IBMS BoneKEy. 2008 November;5(11):454-457
http://www.bonekey-ibms.org/cgi/content/full/ibmske;5/11/454
doi: 10.1138/20080354

MEETING REPORTS

Osteoclasts: Meeting Report from the 30th Annual Meeting of the American Society for Bone and Mineral Research

September 12-16, 2008 in Montréal, Quebéc, Canada

Sakae Tanaka

The University of Tokyo, Faculty of Medicine, Tokyo, Japan

At the 30th Annual Meeting of the ASBMR, the molecular mechanism of the cytoskeletal organization of osteoclasts attracted a great deal of attention. In addition, the signal transduction pathway of osteoclast differentiation was further elucidated. The osteoclast-related abstracts are summarized below.

Regulation of Osteoclastogenesis

Downstream signaling pathways of receptor activator of nuclear factor kappa B (RANK) leading to osteoclastogenesis have been extensively studied, and the central role of transcription factors such as c-Fos, NF-κB and NFATc1 is now well-established (1;2). In contrast, the molecular mechanisms and physiologic and pathologic role of tumor necrosis factor (TNF)-α-induced osteoclast differentiation has not been fully elucidated. TNF-α induces osteoclastogenesis in bone marrow cells although osteoclastogenic activity of TNF-α is much weaker than that of RANKL. Interesting results demonstrating the role of NF-κB2 p100 in TNF-α-mediated osteoclast differentiation were presented (3). TNF-α, but not RANKL, induces NF-κB2 p100 expression in osteoclast progenitors, which may suppress osteoclast differentiation. RANK/nfkb2 or RANKL/nfkb2 double knockout (dKO) mice were generated and TNF-α-induced osteoclastogenesis was examined. Interestingly, TNF-α induced significantly more osteoclasts from bone marrow cells of these mice than from wild type cells. In addition, arthritis occurred earlier and was more severe in TNF-transgenic (TNF-Tg)/*nfkb2(-/-)* mice than in TNF-Tg mice (3). These results indicate a negative regulatory role of NF-κB2 p100 in osteoclastogenesis *in vitro* and bone destruction *in vivo*.

Detailed analysis comparing the cytoplasmic domains of RANK and the TNF receptor was presented (4). RANK contains 3 TRAF-binding motifs and a TRAF-independent motif ($IVVY^{535-538}$) that are required for osteoclast differentiation, and the IVVY motif primes bone marrow macrophages (BMMs) in TNF-α/IL-1-induced osteoclastogenesis (5). Interestingly, when mutated TNFR1 bearing the IVVY motif (TNFR1-I) was expressed in BMMs from *TNFR1(-/-)R2(-/-)* mice, TNF-α was able to form osteoclasts, confirming the role of the IVVY motif in priming BMMs (4). It was also reported that the IVVY motif and TRAF-binding motifs (especially T2 and T3) in RANK exhibit a functional crosstalk between them, indicating that 3 RANK motifs ($IVVY^{535-538}$, $PVQEET^{559-564}$ and $PVQEQG^{604-609}$) play a vital role in osteoclastogenesis and can be targets of anti-resorptive drugs.

Role of Phosphatidylinositol 3 Kinase in Osteoclasts

Previous studies have uncovered the important role of the c-Src tyrosine kinase on the cytoskeletal organization of osteoclasts. C-Cbl is an E3 ubiquitin ligase and is known as one of the downstream target molecules of c-Src in osteoclasts. Tyrosine 731 (Y^{731}) of c-Cbl is phosphorylated by Syk and Src family kinases in response to growth factors, cytokines and integrin activation, and phosphorylated $CblY^{731}$ creates a bindng site for the p85 regulatory subunit of phosphatidylinositol 3 kinase (PI3K).

Knock-in mice (*Cbl*$^{YF/YF}$) in which the PI3K binding site in c-Cbl is ablated were generated and analyzed (6). These mice are smaller in size than wild type mice, and the amount of cancellous bone was increased due to decreased bone resorption. *Cbl*$^{YF/YF}$ osteoclasts are inefficient in resorbing bone although their survival was enhanced, indicating that Cbl-PI3K interaction critically regulates the bone-resorbing function and survival of osteoclasts.

Class IA PI3Ks regulate signaling pathways downstream of receptor-type and non-receptor-type tyrosine kinases such as c-Fms and Src. Class IA PI3Ks consist of an adaptor subunit p85 (α and β), and a catalytic subunit p110 (α, β, δ). To elucidate the role of PI3K *in vivo*, osteoclast-specific p85α and β dKO mice were generated (7). Akt activation in response to M-CSF stimulation was completely abolished in p85 dKO osteoclasts. Radiological and histological analysis showed that p85 dKO mice exhibited a significant increase in bone mass compared to normal littermates due to reduced bone resorption. Osteoclasts differentiated from p85 dKO mouse bone marrow cells showed impaired spreading and actin ring formation, and their bone-resorbing activity was remarkably suppressed. These results clearly demonstrate that class IA PI3Ks are indispensable for osteoclast function by controlling cytoskeletal organization through regulation of Akt activation.

Regulation of Cytoskeletal Organization of Osteoclasts

Many studies were presented regarding the regulatory mechanisms of cytoskeletal organization of osteoclasts. Regulation of cytoskeletal organization is a critical step in generating unique structures called “actin rings” or “podosome belts” in osteoclasts, and plays an essential role in normal osteoclastic bone resorption (8). Integrin signaling is one of the most critical pathways regulating the cytoskeletal organization of osteoclasts, in which the Syk tyrosine kinase and adaptor protein Vav3 play important roles (9). Both Syk and Vav3 are required for integrin-mediated spreading, actin ring formation and bone resorption of osteoclasts, and Syk activation is associated with prominent phosphorylation of tyrosine (Y) 317. It was demonstrated that SykY317F mutation negatively regulates osteoclast function (10). SykY317F disrupts Syk/Cbl association and abolished M-CSF- and integrin-stimulated Syk ubiquitination and degradation, thereby enhancing activity of the downstream cytoskeleton-organizing molecules, SLP-76, Plcγ2 and Vav3.

SLP-adaptor proteins (SLP-76 and BLNK) are Syk substrates in other hematopoietic cells. The role of these molecules was analyzed using KO mice. While *BLNK(-/-)* osteoclasts were normal, *SLP-76(-/-)* osteoclasts exhibited retarded spreading (11). Osteoclasts lacking both BLNK and SLP-76 showed a substantially greater defect in spreading, actin ring formation and bone resorption than those deficient only in SLP-76. The importance of these molecules *in vivo* was further confirmed using radiation chimera mice in which SLP-76 or SLP-76 and BLNK are absent in marrow cells. These observations suggest that SLP-adaptor proteins play an important role in osteoclast function by regulating cytoskeletal organization.

LIM kinase 1 (LIMK1) is a serine/threonine kinase that phosphorylates and inactivates the actin-severing protein, cofilin. The role of LIMK1 in regulating the activity of osteoclasts was investigated using LIMK1 KO mice (12). The KO mice exhibited lower bone mass due to increased bone resorption. Osteoclasts generated from LIMK1 KO mice exhibited higher bone-resorbing activity and a significantly greater increase in cell area in response to treatment with M-CSF than *LIMK1(+/-)* cells, which may result from an increase in the amount of active cofilin in *LIMK1(-/-)* osteoclasts.

Rho family GTP-binding proteins have been known to modulate cytoskeletal organization. Rac in particular is activated in osteoclasts in response to M-CSF and integrin stimulation, and is required for actin ring formation (13;14). Hematopoietic lineage cells express both Rac 1 and Rac 2, and the critical role of these molecules was confirmed by the

observation that mice deficient in both Rac 1 and Rac 2 (Rac dKO) developed severe osteopetrosis (15). Cells isolated from the marrow of Rac dKO mice differentiated into osteoclasts, but the osteoclasts failed to form actin ring structures and ruffled borders. In addition, Rac dKO osteoclast precursors exhibited accelerated apoptosis, which may be due to the inactivation of Akt. These results suggest that Rac 1 and 2, in combination, not only are essential for organization of the osteoclast cytoskeleton, and hence the cells' capacity to resorb bone, but also promote RANKL-mediated survival.

Energy Production and Iron Uptake in Osteoclasts

Interesting observations related to the energy production of osteoclasts were also shared (16). The peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1 (PGC-1) family is a group of transcriptional coactivators that are involved in the regulation of energy production and utilization in metabolic tissues. It was found that PGC-1β expression was markedly increased during osteoclast differentiation in response to increased reactive oxygen species, and that knock-down of PGC-1β inhibited differentiation as well as mitochondrial gene expression in osteoclasts. In parallel, transferrin receptor (TfR) 1 mRNA was markedly induced along with osteoclast differentiation. PGC-1β-deficient mice displayed impaired bone resorption. PGC-1β drives mitochondrial gene expression, coupled with the increased iron uptake through the upregulated TfR1, and plays an important role in bone resorption by coordinating the stimulation of mitochondrial biogenesis and respiration in osteoclasts.

Overall, many novel findings were presented at the 2008 ASBMR Annual Meeting in Montréal. These studies will shed new light on the molecular mechanisms of physiologic and pathologic bone resorption, and lead to the discovery of novel therapeutics against pathologic bone destruction.

Conflict of Interest: None reported.

Peer Review: This article has been peer-reviewed.

References

1. Boyce BF, Yamashita T, Yao Z, Zhang Q, Li F, Xing L. Roles for NF-kappaB and c-Fos in osteoclasts. *J Bone Miner Metab*. 2005;23 Suppl:11-5.

2. Takayanagi H. Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. *Nat Rev Immunol*. 2007 Apr;7(4):292-304.

3. Yao Z, Xing L, Boyce BF. In the absence of NF-κB2, TNF induces osteoclast formation *in vivo* independent of RANKL/RANK and more severe arthritis in TNF-Tg mice. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S4. [Abstract]

4. Jules J, Shi Z, Feng X. Three RANK cytoplasmic motifs, IVVY$^{535-538}$, PVQEET$^{559-564}$ and PVQEQG$^{604-609}$, play a critical role in TNF α /IL-1-mediated osteoclastogenesis. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S34. [Abstract]

5. Xu D, Wang S, Liu W, Liu J, Feng X. A novel receptor activator of NF-kappaB (RANK) cytoplasmic motif plays an essential role in osteoclastogenesis by committing macrophages to the osteoclast lineage. *J Biol Chem*. 2006 Feb 24;281(8):4678-90.

6. Adapala N, Barbe M, Amin M, Safadi F, Popoff S, Sanjay A. Cbl-PI3K interaction regulates osteoclast function, differentiation and survival. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S4. [Abstract]

7. Nakamura M, Iwasawa M, Nagase Y, Nakamura T, Kato S, Ueki K, Luo J, Cantley LC, Nakamura K, Tanaka S. Class IA phosphatidylinositol 3-kinases are indispensable for osteoclast function by regulating cytoskeletal organization and cell death. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S33. [Abstract]

8. Ross JL, Fuller K, Chambers TJ. Formation of podosome belts by osteoclasts on plastic correlates with

resorptive activity. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S259. [Abstract]

9. Faccio R, Teitelbaum SL, Fujikawa K, Chappel J, Zallone A, Tybulewicz VL, Ross FP, Swat W. Vav3 regulates osteoclast function and bone mass. *Nat Med*. 2005 Mar;11(3):284-90.

10. Zou W, Reeve J, Ross FP, Teitelbaum SL. Syk tyrosine 317 negatively regulates osteoclast function. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S35. [Abstract]

11. Reeve J, Zou W, Lu Y, Koretzky G, Ross FP, Teitelbaum SL. SLP-76 couples Syk to the osteoclast cytoskeleton *in vitro* and *in vivo*. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S4. [Abstract]

12. Kawano T, Zhu M, Insogna K. The role of LIM kinase 1 in osteoclast cytoskeletal remodeling and bone resorption. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S34. [Abstract]

13. Fukuda A, Hikita A, Wakeyama H, Akiyama T, Oda H, Nakamura K, Tanaka S. Regulation of osteoclast apoptosis and motility by small GTPase binding protein Rac1. *J Bone Miner Res*. 2005 Dec;20(12):2245-53.

14. Wang Y, Lebowitz D, Sun C, Thang H, Grynpas MD, Glogauer M. Identifying the relative contributions of Rac1 and Rac2 to osteoclastogenesis. *J Bone Miner Res*. 2008 Feb;23(2):260-70.

15. Croke MR, Zhao H, Williams DA, Teitelbaum SL, Ross FP. Rac 1 and 2, in combination, mediate osteoclast function and survival. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S62. [Abstract]

16. Ishii K, Iwai K, Takeshita S, Ito M, Shimohata N, Taketani S, Aburatani H, Lelliott CJ, Vidal-Puig A, Ikeda K. PGC-1β and iron uptake in the mitochondrial activation of osteoclasts. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S62. [Abstract]