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Chapter 2

Sclerostin: current knowledge and future perspectives

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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
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 osteoclast 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 [PINP], 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,
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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).

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**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
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
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 caPTH1 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, dexamethasone 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 teriparatide 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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