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Osteoprotegerin, the bone protector, is a surprising target for β-catenin signaling

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Abstract

Osteoblasts influence bone mass by the amount of bone they synthesize and by regulating osteoclasts, the cells that degrade bone. In the December issue of *Developmental Cell*, Kieslinger et al. (2005) show that immature osteoblasts regulate expression of the osteoclast inhibitor, osteoprotegerin, through an early B cell factor and β-catenin signaling.

As baby boomers age, they face many challenges: increasing health insurance costs, diabetes, the metabolic syndrome, Alzheimer's, and the increasing risk of fracture because of thinning bones. Congress, recognizing the need to investigate the molecular mechanisms regulating the function of bone cells, declared a decade of bone and joint research starting in 2002. This has yielded enormous success, with major advances in the understanding of how the skeleton and bone cells are formed and how bone is remodeled (renewed) in adult life. This is exemplified by findings reported earlier this year indicating that Wnt signaling not only determines if mesenchymal progenitors will become chondrocytes or osteoblasts (Hill et al., 2005) but also regulates bone mass through osteoblastic control of bone resorption. Bone is remodeled throughout life. Pockets or trenches of worn out bone are removed during remodeling by osteoclasts, which dig resorption trenches on bone surfaces. Mature osteoblasts fill in the trenches with new stronger bone, in much the same way as workmen repair roadways. Bone mass, strength, and fracture risk are determined by many factors, including the amount of matrix laid down by osteoblasts and removed by osteoclasts.

Somewhat surprisingly, the osteoblast appears to be the foreman controlling bone remodeling. Osteoblasts orchestrate bone destruction by expressing *R*eceptor *A*ctivator of *N*F-*κ*B (RANK) ligand in response to osteoclast-stimulating hormones and cytokines, such as PTH, TNF, and IL-1, but they also protect the skeleton by secreting osteoprotegerin (OPG) (Simonet et al., 1997). RANKL binds to its receptor, RANK, on osteoclast precursors to mediate osteoclast differentiation and subsequent activation; osteoprotegerin, a secreted nonsignaling decoy receptor for RANKL, inhibits both physiological and pathological bone resorption. Surprisingly, *Opg* expression was found to be regulated by β-catenin signaling through TCF1 (Glass et al., 2005). In the current issue of *Developmental Cell*, Kieslinger et al. (2005) confirm this role for β-catenin by showing that early B cell factor 2 (EBF2), a member of the early B cell factor family, regulates *Opg* expression in synergy with LEF1, another transcription factor mediating β-catenin function.

Wnt proteins play critical roles in carcinogenesis and in early development, controlling mesoderm induction, patterning, cell-fate determination, morphogenesis, and bone development (reviewed by Huelsken and Birchmeier, 2001). Wnts form a dual-receptor complex with Frizzled and low-density-lipoprotein (LDL)-receptor like protein 5 or 6 (LRP5/6) on cell surfaces. This triggers signaling through a large protein complex in the Wnt canonical

pathway, including *g*lycogen *s*ynthase *k*inase 3β(GSK-3β), casein kinase I, and the scaffolding proteins, adenomatous polyposis coli (APC), dishevelled, and axin. This complex has multiple effects on β-catenin: for example, it promotes β-catenin phosphorylation by GSK-3β, enabling it to be detected and destroyed by the proteasome. However, the N-terminal domain of LRP5 mediates its interaction with the Wnt-frizzled ligand-receptor complex, and the intracellular tail of LRP5/6 binds axin, which inhibits β-catenin phosphorylation by GSK-3β allowing β-catenin to move to the nucleus. Nuclear β-catenin combines with the transcription factors TCFs and LEF1 to activate expression of target genes.

Recent studies of mutations in humans have demonstrated that Wnts and proteins associated with Wnt signaling regulate bone mass. For example, patients with osteoporosis pseudoglioma syndrome, an autosomal recessive disorder mapped to a locus at chromosome 11q12-13, have normal bone growth but severe osteoporosis due to inactivating mutations of LRP5. *Lrp5*-deficient mice have a similar phenotype due to decreased bone formation. Interestingly, high bone mass (HBM) syndrome also has been mapped to this chromosomal region and is due to an activating mutation (Gly171Val) in *Lrp5*. These studies spurred a genetic approach in mice to study the role of β-catenin signaling in osteoblastic bone formation. Osteoblast-specific activation of the *β-catenin* gene using $\alpha_1(1)$ collagen-Cre in mice resulted in high bone mass, while deletion of *β-catenin* in osteoblasts led to osteopenia (Glass et al., 2005). Because LRP5 regulates osteoblast function and β-catenin expression is required for the differentiation of mesenchymal progenitors into osteoblasts, it was anticipated that the major role for β-catenin in osteoblasts would be regulation of their formation and function. Surprisingly, the changes in bone mass in the above *β-catenin* mutant mice are due to defects in osteoclast-mediated bone resorption, and *Opg* turns out to be a direct target gene for β-catenin in vitro (Jackson et al., 2005) and in vivo (Glass et al., 2005). Furthermore, conditional deletion of the *Apc* gene in mature osteoblasts by osteocalcin-Cre results in constitutive activation of *β-catenin*, increased bone deposition, and the absence of osteoclasts (Holmen et al., 2005). These mice have increased levels of serum OPG and their osteoblasts display decreased *Rankl* expression. More dramatically, the conditional deletion of *β-catenin* in mature osteoblasts by the same osteocalcin-Cre system leads to osteoporosis, decreased *Opg*, and increased *Rankl* expression (Holmen et al., 2005). Now, Kieslinger et al. (2005) report that the EBF family member, EBF2, activates *Opg* gene transcription in a synergistic manner with the Wnt/β-catenin signaling transcription factor, LEF1, in immature osteoblasts that do not express osteocalcin and thus inhibits osteoclast formation. Consequently, *Ebf2*$^{-/-}$ mice have reduced bone mass and increased numbers of osteoclasts. Their bone formation rate is normal and their osteoblasts express decreased *Opg* and increased *Rankl*. These authors showed that *Opg*, but not *Rankl*, is a target for EBF2.

These new studies suggest that both mature and immature osteoblasts can regulate *Opg* expression in a β-catenin-dependent manner. There are many types of cells in the osteoblast lineage, including progenitors, regulators of hematopoiesis and resorption, matrix secretors, osteocytes, and bone surface lining cells. Kieslinger et al. suggest that immature osteoblasts, which express low levels of alkaline phosphatase and type I collagen and do not express osterix and osteocalcin regulate *Opg* expression, but the Glass and Holmen papers indicate that this is done by more mature osteoblasts which express type I collagen and osteocalcin, respectively, i.e., cells with the capacity to lay down bone matrix. It is not easy to explain these discrepant findings. Intuitively, one would predict that a mature osteoblast busy building bone away from sites where bone is being removed would keep osteoclasts away by secreting OPG and that more immature cells would regulate resorption by expressing RANKL and little OPG. It is not clear if Wnt/β-catenin signaling also regulates transcription of *Rankl*. In osteoblasts in which the *β-catenin* gene is specifically deleted by Col1a1-Cre, *Rankl* expression is normal (Glass et al., 2005), while in more mature osteoblasts in which the *β-catenin* gene is specifically deleted by osteocalcin-Cre, *Rankl* levels are significantly increased (Holmen et al., 2005), although

Opg expression is similar in these two osteoblast-specific *β-catenin* mutants. Is the discrepancy in *Rankl* expression due to differences in the differentiated state of osteoblasts? We will also need to find out which cells regulate osteoblasts in these processes, and whether the osteoblasts are immature or more mature cells.

Selected reading

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