Orchestration of bone remodeling

Moester, M.J.C.

Chapter 2

Sclerostin: current knowledge and future perspectives

M.J.C. Moester
S.E. Papapoulos
C.W.G.M. Löwik
R.L. van Bezooijen

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Abstract

In recent years study of rare human bone disorders led to the identification of important signaling pathways that regulate bone formation. Such diseases include the bone sclerosing dysplasias sclerosteosis and Van Buchem disease, which are due to deficiency of sclerostin, a protein secreted by osteocytes that inhibits bone formation by osteoblasts. The restricted expression pattern of sclerostin in the skeleton and the exclusive bone phenotype of good quality of patients with sclerosteosis and Van Buchem disease provide the basis for the design of therapeutics that stimulate bone formation. We review here current knowledge of the regulation of the expression and formation of sclerostin, its mechanism of action and its potential as a bone building treatment for patients with osteoporosis.

Introduction

Osteoporosis is characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fractures [1]. The balance between bone resorption and bone formation determines the mass and structural integrity of the skeleton and is disturbed in osteoporosis. Current therapies of osteoporosis, with the exception of parathyroid hormone (PTH), decrease the risk of osteoporotic fractures by reducing bone resorption and preserving its architecture but cannot stimulate bone formation. Elucidating the mechanisms regulating bone formation may lead to the development of therapeutics able to rebuild bone mass and architecture.

In recent years, study of rare human bone disorders and of genetically manipulated animal models has led to the identification of signaling pathways that regulate bone formation which provide potential targets for the development of novel therapeutics. Fundamental for this progress have been studies of two rare bone sclerosing dysplasias, sclerosteosis and Van Buchem disease, that led to the identification of sclerostin, an important negative regulator of bone formation.

Sclerosteosis and Van Buchem disease

Sclerosteosis (OMIM 269500) and Van Buchem disease (OMIM 239100) are two rare sclerosing bone disorders, first described in the 1950's as distinct clinical entities, with closely related phenotypes [2]. Sclerosteosis has been mainly diagnosed among Afrikaners of Dutch descent in South Africa, while most patients diagnosed with Van Buchem disease come from a small fishing village in The Netherlands. A few individuals and families with sclerosteosis or Van Buchem disease have been reported in other parts of the world, including Spain, Brazil, USA, Germany, Japan, Switzerland, and Senegal [3].

The skeletal manifestations of sclerosteosis and Van Buchem disease are the result of endosteal hyperostosis and are characterized by progressive generalized osteosclerosis [3-8]. The manifestations are most pronounced in mandible and skull, with characteristic enlargement of the jaw and facial bones leading to facial distortion, increased intracranial pressure and entrapment of cranial nerves, often associated with facial palsy, hearing loss and/or loss of smell (Figure 1). Patients with

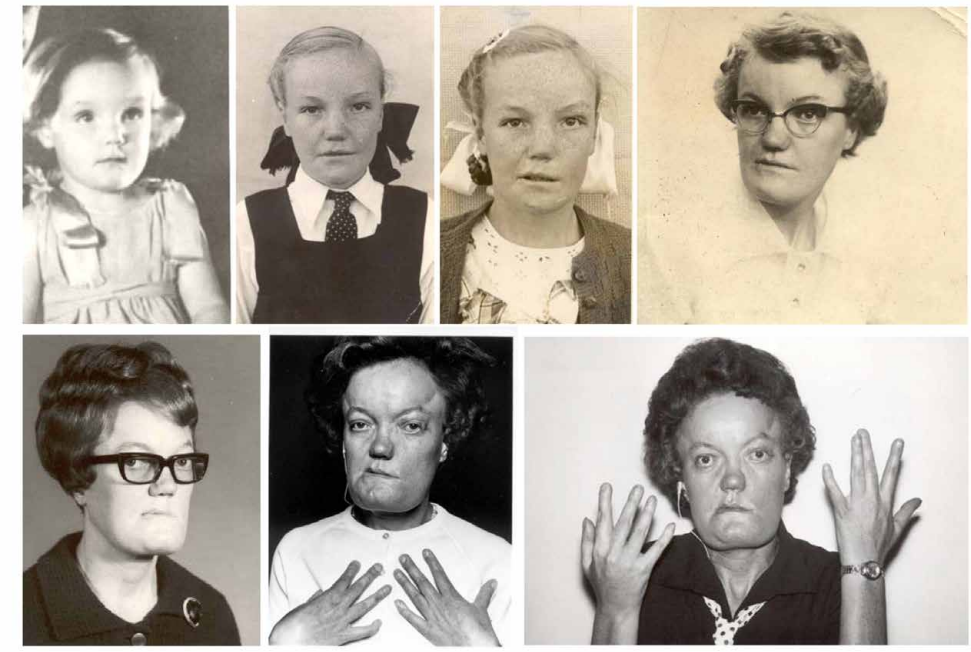

Figure 1. Chronological portraits of a patient with sclerosteosis from the age of 3 years onwards. She was born with syndactyly at both hands and developed facial palsy, deafness, facial distortion, and maxillary overgrowth during childhood. By the age of 30, she had developed proptosis and elevated intracranial pressure due to overgrowth of the calvarium. Craniectomy was performed, but she died nevertheless because of elevated intracranial pressure at the age of 54 years (description of this case was previously published by Epstein *et al.* [14]).

sclerosteosis have a more severe phenotype compared to patients with Van Buchem disease and usually have syndactyly. In a limited number of bone biopsies of affected individuals there is evidence of increased bone formation including predominance of cuboidal active osteoblasts, increased double tetracycline label spacing and increased osteoid that mineralizes normally, while no consistent alteration of osteclast numbers or activity has been reported [9-13]. Information about markers of bone turnover in such patients is also limited. Beighton's group reported elevated serum alkaline phosphatase (AP) activity in the majority of patients with sclerosteosis [14, 15] while Wergedal *et al.* [16] found significantly higher levels of bone formation (AP, procollagen type 1 amino-terminal propeptide [P1NP], osteocalcin) and resorption (urinary amino-terminal type I collagen telopeptide [NTX]) markers in six patients with Van Buchem disease compared to carriers of the disease.

The genetic defect that leads to sclerosteosis was identified in a newly cloned gene called *SOST*, which is located on chromosome 17q12-21 and encodes for the protein

sclerostin. Five mutations have so far been identified in patients with sclerosteosis, of which three introduce a premature termination codon and the others interfere with splicing of the gene [17-20]. No mutations within this gene could be found in patients with Van Buchem disease, but instead a 52kb deletion 35kb downstream of the *SOST* gene was identified [21, 22]. The deleted region was later found to contain regulatory elements for *SOST* transcription explaining its ability to induce a phenotype closely resembling that of patients with sclerosteosis [23]. The different defects of the *SOST* gene cannot readily explain the differences in clinical phenotypes between the two diseases. However, serum sclerostin was severely decreased in patients with Van Buchem disease compared to controls, but could not be detected in patients with sclerosteosis [13, 24]. This difference may give rise to differences in disease severity. A gene-dose effect is also indicated by the fact that serum sclerostin concentrations in carriers of both diseases were significantly higher than in affected individuals, but lower than in controls. With regard to the digit malformations present only in sclerosteosis it may be that the genomic region deleted in Van Buchem disease does not contain regulatory elements required for sclerostin expression during digit formation. This could be the reason for the absence of syndactyly (or other digit malformations) in these patients as opposed to patients with sclerosteosis.

SOST/sclerostin expression

SOST mRNA is, especially during embryogenesis, expressed in many tissues, whereas sclerostin protein expression has only been reported postnatally in terminally differentiated cells embedded within a mineralized matrix, i.e. osteocytes, mineralized hypertrophic chondrocytes and cementocytes [11, 12, 25, 26]. *SOST* mRNA expression in unmineralized tissues has been detected during mouse embryogenesis in the otic vesicle and peridigital or interdigital regions of the limb buds, of which the latter may be implicated in the pathogenesis of syndactyly in patients with sclerosteosis [27]. In humans, *SOST* mRNA is expressed in heart, aorta, liver and at high levels in the kidney [17, 18, 28, 29], but no sclerostin protein has been detected in these organs. Accordingly, patients with sclerosteosis or Van Buchem disease do not have renal or cardiovascular abnormalities [3].

In adult murine and human bone, sclerostin expression is restricted to osteocytes with diffuse staining representing dendrites in osteocytic canaliculi [11, 25, 26,

30]. Osteoclasts, osteoblasts and bone lining cells do not express sclerostin. Due to the difficulties with isolating and culturing osteocytes from mammalian bone, *in vitro* studies of *SOST*/sclerostin expression are technically difficult. Osteogenic cell cultures that form mineralized bone nodules are one of the few available methods for generating osteocyte-like cells *in vitro* [31]. In mouse primary osteogenic bone marrow and mouse mesenchymal KS483 cell cultures, *SOST* mRNA expression is induced at low levels after onset of bone nodule mineralization [11, 32]. Similar to the induction of *SOST* mRNA *in vitro*, newly embedded osteocytes within unmineralized osteoid in humans *in vivo* do not express sclerostin, but become positive for the protein at, or shortly after primary mineralization [26]. When mineralization of osteoid is inhibited by administration of the bisphosphonate etidronate in rats, osteocytes within the unmineralized matrix remain immature and do not express sclerostin [33]. However, *SOST* mRNA is expressed by some osteoblast-like osteosarcoma cell lines [34].

As expected, sclerostin is not expressed by osteocytes in bone biopsies of patients with sclerosteosis [11]. In addition, no sclerostin expression was found in bone biopsies from patients with Van Buchem disease, supporting the function of the genomic region deleted in these patients in the regulation of sclerostin expression in bone [12].

Sclerostin mechanism of action

In patients with sclerosteosis, the combination of high bone mass due to increased bone formation with premature termination codons in the *SOST* gene suggested an inhibitory effect of the gene product sclerostin on bone formation. This is supported by the observation that addition of exogenous sclerostin to osteogenic cultures inhibited proliferation and differentiation of mouse and human osteoblastic cells [11, 25, 35]. In addition, sclerostin was shown to decrease the lifespan of osteoblasts by stimulating their apoptosis [35]. *In vivo*, overexpression of sclerostin using either the osteocalcin promoter or BAC recombination induced osteopenia in mice [23, 25]. Bone formation in these animals was decreased, while bone resorption was unaffected. Furthermore, analysis of *Sost* knockout mice showed significant increases in radiodensity, bone mineral density (BMD), cortical and trabecular bone volume, bone formation rate, and bone strength [36]. Together these data support a negative

effect of sclerostin on bone formation.

Two processes are responsible for construction and reconstruction of the skeleton throughout life, bone remodeling and modeling. Bone remodeling enables constant renewal of the skeleton. In this process, bone resorption by osteoclasts and formation by osteoblasts are tightly coupled within a basic multicellular unit (BMU) and bone resorption always precedes bone formation. Sclerostin expression by newly embedded osteocytes at the onset of mineralization of osteoid may serve as a negative feedback signal on osteoblasts to prevent overfilling of the BMU (Figure 2a)

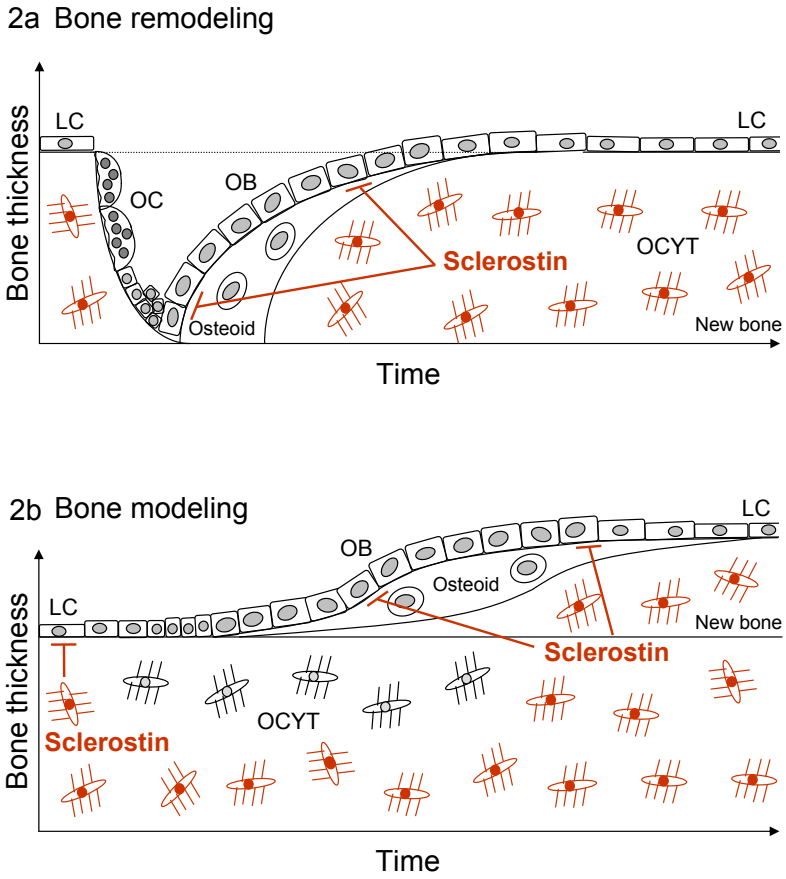

Figure 2. Schematic model of the mechanism of action of sclerostin in bone remodeling and modeling. In remodeling (A), sclerostin produced and secreted by newly embedded osteocytes may be transported to the bone surface where it inhibits osteoblastic bone formation and prevents overfilling of the basic multicellular unit (BMU). In modeling (B), sclerostin may serve two actions. First, it may keep bone lining cells in a state of quiescence and prevent, thereby, initiation of de novo bone formation. In addition, sclerostin produced and secreted by newly embedded osteocytes may inhibit osteoblastic bone formation similar as in a BMU (reproduced from van Bezooijen *et al.* [8]).

[11, 26]. Data on the effect of sclerostin on osteoclastic bone resorption in humans are scarce and inconsistent, reporting unaffected, low or increased bone resorption in patients with sclerosteosis and Van Buchem disease [9, 10, 16]. In addition, during bone modeling sclerostin may keep bone lining cells in a quiescent state [26] and may thereby prevent activation of osteoblasts and bone formation without previous bone resorption (Figure 2b) [8]. Sclerostin expression by osteocytes embedded in newly formed bone by modeling may serve a similar negative feedback mechanism on bone formation as in a BMU.

On the basis of its amino acid sequence, which indicates a cystine knot structure, sclerostin was classified as a member of the DAN (Differential screening-selected gene aberrant in neuroblastoma) family of glycoproteins [6, 17, 18, 37]. This family consists of a group of secreted proteins that share the ability to antagonize bone morphogenetic protein (BMP) activity. The currently available data, however, suggest that sclerostin is not a classical BMP antagonist [11]. Some DAN family members have also been reported to antagonize canonical Wnt signaling, among which Wise is the most closely related to sclerostin [38]. Wnts are secreted glycoproteins that bind to seven transmembrane-spanning receptors of the Frizzled family. Stimulation of these receptors causes the intracellular signaling molecule β-catenin to accumulate and translocate into the nucleus, where it initiates transcription of target genes via complex formation with TCF/Lef1 transcription factors. Conversely, in the absence of Wnt, β-catenin forms a complex with the tumor suppressor proteins APC and Axin, and the kinases glycogen synthase kinase 3 (GSK3) and casein kinase I (CK1), which facilitates phosphorylation and proteosomal degradation of β-catenin [39].

The identification of gain-of-function mutations in the first β-propeller of the low-density lipoprotein receptor-related protein LRP5, an essential membrane bound co-factor in canonical Wnt signaling, in patients with high bone mass (HBM)-phenotype [40, 41] and loss-of-function mutations in LRP5 in patients with the osteoporosis pseudoglioma syndrome (OPPG) [42] demonstrated the importance of LRP5-mediated canonical Wnt signaling in regulating bone formation. Sclerostin has been shown to bind LRP5 and its closely related co-receptor LRP6 and, thereby, inhibit the canonical Wnt signaling via LRP5/6 (Figure 3) [43-45]. However, although sclerostin binds LRP5/6 to antagonize Wnt signaling, sclerostin and Wnts do not appear to compete for binding of this co-receptor [43], and may antagonize different

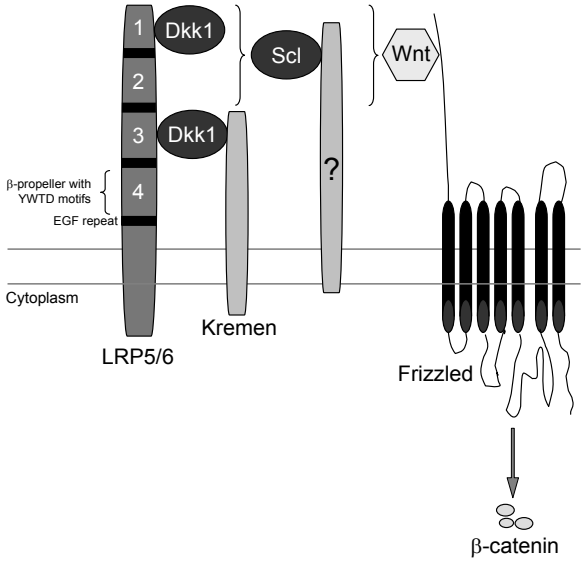

Figure 3. Schematic model of antagonized canonical Wnt signaling. Canonical Wnt signaling involves the formation of complexes of Wnts with Frizzled receptors and LRP5/6 co-receptors, resulting in the accumulation of β-catenin in the cytoplasm and translocation into the nucleus. The antagonist Dkk1 inhibits canonical Wnt signaling by the formation of complexes with LRP5/6 and Kremen, resulting in the removal of LRP5/6 from the membrane. Dkk1 binds to the first and third β-propeller of LRP5/6. The antagonist sclerostin inhibits canonical Wnt signaling by binding to probably the first β-propeller of LRP5/6. Whether sclerostin requires a co-factor like Kremen for Dkk1 to exert its antagonistic effect remains to be established (reproduced from van Bezooijen *et al.* [8]).

Wnts depending on the conformation of LRP5 or 6 [46]. It may be that sclerostin exerts its effect through binding to a co-receptor and inducing internalization of LRP5/6 as has been shown for Dkk1, another Wnt antagonist. Characterization of the structure of sclerostin showed that sclerostin indeed consists out of a cystine knot and three loops [47, 48]. One of these loops is high in positively charged residues, showing a possible site of interaction with the predicted binding site on the first of 6 β-propellers of LRP5, which is negatively charged. The binding site of a neutralizing antibody to sclerostin was mapped to this loop, suggesting a functional role of this region in the inhibition of Wnt signaling. In addition, the loop contains a highly conserved sequence with an NXI motif (in the sequence PNAIG). This motif was also found in the closely related protein WISE, in DKK proteins, and in the interaction between laminin with nidogen, another six-bladed β-propeller containing protein. Mutation of the amino acids in this motif destroyed the ability of sclerostin and Dkk1 to inhibit Wnt signaling [46, 49]. A potential binding site for heparin was

also found within sclerostin, which may mediate localization of sclerostin at the cell surface of target cells and possibly facilitate inhibition of Wnt signaling.

The precise mechanism by which sclerostin secreted by osteocytes inhibits Wnt-mediated bone formation is still unclear. It may be transported to the bone surface via the canaliculi or it may induce another signal in osteocytes that is transported to osteoblasts to inhibit bone formation. In support of the latter, Wnt signaling has been found in osteocytes [50, 51]. Another mechanism was proposed by Krause *et al.* [52]. They found that, even though sclerostin is not a classical BMP antagonist, it could inhibit BMP7-induced responses when both proteins were expressed in the same cell. Sclerostin then bound to BMP7, leading to intracellular retention and proteasomal degradation. The effect of sclerostin may therefore be different in osteocytes that produce the protein, and osteoblasts or sclerostin-negative osteocytes.

Several ELISA methods have become available for the measurement of sclerostin in serum or plasma samples, showing that sclerostin also circulates in the bloodstream. Over the past few years many reports have been published on sclerostin serum concentrations in humans, showing variations in healthy adults [53, 54] and associations between sclerostin and a variety of diseases and conditions. Increased sclerostin concentrations have been found in hypercortisolism [55], type 2 diabetes mellitus (T2DM) [56], atherosclerosis in T2DM [57], immobilization [58], fracture healing [59], thalassemia-associated osteoporosis [60], and high bone turnover as in Paget's disease [61], while sclerostin was decreased in hyperparathyroidism [62], idiopathic osteoporosis in men [63] and ankylosing spondylitis [64, 65]. However, not much is known about the importance of sclerostin in the serum; whether it has a function in circulation, whether serum concentrations reflect changes in the bone, and the temporal resolution of changes in serum concentrations. It is important to elucidate these mechanisms before sclerostin measurements can be routinely used as diagnostic tools in the clinic.

Recently, the role of bone expressed LRP5 in the regulation of bone formation was questioned, since targeted deletion of LRP5 in osteoblasts using the collagen type 1 promoter failed to induce osteopenia and targeted knock-in of LRP5 with a HBM mutation (G171V) using the same promoter did not increase bone mass in mice [66]. It was shown that LRP5-mediated signaling in the duodenum inhibited the expression of Tph1, the rate-limiting enzyme for serotonin production outside the

brain, and, thereby, decreased serum levels of serotonin. Conversely, LRP5 knockout mice that have low bone mass had high serum serotonin levels. In addition, reduction of these elevated serotonin levels by administration of parachlorophenylalanine or a low tryptophan diet normalized bone formation parameters and bone mass. Cui *et al.* [67] could not replicate the results presented by Yadav *et al.* and found no relation between serotonin and bone mass. This discrepancy may be explained by differences in the mouse models that were used. At this point a definitive answer has not been found, and further research may prove if both models can be integrated.

Regulation of *SOST*/sclerostin expression

Due to their location and morphology, osteocytes have been long implicated in mechanosensing and initiation of the bone anabolic response to mechanical load [68, 69]. In support of this, specific ablation of osteocytes in mice resulted in fragile bone and these mice did not respond with bone loss to unloading [70]. Wnt signaling may play an important role in the anabolic response to deformation and loading, since increased Wnt signaling has been found after loading of osteoblastic cells *in vitro* and of tibiae *in vivo* [50, 71, 72]. Wnt signaling and the co-receptor LRP5 were found to be essential for the increase in bone mass after loading [73, 74]. Since sclerostin is produced by osteocytes in bone and inhibits bone formation by antagonizing canonical Wnt signaling, it may play a role in regulating Wnt-signaling in response to mechanical loading. Consistent with this hypothesis, loading decreased *SOST* mRNA and sclerostin levels, while unloading increased *Sost* mRNA expression *in vivo* (Figure 4) [72, 75, 76]. Interestingly, reduction of sclerostin staining intensity was most pronounced in areas with the highest strain, indicating a response to local loading conditions. Furthermore, *Sost* knock-out mice do not exhibit bone loss after unloading [72], and constitutive over-expression of *Sost* severely reduced bone formation after loading [74].

Several systemic and local factors have been suggested as possible regulators of *SOST*/sclerostin expression by osteocytes and the *SOST* promoter region includes Runx2, E-box and C/EBP binding motives [77]. Recombinant human PTH and active fragments of this protein are used in the treatment of osteoporosis [78]. In contrast to the bone resorption stimulating effect of continuous elevation of endogenous PTH as is seen in patients with hyperparathyroidism, intermittent increases of PTH provided

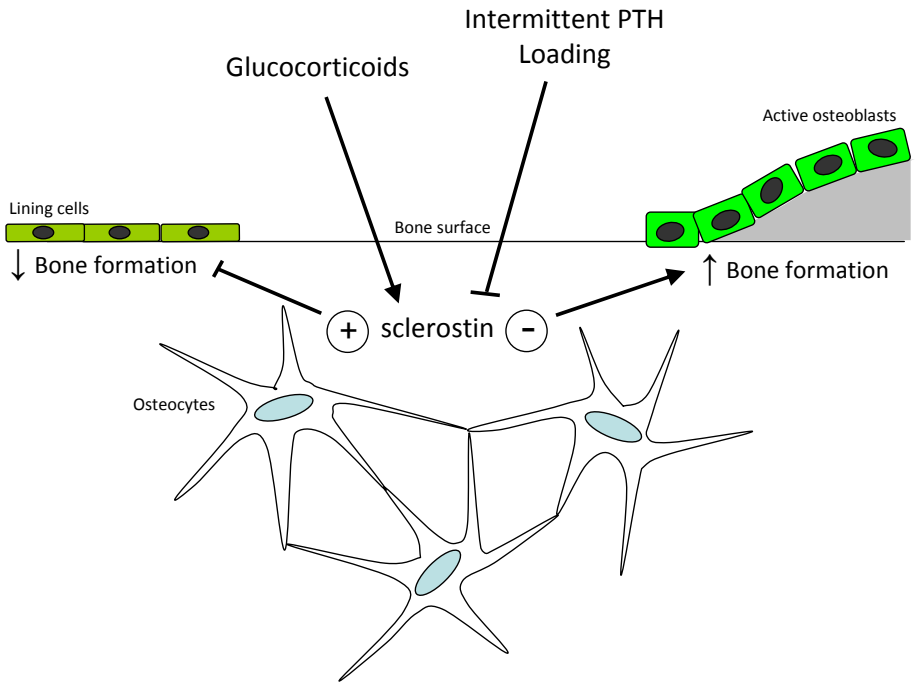

Figure 4. Schematic model for the regulation of the control of bone formation by sclerostin. Sclerostin may exert its inhibitory effect on bone formation by preventing the activation of lining cells as well as the inactivation of active osteoblasts. Glucocorticoids stimulate sclerostin expression and, thereby, inhibit bone formation, whereas intermittent PTH and loading inhibit sclerostin expression in osteocytes and, thereby, stimulate bone formation.

by daily injections are associated with distinct anabolic effects. The mechanisms by which PTH mediates this bone anabolic effect are not completely understood. Part of it may be mediated via sclerostin, as PTH has been shown to inhibit its expression both *in vitro* and *in vivo* (Figure 4). *In vitro*, PTH decreased *SOST* transcription by osteoblastic and osteocytic cells within 4 hours. This was not affected by the protein synthesis inhibitor cyclohexamide, but decreased by the cAMP inducer forskolin [30, 34]. These observations suggest a direct and cAMP dependent regulatory effect of PTH on the expression of *SOST*. Within the 52kb genomic region deleted in Van Buchem disease, a MEF2 response element (ECR5) has been identified that is essential for the PTH-induced downregulation of *SOST* expression [79, 80]. *In vivo*, PTH administration resulted in a decrease in *SOST* mRNA and sclerostin expression in mice and rats [23, 30, 79, 81]. In addition, a constitutively active PTH receptor 1 (caPTHR1) exclusively expressed in osteocytes resulted in increased remodeling with decreased osteoblast apoptosis and suppression of *SOST* expression [82]. This

effect was blunted in mice lacking LRP5, suggesting that the effect of caPTHR1 was mediated by increased Wnt signaling due to suppression of *SOST*. The importance of *SOST* regulation by PTH is further supported by the observations that the anabolic effect of PTH is blunted in *Sost* deficient mice as well as in mice overexpressing *Sost* using a constitutive active promoter [83].

Two other systemic factors have also been shown to affect *SOST*/sclerostin expression. 1,25-Dihydroxyvitamin D3 alone or in combination with retinoic acid increased *SOST* expression in human osteoblastic cells *in vitro* [32, 77]. The specific effect of glucocorticoids on *SOST* expression depends on the experimental conditions. *In vitro*, dexamethason suppressed *SOST* expression in osteoblastic cells [32], while *in vivo* treatment of mice with prednisolone increased *Sost* expression in tibiae, suggesting that suppression of Wnt signaling by the upregulation of sclerostin may account for the glucocorticoid-induced suppression in bone formation (Figure 4) [84].

BMP2, 4, and 6 are local growth factors shown to stimulate *SOST* expression in osteoblastic cells *in vitro* [27, 32], probably by an indirect mechanism [85]. Decreased BMP signaling due to osteoblast specific knockout of Bmpr1a decreased *Sost* mRNA and sclerostin protein expression in embryonic mice calvariae and was associated with increased bone mass [86]. In these mice, however, both bone formation and resorption were inhibited. The authors proposed that the decrease in bone formation was independent of sclerostin expression and a direct result of decreased BMP signaling. The decrease in bone resorption, however, may be an effect of increased Wnt signaling due to the decrease in sclerostin expression. This in turn may be due to upregulation of osteoprotegerin in mature osteoblasts by Wnts and, thereby, inhibition of RANKL-induced osteoclastogenesis [87].

Despite the rapid progress in our understanding of the regulation of the production and function of sclerostin, there are still important questions that need to be addressed in future research. These include the identification of factors that regulate sclerostin/*SOST* expression and determine its highly restricted expression pattern. Furthermore, the mechanism by which sclerostin binding to LRP5/6 interferes with canonical Wnt signaling as well as potential additional functions of sclerostin, besides antagonizing canonical Wnt signaling, should be further explored. While sclerostin measurements in serum revealed some interesting associations

with disease, the precise function and relevance of circulating sclerostin need to be elucidated. More detailed and structured analysis of bone metabolism in patients with sclerosteosis and Van Buchem disease, sclerostin expression in pathological conditions, and a genotype-phenotype characterization of *SOST* are required to better understand its function and regulation in humans.

Therapeutic potential

The identification of sclerostin deficiency as the cause of sclerosteosis and Van Buchem disease and the progress in our understanding of the action of sclerostin on bone formation has opened a new area in bone therapeutics. The restricted expression pattern of sclerostin and the exclusive bone phenotype of good quality of patients with sclerosteosis and Van Buchem disease provide the basis for the design of therapeutics that specifically stimulate bone formation, an action relevant to the treatment of osteoporosis. As sclerostin is a secreted protein, one approach to achieve this is to develop antibodies capable of inhibiting the biological activity of sclerostin, mimicking, thus, its absence in sclerosteosis. Such antibodies have already been shown to increase BMD, bone volume and bone strength in ovariectomized rats [88] and primates [89] and to reverse bone loss in a model of colitis [90] and are currently in Phase III clinical trials (AMG 785, NCT01575834 and NCT01631214 on www.clinicaltrials.gov). Placebo-controlled studies and a recent phase II study comparing sclerostin antibody to alendronate and teriperatide demonstrated a markedly increased BMD at spine, hip and femoral neck that was significantly higher than in patients that were treated with other drugs [91, 92]. Bone formation markers were rapidly increased and bone resorption markers were decreased. While the increase in bone formation markers was transitory, the changes in bone resorption was sustained over the 12-month study period and resulted in a large anabolic window which has not been observed in other osteoporosis therapies.

Other approaches to inhibit sclerostin production or activity are also feasible. However, given the availability of efficacious treatments, any novel treatment for osteoporosis should not only be effective but also devoid of adverse effects. The absence of any extraskeletal complications of patients with sclerosteosis and Van Buchem disease are reassuring. Furthermore, the finding of consistently higher BMD values in carriers of sclerosteosis with no skeletal complications [6] suggests

that the sclerostin inhibition can be titrated and can lead to the desired outcome without any side effects, but safety margins need to be determined. However, there have been concerns that stimulation of bone formation by increasing Wnt signaling may lead to unwanted skeletal effects [93, 94]. The Wnt inhibitor factor 1 (WIF1), for example, has been identified as a candidate tumor suppressor gene in human osteosarcoma, suggesting that the susceptibility to osteosarcoma may be increased in patients receiving novel anabolic treatments targeting Wnt antagonists [95]. This is another issue that needs to be further investigated.

Study of the molecular defects of rare bone disorders such as sclerosteosis and Van Buchem disease can, thus, lead to the development of new bone forming agents allowing to tailor pharmacotherapy to the needs of the individual patient with osteoporosis. In addition, they may help in the management of the small group of patients with sclerosteosis or Van Buchem disease, for whom the only currently available treatment is the technically difficult and often risky removal of excess bone tissue from the skull.

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References

1. National Osteoporosis Foundation. Facts on Osteoporosis. http://www.nof.org/osteoporosis/diseasefacts.htm . 2008.

2. van Buchem FS, Hadders HN, Ubbens R. An uncommon familial systemic disease of the skeleton: hyperostosis corticalis generalisata familiaris. Acta radiol 1955;44:109-20.

3. Hamersma H, Gardner J, Beighton P. The natural history of sclerosteosis. Clin Genet 2003;63:192-7.

4. Beighton P, Barnard A, Hamersma H, van der Wouden A. The syndromic status of sclerosteosis and Van Buchem disease. Clin Genet 1984;25:175-81.

5. Beighton P. Sclerosteosis. J Med Genet 1988;25:200-3.

6. Gardner JC, van Bezooijen RL, Mervis B, Hamdy NA, Löwik CW, Hamersma H *et al.* Bone mineral density in sclerosteosis; affected individuals and gene carriers. J Clin Endocrinol Metab 2005;90:6392-5.

7. van Bezooijen RL, ten Dijke P, Papapoulos SE, Löwik CW. *SOST*/sclerostin, an osteocyte-derived negative regulator of bone formation. Cytokine Growth Factor Rev 2005;16:319-27.

8. van Bezooijen RL, Papapoulos SE, Hamdy NAT, Löwik CWGM. *SOST*/sclerostin; an osteocyte-derived inhibitor of bone formation that antagonizes canonical Wnt signaling. In: Raisz LG, Martin TJ, Bilezikian JP, editors. Principles of bone biology. New York: Academic Press; 2008; p. 139-52.

9. Hill SC, Stein SA, Dwyer A, Altman J, Dorwart R, Doppman J. Cranial CT findings in sclerosteosis. AJNR Am J Neuroradiol 1986;7:505-11.

10. Stein SA, Witkop C, Hill SC, Fallon MD, Viernstein L, Gucer G *et al.* Sclerosteosis: neurogenetic and pathophysiologic analysis of an American kinship. Neurology 1983;33:267-77.

11. van Bezooijen RL, Roelen BA, Visser A, van der Wee-Pals, de Wilt E, Karperien M *et al.* Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. J Exp Med 2004;199:805-14.

12. van Bezooijen RL, Bronckers AL, Gortzak RA, Hogendoorn PC, van der Wee-Pals L, Balemans W *et al.* Sclerostin in mineralized matrices and Van Buchem disease. J Dent Res 2009;88:569-74.

13. van Lierop AH, Hamdy NA, Hamersma H, van Bezooijen RL, Power J, Loveridge N *et al.* Patients with sclerosteosis and disease carriers: human models of the effect of sclerostin on bone turnover. J Bone Miner Res 2011;26:2804-11.

14. Epstein S, Hamersma H, Beighton P. Endocrine function in sclerosteosis. S Afr Med J 1979;55:1105-10.

15. Beighton P, Durr L, Hamersma H. The clinical features of sclerosteosis. A review of the manifestations in twenty-five affected individuals. Ann Intern Med 1976;84:393-7.

16. Wergedal JE, Veskovic K, Hellan M, Nyght C, Balemans W, Libanati C *et al.* Patients with Van Buchem disease, an osteosclerotic genetic disease, have elevated bone formation markers, higher bone density, and greater derived polar moment of inertia than normal. J Clin Endocrinol Metab 2003;88:5778-83.

17. Balemans W, Ebeling M, Patel N, van Hul E, Olson P, Dioszegi M *et al.* Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (*SOST*). Hum Mol Genet 2001;10:537-43.

18. Brunkow ME, Gardner JC, Van Ness J, Paeper BW, Kovacevich BR, Proll S *et al.* Bone dysplasia sclerosteosis results from loss of the *SOST* gene product, a novel cystine knot-containing protein. Am J Hum Genet 2001;68:577-89.

19. Balemans W, Cleiren E, Siebers U, Horst J, van Hul W. A generalized skeletal hyperostosis in two siblings caused by a novel mutation in the *SOST* gene. Bone 2005;36:943-7.

20. Kim CA, Honjo R, Bertola D, Albano L, Oliveira L, Jales S *et al.* A known *SOST* gene mutation causes sclerosteosis in a familial and an isolated case from Brazilian origin. Genet Test 2008;12:475-9.

21. Balemans W, Patel N, Ebeling M, Van Hul E, Wuyts W, Lacza C *et al.* Identification of a 52 kb deletion downstream of the *SOST* gene in patients with Van Buchem disease. J Med Genet 2002;39:91-7.

22. Staehling-Hampton K, Proll S, Paeper BW, Zhao L, Charmley P, Brown A *et al.* A 52-kb deletion in the *SOST*-MEOX1 intergenic region on 17q12-q21 is associated with Van Buchem disease in the Dutch population. Am J Med Genet 2002;110:144-52.

23. Loots GG, Kneissel M, Keller H, Baptist M, Chang J, Collette NM *et al.* Genomic deletion of a long-range bone enhancer misregulates sclerostin in Van Buchem disease. Genome Res 2005;15:928-35.

24. van Lierop AH, Hamdy NA, van Egmond ME, Bakker E, Dikkers FG, Papapoulos SE. Van Buchem disease: Clinical, biochemical, and densitometric features of patients and disease carriers. Journal of bone and mineral research 2013;28:848-54.

25. Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE *et al.* Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J 2003;22:6267-76.

26. Poole KE, van Bezooijen RL, Loveridge N, Hamersma H, Papapoulos SE, Löwik CW *et al.* Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB J 2005;19:1842-4.

27. Ohyama Y, Nifuji A, Maeda Y, Amagasa T, Noda M. Spaciotemporal association and bone morphogenetic protein regulation of sclerostin and osterix expression during embryonic osteogenesis. Endocrinology 2004;145:4685-92.

28. Balemans W, Van Hul W. Extracellular regulation of BMP signaling in vertebrates: a cocktail of modulators. Dev Biol 2002;250:231-50.

29. Kusu N, Laurikkala J, Imanishi M, Usui H, Konishi M, Miyake A *et al.* Sclerostin is a novel secreted osteoclast-derived bone morphogenetic protein antagonist with unique ligand specificity. J Biol Chem 2003;278:24113-7.

30. Bellido T, Ali AA, Gubrij I, Plotkin LI, Fu Q, O'Brien CA *et al.* Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. Endocrinology 2005;146:4577-83.

31. Pockwinse SM, Wilming LG, Conlon DM, Stein GS, Lian JB. Expression of cell growth and bone specific genes at single cell resolution during development of bone tissue-like organization in primary osteoblast cultures. J Cell Biochem 1992;49:310-23.

32. Sutherland MK, Geoghegan JC, Yu C, Winkler DG, Latham JA. Unique regulation of *SOST*, the sclerosteosis gene, by BMPs and steroid hormones in human osteoblasts. Bone 2004;35:448-54.

33. Irie K, Ejiri S, Sakakura Y, Shibui T, Yajima T. Matrix mineralization as a trigger for osteocyte maturation. J Histochem Cytochem 2008;56:561-7.

34. Keller H, Kneissel M. *SOST* is a target gene for PTH in bone. Bone 2005;37:148-58.

35. Sutherland MK, Geoghegan JC, Yu C, Turcott E, Skonier JE, Winkler DG *et al.* Sclerostin promotes the apoptosis of human osteoblastic cells: a novel regulation of bone formation. Bone 2004;35:828-35.

36. Li X, Ominsky MS, Niu QT, Sun N, Daugherty B, D'Agostin D *et al.* Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. J Bone Miner Res 2008;23:860-9.

37. Avsian-Kretchmer O, Hsueh AJ. Comparative genomic analysis of the eight-membered ring cystine knot-containing bone morphogenetic protein antagonists. Mol Endocrinol 2004;18:1-12.

38. Ellies DL, Viviano B, McCarthy J, Rey JP, Itasaki N, Saunders S *et al.* Bone density ligand, Sclerostin, directly interacts with LRP5 but not LRP5G171V to modulate Wnt activity. J Bone Miner Res 2006;21:1738-49.

39. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell 2006;127:469-80.

40. Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA *et al.* High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med 2002;346:1513-21.

41. Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C *et al.* A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. Am J Hum Genet 2002;70:11-9.

42. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM *et al.* LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 2001;107:513-23.

43. Li X, Zhang Y, Kang H, Liu W, Liu P, Zhang J *et al.* Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. J Biol Chem 2005;280:19883-7.

44. Semenov M, Tamai K, He X. *SOST* is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. J Biol Chem 2005;280:26770-5.

45. van Bezooijen RL, Svensson JP, Eefting D, Visser A, van der Horst G, Karperien M *et al.* Wnt but not BMP signaling is involved in the inhibitory action of sclerostin on BMP-stimulated bone formation. J Bone Miner Res 2007;22:19-28.

46. Holdsworth G, Slocombe P, Doyle C, Sweeney B, Veverka V, Le Riche K *et al.* Characterization of the interaction of sclerostin with the low density lipoprotein receptor-related protein (LRP) family of Wnt co-receptors. J Biol Chem 2012;287:26464-77.

47. Veverka V, Henry AJ, Slocombe PM, Ventom A, Mulloy B, Muskett FW *et al.* Characterization of the structural features and interactions of sclerostin: molecular insight into a key regulator of Wnt-mediated bone formation. J Biol Chem 2009;284:10890-900.

48. Weidauer SE, Schmieder P, Beerbaum M, Schmitz W, Oschkinat H, Mueller TD. NMR structure of the Wnt modulator protein Sclerostin. Biochem Biophys Res Commun 2009;380:160-5.

49. Bourhis E, Wang W, Tam C, Hwang J, Zhang Y, Spittler D *et al.* Wnt antagonists bind through a short peptide to the first beta-propeller domain of LRP5/6. Structure 2011;19:1433-42.

50. Hens JR, Wilson KM, Dann P, Chen X, Horowitz MC, Wysolmerski JJ. TOPGAL mice show that the canonical Wnt signaling pathway is active during bone development and growth and is activated by mechanical loading *in vitro*. J Bone Miner Res 2005;20:1103-13.

51. Bonewald LF, Johnson ML. Osteocytes, mechanosensing and Wnt signaling. Bone 2008;42:606-15.

52. Krause C, Korchynskyi O, de Rooij K, Weidauer SE, de Gorter DJ, van Bezooijen RL *et al.* Distinct modes of inhibition by sclerostin on bone morphogenetic protein and Wnt signaling pathways. J Biol Chem 2010;285:41614-26.

53. Amrein K, Amrein S, Drexler C, Dimai HP, Dobnig H, Pfeifer K *et al.* Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. J Clin Endocrinol Metab 2012;97:148-54.

54. Modder UI, Hoey KA, Amin S, McCready LK, Achenbach SJ, Riggs BL *et al.* Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res 2011;26:373-9.

55. Belaya ZE, Rozhinskaya LY, Melnichenko GA, Solodovnikov AG, Dragunova NV, Iljin AV *et al.* Serum extracellular secreted antagonists of the canonical Wnt/beta-catenin signaling pathway in patients with Cushing's syndrome. Osteoporos Int 2013.

56. Garcia-Martin A, Rozas-Moreno P, Reyes-Garcia R, Morales-Santana S, Garcia-Fontana B, Garcia-Salcedo JA *et al.* Circulating levels of sclerostin are increased in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab 2012;97:234-41.

57. Morales-Santana S, Garcia-Fontana B, Garcia-Martin A, Rozas-Moreno P, Garcia-Salcedo JA, Reyes-Garcia R *et al.* Atherosclerotic Disease in Type 2 Diabetes Is Associated With an Increase of Sclerostin Levels. Diabetes Care 2013.

58. Gaudio A, Pennisi P, Bratengeier C, Torrisi V, Lindner B, Mangiafico RA *et al.* Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. J Clin Endocrinol Metab 2010;95:2248-53.

59. Sarahrudi K, Thomas A, Albrecht C, Aharinejad S. Strongly enhanced levels of sclerostin during human fracture healing. J Orthop Res 2012;30:1549-55.

60. Voskaridou E, Christoulas D, Plata E, Bratengeier C, Anastasilakis AD, Komninaka V *et al.* High circulating sclerostin is present in patients with thalassemia-associated osteoporosis and correlates with bone mineral density. Horm Metab Res 2012;44:909-13.

61. Yavropoulou MP, van Lierop AH, Hamdy NA, Rizzoli R, Papapoulos SE. Serum sclerostin levels in Paget's disease and prostate cancer with bone metastases with a wide range of bone turnover. Bone 2012;51:153-7.

62. van Lierop AH, Witteveen JE, Hamdy NA, Papapoulos SE. Patients with primary hyperparathyroidism have lower circulating sclerostin levels than euparathyroid controls. Eur J Endocrinol 2010;163:833-7.

63. Lapauw BM, Vandewalle S, Taes Y, Goemaere S, Zmierczak HG, Collette J *et al.* Serum Sclerostin Levels In Men With Idiopathic Osteoporosis. Eur J Endocrinol 2013.

64. Appel H, Ruiz-Heiland G, Listing J, Zwerina J, Herrmann M, Mueller R *et al.* Altered skeletal expression of sclerostin and its link to radiographic progression in ankylosing spondylitis. Arthritis Rheum 2009;60:3257-62.

65. Saad CG, Ribeiro AC, Moraes JC, Takayama L, Goncalves CR, Rodrigues MB *et al.* Low sclerostin levels: a predictive marker of persistent inflammation in ankylosing spondylitis during anti-tumor necrosis factor therapy? Arthritis Res Ther 2012;14:R216.

66. Yadav VK, Ryu JH, Suda N, Tanaka KF, Gingrich JA, Schutz G *et al.* Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. Cell 2008;135:825-37.

67. Cui Y, Niziolek PJ, MacDonald BT, Zylstra CR, Alenina N, Robinson DR *et al.* Lrp5 functions in bone to regulate bone mass. Nat Med 2011;17:684-91.

68. Knothe Tate ML, Adamson JR, Tami AE, Bauer TW. The osteocyte. Int J Biochem Cell Biol 2004;36:1-8.

69. Han Y, Cowin SC, Schaffler MB, Weinbaum S. Mechanotransduction and strain amplification in osteocyte cell processes. Proc Natl Acad Sci U S A 2004;101:16689-94.

70. Tatsumi S, Ishii K, Amizuka N, Li M, Kobayashi T, Kohno K *et al.* Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. Cell Metab 2007;5:464-75.

71. Robinson JA, Chatterjee-Kishore M, Yaworsky PJ, Cullen DM, Zhao W, Li C *et al.* Wnt/beta-catenin signaling is a normal physiological response to mechanical loading in bone. J Biol Chem 2006;281:31720-8.

72. Lin C, Jiang X, Dai Z, Guo X, Weng T, Wang J *et al.* Sclerostin mediates bone response to mechanical unloading through antagonizing Wnt/beta-catenin signaling. J Bone Miner Res 2009;24:1651-61.

73. Sawakami K, Robling AG, Ai M, Pitner ND, Liu D, Warden SJ *et al.* The Wnt co-receptor LRP5 is essential for skeletal mechanotransduction but not for the anabolic bone response to parathyroid hormone treatment. J Biol Chem 2006;281:23698-711.

74. Tu X, Rhee Y, Condon KW, Bivi N, Allen MR, Dwyer D *et al. Sost* downregulation and local Wnt signaling are required for the osteogenic response to mechanical loading. Bone 2012;50:209-17.

75. 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;283:5866-75.

76. Moustafa A, Sugiyama T, Saxon LK, Zaman G, Sunters A, Armstrong VJ *et al.* The mouse fibula as a suitable bone for the study of functional adaptation to mechanical loading. Bone 2009;44:930-5.

77. Sevetson B, Taylor S, Pan Y. Cbfa1/RUNX2 directs specific expression of the sclerosteosis gene (*SOST*). J Biol Chem 2004;279:13849-58.

78. Pleiner-Duxneuner J, Zwettler E, Paschalis E, Roschger P, Nell-Duxneuner V, Klaushofer K. Treatment of osteoporosis with parathyroid hormone and teriparatide. Calcif Tissue Int 2009;84:159-70.

79. Leupin O, Kramer I, Collette NM, Loots GG, Natt F, Kneissel M *et al.* Control of the *SOST* bone enhancer by PTH using MEF2 transcription factors. J Bone Miner Res 2007;22:1957-67.

80. Collette NM, Genetos DC, Economides AN, Xie L, Shahnazari M, Yao W *et al.* Targeted deletion of *Sost* distal enhancer increases bone formation and bone mass. Proc Natl Acad Sci U S A 2012;109:14092-7.

81. Silvestrini G, Ballanti P, Leopizzi M, Sebastiani M, Berni S, Di VM *et al.* Effects of intermittent parathyroid hormone (PTH) administration on *SOST* mRNA and protein in rat bone. J Mol Histol 2007;38:261-9.

82. O'Brien CA, Plotkin LI, Galli C, Goellner JJ, Gortazar AR, Allen MR *et al.* Control of bone mass and remodeling by PTH receptor signaling in osteocytes. PLoS One 2008;3:e2942.

83. Kramer I, Loots GG, Studer A, Keller H, Kneissel M. Parathyroid hormone (PTH)-induced bone gain is blunted in *SOST* overexpressing and deficient mice. J Bone Miner Res 2010;25:178-89.

84. Yao W, Cheng Z, Pham A, Busse C, Zimmermann EA, Ritchie RO *et al.* Glucocorticoid-induced bone loss in mice can be reversed by the actions of parathyroid hormone and risedronate on different pathways for bone formation and mineralization. Arthritis Rheum 2008;58:3485-97.

85. Yu L, van der Valk M, Cao J, Han CY, Juan T, Bass MB *et al.* Sclerostin expression is induced by BMPs in human Saos-2 osteosarcoma cells but not via direct effects on the sclerostin gene promoter or ECR5 element. Bone 2011;49:1131-40.

86. Kamiya N, Ye L, Kobayashi T, Lucas DJ, Mochida Y, Yamauchi M *et al.* Disruption of BMP signaling in osteoblasts through type IA receptor (BMPRIA) increases bone mass. J Bone Miner Res 2008;23:2007-17.

87. Goldring SR, Goldring MB. Eating bone or adding it: the Wnt pathway decides. Nat Med 2007;13:133-4.

88. Li X, Ominsky MS, Warmington KS, Morony S, Gong J, Cao J *et al.* Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. J Bone Miner Res 2009;24:578-88.

89. Ominsky M, Stouch B, Doellgast G, Gong J, Cao J, Gao Y *et al.* Administration of Sclerostin Monoclonal Antibodies to Female Cynomolgus Monkeys Results in Increased Bone Formation, Bone Mineral Density and Bone Strength. Proc Am Soc Bone Miner Res . 2006.

90. Eddleston A, Marenzana M, Moore AR, Stephens P, Muzylak M, Marshall D *et al.* A Short Treatment with an Antibody to Sclerostin can Inhibit Bone Loss in an Ongoing Model of Colitis. J Bone Miner Res 2009.

91. Padhi D, Jang G, Stouch B, Fang L, Posvar E. Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. J Bone Miner Res 2011;26:19-26.

92. McClung MR, Grauer A, Boonen S, Bolognese MA, Brown JP, Diez-Perez A *et al.* Romosozumab in postmenopausal women with low bone mineral density. N Engl J Med 2014;370:412-20.

93. Whyte MP, Reinus WH, Mumm S. High-bone-mass disease and LRP5. N Engl J Med 2004;350:2096-9.

94. Rickels MR, Zhang X, Mumm S, Whyte MP. Oropharyngeal skeletal disease accompanying high bone mass and novel LRP5 mutation. J Bone Miner Res 2005;20:878-85.

95. Kansara M, Tsang M, Kodjabachian L, Sims NA, Trivett MK, Ehrich M *et al.* Wnt inhibitory factor 1 is epigenetically silenced in human osteosarcoma, and targeted disruption accelerates osteosarcomagenesis in mice. J Clin Invest 2009;119:837-51.