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COMMENTARY

Wnt5a non-canonical signaling through Ror2; a novel co-stimulatory mechanism to enhance RANKL-induced osteoclastogenesis

Brendan F Boyce

Director of Anatomic Pathology, University of Rochester Medical Center, Rochester, NY, USA.

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Commentary on: Maeda K, Kobayashi Y, Udagawa N, Uehara S, Ishihara A, Mizoguchi T, *et al.* Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. Nat. Med. 2012; **18**(3):405-412.

Wnt/β-catenin signaling regulates bone mass through the canonical Wnt pathway predominantly by promoting osteoblast (OB) differentiation from mesenchymal stem cells, but it also limits OB-mediated receptor activator of NF-κB ligand (RANKL) induction of osteoclast (OC) formation and bone resorption by inducing expression of osteoprotegerin. Over the past few years several mechanisms whereby osteoblastic and osteoclastic cells interact with one another have been identified, and these positively and negatively affect the formation and functions of both the cell types. A recent report from Maeda *et al.*[1] adds to this growing list of regulatory mechanisms and identifies a novel role for Wnt5a expressed by OB lineage cells to enhance RANKL-induced OC formation through Wnt non-canonical signaling by binding to the receptor tyrosine kinase-like orphan receptor 2 (Ror2) expressed by OC precursors (OCPs). The findings identify Wnt5a-Ror2 signaling as a novel pathway in physiologic and pathologic osteoclastogenesis and as a potential new therapeutic target for destructive bone diseases, including rheumatoid arthritis.

Maeda *et al.*[1] were aware that OB-lineage cells have a key role in the maintenance of hematopoietic stem cells by providing signals to these cells in osteoblastic niches on bone surfaces.[2] They also knew that RANK-expressing OCPs are found in the vicinity of OB-lineage cells on bone surfaces,[3] suggesting that OBs may provide an endosteal niche for OCPs where these cells could interact and influence one another's functions. Canonical Wnt/β-catenin signaling is crucial for osteoblastogenesis through LRP5-mediated OB precursor differentiation.[4] Non-canonical signaling is mediated through Wnt-Ca^{2+} and Wnt-Jun kinase pathways, both of which are activated by Wnt5a, the latter being mediated by Ror2.[5] Overexpression of Ror2 in human mesenchymal stem cells or MC3T3-E1 osteoblastic cells increases their differentiation into OBs;[6] Maeda *et al.*[1] hypothesized that Ror2-mediated signaling might also regulate osteoclastogenesis. They first identified that Wnt5a, but not Wnt3a or -10, was expressed in both immature and mature OBs and that Ror2, but not Ror1, was expressed in bone marrow macrophages, suggesting that Ror2-mediated signaling might regulate osteoblastogenesis and osteoclastogenesis. Then they examined bones from *Wnt5a* −/− and *Ror2* −/− mice at embryonic day 18.5 (both knockout mice die soon after birth) and found that osteoclastogenesis was impaired in both mutant mice.

The authors next investigated the possibility that Wnt5a secreted by OB-lineage cells might enhance differentiation of OCPs through Ror2. They found that Wnt5a did not induce OC formation by itself, but it enhanced RANKL-induced osteoclastogenesis from WT bone marrow macrophages. Notably, Wnt5a did not induce OC formation from *Ror2* −/− OCPs, suggesting that Ror2-mediated signaling is required for Wnt5a-induced osteoclastogenesis. They generated mice with Ror2 deleted specifically in OCPs by crossing floxed Ror2 +/- mice with Rank-Cre mice and found that these mice had osteopetrosis associated with reduced OC formation. Mutant mice lacking one allele of *Wnt5a* in all cells and one allele of *Ror2* in OCPs (*Wnt5a* +/−; *Ror2*fl/+; *Rank*-Cre/+ mice) that they generated had reduced OC numbers, confirming that Wnt5a secreted by OB-lineage cells induces osteoclastogenesis through Ror2 in OCPs. Additional studies showed that Wnt5a induced mRNA expression of *Rank*, but not *Csfr* (the receptor for macrophage-colony stimulating factor, which like RANKL is essential for OC formation) in OCPs; this is mediated by c-Jun, which unlike JunB, JunD and ATF2 (other potential mediators), was recruited to the Rank promoter. Finally, Maeda *et al.*[1] prepared a glutathione S-transferase (GST)-soluble Ror2 fusion protein (GST-sRor2) composed of the extracellular region of Ror2, which blocked Wnt5a-mediated signaling as a decoy receptor in several assay systems. They used GST-sRor2 in a collagen-induced and a collagen-specific antibody-induced arthritis mouse model because they knew that synovial cells produced Wnt5a; indeed, they observed intense Wnt5a expression in the inflammatory pannus of collagen-induced mice. They found that GST-sRor2 suppressed bone resorption in the joints of both

mouse models, but it did not affect the intensity of the synovitis or pannus formation. These findings support a role for Wnt5a activation of Ror2 in OCPs to induce joint destruction, but not inflammation in inflammatory arthritis, presumably by upregulating Rank expression in OCPs. The inhibition of resorption without affecting inflammation in models of rheumatoid arthritis is similar to that seen in response to osteoprotegerin,[7] which blocks RANKL-induced osteoclastogenesis.

OCs resorb bone in a variety of clinical settings in response to cytokines, hormones, growth factors and mechanical stress, most of which induce osteoclastogenesis indirectly by upregulating expression of RANKL in OB lineage cells.[8] Some cytokines, such as tumor necrosis factor, can also induce OC formation directly without the requirement of RANKL,[9] and tumor necrosis factor mediates inflammation and joint destruction in many patients with rheumatoid arthritis. Tumor necrosis factor induces expression of CSFR on OCPs and of macrophage-colony stimulating factor by T cells, which induces RANK expression by OCPs, similar to the effect of Wnt5a. These effects further enhance osteoclastogenesis in inflammatory bone diseases, such as rheumatoid arthritis. The report by Maeda *et al.*[1] identify Wnt5a as another co-stimulatory molecule that can enhance RANKL-induced OC formation.

Co-stimulatory signaling is activated in numerous cell types in immune responses, and in OCPs it typically induces osteoclastogenesis independently of RANKL/RANK signaling.[10,11] Osteoblastic and immune cells express co-stimulatory molecules.[11] These are mainly unidentified ligands for immunoglobulin-like receptors, such as triggering receptor expressed in myeloid cells-2 and OC-associated receptor to which immunoreceptor tyrosine-based activation motif-containing molecules bind in OCPs to induce osteoclastogenic signals.[10,11] These signals activate phospholipase Cγ/calcium-mediated signaling similar to RANK and thus upregulate expression of nuclear factor of activated T cells, the master regulator of OC formation.[10,11] Interestingly co-stimulatory and Rank signaling appear to be linked directly in OCPs[12] and likely augment osteoclastogenesis in inflammatory bone diseases, such as rheumatoid arthritis and periodontitis. Wnt5a/cJun co-stimulatory signaling upregulates Rank expression in OCPs and thus appears to function differently from other co-stimulatory signaling pathways identified to date.

Several studies in the past few years[13] and now this study by Maeda *et al.*[1] have identified additional mechanisms whereby osteoblastic and osteoclastic cells interact with one another to positively and negatively regulate their formation and/or functions, thus changing the long-held view that OB/OC interactions worked one way with OBs having the dominant role. For example, ephrin B2, a protein ligand expressed by OCPs, and its receptor, Eph 4, on OB precursors inhibits OC formation through reverse signaling back through ephrin B2 that downregulates c-Fos activation of nuclear factor of activated T cells and in this way inhibits OCP differentiation.[14] In contrast, conventional forward signaling through Eph4 in OB precursors stimulates OB differentiation through EphB4-mediated RhoA inactivation. Thus, this bi-directional signaling could stop OC formation in the bases of bone remodeling units away from the active resorption surface and stimulate new bone formation at this site to which OB precursors need to be attracted during normal or pathologic bone remodeling. Interestingly, recent studies have suggested that sphingosine-1 phosphate secreted by OCs,[15,16] but not OCPs, might attract OB precursors from the bloodstream to remodeling units to fulfill this function. However, this does not fit well with a model in which OB precursors should be attracted to the bases of remodeling units. Other studies showed that Atp6v0d2, a subunit of V-ATPase, a component of the V-type H^+ ATP6i proton pump complex that secretes H^+ from actively resorbing OCs, inhibits OB precursor differentiation.[17] In addition, semaphorin 4D produced by OCs binds to its receptor, Plexin-B1, on OBs, resulting in RhoA association with Plexin-B1, and inhibition of Rho-A/cadherin-11-mediated migration and insulin receptor substrate 1/insulin-like growth factor-1-mediated OB precursor differentiation.[18] Intuitively, these latter two findings would make sense and provide a mechanism to keep OB precursors away from the site of active resorption in lacunae.

Exactly how OCs and OCPs regulate the attraction and differentiation of OB precursors and precisely where OB and OC precursor interactions in remodeling units, such as those above and through Wnt5a/Ror2 signaling, take place to induce OB formation will require further study. Nonetheless, these studies suggest that there may be some consequences of inhibiting OCP formation and differentiation that could negatively impact bone mass by inhibiting OB differentiation. That Wnt/Ror2 signaling appears to affect only rank expression in OCPs (Wnt5a does not affect osteoprotegerin expression by osteoblastic cells) suggests that a GST-Ror2 decoy receptor could have a defined, specific effect on osteoclastogenesis without affecting OBs. It would seem unlikely that GST-Ror2 could be more effective than denosumab (a monoclonal antibody that binds to RANKL to prevent it inducing OC formation[19]) to inhibit bone loss in inflammatory bone diseases, but this will require further study.

Conflict of Interest

The author declares no conflict of interest.

References

1. Maeda K, Kobayashi Y, Udagawa N, Uehara S, Ishihara A, Mizoguchi T *et al.* Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. *Nat Med* 2012;**18**:405–412.
2. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC *et al.* Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* 2003;**425**:841–846.
3. Mizoguchi T, Muto A, Udagawa N, Arai A, Yamashita T, Hosoya A *et al.* Identification of cell cycle-arrested quiescent osteoclast precursors in vivo. *J Cell Biol* 2009;**184**:541–554.
4. Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Dev Cell* 2005;**8**:739–750.
5. Oishi I, Suzuki H, Onishi N, Takada R, Kani S, Ohkawara B *et al.* The receptor tyrosine kinase Ror2 is involved in non-canonical Wnt5a/JNK signalling pathway. *Gene Cells* 2003;**8**: 645–654.
6. Liu Y, Bhat RA, Seestaller-Wehr LM, Fukayama S, Mangine A, Moran RA *et al.* The orphan receptor tyrosine kinase Ror2 promotes osteoblast differentiation and enhances ex vivo bone formation. *Mol Endocrinol (Baltimore, Md)* 2007;**21**:376–387.
7. Redlich K, Hayer S, Maier A, Dunstan CR, Tohidast-Akrad M, Lang S *et al.* Tumor necrosis factor alpha-mediated joint destruction is inhibited by targeting osteoclasts with osteoprotegerin. *Arthr Rheum* 2002;**46**:785–792.
8. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Arch Biochem Biophys* 2008;**473**:139–146.
9. Yao Z, Xing L, Boyce BF. NF-kappaB p100 limits TNF-induced bone resorption in mice by a TRAF3-dependent mechanism. *J Clin Invest* 2009;**119**:3024–3034 Epub 23 September 2009.
10. Nakashima T, Takayanagi H. New regulation mechanisms of osteoclast differentiation. *Annl NY Acad Sci* 2011;**1240**:E13–E18.
11. Koga T, Inui M, Inoue K, Kim S, Suematsu A, Kobayashi E *et al.* Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. *Nature* 2004;**428** :758–763.

12. Shinohara M, Koga T, Okamoto K, Sakaguchi S, Arai K, Yasuda H *et al.* Tyrosine kinases Btk and Tec regulate osteoclast differentiation by linking RANK and ITAM signals. *Cell* 2008;**132**: 794–806.
13. Boyce BF, Yao Z, Xing L. Osteoclasts have multiple roles in bone in addition to bone resorption. *Crit Rev Eukaryot Gene Expr* 2009;**19**:171–180.
14. Zhao C, Irie N, Takada Y, Shimoda K, Miyamoto T, Nishiwaki T *et al.* Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab* 2006;**4**:111–121.
15. Ishii M, Egen JG, Klauschen F, Meier-Schellersheim M, Saeki Y, Vacher J *et al.* Sphingosine-1-phosphate mobilizes osteoclast precursors and regulates bone homeostasis. *Nature* 2009;**458**: 524–528.
16. Pederson L, Ruan M, Westendorf JJ, Khosla S, Oursler MJ. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. *Proc Natl Acad Sci USA* 2008;**105**:20764–20769.
17. Lee SH, Rho J, Jeong D, Sul JY, Kim T, Kim N *et al.* v-ATPase V0 subunit d2-deficient mice exhibit impaired osteoclast fusion and increased bone formation. *Nat Med* 2006;**12**:1403–1409.
18. Negishi-Koga T, Shinohara M, Komatsu N, Bito H, Kodama T, Friedel RH *et al.* Suppression of bone formation by osteoclastic expression of semaphorin 4D. *Nat Med* 2011;**17**:1473–1480.
19. Cummings SR, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR *et al.* Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med* 2009;**361**: 756–765.