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PERSPECTIVES

Control of Bone Formation by Osteocytes? Lessons from the Rare Skeletal Disorders Sclerosteosis and van Buchem Disease

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In the adult, bone homeostasis is maintained by a tight balance between the supply, activity, and life span of osteoclasts and osteoblasts within the basic multicellular unit (BMU), a temporary anatomic structure within which bone remodeling occurs. Hormones, cytokines, and mechanical factors are known to control this balance. The exact role of osteocytes, the most abundant cell type in bone, remains unclear. Osteocytes are thought to be mechanosensor cells that control the activity of osteoblasts and osteoclasts within a BMU. Marotti and Martin suggested that osteocytes produce an inhibitory factor that signals to osteoblasts at a bone forming surface, causing them to slow down osteoid formation (1;2), but direct evidence for this is lacking. Recent data obtained from detailed analysis of the two closely related, rare skeletal disorders sclerosteosis and van Buchem disease demonstrated that osteocytes indeed produce a negative regulator of bone formation (3;4).

Patients with sclerosteosis and van Buchem disease have similar phenotypes, and both disorders are characterized by a substantial increase in bone mass (5-7). Sclerosteosis is due to premature termination mutations in the *SOST* gene on chromosome 17q12-q21 (8;9), whereas a 52 kb homozygous deletion downstream of the *SOST* gene is associated with van Buchem disease (10;11). The *SOST* gene encodes a protein, named sclerostin, the expression of which is highly restricted to osteocytes in the adult (3;4). Sclerostin is specifically localized in mature osteocytes in mineralized cortical and cancellous bone (12). In patients with sclerosteosis and van Buchem disease, sclerostin is not present in bone (3;7). Using transgenesis and *in vitro* transfection studies, a candidate enhancer element that may drive *SOST* expression in bone, but not during digit development, was identified within the van Buchem deletion (13). Syndactyly is a feature of the phenotype in sclerosteosis. Its absence in van Buchem disease may be explained by the absence of regulatory sequences in the van Buchem deletion that determine *SOST* expression in the zone of digit development.

Increased bone mass in these two rare skeletal diseases is due to increased osteoblast activity, as demonstrated by the following histological features in bone biopsies of affected individuals: predominance of cuboibal, active-appearing osteoblasts, increased double tetracycline label spacing, and increased osteoid that mineralizes normally (3;14;15). Osteoclast numbers appear not to be affected. *In vitro* studies confirm that sclerostin is a negative regulator of bone formation. Transgenic mice with overexpression of sclerostin have been found to be osteopenic (4;13).

Sclerostin Is Not a Classical BMP Antagonist

Based on amino acid sequence similarity, sclerostin was suggested to be a member of the DAN family of glycoproteins (9). This family of proteins includes wise, cerberus, DAN, coco, caronte, gremlin, dante, and protein related to DAN and cerberus (PRDC) that share as their main characteristic the ability to antagonize bone morphogenetic protein (BMP) activity. Of the BMP antagonists described so far, chordin and noggin have been shown to antagonize BMP signaling by blocking the binding of BMPs to their receptors (16;17). A similar mechanism has been proposed for members of the DAN family.

BMPs are secreted cytokines that were originally identified because of their ability to induce ectopic bone and cartilage formation (18). They exert their effects through distinct combinations of two different types of serine/threonine kinase receptors, *i.e*., type I and type II receptors (19;20). In initial studies, sclerostin was found to bind BMPs and to antagonize BMP-stimulated alkaline phosphatase activity in osteoblastic cells (3;4;21). However, detailed analysis of the mechanism by which sclerostin antagonizes BMP-stimulated bone formation revealed that the protein is not a classical BMP antagonist (3).

The capacity of sclerostin to inhibit late BMP responses such as bone formation, without antagonizing early BMP responses such as Smad phosphorylation and BMP reporter construct activation, could be achieved via a BMP-induced co-factor that allows sclerostin to function as a BMP antagonist. This hypothetical co-factor may, for example, increase the relative low binding affinity (10^{-8} M) of sclerostin to BMPs (4;21), an effect similar to that of twisted gastrulation for the binding of chordin to BMPs (22). However, we recently found that even after a previous BMP stimulation, sclerostin still did not antagonize BMP signaling (23). Available data thus strongly suggest that sclerostin is not a classical BMP antagonist in osteoblastic cells, although an effect on BMPs that have not been tested thus far cannot be excluded.

Sclerostin Is a Wnt Antagonist

As an alternative to antagonizing BMP signaling, sclerostin may inhibit late BMP responses by antagonizing other factors that cooperate with BMPs to stimulate bone formation. Xenopus cerberus, coco, and wise, three DAN family members of which wise has the highest amino acid similarity with sclerostin, have been found to antagonize Wnt activity. Wnts are known to cooperate with BMPs in stimulating bone formation (24-26). Like BMPs, Wnts are secreted cytokines with pivotal roles in a variety of cellular activities, including cell fate determination, proliferation, migration, polarity, and differentiation (27;28).

Recently, sclerostin was found to bind to the first two YWTD-EGF repeats of Wnt co-receptors LRP5 and LRP6 and to antagonize Wnt1- and Wnt3-stimulated activation of a canonical Wnt reporter construct (29;30). Similarly, we showed in a preliminary short report that sclerostin antagonized Wnt reporter construct activation by both BMPs and ligand independent constitutive active BMP receptors in osteoblastic cells, suggesting that sclerostin antagonized Wnt activity (23). However, sclerostin appears not to antagonize Wnt3A-induced β–catenin stabilization in mouse mesenchymal C3H10T1/2 cells (31), which is consistent with our own observations when we used recombinant Wnt3A. However, when we used Wnt expression vectors to stimulate Wnt signaling, sclerostin did antagonize Wnt signaling (23). In all studies reported so far in which sclerostin antagonized Wnt signaling, Wnt expression vectors were used as the source of Wnt activity. The differences between the effects of Wnts produced by transiently transfected cells and recombinant Wnts suggests that these molecules are differentially recognized by sclerostin. This may be explained by differences in tertiary structure, glycosylation, and/or other characteristics of the Wnts. Alternatively, Wnts produced by transient transfection may be membrane-bound, and this may be important for the interference by sclerostin. The mechanism by which sclerostin binding to LRP5 and 6 antagonizes Wnt signaling is currently unclear, although, for example, Wnt3 did not appear to compete with sclerostin for binding to LRP5 (29).

A Common Signaling Pathway is Affected in Sclerosteosis, van Buchem Disease, and Human High Bone Mass Phenotype

The human high bone mass (HBM) phenotype is an autosomal dominant condition that, like sclerosteosis and van Buchem disease, is characterized by increased bone mass due to enhanced bone formation in the presence of normal bone resorption (32). In two North American Caucasian families with the HBM phenotype, a G/T substitution at position 512 in exon 3, encoding a glycine/valine substitution at amino acid residue 171 (G171V) of the *LRP5* gene that makes it resistant to Dkk1-mediated inhibition, was identified as the underlying genetic defect (33-35). The G171V mutation and other mutations in *LRP5* associated with the HBM phenotype were recently shown to have little

effect on LRP5 transit to the cell surface, but instead acted by reducing the affinity for and inhibition by Dkk1, thereby increasing Wnt signaling (36).

Although sclerosteosis, van Buchem disease, and HBM have similar skeletal phenotypes, two distinct molecular mechanisms, increased BMP and Wnt signaling, were believed to cause these disorders. The recent observations that sclerostin antagonizes Wnt signaling rather than BMP signaling raises the possibility that the skeletal disorders sclerosteosis and van Buchem disease, as well as HBM, are due to increased activity of the same signaling pathway: LRP5-mediated canonical Wnt signaling. In the HBM phenotype, the inability of Dkk1 to inhibit LPR5-mediated Wnt signaling increases bone formation, while in sclerosteosis and van Buchem disease, the phenotype is due to the inability of sclerostin to inhibit LPR5-mediated Wnt signaling. *LRP5* mutations associated with the HBM phenotype may cause reduced binding and inhibition by sclerostin, in addition to reduced affinity and inhibition by Dkk1.

Conclusion

Evidence obtained during the past 2 years indicates that sclerostin is an osteocyte-expressed protein that inhibits the activity of osteoblasts and prevents them from promoting excessive bone formation. Sclerostin may be transported by the canaliculi to the bone surface, where it inhibits the bone-forming activity of osteoblasts. In this respect, it serves the function of the unknown inhibitory factor, proposed by Marotti and Martin, that is secreted by mature osteocytes and communicates with osteoblasts at a forming surface, causing them to slow osteoid formation (1;2). Alternatively, sclerostin may have an autocrine negative regulatory effect on Wnt signaling in osteocytes and, thereby, indirectly inhibit osteocyte-directed osteoblastic bone formation. Wnt signaling was recently reported in osteocytes using Wnt activity reporter mice (37).

Disturbances in bone remodeling balance constitute the pathophysiological basis of common skeletal disorders such as osteoporosis. It is obvious that inhibition of the activity or production of sclerostin is a promising strategy for the development of therapeutics that stimulate bone formation, thereby increasing bone mass. As sclerostin is a secreted protein, one approach to achieve this is to develop humanized neutralizing monoclonal antibodies capable of inhibiting the biological activity of sclerostin. A preliminary short report indicates that such an approach has been successful in rats (38). There have been concerns, however, about attempts to stimulate bone formation by targeting the sclerostin/LRP5 axis, as such therapeutics may lead to unwanted skeletal side effects. We have recently shown that heterozygous carriers of sclerosteosis have bone mineral density values consistently higher than healthy subjects, without any of the bone complications encountered in homozygotes (39). This suggests that the production and/or activity of sclerostin might be titrated *in vivo* to promote increases in bone mass without necessarily leading to unwanted skeletal side effects.

The only currently available therapy to stimulate bone formation in humans, intermittent parathyroid hormone (PTH) administration, was recently shown to reduce *SOST* mRNA and sclerostin protein expression in rats and mice (40;41). This suggests that inhibition of sclerostin expression, thereby removing a negative regulator of Wnt-stimulated bone formation, may play a role in the bone formation stimulating effect of intermittent PTH therapy.

Understanding the molecular mechanism of sclerostin action may not only provide the basis for addressing issues in the management of individuals with osteoporosis, but may also help in the management of affected individuals with sclerosteosis or van Buchem disease, for whom the only currently available treatment is surgical removal of excess bone, a difficult and risky procedure because of its anatomical location.

Conflict of Interest: The authors report that no conflicts of interest exist.

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