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COMMENTARIES

Amazing Multifunctionality of Calcineurin and NFAT Signaling in Bone Homeostasis

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Commentary on: Winslow MM, Pan M, Starbuck M, Gallo EM, Deng L, Karsenty G, Crabtree GR. Calcineurin/NFAT signaling in osteoblasts regulates bone mass. *Dev Cell*. 2006 Jun;10(6): 771-82.

The nuclear factor of activated T cells (NFAT) family of transcription factors was originally discovered in T cells as a regulator of cytokines such as interleukin-2, which induces T cell proliferation and differentiation (1;2). Calcineurin and NFAT have also emerged as important new regulators of bone homeostasis in both osteoclast differentiation and osteoblastic bone formation. The paper by Winslow *et al*. sheds light on novel aspects of the multiple functions of calcineurin and NFAT in the context of bone homeostasis using two intriguing genetic models (3). This report is of great importance not only because it supports the bifunctional role of *NFATc1* in both bone resorption and formation, but also because it provides the novel insight that chemokines regulated by NFAT may contribute to the osteoblast-mediated regulation of osteoclastogenesis.

The NFAT family comprises five members including NFATc1 (NFAT2), NFATc2 (NFAT1), NFATc3 (NFAT4), NFATc4 (NFAT3) and NFAT5. These family members play important roles not only in the regulation of the immune system, but also in a variety of other systems including the cardiovascular and muscular systems. In response to an increase in intracellular calcium concentration, calcium/calmodulin activate the heterodimeric serine/threonine phosphatase calcineurin, composed of calcineurin A (α/β/γ isoforms) and B (α/β isoforms) which dephosphorylates NFATc1/c2/c3/c4. Based on the critical role of calcineurin in T cell-mediated immune responses, calcineurin inhibitors such as FK506 and cyclosporin A are widely used as immunosuppressants in the clinic.

The *in vivo* function of NFATs and calcineurin in the immune system has been well-analyzed by gene disruption studies (1), but more recent studies are highlighting their roles as multifunctional regulators of diverse phases of bone metabolism. Genome-wide screening of RANKL-inducible genes in osteoclasts first shed light on the unexpected function of NFAT in osteoclast differentiation (4). The necessary and sufficient role of *NFATc1* in osteoclastogenesis was suggested by *in vitro* observations that *NFATc1*(-/-) embryonic stem cells do not differentiate into osteoclasts and that ectopic expression of NFATc1 causes bone marrow-derived precursor cells to undergo osteoclast differentiation in the absence of RANKL. Although analysis of *NFATc1*-deficient mice has been hampered by embryonic lethality owing to a defect in cardiac valve formation, recent novel studies with osteoclast-deficient mice (involving adoptive transfer of hematopoietic stem cells (HSCs) to *c-fos*(-/-) mice (1) and *c-fos*-deficient blastocyst complementation(2)) have clearly demonstrated that *NFATc1* is essential for osteoclast differentiation *in vivo* (5).

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In vivo suppression of calcineurin or NFAT activity also affects another aspect of bone remodeling, osteoblastic bone formation. Although the *in vitro* effects of calcineurin inhibitors have been contradictory and the role of calcineurin and NFAT in bone formation is hence controversial, recent analyses using *NFATc1*(-/-) embryonic fibroblasts (6), *NFATc2*(-/-) mice (6) and *calcineurin* Aα(-/-) mice (7) have unambiguously demonstrated that calcineurin and NFAT activity are crucial for osteoblast differentiation and bone formation. This is consistent with the observation that patients treated for extended periods with calcineurin inhibitors develop osteoporosis (8). Calcineurin and NFAT thus play important roles in the regulation of bone homeostasis in both aspects of bone remodeling. It is worth noting that members of the NFAT family have redundant roles in osteoblasts while NFATc1 plays an exclusive role in osteoclasts.

In the paper by Winslow *et al*. (3), transgenic mice in which constitutively active NFATc1 is overexpressed (*NFATc1*(nuc) mice) exhibited a high bone mass phenotype due to an enhanced proliferation of osteoblasts. This is possibly mediated by activation of Wnt signals, since the expression of Wnt4 and Frizzled9 are upregulated and Dickkopf2 (an inhibitory protein of Wnt) is downregulated. Although differentiation of osteoclasts is also accelerated, the authors show that constitutively active NFATc1 is not expressed by osteoclast precursor cells. They propose that the chemokines expressed by osteoblasts such as CCL8/MCP-2 and CCL6 are involved in the enhanced recruitment of osteoclast precursor cells to bone marrow, which causes the enhanced differentiation of osteoclasts. In contrast, the recent report by Ikeda *et al*. showed that overexpression of *NFATc2* in the osteoclast lineage (TRAP promoter-driven *NFATc2* transgenic mice) results in enhanced osteoclastogenesis associated with an osteoporotic phenotype (9). It is also notable that bone resorbing activity is enhanced in this strain, suggesting that NFAT regulates not only the differentiation but also the function of osteoclasts.

The authors performed another important genetic study by generating viable *NFATc1*(-/-) mice. As mentioned above, *NFATc1*(-/-) mice die during embryogenesis due to impaired heart valve morphogenesis caused by a defect in the development of cardiac endothelial cells. To avoid the lethality, the authors first generated transgenic mice in which *NFATc1* is overexpressed in the endothelial lineage by placing it under the control of the Tie2 promoter (Tie2-*NFATc1*(+) mice). They then crossed these mice with *NFATc1*(-/-) mice to obtain *NFATc*1(-/-);Tie2-*NFATc1*(+) mice. As expected, *NFATc*1(-/-);Tie2-*NFATc1*(+) mice were viable. Bone analyses revealed that these mice developed severe osteopetrosis (no formation of bone marrow and no tooth eruption) due to lack of osteoclasts, indicating the essential role of *NFATc1* in osteoclastogenesis. This is consistent with the previous observations in adoptive transfer of *NFATc*1(-/-) HSCs to *c-fos*(-/-) mice and *c-fos*-deficient blastocyst complementation (5). Despite the difficulty in analyzing bone formation in osteopetrotic mice, the authors demonstrated that bone formation in *NFATc*1(-/-);Tie2-*NFATc1*(+) mice is decreased by showing delayed calvaria formation, suggesting that *NFATc1* is also important for osteoblastic bone formation *in vivo*.

The study by Winslow *et al*., in addition to supporting the bifunctional role of NFATc1 in both bone resorption and formation, also demonstrates that chemokines regulated by NFAT may be involved in the regulation of osteoclastogenesis by osteoblasts, and is thus of great significance. It is surprising that calcineurin and NFAT are involved in the third aspect of the regulation of bone homeostasis: the coupling mechanism of osteoblasts and osteoclasts. Importantly, the contribution of CCL8 and other chemokines should be further explored in *in vivo* models in the future. Calcineurin and NFAT transcription factors play such an important role in the immune system that the major immunosuppressants used clinically

commonly target this pathway, but they also play multiple crucial roles in bone metabolism. Since the multifunctionality of this pathway may underlie not only the beneficial effects of immunosuppressants but also their adverse effects, the development of cell type-specific methods to suppress this pathway will be of great clinical relevance.

Clearly, calcineurin and NFAT regulate several phases of osteoblastogenesis. NFAT binds Osterix and regulates its transcriptional activity (6) and the reduction of type I collagen synthesis in FK506-treated cells or *NFAT*-deficient cells can be explained by the decreased activity of Osterix. Thus, NFATs regulate the relatively late phase of osteoblastogenesis. However, the current study shows that the proliferation of osteoblasts is increased in *NFATc1*(nuc) mice and this may be caused by the activation of Wnt signaling (3). Therefore, calcineurin and NFAT also regulate the early proliferation phase of osteoblastogenesis. Although Runx2 expression is decreased in *calcineurin Aα*(-/-) mice (7), Runx2 is expressed at the normal level in *NFATc1*(nuc) mice (3) and FK506-treated osteoblasts (6). Further studies are needed to obtain a complete understanding of the mechanism by which NFATs regulate bone formation. Whatever the mechanisms, the evidence is compelling that calcineurin and NFATs play a positive role in the regulation of bone formation.

The key roles of NFAT transcription factor activity in both the immune and skeletal systems suggest that these two systems utilize the same signaling apparatus, possibly because these systems developed simultaneously in the course of vertebrate evolution. Recently, the interplay of these systems and the interdisciplinary field called osteoimmunology have attracted much attention (10;11). The calcineurin-NFAT system may be one of the best examples of the same signaling pathway playing distinct roles in two different systems, leading us to a better realization of the amazing complexity of biological systems in general.

Conflict of Interest: The author reports that no conflict of interest exists.

References

1. Crabtree GR, Olson EN. NFAT signaling: choreographing the social lives of cells. *Cell*. 2002 Apr;109(Suppl):S67-79.

2. Hogan PG, Chen L, Nardone J, Rao A. Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev*. 2003 Sep 15;17(18):2205-32.

3. Winslow MM, Pan M, Starbuck M, Gallo EM, Deng L, Karsenty G, Crabtree GR. Calcineurin/NFAT signaling in osteoblasts regulates bone mass. *Dev Cell*. 2006 Jun;10(6):771-82.

4. Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, Wagner EF, Mak TW, Kodama T, Taniguchi T. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling for terminal differentiation of osteoclasts. *Dev Cell*. 2002 Dec;3(6):889-901.

5. Asagiri M, Sato K, Usami T, Ochi S, Nishina H, Yoshida H, Morita I, Wagner EF, Mak TW, Serfling E, Takayanagi H. Autoamplification of NFATc1 expression determines its essential role in bone homeostasis. *J Exp Med*. 2005 Nov 7;202(9):1261-9.

6. Koga T, Matsui Y, Asagiri M, Kodama T, de Crombrugghe B, Nakashima K, Takayanagi H. NFAT and Osterix cooperatively regulate bone formation. *Nat Med*. 2005 Aug;11(8):880-5.

7. Sun L, Blair HC, Peng Y, Zaidi N, Adebanjo OA, Wu XB, Wu XY, Iqbal J, Epstein S, Abe E, Moonga BS, Zaidi M. Calcineurin regulates bone formation by the osteoblast. *Proc Natl Acad Sci U S A*. 2005 Nov 22;102(47):17130-5.

8. Rodino MA, Shane E. Osteoporosis after organ transplantation. *Am J Med*. 1998 May;104(5):459-69.

9. Ikeda F, Nishimura R, Matsubara T, Hata K, Reddy SV, Yoneda T. Activation of NFAT signal in vivo leads to osteopenia associated with increased osteoclastogenesis and bone-resorbing activity. *J Immunol*. 2006 Aug 15;177(4):2384-90.

10. Walsh MC, Kim N, Kadono Y, Rho J, Lee SY, Lorenzo J, Choi Y. Osteoimmunology: interplay between the immune system and bone metabolism. *Annu Rev Immunol*. 2006;24:33-63.

11. Sato K, Takayanagi H. Osteoclasts, rheumatoid arthritis, and osteoimmunology. *Curr Opin Rheumatol*. 2006 Jul;18(4):419-26.