Citation: IBMS BoneKEy **9,** Article number: 39 (2012) | doi:10.1038/bonekey.2012.36
© 2012 International Bone & Mineral Society All rights reserved 1940-8692/12
www.nature.com/bonekey

COMMENTARY

Osteocytes communicate with osteoclast lineage cells via RANKL

Koichi Matsuo

Laboratory of Cell and Tissue Biology, School of Medicine, Keio University, Tokyo, Japan.

IBMS BoneKEy **9**, Article number: 39 (2012) | doi:10.1038/bonekey.2012.36; published online 29 February 2012

Commentary on: Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, Bonewald LF, Kodama T, Wutz A, Wagner EF, Penninger JM, Takayanagi H. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. Nat Med. 2011; **17**(10):1231–1234 and Xiong J, Onal M, Jilka RL, Weinstein RS, Manolagas SC, O'Brien CA. Matrix-embedded cells control osteoclast formation. Nat Med. 2011; **17**(10):1235–1241.

Using conditional knockout mice, two groups recently reported phenotypes of mice with osteocyte-specific deletion of the receptor activator of nuclear factor-κB ligand (RANKL), the cytokine essential for osteoclast differentiation. Both groups, Nakashima *et al.*[1] and Xiong *et al.*[2], found that mice lacking RANKL (encoded by *Tnfsf11*) in osteocytes develop postnatal osteopetrosis, indicating that matrix-embedded osteocytes induce RANKL-dependent osteoclast formation in bone remodeling. These are indeed paradigm-shifting studies.[3]

Previously, many of us believed that osteoblasts or stromal cells are the major source of RANKL throughout life, although many other cell types including T lymphocytes and hypertrophic chondrocytes express RANKL. The prevailing idea that osteoblasts direct osteoclastogenesis has been reinforced by widely used co-culture methods in which osteoclast formation from hematopoietic precursor cells is supported by 'calvarial osteoblasts', which in hindsight most likely contain osteocytes.[4] Nakashima *et al.* observed high RANKL expression in both primary cells purified by alternate collagenase-EDTA extractions and dentin matrix protein 1 (Dmp1)-positive osteocytes purified from mouse calvarial and cortical bone by cell sorting. They demonstrated that purified osteocytes more potently support osteoclastogenesis than do osteoblasts and bone marrow stromal cells in co-culture.[1] Nakashima *et al.* proposed that osteocytes derived from osteoblasts might provide RANKL to osteoclast precursor cells. This laboratory had been interested in whether T lymphocytes are a major RANKL source under pathological conditions, such as in joints of rheumatoid arthritis patients.[5,6] LoxP sites were introduced flanking *Tnfsf11* exons 3 and 4, and 'floxed' mice were bred with mice expressing Cre recombinase driven by the osteocyte-specific *Dmp1* promoter or the T-cell-specific *Lck* promoter. Although the T-cell-specific knockout mice showed no phenotype under physiological conditions, osteocyte-specific knockout mice developed osteopetrosis postnatally.

Independently, O'Brien and co-workers[2] generated an allele carrying floxed exons 3 and 4 of *Tnfsf11*, and crossed resultant mice with various 'deleter mice', such as Prx1-Cre (for excision in early limb bud mesenchyme and in a subset of craniofacial mesenchyme, but not in the axial skeleton), Osterix 1 (Osx1)-Cre (early osteoblasts and late-proliferating and hypertrophic chondrocytes), Osteocalcin (Ocn)-Cre (mature osteoblasts and hypertrophic chondrocytes), Dmp1-Cre (osteocytes) and collagen X (ColX)-Cre (hypertrophic chondrocytes). Cre-recombinase-mediated deletion is irreversible; once a gene is deleted in the osteoblasts, osteocytes derived from these osteoblasts carry deleted alleles. Following careful analysis of phenotypes of various conditional knockout mice, both Nakashima *et al.* and Xiong *et al.* found that osteocyte-specific RANKL deletion resulted in postnatal osteopetrosis, suggesting that osteocytes are a major source of RANKL during bone remodeling. Xiong *et al.* demonstrated that Dmp1-Cre-mediated deletion occurs not only in osteocytes, but also in osteoblasts. However, they also showed that RANKL deletion mediated by doxycycline withdrawal over a period of 2 months in osteoprogenitors of adult Osx1-Cre mice (via the Tet-off system) did not alter bone remodeling, suggesting that osteoprogenitor RANKL does not contribute to bone remodeling. By contrast, in bone modeling characterized by bone growth associated with changes in shape during development, resorption of the primary spongiosa is dependent on RANKL produced by mature osteoblasts or hypertrophic chondrocytes, as illustrated by unresorbed calcified cartilage seen in conditional RANKL-knockout mice carrying either Osx1-Cre, Ocn-Cre or ColX-Cre.[2] Thus, for the first time, osteoblast/hypertrophic cartilage-derived RANKL has been linked to osteoclastogenesis during 'bone modeling', whereas osteocyte-derived RANKL is associated with 'bone remodeling' in maintenance of bone.

What is the significance of RANKL production by osteocytes in bone remodeling, particularly in coupling of bone resorption and formation? Osteocytes communicate with osteoblasts and negatively regulate osteoblast differentiation by secretion of sclerostin (encoded by *Sost*) through dendrites extended into canaliculi.[7] Sclerostin is a potent inhibitor of osteoblast differentiation, which binds to LRP5/6 receptors and antagonizes Wnt signaling.[8] As it is now clear that osteocytes communicate

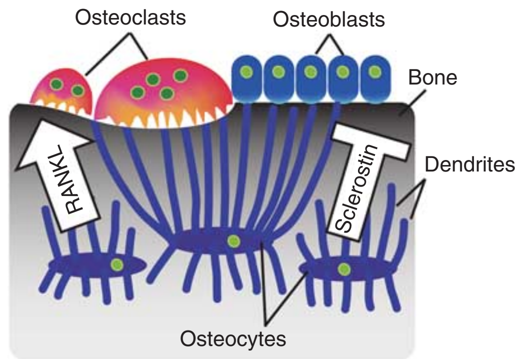

Figure 1 The tri-partite relationship of osteocytes, osteoclasts and osteoblasts during bone remodeling. Multinucleated osteoclasts (red) resorb bone surface, whereas osteoblasts (blue) form bone (grey/black). Osteocytes with multiple dendrites (purple) are embedded in bone. Osteocytes and their dendrites are embedded in lacunae and canaliculi, respectively (not shown), in the mineralized bone matrix. Osteocytes produce RANKL to induce osteoclast formation and suppress osteoblast differentiation through sclerostin secretion.

with osteoclast lineage cells through RANKL, both osteoclasts and osteoblasts are likely controlled by osteocytes during bone remodeling (**Figure 1**). On the other hand, osteoclast-derived hypothetical 'coupling factors' facilitate transition from bone resorption to bone formation.[9] How is this three-part relationship regulated to maintain bone integrity? It is plausible that bone resorption in the absence of bone formation occurs when osteocytes produce both RANKL and sclerostin at high levels. When coupling occurs, osteocytes likely stop producing both to allow the transition from bone resorption to formation. Further studies will be required to determine whether osteoclast-derived coupling factors, which by definition activate osteoblastic bone formation, regulate this switching in osteocytes.

Numerous interesting questions for future study emerge from these highly stimulating papers.

- How is RANKL expression in osteocytes induced by unloading? Xiong *et al.*[2] demonstrated that RANKL mRNA levels and osteoclast number were elevated in control but not conditional Dmp1-Cre mice, based on 3 weeks of tail suspension experiments. If osteocytes sense mechanical loading in the form of fluid-flow shear stress, how does loss of fluid-flow shear stress result in elevated RANKL expression? As sclerostin stimulates RANKL expression in osteocytes,[10] and unloading increases osteocyte expression of sclerostin,[11] unloading may induce RANKL expression through sclerostin. Is there concerted regulation of RANKL expression in multiple interconnected osteocytes by dendrites? Conversely, do multiple dendrites from a single osteocyte deliver different levels of RANKL depending upon orientation or geometry of the lacuna-canalicular system? Technology is currently being developed to analyze the lacuna-canalicular system three-dimensionally by X-ray imaging,[12] which can be combined with immunogold staining of RANKL.
- Do parathyroid hormone,[13] 1,25-dihydroxyvitamin D_3,[14] bisphosphonate[15] or other molecules regulate osteocytic RANKL expression *in vivo*? Do osteocytes express RANK for RANKL to function in an autocrine manner? If so, what is the function of this autocrine loop?
- There may be spatial, temporal and species variations in osteocyte RANKL production. How do osteocytes differ between cortical and cancellous bone in terms of RANKL expression? Is RANKL not expressed in osteocytes in developing animals? Do new and old osteocytes in adult animals express similar amounts of RANKL? A simple hypothesis is that old osteocytes function to induce osteoclastogenesis to replace old bone. If so, how do aged osteocytes produce more RANKL? Does RANKL expression by apoptotic osteocytes regulate osteoclast precursor differentiation?[16,17] Do osteocytes ever dedifferentiate into osteoblasts *ex vivo*? How is RANKL production regulated in 'acellular' (lacking osteocytes) bone during bone remodeling, as occurs in some teleost fish such as medaka?[18]
- Which among two membrane-bound forms of RANKL and a secreted form[19] are produced by osteocytes? How does RANKL reach the bone surface? At least in osteoblastic cells, RANKL is localized to secretory lysosomes and stimulation with RANK-Fc-conjugated beads promotes its translocation to the cell surface mediated by Rab 27 small GTPases.[20]

The regulatory system depicted in **Figure 1** is further complicated by osteoprotegerin (OPG), a soluble decoy receptor for RANKL that blocks osteoclast formation by inhibiting binding of RANKL to RANK. As OPG is produced by osteocytes as a target of Wnt signaling,[21,22] it is reasonable to assume that when sclerostin (and RANKL) production is reduced, OPG expression is elevated. Osteocytes are a source of constant surprise to bone biologists. Previously, we were astonished when we learned that osteocytes are hormone-producing cells that communicate with kidneys at a distance by secreting the hormone fibroblast growth factor 23, which regulates phosphate metabolism.[23,24] What will be the next unanticipated finding in bone metabolism? Major bone resorption by osteocytes under certain conditions? Morphogenesis controlled by osteocytes? Osteocyte-targeted therapy? Whatever happens, osteocytes' waving arms serve to attract many bone scientists.

Conflict of Interest

The author declares no conflict of interest.

References

1. Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ *et al.* Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat Med* 2011;**17**: 1231–1234.
2. Xiong J, Onal M, Jilka RL, Weinstein RS, Manolagas SC, O'Brien CA. Matrix-embedded cells control osteoclast formation. *Nat Med* 2011;**17**:1235–1241.
3. Killock D. Bone: Osteocyte RANKL in bone homeostasis: a paradigm shift? *Nat Rev Rheumatol* 2011;**7**:619.
4. Takahashi N, Akatsu T, Udagawa N, Sasaki T, Yamaguchi A, Moseley JM *et al.* Osteoblastic cells are involved in osteoclast formation. *Endocrinology* 1988;**123**:2600–2602.
5. Takayanagi H, Ogasawara K, Hida S, Chiba T, Murata S, Sato K *et al.* T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-γ. *Nature* 2000; **408**:600–605.
6. Takayanagi H. New immune connections in osteoclast formation. *Ann NY Acad Sci* 2010; **1192**:117–123.
7. Bonewald LF. The amazing osteocyte. *J Bone Miner Res* 2011;**26**:229–238.
8. Monroe DG, McGee-Lawrence ME, Oursler MJ, Westendorf JJ. Update on Wnt signaling in bone cell biology and bone disease. *Gene* 2011;**492**:1–18.

9. Matsuo K, Irie N. Osteoclast-osteoblast communication. *Arch Biochem Biophys* 2008;**473**: 201–209.
10. Wijenayaka AR, Kogawa M, Lim HP, Bonewald LF, Findlay DM, Atkins GJ. Sclerostin stimulates osteocyte support of osteoclast activity by a RANKL-dependent pathway. *PLoS ONE* 2011;**6**:e25900.
11. Robling AG, Niziolek PJ, Baldridge LA, Condon KW, Allen MR, Alam I *et al.* Mechanical stimulation of bone *in vivo* reduces osteocyte expression of Sost/sclerostin. *J Biol Chem* 2008;**283**:5866–5875.
12. Schneider P, Meier M, Wepf R, Müller R. Towards quantitative 3D imaging of the osteocyte lacuno-canalicular network. *Bone* 2010;**47**:848–858.
13. Rhee Y, Bivi N, Farrow E, Lezcano V, Plotkin LI, White KE *et al.* Parathyroid hormone receptor signaling in osteocytes increases the expression of fibroblast growth factor-23 *in vitro* and *in vivo*. *Bone* 2011;**49**:636–643.
14. Masuyama R, Stockmans I, Torrekens S, Van Looveren R, Maes C, Carmeliet P *et al.* Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. *J Clin Invest* 2006;**116**:3150–3159.
15. Bivi N, Picotti P, Muller LN, Romanello M, Moro L, Quadrifoglio F *et al.* Shotgun proteomics analysis reveals new unsuspected molecular effectors of nitrogen-containing bisphosphonates in osteocytes. *J Proteomics* 2011;**74**:1113–1122.
16. Kogianni G, Mann V, Noble BS. Apoptotic bodies convey activity capable of initiating osteoclastogenesis and localized bone destruction. *J Bone Miner Res* 2008;**23**:915–927.
17. Al-Dujaili SA, Lau E, Al-Dujaili H, Tsang K, Guenther A, You L. Apoptotic osteocytes regulate osteoclast precursor recruitment and differentiation *in vitro*. *J Cell Biochem* 2011;**112**: 2412–2423.
18. To TT, Witten PE, Renn J, Bhattacharya D, Huysseune A, Winkler C. Rankl-induced osteoclastogenesis leads to loss of mineralization in a medaka osteoporosis model. *Development* 2011;**139**:141–150.
19. Ikeda T, Kasai M, Utsuyama M, Hirokawa K. Determination of three isoforms of the receptor activator of nuclear factor-κB ligand and their differential expression in bone and thymus. *Endocrinology* 2001;**142**:1419–1426.
20. Kariya Y, Honma M, Hanamura A, Aoki S, Ninomiya T, Nakamichi Y *et al.* Rab27a and Rab27b are involved in stimulation-dependent RANKL release from secretory lysosomes in osteoblastic cells. *J Bone Miner Res* 2011;**26**:689–703.
21. Kramer I, Halleux C, Keller H, Pegurri M, Gooi JH, Weber PB *et al.* Osteocyte Wnt/β-catenin signaling is required for normal bone homeostasis. *Mol Cell Biol* 2010;**30**:3071–3085.
22. Glass II DA, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H *et al.* Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell* 2005;**8**:751–764.
23. Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B *et al.* Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nat Genet* 2006;**38**:1310–1315.
24. Pereira RC, Jüppner H, Azucena-Serrano CE, Yadin O, Salusky IB, Wesseling-Perry K. Patterns of FGF-23, DMP1, and MEPE expression in patients with chronic kidney disease. *Bone* 2009;**45**:1161–1168.