Editorial

A potential osteoporosis target in the FAS ligand/FAS pathway of osteoblast to osteoclast signaling

Daniel R. Jones

Division of Natural Sciences, Indiana Wesleyan University, Marion, Indiana 46953, USA

Correspondence to: Daniel R. Jones. Division of Natural Sciences, Indiana Wesleyan University, Marion, Indiana 46953, USA. Email: dan.jones@indwes.edu.

Submitted Jul 01, 2015. Accepted for publication Jul 02, 2015.
doi: 10.3978/j.issn.2305-5839.2015.07.01
View this article at: http://dx.doi.org/10.3978/j.issn.2305-5839.2015.07.01

One of the pivotal factors in the health and maintenance of dense bone is the coordinated activity of osteoblasts and osteoclasts. Research scientists are developing an enormous knowledge base about the signaling that occurs between these two cell types with the goal of understanding the bone microenvironment under normal as well as resorptive disease states. This knowledge base has already led to the development of successful therapies for osteoporosis. The monoclonal antibody to receptor activator of nuclear factor-κB ligand (RANKL), known as Prolia® (denosumab), is a recent example (1). RANKL is a cytokine expressed on the surface of the osteoblast in response to bone resorption cues like 1α,25-dihydroxyvitamin D3, parathyroid hormone (PTH), prostaglandin E_2 (PGE_2), and interleukin 11 (IL-11) (2). A second cytokine, macrophage colony-stimulating factor (M-CSF), is released by the osteoblast on a continual basis with both cytokines promoting differentiation of osteoclast precursors. The osteoclast precursors express receptors for the two cytokines, c-Fms (M-CSF receptor) and RANK (RANKL receptor). Another ligand for c-Fms is IL-34, expressed primarily by splenic red pulp, which appears to act in concert with M-CSF and RANKL (3,4). Osteoblasts express a third factor which is a soluble tumor necrosis factor (TNF) receptor family member, osteoprotegrin (OPG), that serves as a "decoy" receptor for RANKL and thereby blocks RANKL-RANK binding to inhibit osteoclastogenesis (2). A construct of OPG in which the heparin-binding and death homology domains are removed and the remaining peptide is fused to the F_c domain of the human immunoglobulin G1 (IgG1) is used for experimental therapeutic purposes. The resulting fusion protein construct is called OPG-F_c, and it neutralizes RANKL (5). In a study of ovariectomized (OVX) rats, the combined use of OPG-F_c and alendronate (a commonly used bisphosphonate) significantly increased the mechanical strength properties of femurs and lumbar vertebrae bodies compared to treatment with either OPG-F_c or alendronate alone (6).

Osteoblasts also produce factors encoded by a gene family collectively called the *Wnt* gene family. The family received its name from a combination of the names for two homologous genes from other organisms: Wg (the wingless gene in *Drosophila*) and int-1 (the integration-1 gene in mouse) (7). Wnt5a binds to 2 different osteoclast precursor receptor complexes. The binding of one of those complexes, Frizzled and low density lipoprotein receptor-related protein 5/6 (LRP5/6), by Wnt5a acts through β-catenin to produce needed signaling for osteoblastogenesis and osteoclastogenesis (2,8). The osteoblastogenesis branch of Wnt5a signaling is negatively regulated by a product of the osteoblast, the glycoprotein Dickkopf-1 (DKK1). DKK1 also activates osteoclast formation. An antibody to DKK1 (BHQ880) increased osteoblast activity and reduced myeloma bone disease in a mouse model (9).

Wnt5a binding to the other complex, Frizzled and receptor tyrosine kinase-like orphan receptor 2 (Ror2), appears to increase RANK expression in osteoclasts, sensitizing them to RANKL (10). Several studies suggest that Wnt5a signaling is associated with inflammatory joint conditions as well as pathologic bone resorption (10-12). Although not produced by the osteoblast, sclerostin is a factor that influences the Wnt signaling pathway, favoring increased bone resorption by binding to LRP5/6. Sclerostin is expressed by mineralized hypertrophic chondrocytes, cementocytes, and osteocytes. Two monoclonal antibodies to sclerostin, blosozumab

and romosozumab, effectively increase bone density. Romosozumab is currently in five different Phase III clinical trials, four in postmenopausal, osteoporotic women and one in male osteoporosis patients (13).

A further level of complexity exists in osteoblast to osteoclast precursor signaling through the expression and action of osteoblast semaphorins. Semaphorin 3A (Sema3A) binds to a receptor complex on the precursor comprised of neurophilin-1 (Nrp1) and Plexin-A1 to inhibit osteoclast differentiation (14). A study by Takayanagi revealed that administration of recombinant Sema3A to ovariectomized, osteoporotic mice increased bone volume and regeneration (14). Thus Sema3A is considered an anabolic factor in bone, suggesting the potential for its investigation as a therapeutic. Other members of the semaphorin family, Sema6C/6D, are osteoblast transmembrane proteins that bind to a receptor complex on the osteoclast precursor to stimulate immunoreceptor tyrosine-based activation motifs (ITAMs), thereby enhancing RANK signaling and osteoclastogenesis (15).

Recently Wang *et al*. (a group of authors from across the world including departments of The Fourth Military Medical University in China, INSERM in France, and the School of Dental Medicine at the University of Pennsylvania in the United States) published a remarkable work that carefully elucidates a pathway in the osteoblast-osteoclast axis, that of FAS ligand/FAS (16). It is well understood that FAS ligand (FASL) is a transmembrane protein that binds to its death receptor, FAS, on target cells to trigger the extrinsic apoptosis pathway in the target cell. This is true of many cell types and is critical for many phenomena, including immune cell regulation as well as cancer cell progression (17-19). Wang's publication in *Cell Death and Differentiation* on March 6th, 2015 was not the first to investigate this pathway in osteoblasts. Studies to characterize the osteoprotective effects of estrogen revealed that estrogen increases FASL expression in osteoblasts, resulting in increased osteoclast precursor apoptosis (20). More details emerged in a later study showing that estrogen, acting through estrogen receptor alpha (ERα), induced MMP3 cleavage of FASL in osteoblasts, thus releasing soluble FASL to bind to FAS receptors of osteoclast precursors. The resulting FASL-FAS interaction explains the mechanism of reduced osteoclast number in the presence of estrogen (21). The importance of Wang's work includes the elaboration of the paracrine pathway in which FASL from osteoblasts affects bone mass in health and disease (16), increasing our understanding of bone homeostasis and potentially representing a therapeutic target in post-menopausal osteoporosis.

Wang and colleagues constructed mice that expressed FASL in every tissue except osteoblasts. To achieve this mice with conditional FASL knockout alleles (cKO) were crossed to transgenic mice in which the Cre recombinase gene had been inserted at the FASL locus under the control of the SP7 promoter and only expressed in osteoblasts. The adult cKO mice were confirmed to have an osteoblast-specific FASL deficiency. These mice had an increased number of osteoclasts as well as increased osteoclast function. Osteoclast function was assessed through micro-CT scan as well as von Kossa staining. Decreased bone mineral density and bone volume to total volume ratio occurred as determined by micro-CT scan of femurs. Decreased trabeculation occurred as measured by histomorphometric analyses of von Kossa-stained femurs (16). The experimenters went an additional step to show that OVX, osteoporotic mice had decreased FASL expression. The mechanism appeared to be through the interferon gamma-(IFN-γ-) and TNF-α-activated nuclear factor-kappa B (NF-κB) pathway. This was confirmed by administering neutralizing antibodies to either IFN-γ or TNF-α. In the OVX animals which received the neutralizing antibodies, osteoblast FASL expression was restored and the osteoporotic phenotype was markedly reduced. Another layer of the experimenters' research involved the findings that OVX FASL cKO mice had even greater bone loss than OVX mice. Further, the RANKL and OPG levels remained constant in OVX FASL cKOs. The authors put forward the interpretation that RANKL/OPG functions in regulation of osteoclast differentiation whereas FASL/FAS functions in the disposal of osteoclasts that are mature. The authors concluded that the FASL/FAS signaling from osteoblast to osteoclast is important during normal bone remodeling and during OVX-induced osteoporosis. The authors feel that these findings raise hope for investigations toward a potential therapeutic intervention in which a synchronous modification of the interaction between these two cell types takes center stage. Wang's group hints at introducing monoclonal antibodies that target pro-inflammatory IFN-γ or TNF-α in order to increase osteoblastic FASL expression and hence rescue impaired bone formation (16).

The trend toward specifically blocking members of signal transduction pathways in bone through inhibitory monoclonal antibodies has arisen because current established osteoporosis treatments are known

to be accompanied by side effects: the bisphosphonates can be accompanied by osteonecrosis of the jaw and subtrochanteric femur fractures (22,23). Raloxifene (a selective estrogen receptor modulator) and strontium ranelate are associated with thromboembolic disease, and both teriparatide and PTH 1-84 can produce the side effects of hypercalcemia, nausea, and diarrhea (22,23). In spite of the recent advances with monoclonal antibody treatments, namely denosumab, unwanted peripheral effects are still produced from their usage. Denosumab administration can be associated with osteonecrosis of the jaw, hypocalcemia, hypersensitivity, and atypical femoral fracture (24,25). Consequently, it is imperative to pursue new therapies beyond the existing ones (existing combination therapies included).

As Wang's group intimated, IFN-γ and TNF-α neutralizing antibodies may be the next monoclonal development project for bone. IFN-γ is an antiviral protein synthesized by many somatic cells to regulate the immune response (26). Likewise, TNF-α plays a vital role in the immune system through regulation of immune cells, involvement in the acute phase reaction, and inhibition of tumorigenesis (27). Specific delivery of neutralizing antibodies to the osteoblast would be needed to prevent potentially devastating effects to immune system function. Wang's work did not address whether the OVX mice that received the neutralizing antibodies were challenged immunologically; however, those parameters were not the focus of the Wang group study. Anti-TNF-α and anti-IL-6 antibodies delivered to joints of rheumatoid arthritis patients have been effective at decreasing bone resorption and inflammation in the confines of a diseased joint (28). It is a different scenario to administer pro-inflammatory cytokine antibodies on a skeletal system-wide scale.

Parallel limitations also exist for targeting FAS or FASL directly. Previous attempts to target the FAS/FASL system in mice with systemic treatments utilizing antibodies to FAS or to FASL met with fibrosis, hepato-toxicity, and pulmonary inflammation (29,30). The Wang group study focused on increasing FASL expression from osteoblasts to act upon osteoclast precursor FAS to initiate apoptosis and rescue the osteoporotic phenotype. The picture is more complicated because both FAS and FASL are each expressed on osteoblasts and osteoclasts in response to cytokines. Plus the expression levels of FAS and FASL in these two cell types fluctuate with the different stages of both osteoblast and osteoclast differentiation. Considering these details, inhibition of FAS on osteoblasts would be another theoretical target. To avoid systemic problems, targeting FAS and/or FASL could involve a couple of approaches. Kovacic *et al*. suggest *ex vivo* tweaking of osteoblast, osteoclast, or osteoblast/osteoclast progenitor FAS expression. Another approach would be targeting osteoblast- or osteoclast-specific intracellular signaling molecules downstream of FAS (31).

The successful treatment and prevention of osteoporosis, like many other diseases, requires the patient to work with health professionals to eliminate underlying pathologies and deficiencies as well as limit risk factors. The patient must be compliant in modifying harmful lifestyle habits like smoking and excessive alcohol use. Tackling this disease entity requires adherence to a balanced diet that is sufficient in all the nutrients, especially calcium, vitamin D, and protein (32). A commitment to appropriate levels of weight-bearing exercise and adjustments for hormonal deficiencies are also a must. Depending on the specific patient, the multi-faceted approach to treatment and prevention may very well additionally include pharmacological intervention to right the imbalanced molecular factors that have upset bone homeostasis. Fortunately in today's practice of personalized medicine, physicians and their patients have valuable genomic tools to determine not just individual genetic predisposition to disease but increasingly to determine probability of ineffective or adverse drug response (33,34). Consequently, not only are pharmacological approaches becoming more specific for their molecular targets, but they can be more specifically tailored for variations in the individual patient as well. These approaches coupled with the application of new insights from cell biology-based research should translate into improved patient care. As further details of the molecular interworkings of osteoblasts and osteoclasts are uncovered like those brought to light by Wang and colleagues, it becomes more realistic to envision a future in which osteoporosis can be managed or even eliminated without detrimental side effects.

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Choi HJ. New antiresorptive therapies for postmenopausal osteoporosis. J Menopausal Med 2015;21:1-11.
2. Yamashita T, Takahashi N, Udagawa N. New roles of osteoblasts involved in osteoclast differentiation. World J Orthop 2012;3:175-81.
3. Lin H, Lee E, Hestir K, et al. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. Science 2008;320:807-11.
4. Chen Z, Buki K, Vääräniemi J, et al. The critical role of IL-34 in osteoclastogenesis. PLoS One 2011;6:e18689.
5. Zauli G, Melloni E, Capitani S, et al. Role of full-length osteoprotegerin in tumor cell biology. Cell Mol Life Sci 2009;66:841-51.
6. Wang Y, Huang P, Tang PF, et al. Alendronate (ALN) combined with osteoprotegerin (OPG) significantly improves mechanical properties of long bone than the single use of ALN or OPG in the ovariectomized rats. J Orthop Surg Res 2011;6:34.
7. Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer 2008;8:387-98.
8. Glass DA 2nd, Bialek P, Ahn JD, et al. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell 2005;8:751-64.
9. Hameed A, Brady JJ, Dowling P, et al. Bone disease in multiple myeloma: pathophysiology and management. Cancer Growth Metastasis 2014;7:33-42.
10. Maeda K, Kobayashi Y, Udagawa N, et al. Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. Nat Med 2012;18:405-12.
11. Sen M, Lauterbach K, El-Gabalawy H, et al. Expression and function of wingless and frizzled homologs in rheumatoid arthritis. Proc Natl Acad Sci U S A 2000;97:2791-6.
12. Sen M, Chamorro M, Reifert J, et al. Blockade of Wnt-5A/frizzled 5 signaling inhibits rheumatoid synoviocyte activation. Arthritis Rheum 2001;44:772-81.
13. Shah AD, Shoback D, Lewiecki EM. Sclerostin inhibition: a novel therapeutic approach in the treatment of osteoporosis. Int J Womens Health 2015;7:565-80.
14. Hayashi M, Nakashima T, Taniguchi M, et al. Osteoprotection by semaphorin 3A. Nature 2012;485:69-74.
15. Koga T, Inui M, Inoue K, et al. Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. Nature 2004;428:758-63.
16. Wang L, Liu S, Zhao Y, et al. Osteoblast-induced osteoclast apoptosis by fas ligand/FAS pathway is required for maintenance of bone mass. Cell Death Differ 2015. [Epub ahead of print].
17. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 2003;114:181-90.
18. Zhang Y, Xu G, Zhang L, et al. Th17 cells undergo Fas-mediated activation-induced cell death independent of IFN-gamma. J Immunol 2008;181:190-6.
19. Hohlbaum AM, Moe S, Marshak-Rothstein A. Opposing effects of transmembrane and soluble Fas ligand expression on inflammation and tumor cell survival. J Exp Med 2000;191:1209-20.
20. Krum SA, Miranda-Carboni GA, Hauschka PV, et al. Estrogen protects bone by inducing Fas ligand in osteoblasts to regulate osteoclast survival. EMBO J 2008;27:535-45.
21. Garcia AJ, Tom C, Guemes M, et al. ERα signaling regulates MMP3 expression to induce FasL cleavage and osteoclast apoptosis. J Bone Miner Res 2013;28:283-90.
22. Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. Lancet 2011;377:1276-87.
23. Lewiecki EM. New targets for intervention in the treatment of postmenopausal osteoporosis. Nat Rev Rheumatol 2011;7:631-8.
24. Katsarelis H, Shah NP, Dhariwal DK, et al. Infection and medication-related osteonecrosis of the jaw. J Dent Res 2015;94:534-9.
25. Zhou Z, Chen C, Zhang J, et al. Safety of denosumab in postmenopausal women with osteoporosis or low bone mineral density: a meta-analysis. Int J Clin Exp Pathol 2014;7:2113-22.
26. Wietzerbin J, Stefanos S, Falcoff R, et al. Immune interferon induced by phytohemagglutinin in nude mouse spleen cells. Infect Immun 1978;21:966-72.
27. Williamson BD, Carswell EA, Rubin BY, et al. Human tumor necrosis factor produced by human B-cell lines: synergistic cytotoxic interaction with human interferon. Proc Natl Acad Sci U S A 1983;80:5397-401.
28. Taylor PC, Feldmann M. Anti-TNF biologic agents: still the therapy of choice for rheumatoid arthritis. Nat Rev Rheumatol 2009;5:578-82.
29. Hagimoto N, Kuwano K, Miyazaki H, et al. Induction of apoptosis and pulmonary fibrosis in mice in response to ligation of Fas antigen. Am J Respir Cell Mol Biol 1997;17:272-8.
30. Ogasawara J, Watanabe-Fukunaga R, Adachi M, et al.

Lethal effect of the anti-Fas antibody in mice. Nature 1993;364:806-9.
31. Kovacic N, Grcevic D, Katavic V, et al. Targeting Fas in osteoresorptive disorders. Expert Opin Ther Targets 2010;14:1121-34.
32. Mosekilde L, Vestergaard P, Rejnmark L. The pathogenesis, treatment and prevention of osteoporosis in men. Drugs 2013;73:15-29.
33. Boron D, Czerny B, Bartkowiak-Wieczorek J, et al. Omentin Polymorphism and its Relations to Bone Mineral Density in Women. Arch Med Res 2015;46:173-80.
34. Sánchez-Lázaro I, Herrero MJ, Jordán-De Luna C, et al. Association of SNPs with the efficacy and safety of immunosuppressant therapy after heart transplantation. Pharmacogenomics 2015;16:971-9.

Cite this article as: Jones DR. A potential osteoporosis target in the FAS ligand/FAS pathway of osteoblast to osteoclast signaling. Ann Transl Med 2015;3(14):189. doi: 10.3978/j.issn.2305-5839.2015.07.01

