulation and formed a complex with RANK, the adapter protein BLNK and the ITAM-harboring adaptors, which mediated PLCγ-mediated NFATc1 upregulation during osteoclastogenesis. Importantly, *in vivo* studies showed that these mice are protected from ovariectomy-induced bone loss. In light of these findings, results from another group appeared very intriguing (14). In fact, in contrast to *Tec*(-/-)*Btk*(-/-) mice, ITAM-containing receptor FcRγ/Dap12 double null mice, which have a severe osteopetrotic phenotype due to a blockade in OC development, responded to ovariectomy with bone loss in both femurs and tibias of approximately 40% relative to basal bone volumes. Thus, this study suggests that whereas ITAM signaling is critical for basal bone remodeling, estrogen deficiency induces an ITAM-independent bypass mechanism allowing for increased osteoclastogenesis and activation in specific bony microenvironments.

The interaction between the immune and bone systems is becoming increasingly recognized. New research demonstrated that T-lymphocytes amplify the anabolic action of intermittent PTH (iPTH) treatment by regulating OC formation (15). Mechanistically, using T cell receptor β (*TCRβ*)(-/-) mice, researchers demonstrated that the deficient mice had decreased bone mineral density (BMD) measured by DEXA and less of an increase in BV/TV when treated with intermittent PTH than WT mice. 4-point bending tests also showed that iPTH increased femoral stiffness in WT mice but not in TCRβ null animals. These observations correlated with decreased numbers of CFU-ALP colonies, an index of the number of stromal cells (SC) with osteogenic potentials and a 3-fold lower increase in *ex-vivo* formation of OCs in *TCRβ*(-/-) mice as compared to WT, indicating that T cells potentiated the capacity of iPTH to stimulate both osteoblasts and OCs. Importantly, the paper showed that iPTH stimulated RANKL expression of T cells by targeting stromal cells. This work is another demonstration of

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the importance of T cells in modulating bone cell development.

Overall, several intriguing findings were reported at the 2007 ASBMR annual meeting in Honolulu. These exciting studies will certainly open new roads toward understanding the mechanisms of osteoclast recruitment, differentiation and activation in pathological conditions of bone loss, with the hope of soon finding new effective anti-osteoclastogenic therapeutic targets.

Conflict of Interest: None reported.

References

1. Duong LT, Rodan GA. The role of integrins in osteoclast function. *J Bone Miner Metab*. 1999;17(1):1-6.

2. Faccio R, Novack DV, Zallone A, Ross FP, Teitelbaum SL. Dynamic changes in the osteoclast cytoskeleton in response to growth factors and cell attachment are controlled by beta3 integrin. *J Cell Biol*. 2003 Aug 4;162(3):499-509.

3. Sanjay A, Houghton A, Neff L, Didomenico E, Bardelay C, Antoine E, Levy J, Gailit J, Bowtell D, Horne WC, Baron R. Cbl associates with Pyk2 and Src to regulate Src kinase activity, alpha(v)beta(3) integrin-mediated signaling, cell adhesion, and osteoclast motility. *J Cell Biol*. 2001 Jan 8;152(1):181-95.

4. Lu G, Patrene K, Garcia-Palacios V, Anderson J, Boykin C, Del Prete D, Windle JJ, Roodman GD. ADAM8 binds $\alpha_9\beta_1$ to increase OCL formation and function by interacting with Pyk2 and activating paxillin. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S29. [Abstract]

5. Li M, Crawford DT, Qi H, Simmons HA, Li Y, Healy DR, Smith EA, Buchbinder L, Brown TA. Deletion of PYK2 led to high bone mass through a positive balance between bone formation and resorption in aged mice. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S5. [Abstract]

6. Gil-Henn H, Destaing O, Sims NA, Aoki K, Alles N, Neff L, Sanjay A, Bruzzaniti A, De Camilli P, Baron R, Schlessinger J. Defective microtubule-dependent podosome organization in osteoclasts leads to increased bone density in Pyk2(-/-) mice. *J Cell Biol*. 2007 Sep 10;178(6):1053-64.

7. Ito Y, Zheng Y, Johnson JF, Yang L, Teitelbaum SL, Ross FP, Zhao H. The small GTPase cdc42 enhances osteoclastogenesis and bone resorption. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S42. [Abstract]

8. Lam J, Takeshita S, Barker JE, Kanagawa O, Ross FP, Teitelbaum SL. TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *J Clin Invest*. 2000 Dec;106(12):1481-8.

9. Yao Z, Xing L, Boyce B. NFκ-B2 limits TNF-induced osteoclastogenesis through precursor cell cycle regulation. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S42. [Abstract]

10. Novack DV, Yin L, Hagen-Stapleton A, Schreiber RD, Goeddel DV, Ross FP, Teitelbaum SL. The IkappaB function of NF-kappaB2 p100 controls stimulated osteoclastogenesis. *J Exp Med*. 2003 Sep 1;198(5):771-81.

11. Otero JE, Al-hawagri M, Dai S, Vacher J, Pasparakis M, Abu-Amer Y. Mice with a myeloid lineage-specific deletion of IKKβ demonstrate both in vivo and in vitro defects in osteoclastogenesis. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S82. [Abstract]

12. Koga T, Inui M, Inoue K, Kim S, Suematsu A, Kobayashi E, Iwata T, Ohnishi H, Matozaki T, Kodama T, Taniguchi T, Takayanagi H, Takai T. Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. *Nature*. 2004 Apr 15;428(6984):758-63.

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http://www.bonekey-ibms.org/cgi/content/full/ibmske;4/12/333
DOI: 10.1138/20070285

13. Shinohara M, Koga T, Okamoto K, Takayanagi H. Tec tyrosine kinases are essential for osteoclast differentiation. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S81. [Abstract]

14. Wu Y, Torchia J, Yao W, Lane NE, Lanier LL, Nakamura MC, Humphrey MB. Distinct roles of ITAM signaling in bone remodeling in various bony microenvironments. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S81. [Abstract]

15. Terauchi M, Gao Y, Galley S, Yang X, Page K, Shah E, Weitzmann MN, Pacifici R. T-lymphocytes amplify the anabolic action of intermittent PTH treatment by regulating osteoclast formation. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S83. [Abstract]

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