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Role and mechanism of action of Sclerostin in bone

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Abstract

After discovering that lack of Sost/sclerostin expression is the cause of the high bone mass human syndromes Van Buchem disease and sclerosteosis, extensive animal experimentation and clinical studies demonstrated that sclerostin plays a critical role in bone homeostasis and that its deficiency or pharmacological neutralization increases bone formation. Dysregulation of sclerostin expression also underlies the pathophysiology of skeletal disorders characterized by loss of bone mass as well as the damaging effects of some cancers in bone. Thus, sclerostin has quickly become a promising molecular target for the treatment of osteoporosis and other skeletal diseases, and beneficial skeletal outcomes are observed in animal studies and clinical trials using neutralizing antibodies against sclerostin. However, the anabolic effect of blocking sclerostin decreases with time, bone mass accrual is also accompanied by anti-catabolic effects, and there is bone loss over time after therapy discontinuation. Further, the cellular source of sclerostin in the bone/bone marrow microenvironment under physiological and pathological conditions, the pathways that regulate sclerostin expression and the mechanisms by which sclerostin modulates the activity of osteocytes, osteoblasts, and osteoclasts remain unclear. In this review, we highlight the current knowledge on the regulation of Sost/sclerotin expression and its mechanism(s) of action, discuss novel observations regarding its role in signaling pathways activated by hormones and mechanical stimuli in bone, and propose future research needed to understand the full potential of therapeutic interventions that modulate Sost/sclerostin expression.

Keywords

Wnt/βcatenin signaling; bone formation; bone resorption; osteocytes; anabolism; PTH; glucocorticoids; multiple myeloma

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CONFLICT OF INTEREST

Authors have no conflicts of interest to report.

1. SOST/SCLEROSTIN, CANONICAL WNT SIGNALING, AND BONE MASS

A critical advance in our understanding of skeletal biology of the last few years was the discovery of the role of Wnt/βcatenin signaling in bone (1). Wnt/βcatenin signaling is activated by binding of Wnt proteins to receptor complexes composed of frizzled receptors and co-receptors of the low density lipoprotein receptor-related protein (LRP) family, LRP5 and 6. This event stabilizes βcatenin, induces its translocation to the nucleus, and activates gene transcription. This so-called canonical Wnt signaling pathway controls differentiation of mesenchymal stem cells (MSC) restraining chondrogenic and adipogenic differentiation and favoring osteoblastic differentiation. Canonical Wnt signaling also promotes osteoblast maturation and survival of osteoblasts and osteocytes, and inhibits osteoclast generation by increasing the expression in osteoblasts and osteocytes of osteoprotegerin (Opg), the decoy receptor of the receptor activator of Nfκb ligand (Rankl). Thus, activation of this pathway is critical for bone acquisition and maintenance through increased bone formation and decreased resorption.

Osteocytes are key players in the regulation of the canonical Wnt signaling pathway as producers and targets of Wnt ligands and as secretors of molecules that modulate Wnt actions (1;2). A potent antagonist of Wnt signaling secreted by osteocytes is sclerostin, a protein encoded by the Sost gene primarily expressed by mature osteocytes but not by early osteocytes or osteoblasts (3). Sclerostin binds to the Wnt co-receptors LRP5/6 antagonizing downstream signaling (4). Sclerostin also interacts with LRP4, another member of the LRP family of proteins, which acts as a chaperone and is required for the inhibitory action of sclerostin on Wnt/βcatenin signaling (5).

Absence of sclerostin expression or secretion in humans causes inherited high bone mass conditions characterized by exaggerated bone formation, including sclerosteosis, van Buchem disease, and craniodiaphyseal dysplasia (1). Consistent with the requirement of LRP4 for the inhibitory function of sclerostin, individuals with LRP4 inactivating mutations exhibit high bone mass. Moreover, genetic deletion of Sost or LRP4 in mice or neutralizing antibodies for sclerostin or LRP4 reproduce the high bone mass phenotype found in humans lacking sclerostin or LRP4 activity (6–10). In contrast, overexpression of Sost/sclerostin decreases bone mass (11–14). In addition, mice expressing LRP5 mutants with deficient binding to sclerostin exhibit high bone mass, demonstrating a central role of the inhibitory action of sclerostin in the modulation of bone maintenance (13).

2. SOST/SCLEROSTIN EXPRESSION AND ITS REGULATION

Cellular sources of Sost/sclerostin

Immunohistochemical approaches performed in human and rodent bone demonstrate that sclerostin is expressed in osteocytes, but not in osteoblasts or lining cells. Consistent with being a secreted protein, sclerostin is detected in osteocytic lacunae and along osteocytic canaliculi. Sost/sclerostin is a marker of mature osteocytes and its expression progressively increases as osteocytes mature and acquire their full molecular signature (15). Thus, sclerostin is rarely detected in recently embedded osteocytes (osteoid osteocytes) or in

osteocytes close to bone forming surfaces. In contrast, high levels of sclerostin are found in osteocytes surrounded by mineralized bone and distant from active bone surfaces (3;16–18).

Although the current evidence indicates that sclerostin is predominately produced by osteocytes in bone in the adult skeleton, new studies suggest that other cells of bone and the bone microenvironment might express the Sost gene in the developing skeleton or under pathological conditions. Recently, mice expressing Cre recombinase under the control of the second exon of the murine Sost gene (Sost-Cre mice) were crossed with Cre-reporter mice expressing tdTomato fluorescent protein. The progeny of this cross exhibited tdTomato expression in osteocytes, but not osteoblasts or lining cells, confirming the osteocyte-specific expression of Sost (19). However, reporter gene activation was also observed in bone marrow cells of the hematopoietic lineage, although Sost mRNA expression was not detected in the marrow (19). This apparent contradiction could be explained by transient expression of the Sost-Cre transgene in marrow cells during the mouse development, which resulted in Cre-mediated expression of the fluorescent protein. These findings highlight that interpretation of studies using Cre-reporter mice is challenging because a Cre-mediated recombination event at an earlier time point persists in all daughter cells, regardless of whether these cells continue to express Cre. Moreover, they caution that the spatiotemporal profile of Cre expression by examination of reporter gene expression needs to be validated by detecting mRNA/protein expression of the actual gene of interest in targeted tissues.

Sost/Sclerostin expression has been also detected in bone marrow derived-osteoclast precursors and found to decrease as osteoclasts are formed in vitro (20). In addition, higher levels of sclerostin are produced by osteoclast cultures established from bone marrow of old compared to young mice, suggesting that osteoclast-derived sclerostin may contribute to the decrease in bone formation that ensues with aging (21). Sclerostin-positive osteoblasts, cementocytes and mineralized hypertrophic chondrocytes have been reported in different studies (22–25). However, some of these studies lack proper controls and thus the biological relevance of these findings is uncertain. Sclerostin has also been detected in calcifying vascular tissues (26–28). Moreover, fibroblast-like synoviocytes have been also proposed to be a major source of sclerostin in rheumatoid arthritis (29), and multiple myeloma cells appear to also express the protein (30;31), as it will be discussed below. All this evidence notwithstanding, the contribution of non-osteocytic sclerostin to the skeletal effects attributed to this protein remains to be determined.

Regulation of Sost transcription and sclerostin synthesis

Sclerostin is encoded by Sost, a gene identified through genetic linkage analyses in sclerosteosis and van Buchem’s disease patients (7;32). It is now recognized that several regulatory elements in the Sost gene tightly regulate its transcription in bone. The first evidence of a mechanism regulating Sost transcription derived from the observation that Van Buchem’s patients exhibit a homozygous deletion of a 52-kb noncoding located 35 kb downstream of the SOST transcription start site (33;34). This finding led to the identification of an enhancer element essential for Sost expression, evolutionarily conserved region 5 (ECR5), and the regulation of Sost transcriptional activity by myocyte enhancer factor-2 (Mef2c), a transcription factor that binds to ECR5 (35;36).

Growing evidence supports that epigenetic modifications in the Sost proximal promoter (~1.4Kb upstream the SOST gene) also control expression of this gene, one of them being DNA methylation (37). Accumulation of DNA methyl marks in the proximal promoter of Sost prevents binding of transcription factors and inhibits Sost gene expression in osteoblasts (38). Conversely, elimination of DNA methyl marks during osteoblast-osteocyte transition allows osteocytes to express the gene (38). In addition, sirtuin 1, a histone deacetylase (HDAC) that remodels chromatin to silence gene expression, directly and negatively regulates Sost gene expression by deacetylation of histone 3 at lysine 9 at the Sost promoter (39). In contrast, silencing of class I HDACs 1, 2, and 3 inhibits constitutive expression of Sost (40), suggesting that different HDACs can have different actions on Sost expression. Taken together these findings suggest that Sost expression is regulated by epigenetic mechanisms that act as an on-off switch to restrict the expression of this gene to particular cell types.

A number of transcription factors activated in response to cytokines and growth factors also bind to regulatory regions of the Sost gene. Sost transcription is negatively regulated by parathyroid hormone (PTH) as discussed below, via HDAC5 and Mef2c through interactions with response elements located in the ECR5 regulatory region (35;41–43). In addition, Smads can bind to ECR5 and mediate the regulation of Sost expression by transforming growth factor beta and activin-A (44), and most likely bone morphogenetic proteins (44–46). Osterix and Runx2 also bind to response elements located upstream of the Sost transcription start site and are positive regulators of Sost/sclerostin production (47); whereas osteocytes cultured under hypoxia exhibit decreased Sost and sclerostin expression (48). Moreover, mechanical loading negatively regulates Sost expression by mechanisms that might involve prostaglandin E2 production, nitric oxide, and periostin expression (11;49–51). In vitro studies using a reporter construct carrying the proximal Sost promoter also identified forskolin and oncostatin M as potential regulators of Sost expression (52;53). In addition to PTH, other systemic hormones have a strong impact on Sost regulation. Androgens and estrogens inhibit Sost expression (54;55), although the mechanisms are still unclear. In contrast, excess of glucocorticoids increases Sost and sclerostin production in osteocytes (56), as will be discussed below. Vitamin D can also regulate Sost expression, although contradictory data has been published. Thus, using Saos-2 cells, Wijenayaka et al showed that 1α,25-dihydroxyvitamin D3 induces the expression of Sost/Sclerostin (57), whereas St John et al found that it decreases the transcriptional activity of the Sost promoter (52). Similarly conflicting clinical data on the effects of vitamin D status on circulating sclerostin levels have been reported (58–62). Furthermore, tumor necrosis factor alpha and Tnf-related weak inducer of apoptosis increase Sost expression (63). In addition, glucose and advanced glycation end products increase Sost expression in vitro (64), and Sost/sclerostin expression is increased in mice with diabetes type 1 (65).

Unresolved enigmas in the study of the Sost/sclerostin regulation

The mechanisms controlling osteoblast differentiation towards mature osteocytes remain unclear. In vitro osteoblast to osteocyte differentiation is complex and give rise to cell populations with few of osteocytes, even after long periods of culture. Thus, one of the problems faced when studying the mechanisms regulating Sost expression is the limitations

of the cellular systems used for in vitro studies. Initial experiments were done with the rat UMR-106 cells (66;67) and human Saos-2 cells (68;69), which constitutively express Sost/sclerostin. However, the fact that these cells are derived from osteosarcoma limit the impact and interpretation of the results. Cell lines established from murine bone were developed and have been extensively used, but also present limitations. The murine MLO-Y4 and MLO-A5 cells exhibit very low expression of Sost/sclerostin and the IDG-SW3 and Ocy454 cells require several days of culture to achieve detectable Sost mRNA transcripts and sclerostin protein (70–72). Human osteoblastic cell lines capable to differentiate and express Sost/Sclerostin in vivo have been established (73) and artificial expression of Sost/Sclerostin has been achieved in human dermal fibroblasts (74). These approaches might prove useful in the future. An alternative to the cell lines is the use of ex vivo bone organ cultures in which the tridimensional living structure of the osteocyte network is maintained. This approach recapitulates closely the in vivo responses of Sost (and other osteocytic genes) to PTH, glucocorticoids and Wnt/βcatenin (56;75;76).

The exact mechanisms controlling sclerostin protein synthesis remain unclear. Sclerostin was originally described as a glycoprotein of ~27 KDa that is secreted in a monomeric form (77;78). However, sclerostin is rarely detected at this molecular weight; instead higher molecular weight forms are found, potentially dimers and trimers, although their functions are yet to be determined (79). Moreover, there is poor correlation between mRNA Sost levels and sclerostin protein expression. For instance, Sost gene expression has been detected in other organs besides bone, such as kidney, lung, heart, and aorta; however, sclerostin protein is rarely detected in these tissues (7;78). In fact, although the ECR5 is required for a normal production of Sclerostin by osteocytes in bone, it does not contribute to the regulation of Sclerostin expression in cells from other tissues (i.e. kidney, lung, or aorta) suggesting that tissue-specific mechanisms may regulate the expression of this gene (36).

Another unresolved issue regarding the Sost/sclerostin system is that the Sost/sclerostin expression levels in bone do not always correlate with sclerostin levels in the circulation. For example, serum sclerostin increases with age in humans, but Sost mRNA levels in bone are not different between old and young subjects (80;81). Similarly, sclerostin is high in the serum of osteoporotic patients, whereas no differences in Sost mRNA or sclerostin expression in bone are detected between normal and osteoporotic subjects (16;58;82). Some of these discrepancies may reflect limitations of the available assays used to measure sclerostin or differences in the material used in the analysis (serum versus plasma) (83). Nevertheless, these inconsistencies question the value of relying on circulating sclerostin levels to predict bone formation or bone mass. A clear example of this dichotomy is represented by the aforementioned genetic deletion of LRP4 from the mouse genome. LRP4 KO mice exhibit increased bone formation and mass despite the high levels of serum sclerostin (9;84), confirming that local rather than circulating sclerostin levels better reflect the skeletal actions of the protein. In contrast, another study reported that high levels of sclerostin in the blood achieved by using the ΦC31 integrase system to overexpress the protein, resulted in trabecular bone loss (85). Further research to unravel how sclerostin production is controlled and to determine the local versus systemic actions of the protein is needed.

3. SOST/SCLEROSTIN FUNCTION

Sost/sclerostin potently inhibits bone formation

Studies with genetically modified mice provided insights into the mechanism of action of Sost/sclerostin in bone. Deletion of the Sost gene from the murine genome reproduced the high bone mass hallmark of humans with inheritable sclerostin deficiency as in cases of sclerosteosis or Van Buchem disease (5;7;8;86). This phenotype is driven by increased bone formation on all bone surfaces, without changes in osteoclasts or resorption markers compared to age-matched wild type (WT) mice (8). The anabolic effect of Sost deletion (Sost KO) results in up to >50% gain in vertebral and long bone BMD in mice of both genders and enhanced structural properties in cancellous and cortical bone. In contrast to high bone mass phenotypes due to osteopetrosis that present with increased bone fragility and compromised material properties, the gain in bone mass in the Sost KO mice results in increased structural material properties of maximum load, stiffness, and energy to failure (8). Increased strength of sclerostin deficient bones might be, at least in part, due to favorable alterations in bone composition, as bones from Sost KO and sclerosteosis patients have enhanced relative proteoglycan content, lower apatite crystal maturity/crystallinity, and lower matrix mineralization (87), all properties associated with increased toughness and decreased brittleness.

Conversely, mice overexpressing Sost exhibit overall suppressed bone formation resulting in notable reductions of bone mass and volume (12;33;77). However, some differences are found depending on the promoter used to overexpress the gene. Thus, when the human osteocalcin promoter was used, the osteopenic phenotype was observed in cancellous bone and it was accompanied by increased chondrodysplasia as evidenced by lack of lamellar bone formation with increased hypertrophic chondrocyte and calcified cartilage area (77). Reduced appositional bone and increased osteoid was also observed in calvarial bone. Consequently, marked decreased bone strength was exhibited in lumbar vertebra, femoral head, and the whole femur (77). Mice overexpressing a transgene in which Sost is driven by the entire human SOST promoter (BAC-SOST mice) also displayed reduced cancellous bone of the axial and appendicular skeleton due to reductions in bone formation (14;33;77). In addition, these mice, as well as mice expressing a transgene with a Van Buchem's like deletion of the noncoding SOST-specific regulatory element, exhibited impaired limb bud development and syndactyly (33). This feature is observed in sclerosteosis patients with loss-of-function SOST mutations, but not in patients of Van Buchem's disease in which the enhancer element involved in adult SOST expression is absent (5;7;86). Lastly, DMP1-SOST transgenic mice overexpressing human Sost in osteocytes under the control of the DMP1–8kb promoter fragment showed a potent inhibition of bone formation and decreased bone mass in cancellous bone of the vertebra or the distal femur (12). However, no decreases in bone formation on periosteal or endocortical surfaces or changes in the geometry of cortical bone were observed in DMP1-SOST mice. Nevertheless, bone apposition on the periosteal surface of the ulnae in response to loading is blunted in DMP1-SOST mice (11), as it will be discussed below. Moreover, the increase in bone formation on both periosteal and endocortical surfaces induced by osteocytic PTH receptor signaling in DMP1-caPTHR transgenic mice is abolished in double transgenic mice also expressing the DMP1-SOST

transgene (12). These findings indicate that sclerostin is a potent negative regulator of bone formation and a critical mediator in the anabolic response of the skeleton to mechanical force and signaling activated by the PTH receptor in osteocytes.

Sost/sclerostin stimulates bone resorption

Osteoprotegerin (Opg), the decoy receptor for Rankl and thus inhibitor of bone resorption (88), is a Wnt/βcatenin target gene (1). Thus, genetic manipulation of Wnt/βcatenin signaling leads to marked changes in Opg expression with the consequent effects on resorption. Specifically, inactivation of Wnt/βcatenin in mature osteoblasts/osteocytes decreases Opg and increases osteoclast differentiation and bone resorption (89–91). Conversely, activation of Wnt/βcatenin in osteoblasts increases Opg expression and reduces bone resorption (89;90).

Because sclerostin antagonizes the Wnt/βcatenin signaling pathway, it is not unexpected that changes in Sost/sclerostin expression could also modulate resorption by regulating Opg. In fact, neutralizing anti-sclerostin antibodies increase bone formation and decrease bone resorption markers in experimental animals and humans, suggesting that the bone gain achieved results from the combination of enhanced bone formation and decreased bone resorption (92).

Consistent with this notion, overexpression of Sost in osteocytes exacerbate intracortical remodeling and the increase in osteoclasts displayed by DMP1-8kb-caPTHR1 mice (12). This enhanced bone resorption in double transgenic DMP1-8kb-caPTHR1/DMP1-SOST mice is accompanied by a marked decrease in Opg expression. In addition, overexpression of Sost in DMP1-SOST mice is sufficient to increased Rankl levels in bone (12;75). Activation of Wnt/βcatenin signaling in osteocytes in knockin daβcat^{Ot} mice also increases Opg and increases Rankl expression (75). However, in contrast to mice with activation of the pathway in osteoblasts, Rankl expression also increased and to a greater extent than Opg in daβcat^{Ot} mice, resulting in enhanced Rankl/Opg ratio, increased osteoclasts and elevated resorption (75). The increased in Rankl induced by activation of Wnt/βcatenin in osteocytes is driven by Sost/sclerostin production and neutralization of sclerostin activity abolished Rank increase displayed bone by daβcat^{Ot} mice (75). Moreover, Sost KO mice exhibit the expected increase in Opg but no changes in Rankl demonstrating that Sost expression is needed to increase Rankl by Wnt/βcatenin signaling (75). These results are in line with earlier findings indicating that recombinant sclerostin upregulates Rankl and increases osteoclast formation in vitro (93). This evidence supports the notion that sclerostin exhibits autocrine effects in osteocytes. The mechanisms underlying the regulation of Rankl expression by Sost/sclerostin in osteocytes remain to be determined.

Wnt/βcatenin signaling inhibits osteoclast formation directly by acting on osteoclast precursors, as knockout of βcatenin in these cells increases osteoclast number and enhances resorption (94). This evidence suggest that sclerostin might have a direct role on osteoclast differentiation that is independent of Rankl and Opg in osteoclast support cells. Furthermore, sclerostin upregulates in osteocytes in vitro the expression of cathepsin K, TRAP, and carbonic anhydrase-2, proteins involved in the remodeling of extracellular matrix

surrounding osteocytes (93). These findings suggest that sclerostin might also influence the release of mineral from bone in the process called osteocytic osteolysis.

Wnt/β-catenin-independent and non-skeletal actions of sclerostin

Previous work demonstrated that Sclerostin directly interacts with several members of the BMP and Wnt pathways such as BMP4, BMP2 and Lrp 4/5/6 (4;77;95;96). More recently, using a sclerostin affinity capture technique and mass spectrometry, it was shown that alkaline phosphatase, carbonic anhydrase, gremlin-1, fetuin A, midkine, annexin A1 and A2, and collagen α1, bind to sclerostin (97). Further, in this study, binding between sclerostin and other protein members of the Wnt signaling cascade such as casein kinase II and secreted frizzled related protein 4 was found. Thus, while inhibition of Wnt/β-catenin via interaction with Lrp4/5/6 appears to be the main mechanism of action of sclerostin, it is possible that interactions between sclerostin and other proteins in bone contribute to alter bone formation and resorption.

The lack of extraskeletal complications in sclerosteosis or van Buchem patients or after anti-sclerostin therapy indicate that the main actions of sclerostin occur in the skeleton. However, several reports has suggested that sclerostin might also have non-skeletal actions. For instance, Sost deficiency induces B cells apoptosis potentially by inducing a decrease in the expression of the growth stimulating factor CXCL12 (98). Moreover, circulating sclerostin levels correlate with vascular calcifications in humans with and without renal disease; and sclerostin expression is found in areas with ectopic calcification (26;99). Moreover, treatment with anti-sclerostin in a mouse model of chronic kidney disease showed a trend towards reduction in calcification (100). However, whether sclerostin plays a role in vasculature calcification or its expression is simply a biomarker for valve calcification of remains to be seen. It has been also suggested that sclerostin regulates adipocyte differentiation and/or fat production. In fact, several studies show a positive correlation between the percentage of abdominal fat, gynoid fat, and fat mass (82;101;102); and in vitro studies demonstrate that sclerostin enhances adipocyte differentiation in 3T3-L1 cells (103). Further research is warranted to determine the potential role of Wnt-βcatenin-independent actions of sclerostin and to examine the therapeutic potential of pharmacological inhibition sclerostin in non-skeletal tissues.

4. ROLE OF SCLEROSTIN IN THE SKELETAL RESPONSE TO MECHANICAL STIMULATION

The abundance and strategic location of osteocytes makes them the most suitable candidate cells for detecting variations in strain and for distributing signals leading to adaptation of the skeleton to meet mechanical needs by changing its mass, shape, and microarchitecture (104;105). However, the molecular mediator(s) had remained elusive until it was demonstrated that mechanical loading decreases the expression of Sost/sclerostin in osteocytes, and that conversely, unloading increases it. Moreover, areas of cortical bone receiving greater strain stimuli and higher bone formation exhibit a greater reduction in the number of sclerostin-positive osteocytes and in the intensity of sclerostin staining per osteocyte (106). These findings support the notion that osteocytes coordinate the osteogenic

response to mechanical force by downregulating sclerostin, thereby locally unleashing Wnt signaling. Indeed, the osteogenic response to mechanical stimulation is disrupted in mice overexpressing in osteocytes a human SOST transgene that cannot be downregulated by loading (DMP1-SOST) (11). Thus, whereas WT mice showed the expected decrease in sclerostin expression in osteocytes and a robust and dose-dependent increased bone formation upon ulnae loading, DMP1-SOST mice exhibited an overall reduced sensitivity to mechanical stimulation. Moreover, loading increased the expression of several genes associated with activation of Wnt signaling in WT mice, whereas this effect was absent in DMP1-SOST mice (107;108). Thus, Sost/sclerostin downregulation is an obligatory step for mechanotransduction, and for activation of the Wnt pathway and the increase in bone formation induced by mechanical force.

5. ROLE OF SCLEROSTIN IN THE BONE ANABOLIC ACTIONS OF PTH

Osteocytes are crucial target cells for the skeletal actions of PTH as evidenced by the downregulation of Sost expression as well as the modulation by the hormone of the expression of several other osteocytic genes that impact skeletal homeostasis, including RANKL, FGF23, and OPG (42). This notion is supported by the evidence that mice lacking the PTH receptor in osteocytes exhibit impaired bone gain in response to intermittent PTH administration (42;109;110). Furthermore, the increase in bone formation exhibited by DMP1-8kb-caPTHR1 is abolished in double transgenic mice also overexpressing human SOST in osteocytes (DMP1-SOST), demonstrating the requirement of Sost downregulation for bone gain in this transgenic mouse model (111). Moreover, similar to daily injections of PTH, mechanical loading failed to increase cancellous and cortical bone mass and to downregulate Sost/sclerostin or stimulate Wnt/βcatenin signaling in mice lacking the PTH receptor in osteocytes. These findings suggested a potential role of osteocytes in bone anabolism induced by PTH through inhibition of Sost/sclerostin. Remarkably, however, PTH induced equivalent increases in cortical and cancellous bone mass, similar activation of canonical Wnt signaling, and increased bone forming activity of osteoblasts in DMP1-SOST compared to WT control littermates (112). The lack of requirement of Sost/sclerostin for PTH anabolic actions is in line with earlier studies showing normal or even enhanced response to anabolic regimens of PTH in Sost knockout mice (113). In contrast, Kramer et al. reported that PTH-induced bone gain is reduced in mice harboring the entire human SOST gene (BAC-SOST), including its regulatory elements, to systemically overexpress Sost (14;33). The discrepancy between the latter study and the findings with the DMP1-SOST mice could be explained by the use of different transgenic mouse models, and the fact that the BAC-SOST transgene is still downregulated by PTH hindering the interpretation of the results. Thus, whereas the levels of the human SOST transgene remained unchanged by PTH administration to DMP1-SOST mice, they were reduced about 50% in PTH-treated BAC-SOST mice.

All together, these findings demonstrate that the osteocytic PTHR1 is central for the increased bone formation induced by PTH and mechanical loading; however, the molecular mediator(s) downstream PTHR1 signaling are different. Sost/sclerostin downregulation is required for the anabolic actions of mechanical loading, whereas Sost/sclerostin

downregulation is dispensable for the anabolic effects of PTH. Future studies are warranted to unravel alternative pathways activated by PTH in osteocytes that lead to bone anabolism.

6. ROLE OF SCLEROTIN IN THE BONE LOSS INDUCED BY GLUCOCORTICOIDS

Glucocorticoid excess is a leading cause of bone fragility worldwide (114), and the mechanisms activated by the hormone and how to interfere with its actions in bone are a matter of intense investigation. Recent findings demonstrate that glucocorticoids hinder the expression of target genes of the Wnt/βcatenin pathway, regardless of whether they are associated with bone anabolism (i.e. bone gain) or anti-catabolism (i.e. inhibition of bone loss) (56). This action of the hormone is exerted, at least in part, by increasing the expression of Sost/sclerostin. However, it is unclear whether this phenomenon is due to direct regulation of transcription of the Sost gene or to indirect mechanisms. Nevertheless, Sost/sclerostin deficiency protects against the loss of bone mass, deterioration of microarchitecture, and reduction of extrinsic/structural and intrinsic/material mechanical properties induced by glucocorticoids. Remarkably, however, the bone protective effect of Sost/sclerostin deficiency against glucocorticoids is not due to an opposing action to increase bone formation and maintain anabolic signaling. Instead, it is due to preservation of the Wnt/βcatenin anti-catabolic cellular and molecular signature. Therefore, this pathway which is predominantly anabolic for bone is switched to anti-catabolic in the frame of glucocorticoid excess. These findings suggest that therapeutic interventions targeting sclerostin and activating Wnt/βcatenin signaling could effectively halt the high bone resorption responsible for the profound and rapid bone loss induced by glucocorticoids, which in humans can reach up to 12% during the first year of treatment (115).

Consistent with these findings in skeletally mature Sost/sclerostin deficient mice, pharmacologic inhibition of sclerostin with a neutralizing antibody opposed the lack of bone gain and the loss of strength induced by glucocorticoids in growing mice (116;117). Although it was proposed that these effects were due to preservation of osteoblast activity (117), mice treated with glucocorticoids and the anti-sclerostin antibody in these earlier studies exhibited lower circulating TRAP5b (116) or CTX-1 (117), but still markedly reduced bone formation markers osteocalcin and P1NP (116), compared to the corresponding mice treated with glucocorticoids alone. Likewise, sustained activation of the Wnt/βcatenin signaling in Sost deficient mice abolished the increase in resorption induced by glucocorticoids but not the decreased bone formation (56). Moreover, the decrease in Opg and increase the Rankl/Opg ratio induced by glucocorticoids is abolished in bones from Sost−/− mice or in WT bones treated with an anti-sclerostin antibody. Taking together, these findings demonstrate that Sost/sclerostin deficiency, either genetic or pharmacologically achieved, maintains bone mass and strength in conditions of glucocorticoid excess by inhibiting bone resorption, through sustained anti-catabolic signaling driven by Opg.

A clinical case reported that glucocorticoids stop the exaggerated bone gain and reduced the high circulating P1NP in a patient with Van Buchem disease, in which impaired production of sclerostin expression leads to continuous bone anabolism causing life-threatening

increased intracranial pressure (118). The findings that bone formation and Wnt/βcatenin anabolic signaling is still decreased in Sost/sclerostin deficient mice treated with glucocorticoids (56) provide a mechanistic explanation for these clinical findings. Taken together, these pieces of evidence demonstrate that glucocorticoids oppose the effects of Sost/sclerostin deficiency on bone formation in both humans and mice.

Another unwanted consequence of glucocorticoid excess, either endogenous or iatrogenic, is muscle weakness; which reduces body balance and, when combined with lower bone mass, increases the risk of bone fractures. Due to their intimate association as a mechanical unit, changes in bone could potentially impact skeletal muscle and vice versa. The mouse model of glucocorticoid excess faithfully reproduces the bone and skeletal muscle atrophy exhibited by humans (56). However, the bone preservation resulting from Sost/sclerostin deficiency did not protect from muscle atrophy; and conversely, the marked loss of muscle mass experienced by the Sost deficient mice did not translate into apparent detrimental effects on bone volume or mechanical properties. These findings demonstrate that Sost/sclerostin deficiency protects exclusively bone, but not muscle, from the action of glucocorticoids, and show a lack of crosstalk between these two tissues in the frame of glucocorticoid-induced musculoskeletal atrophy. Future studies are warranted to investigate whether muscle-derived factors contribute to the low bone formation and high prevalence of osteoblast and osteocyte apoptosis still exhibited by Sost/sclerostin deficient mice treated with glucocorticoids

7. ROLE OF SCLEROSTIN (AND DKK1) AS NEGATIVE FEEDBACK INHIBITORS OF WNT SIGNALING-DRIVEN BONE FORMATION

We recently showed that genetic activation of βcatenin signaling in osteocytes increases bone formation and resorption leading to bone gain (75). Bone anabolism is achieved by enhancing osteoblast to osteocyte differentiation, and, consequently, the expression of osteocyte markers, including Sost/sclerostin and Dkk1, is increased. Similarly, the activation of Wnt/βcatenin signaling and increased bone formation exhibited by rodents receiving neutralizing antibodies against sclerostin is also accompanied by increased expression of both Sost and Dkk1 (92;119). Upregulation of the Wnt antagonists might temper the anabolic response triggered by activation of the pathway to protect from excessive bone gain and could explain at least partially the reduced anabolic potency that presents with prolonged sclerostin inhibition (92) and the decline of bone formation makers after each dose of anti-sclerostin antibody (120;121). Consistent with this notion, dual inhibition of sclerostin and Dkk-1 with a novel bispecific antibody results in greater increases bone formation when compared to neutralization of each of the Wnt antagonists alone (119). Taken together, these findings suggest that Sost and Dkk1 act as a negative feedback mechanism that limits Wnt-driven bone formation.

8. ROLE OF SOST/SCLEROSTIN IN MULTIPLE MYELOMA BONE DISEASE

In multiple myeloma (MM) increased numbers of monoclonal plasma cells in the bone marrow induce localized osteolytic lesions that rarely heal, due to increased bone resorption and suppressed bone formation (122;123). Serum sclerostin levels are elevated in MM

patients, which correlate with reduced osteoblast function and poor survival (124). Recent studies demonstrate that osteocytes contribute to MM generating a microenvironoment that is conducive to enhanced bone resorption and suppressed bone formation, and that osteocytes in mice bearing MM tumors express elevated Sost/sclerostin (125). Further, direct cell-to-cell contact with MM cells increases Sost/sclerostin expression in osteocytes and sclerostin accumulated in culture media decreases Wnt signaling and inhibits osteoblastic gene expression. Sost/sclerostin expression has also been detected in CD138+ cells from MM patients (30;31); and more recently it has been suggested that osteoblasts could also be a potential source of this protein in MM (22). Regardless of the cell source, recent findings suggest that blockade of Sost/sclerostin action could ameliorate MM bone disease. Thus, genetic deletion of the Sost gene prevented the bone loss and decreased osteocytic lesions in an immunodeficient/SCID MM model of MM (126). Moreover, administration of a neutralizing anti-sclerostin antibody reduced the development of osteolytic lesions and the decrease in circulating bone formation markers exhibited by immunocompetent mice with established MM (126). In these studies, Sost deficiency or pharmacological inhibition of sclerostin did not alter tumor burden. Similarly, pharmacologic inhibition of sclerostin reversed the osteolytic bone disease in a humanized MM xenograft mouse model bearing human MM cells, also without affecting tumor growth (22); and combination of anti-sclerostin therapy with the anti-tumor drug Bortezomib decreased tumor burden and improve bone disease. Further, simultaneous injection of MM cells together with an anti-sclerostin antibody prevented the development of MM bone disease and suppressed tumor growth (127). Future research is warranted to identify the factors involved in the dysregulation of Sost/sclerostin in MM and the mechanisms underlying the protective effects on bone of inhibition of sclerostin in these preclinical models of MM and to develop therapeutic approaches based on pharmacological inhibition of sclerostin in combination with other anti-tumor drugs to synergistically decrease MM growth and prevent bone disease.

In addition, sclerostin may play a role in other cancers that grow in bone, as patients with prostate cancer exhibit high serum sclerostin levels compared to healthy subjects (128); and breast cancer cells have been reported to produce sclerostin and inhibit osteoblast differentiation (129). Thus, future studies are also needed to investigate the contribution of sclerostin to other cancers that grown in bone and weaken the skeleton.

9. CONCLUSION AND FUTURE DIRECTIONS

Advances during the last decade provided relevant information on the regulation of Sost/sclerostin and its mechanism(s) of action. Several stimuli have been reported to regulate Sost/Sclerostin expression, however how these factors interplay to regulate the expression of this gene in a spatiotemporal manner is unknown. Animal studies demonstrate that sclerostin is key for skeletal homeostasis, and required for the bone anabolic response to mechanical loading although appears dispensable for PTH-induced bone gain. The knowledge provided by preclinical investigations resulted into clinical trials based on the neutralization of sclerostin activity as novel osteoanabolic therapeutic approach (130–133). It is now clear that sclerostin is capable of uncoupling bone formation and bone resorption, by inhibiting osteoblast function while stimulating osteoclast function, as the bone gain achieved by pharmacologic inhibition of sclerostin results from stimulation of osteoblast activity and

inhibition of bone resorption. Furthermore, the recent observations show that activation of βcatenin in osteocytes increases bone resorption and Rankl production in a sclerostin-dependent manner. Anti-sclerostin therapy has shown beneficial skeletal outcomes in osteoporotic patients, however more recent evidence shows that the anabolic effects of this therapy attenuate with time and that after discontinuation BMD returns to pretreatment levels over time. The new evidence showing increased levels of Sost/sclerostin (and Dkk1) after activation of Wnt-βcatenin signaling suggest that sclerostin (and Dkk1) act as a negative feedback limiting bone formation stimulated by this pathway. Preclinical models demonstrate that sclerostin production is also altered in cancers that grow in bone and that its inhibition prevents cancer-induced bone loss, raising the possibility of using neutralizing antibodies against sclerostin as new therapeutic approach for cancers that target bone. Future studies are required to identify the exact source of sclerostin, the events leading to its aberrant production in skeletal pathologies, the mechanisms underlying its actions on osteoblasts and osteoclasts, and its autocrine effects on osteocytes. Full understanding of the biology and pathophysiology of sclerostin will lead to improved current therapies and the identification of novel targets to treat skeletal diseases.

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HIGHLIGHTS

- Sclerostin is a key molecular coordinator of both bone formation and bone resorption.
- Binding to the chaperone LRP4 and the co-receptors of canonical Wnt signaling LRP5 and 6 and the consequent inhibition of Wnt/βcatenin signaling mediates the skeletal actions of sclerostin.
- Sost/sclerostin expression is regulated by complex mechanisms that involve crosstalk between hormones and mechanical stimuli, through activation of transcription factors and chromatin epigenetic modifications.
- Sost/sclerostin expression is altered during aging, in several skeletal diseases and cancers that grow in bone.
- Genetic and pharmacologic inhibition of Sost/sclerostin expression markedly affects skeletal homeostasis resulting from potent stimulation of bone formation and inhibition of bone resorption.
- Identification of the exact factors controlling Sost/Sclerostin expression and full understanding of the mechanisms of action of this gene/protein will provide the basis for developing new therapeutic strategies to treat bone diseases.

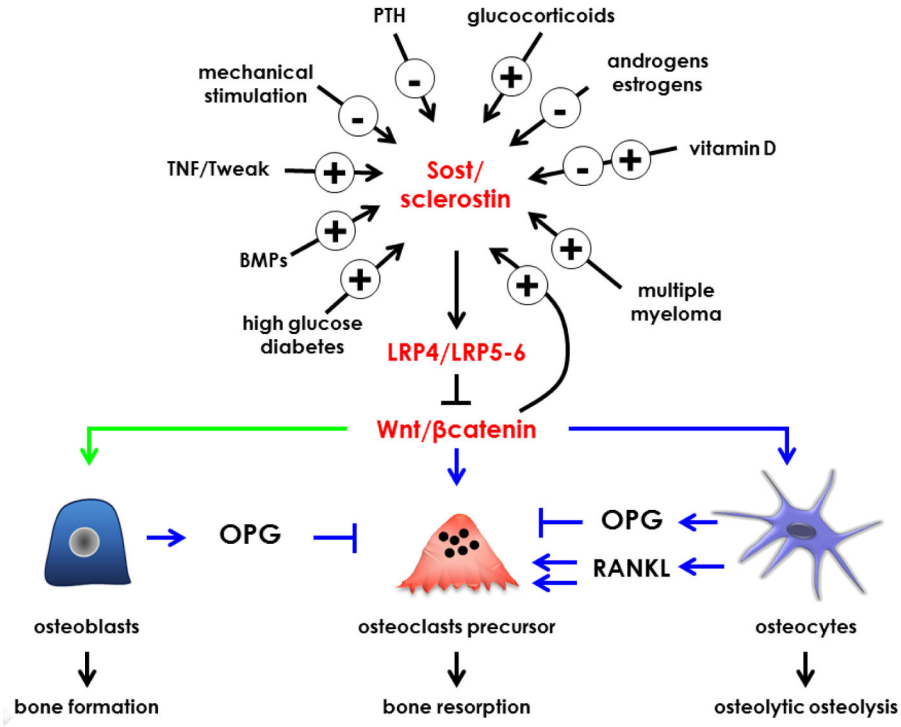

Figure 1. Role and mechanism of action of Sost/sclerostin in bone

The expression of Sost/sclerostin is tightly regulated by complex mechanisms involving crosstalk between systemic hormones, cytokines and mechanical stimuli (black lines). The chaperone LRP4 presents sclerostin to the Wnt co-receptors LRP5/6, thus facilitating sclerostin inhibition of Wnt/βcatenin signaling. The consequent inhibition of the Wnt/βcatenin pathway leads to decreased bone formation due to impaired osteoblastogenesis and decreased osteoblast survival (green line). Novel findings suggest that activation of osteocytic Wnt/βcatenin signaling itself increases the expression of Sost/sclerostin, which in turn acts in a negative feedback limiting bone formation driven by the pathway. Sclerostin not only regulates bone formation, but also bone resorption (blue lines). Inhibition of Wnt/βcatenin signaling in osteoblasts and osteocytes decreases the expression of Opg, and direct actions of sclerostin on osteocytes increase the expression of RANKL. In addition, inhibition of Wnt/βcatenin signaling in osteoclast precursors directly favors their differentiation. Thus, by antagonizing Wnt/βcatenin signaling, sclerostin has the potential to stimulate osteoclast differentiation and enhance bone resorption. Further, sclerostin increases in osteocytes the expression and activity of enzymes that remodel perilacunar matrix leading to osteolytic osteolysis.