IBMS BoneKEy. 2009 December;6(12):485-489
http://www.bonekey-ibms.org/cgi/content/full/ibmske;6/12/485
doi: 10.1138/20090419

MEETING REPORTS

Osteoclasts: Meeting Report from the 31st Annual Meeting of the American Society for Bone and Mineral Research

September 11-15, 2009 in Denver, Colorado

Sakae Tanaka

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

At the 31st Annual Meeting of ASBMR, many interesting papers demonstrated various modulators of osteoclast differentiation and function. Topics included negative regulators of osteoclast differentiation, cathepsin K, angiogenesis, IKK2-NF-κB pathways, IL-34, and the TGF-β superfamily. The most interesting osteoclast-related abstracts are summarized below.

Negative Regulators of Osteoclast Differentiation

As evidenced by *in vitro* and *in vivo* experiments as well as clinical trials using the anti-RANKL antibody denosumab, the RANKL-RANK axis primarily regulates osteoclast development in human as well as murine skeletal tissues (1;2). At the meeting, several important negative regulators of osteoclast differentiation were revealed. Interferon regulatory factor 8 (IRF8), a transcription factor expressed in immune cells, was found to be downregulated in mouse and human osteoclast precursors in response to RANKL stimulation at the early stage of differentiation (3). Gene deletion of IRF8 stimulated, while overexpression of the molecule strongly suppressed RANKL-induced osteoclast differentiation (4). IRF8-deficient mice exhibited severe osteoporosis caused by increased bone resorption, and they showed enhanced bone destruction following lipopolysaccharide administration due to enhanced osteoclast formation. IRF8 suppressed osteoclast differentiation by suppressing the transcriptional activity and expression of NFATc1. These results clearly indicate that IRF8 is a negative regulator of osteoclastogenesis (3).

Interferon-β (IFN-β) is a potent negative regulator of osteoclast differentiation acting downstream of RANKL-RANK signaling, and the negative feedback regulation by IFN-β requires IRF9 expression. IFN-β-induced inhibition of osteoclast differentiation was abrogated by a selective iNOS inhibitor (L-NIL) or in *IRF9*(-/-) cells, but iNOS induction was rather enhanced in *IRF9*(-/-) cells. These results suggest that the negative feedback regulation of RANKL-induced osteoclastogenesis through IFN-β is mediated by two different pathways: IRF9 and iNOS/NO (5).

Cathepsin K Is a Negative Regulator of Bone Formation

Cathepsin K is highly expressed in osteoclasts and critically regulates type I collagen degradation during bone resorption. Mice globally lacking the cathepsin K gene had mild osteopetrosis and high numbers of poorly functional osteoclasts, and interestingly, these mice also exhibited increased bone formation rates (BFR) (6;7). To investigate which cell types are responsible for the increased BFR in these mice, conditional deletion of the cathepsin K gene was achieved by mating $CKLP^{fl/fl}$ mice, in which exon 5 of the cathepsin K gene was flanked by lox P sites, with Mx1-Cre mice (Mx1-Cre;$CKLP^{fl/fl}$) or osterix-Cre mice (Osx-Cre;$CKLP^{fl/fl}$). When the cathepsin K gene was deleted in osteoclasts by injecting Mx1-Cre;$CKLP^{fl/fl}$ mice with polyinosinic-polycytidylic acid, the mice exhibited moderate osteopetrosis with an increase in cancellous bone volume (+163%) and osteoclast number (+400%). Importantly, BFR and osteoblast number in the mice

were also markedly increased (+47% and +214%, respectively) compared to controls, and osteoblasts obtained from the mice showed increased alkaline phosphatase and mineralization activity. In contrast, Osx-Cre;CKLP$^{fl/fl}$ mice failed to show any skeletal phenotype, strongly suggesting that deletion of cathepsin K in the osteoblast lineage has no effect on bone resorption or on bone formation, providing genetic evidence that cathepsin K produced by osteoclasts may impair the coupling message from osteoclasts to osteoblasts (8). These intriguing observations may explain why the mechanisms of action of cathepsin K inhibitors appear distinct from those of bisphosphonates in the skeletal tissues of ovariectomized animals and osteoporosis patients (9-11).

Angiogenesis is Regulated by Osteoclasts

A novel role of osteoclasts in skeletal angiogenesis was demonstrated. Angiogenesis is a crucial step for skeletal development, and it was previously reported that matrix metalloproteinase 9 (MMP-9) produced by osteoclasts plays an important role in growth plate angiogenesis (12). Using the fetal mouse metatarsal angiogenesis assay, it was revealed that continuous PTHrP treatment increased both the area covered by endothelial cells and osteoclastic resorption in metatarsal explants, which was abrogated by osteoprotegerin treatment or in osteoclast-deficient *op/op* mouse bones, showing that the angiogenic effect of PTHrP requires osteoclasts. In addition, the proangiogenic effect of PTHrP is blunted in metatarsal explants from *MMP-9*(-/-) mice. These results suggest that the presence of osteoclasts and MMP-9 produced by osteoclasts is essential for skeletal angiogenesis (13).

Constitutive Activation of IKK Is Sufficient for Osteoclast Differentiation

NF-κB is a collective term referring to dimeric transcription factors that belong to the Rel family, and NF-κB is involved in various aspects of physiologic and pathologic events. The essential role of NF-κB in osteoclast development was first observed in knockout mice. Although targeted disruption of either *Nfkb1* or *Nfkb2* alone did not affect skeletal development, double knockout of these genes induced osteopetrosis in mice due to a defect in osteoclast differentiation (14;15). These results suggest that there are redundant roles of the canonical and noncanonical NF-κB pathways in osteoclast differentiation. Interesting results were presented on the role of the NF-κB pathways in osteoclastogenesis. It was shown that constitutively active IKK2 (IKK2^{SSEE}) was sufficient for osteoclast differentiation independent of RANKL (16). Osteoclasts were induced by IKK2^{SSEE} introduction even in the presence of osteoprotegerin or in RANK knockout cells, indicating that the effect is independent of RANKL-RANK pathways. Double knockout cells missing *Nfkb1* or *Nfkb2* were resistant to IKK^{SSEE}-induced osteoclastogenesis, while NEMO, IKKα or RelB deficiency did not affect IKK^{SSEE}-induced osteoclast differentiation. In addition, adenoviral transfer of the IKK^{SSEE} gene induced massive osteolysis in mice (16). It was also reported that tyrosine-mutated or ubiquitin-like domain-deleted IKK2 failed to support RANKL-induced osteoclast differentiation (17). These results indicate an essential role of IKK2-NF-κB pathways in osteoclast differentiation.

The Role of Splenic IL-34 in CSF-1-Independent Osteoclastogenesis

Osteoclast differentiation is critically regulated by two cytokines produced by osteoblasts: RANKL and macrophage colony-stimulating factor (M-CSF). However, the defective osteoclast differentiation in M-CSF-deficient *op/op* mice is recovered in an age-dependent manner, although the mechanisms underlying this effect remain unknown (18). Recently, interleukin-34 (IL-34) was discovered as a novel ligand for the M-CSF receptor, and highly expressed in the spleen (19). It was shown that IL-34 substituted for M-CSF in a murine osteoclastogenesis assay (20). When a highly potent 1,25-dihydroxyvitamin D_3 analog, 2MD, was administered to

3-week-old *op/op* mice, many tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts were observed in tibiae. Prior to the appearance of osteoclasts in bone, the expression of IL-34 in the spleen was increased in response to 2MD, which was followed by the appearance of TRAP-positive mononuclear cells. In addition, 2MD failed to induce TRAP-positive osteoclasts in bone in splenectomized *op/op* mice. These results suggest that IL-34 is a novel regulator of osteoclast differentiation and that the spleen is a source of osteoclast progenitors, which are differentiated in response to IL-34 induced by 1,25-dihydroxyvitamin D_3 (20).

Role of the TGF-β Superfamily in Osteoclastogenesis

The direct effect of TGF-β and bone morphogenetic proteins (BMPs) on osteoclast differentiation was reported. It was previously found that TGF-β promoted RANKL-induced osteoclastogenesis (21;22). At this year's meeting, it was shown that exogenous BMP2 synergizes with sub-optimal levels of RANKL to enhance *in vitro* differentiation of osteoclast precursors (23). In addition, when TGF-β signals were blocked either by administration of a specific inhibitor or by introducing a dominant negative mutant of the TGF-β type II receptor, RANKL-induced osteoclastogenesis was almost completely suppressed both *in vitro* and *in vivo*, and the effect of TGF-β on RANKL-induced osteoclast differentiation was partially explained by the direct interaction between Smad2/3 and TRAF6 (24).

Conclusion

More than 10 years have passed since the discovery of the RANKL-RANK system as a master regulator of osteoclast differentiation. However, many questions remain answered. In particular, the cellular and molecular interaction between bone formation and bone resorption has attracted a great deal of attention. For example, it was reported that osteocytes produce osteoprotegerin in a Wnt-β-catenin signal-dependent manner, indicating a tight connection between osteocyte activity and bone resorption (M. Kneissel, plenary symposium II). It will become increasingly important to understand osteoclastic bone resorption in relation to entire skeletal homeostasis, which will open a new field of research into the osteoclast.

Conflict of Interest: None reported.

Peer Review: This article has been peer-reviewed.

References

1. Cummings SR, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, Delmas P, Zoog HB, Austin M, Wang A, Kutilek S, Adami S, Zanchetta J, Libanati C, Siddhanti S, Christiansen C; FREEDOM Trial. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med*. 2009 Aug 20;361(8):756-65.

2. Tanaka S, Nakamura K, Takahasi N, Suda T. Role of RANKL in physiological and pathological bone resorption and therapeutics targeting the RANKL-RANK signaling system. *Immunol Rev*. 2005 Dec;208:30-49.

3. Zhao B, Takami M, Yamada A, Hu X, Choi Y, Takayanagi H, Ivashkiv L, Kamijo R. Interferon regulatory factor 8 autonomously suppresses osteoclastogenesis in mouse and human systems. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

4. Zhao B, Takami M, Yamada A, Wang X, Koga T, Hu X, Tamura T, Ozato K, Choi Y, Ivashkiv LB, Takayanagi H, Kamijo R. Interferon regulatory factor-8 regulates bone metabolism by suppressing osteoclastogenesis. *Nat Med*. 2009 Sep;15(9):1066-71.

5. Collin-Osdoby P, Rothe L, Osdoby P. Divergent pathways of RANKL induced negative feedback inhibition by IRF9 and iNOS/NO downstream of IFNβ during osteoclastogenesis. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

6. Kiviranta R, Morko J, Alatalo SL, NicAmhlaoibh R, Risteli J, Laitala-Leinonen T, Vuorio E. Impaired bone resorption in cathepsin K-deficient mice is partially compensated for by enhanced osteoclastogenesis and increased expression of other proteases via an increased RANKL/OPG ratio. *Bone*. 2005 Jan;36(1):159-72.

7. Pennypacker B, Shea M, Liu Q, Masarachia P, Saftig P, Rodan S, Rodan G, Kimmel D. Bone density, strength, and formation in adult cathepsin K (-/-) mice. *Bone*. 2009 Feb;44(2):199-207.

8. Lotinun S, Kiviranta R, Alzate J, Campard G, Vuorio E, Horne W, Sabatakos G, Baron R. Osteoclast-targeted deletion of cathepsin K in mice increases bone formation whereas deletion in osteoblasts has no effect. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

9. Yamada H, Mori H, Nakanishi Y, Kunishige A, Nishikawa S, Tanaka M, Shiroya T. Orally active cathepsin K inhibitor, ONO-5334, potently improved bone mineral density not only in trabecular bone but also in cortical bone in ovariectomized cynomolgus monkeys. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

10. Cusick T, Pennypacker B, Scott K, Duong L, Kimmel D. Effects of odanacatib on bone mass, turnover and strength in the femoral neck of estrogen deficient adult rhesus monkeys. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

11. Eisman J, Bone H, Hosking D, McClung M, Reid I, Rizzoli R, Resch H, Verbruggen N, Hustad C, DaSilva C, Santora A, Ince B, Lombardi A. Odanacatib in the treatment of postmenopausal women with low bone mineral density: long-term continued therapy and resolution of effect. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

12. Vu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, Shapiro SD, Senior RM, Werb Z. MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell*. 1998 May 1;93(3):411-22.

13. Cackowski F, Anderson J, Choksi R, Patrene K, Yao G, Insogna K, Shapiro S, Blair H, Stolz D, Windle J, Roodman G. Osteoclasts are important for bone angiogenesis. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

14. Franzoso G, Carlson L, Xing L, Poljak L, Shores EW, Brown KD, Leonardi A, Tran T, Boyce BF, Siebenlist U. Requirement for NF-kappaB in osteoclast and B-cell development. *Genes Dev*. 1997 Dec 15;11(24):3482-96.

15. Iotsova V, Caamaño J, Loy J, Yang Y, Lewin A, Bravo R. Osteopetrosis in mice lacking NF-kappaB1 and NF-kappaB2. *Nat Med*. 1997 Nov;3(11):1285-9.

16. Otero J, Alhawagri M, Lai K, Darwech I, Abu-Amer Y. Constitutive activation of IKKβ is sufficient for RANK-independent osteoclast differentiation and osteolysis. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

17. Darwech I, Otero J, Abu-Amer Y. Modulation of osteoclastogenesis by targeting IKK2 domains. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

18. Begg SK, Radley JM, Pollard JW, Chisholm OT, Stanley ER, Bertoncello I. Delayed hematopoietic development in osteopetrotic (op/op) mice. *J Exp Med*. 1993 Jan 1;177(1):237-42.

19. Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, Halenbeck R, Wu G, Zhou A, Behrens D, Hollenbaugh D, Linnemann T, Qin M, Wong J, Chu K, Doberstein SK, Williams LT. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. *Science*. 2008 May 9;320(5877):807-11.

20. Nakamichi Y, Udagawa N, Mizoguchi T, Kobayashi Y, Takahashi N. The role of

splenic IL-34 in CSF-1-independent osteoclastogenesis induced by active vitamin D_3. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

21. Fuller K, Lean JM, Bayley KE, Wani MR, Chambers TJ. A role for TGFbeta(1) in osteoclast differentiation and survival. *J Cell Sci*. 2000 Jul;113(Pt 13):2445-53.

22. Kaneda T, Nojima T, Nakagawa M, Ogasawara A, Kaneko H, Sato T, Mano H, Kumegawa M, Hakeda Y. Endogenous production of TGF-beta is essential for osteoclastogenesis induced by a combination of receptor activator of NF-kappa B ligand and macrophage-colony-stimulating factor. *J Immunol*. 2000 Oct 15;165(8):4254-63.

23. Jensen E, Pham L, Espe K, Carlson A, Gopalakrishnan R, Mansky K. BMP signaling directly enhances in vitro osteoclast differentiation. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]

24. Yasui T, Yasui T, Kadono Y, Matsumoto T, Masuda H, Hikita A, Choi Y, Yasuda H, Nakamura K, Tanaka S. Regulation of RANKL-induced osteoclastogenesis by TGF-β through molecular interaction between Smad2/3 and TRAF6. *J Bone Miner Res*. 2009;24(Suppl 1). [Abstract]