Sclerostin and Bone Aging: A Mini-Review

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Abstract
Sclerostin, mainly produced by osteocytes, is now considered a major regulator of bone formation. Identified from patients with a low bone mass, sclerostin inhibits the Wnt pathway by binding to LRP5/6 and subsequently increases bone formation. Sclerostin may also play a role in the mediation of systemic and local factors such as calcitriol, PTH, glucocorticoids and tumor necrosis factor-alpha. Circulating sclerostin levels increase with age and with the decline of kidney function. However, they are surprisingly higher in patients with a high bone mineral density, suggesting that sclerostin may be a relevant marker of the pool of mature osteocytes. The anti-anabolic properties lead to the development of anti-sclerostin biotherapies that are under current evaluation. The results of these clinical trials will open new promising opportunities for the treatment of osteoporosis and bone fragility fractures.

Introduction
Osteoporosis is a skeletal disorder characterized by diminished bone strength that is responsible for an increased fracture risk. Bone strength results from bone quality and quantity of bone mass assessed by bone mineral density (BMD). Osteoporosis affects mainly postmenopausal women, although 30% of the men will experience a fracture. Accelerated bone loss occurs in women after the menopause as a result of estrogen deficiency associated with the loss of ovarian function. The bone loss is sustained with increasing age and then predisposes elderly patients to significant increase in fracture risk [1]. In the elderly, there is an additional bone loss related to a reduced capacity of bone formation in relation with an impaired osteoblast differentiation and function [2]. Thus, failure of osteoblast capacity results in a net bone loss because bone formation does not compensate bone resorption. To date, most of the therapies available are anti-catabolic agents, which have been approved for their anti-fracture efficacy. By reducing bone resorption and bone remodeling, anti-resorbing agents prevent fractures through the increase in bone mineralization rather than the increase in bone mass [3]. To date, teriparatide is the only anabolic agent available promoting bone formation and thereby reducing the rate of fractures. However, teriparatide is limited to patients with severe forms, requires subcutaneous injections and should be followed by bone resorption inhibitors. Such limitations illustrate the need for anabolic agents, which is a real challenge for osteoporosis in the near future. The recent discovery of sclerostin has changed the understanding of the control of bone mass and has provided new insights in regulating factors of bone remodeling. In addition, the mode of action of sclerostin has offered new opportunities for the treatment of bone fragility.
Identification of Sclerostin

Sclerostin was described in 2 rare genetic diseases that have identified SOST gene and thereafter delineate new pathways of bone remodeling. The first is sclerosteosis (MIM 269500), an autosomal recessive disease characterized by a high bone mass, cortical thickening, gigantism and face and skull deformities that appear in early childhood. These features continue steadily with age [4]. Sclerosteosis, reported in Caucasian patients living in South Africa and having a common ancestor of German origin, was found to be caused by inactivating mutations of the SOST gene [5]. Their skeleton is resistant to fracture. The SOST gene is located on the long arm of chromosome 17 (17q12–q21) [6] and encodes for the protein called sclerostin. Heterozygous carriers have a normal phenotype and low circulating levels of sclerostin in addition to higher BMD and higher levels of biomarkers of bone formation compared to the general population [7]. Meanwhile, van Buchem disease (MIM 239100) was also reported in a small fishing village in the Netherlands and Germany [6, 8]. The phenotype of patients with van Buchem disease is close to sclerosteosis, characterized by a high bone mass but no gigantism. Van Buchem disease was shown to be related to the 52-Kb downstream deletion of the SOST gene, leading to the production of a truncated protein found in very low serum concentrations [9]. Thereafter, genetic manipulation in mice recapitulated the human phenotype and demonstrated the considerable impact of this gene on the control of bone. Mice lacking the SOST gene showed elevated bone mass BMD, while overexpression of the SOST gene in transgenic mice led to osteoporosis.

Function of Sclerostin in the Wnt Pathway

The SOST gene is mainly expressed in bone cells, although it is also expressed in several tissues including cartilage, bone marrow, pancreas, heart, aorta, liver and kidney during development. However, postnatal expression of sclerostin is limited to osteocytes, chondrocytes and cementocytes [10]. In the mature skeleton, sclerostin is mainly synthesized by differentiated osteocytes within the mineralized matrix, while immature osteocytes embedded in osteoid do not express sclerostin. Lining cells and osteoblasts do not express sclerostin. Osteocytes represent 90% of the cells in bone and are derived from mature osteoblasts. Once embedded, osteocytes develop dendrites in order to communicate with other osteocytes and osteoblasts at the bone surface and to release their products in the bloodstream. Osteocytes are mechanical, sensitive cells able to integrate mechanical loading applied to bone and reduces the secretion of sclerostin [11]. In healthy rodents, mechanical stimulation of the ulnar by a loading resulted in a dramatic decrease of sclerostin by osteocytes, with an increase of bone formation.

Sclerostin appeared rapidly as a potent inhibitor of Wnt canonical signaling. Involved in embryogenesis and morphogenesis, sclerostin is crucial for the differentiation and proliferation of stem cells. The activation of the Wnt pathway triggers either the canonical signaling by the stabilization of β-catenin or the non-canonical signaling pathway independently of β-catenin [12]. Wnt molecules bind to the receptors of the Frizzled family and to low-density lipoprotein receptor-related protein 5 or 6 (LRP5/6) co-receptors [13]. The binding inhibits the glycogen synthase kinase (GSK)-3β, reduces the phosphorylation of β-catenin, then leading to its intracellular accumulation and to its translocation to the nucleus (fig. 1). Once translocated, β-catenin activates the transcription of target genes by the binding of T-cell factor/lymphoid enhancer factor [14]. Differentiation of osteoblasts is then stimulated, promoting the bone formation. The regulation of the Wnt pathway in osteoblasts is ensured physiologically by the DKK-1 and sclerostin antagonists [15]. As DKK-1, sclerostin directly inhibits the Wnt pathway by binding to LRP5/6 [16]. Thus, in the absence of Wnt signal, β-catenin is phosphorylated, enabling the recognition by ubiquitin, and then leads to the degradation in the proteasome.

Because LRP5 is a major gene in osteoblast function, sclerostin emerged rapidly as a potent inhibitor of Wnt signaling pathway in osteoblasts [16]. Indeed, sclerostin inhibits the differentiation of osteoblasts and reduces bone formation. In addition to the anti-anabolic action, sclerostin is capable of stimulating osteoclast differentiation in a RANKL-dependent manner and therefore has an indirect activity in bone resorption [17]. Interestingly, osteocyte is able to directly generate a process of osteolysis because sclerostin controls the acidification of the extracellular matrix [18].

Finally, sclerostin negatively regulates the mineralization process either directly or indirectly by regulating FGF23, another molecule produced by osteocytes and involved in mineralization. Indeed, sclerostin knockout mice have decreased levels of FGF23 and urinary excretion of calcium [19]. Sclerostin and FGF23 could act synchronously on the renal tubules to increase the urinary
excretion of calcium and phosphorus, thereby reducing the availability of these ions at the mineralization front. Sclerostin expression is upregulated by several systemic and local factors such as calcitriol alone or in combination with glucocorticoids, tumor necrosis factor-alpha and bone morphogenic proteins. Conversely, PTH reduces the osteocytic expression of sclerostin in cell lines and in mice [20].

**Sclerostin and Bone Fragility in Aging**

Due to its inhibitory action in osteoblast function, sclerostin is a potential candidate as a biomarker of bone formation. Serum sclerostin levels are higher in men than in women and increase with the decline of renal function and age [21]. The increase in circulating levels with age suggests that the enhanced production may be part of the age-related impairment in bone formation. However, levels of sclerostin mRNA are similar in bone of 70-year-old...
women compared to 30-year-old women, along with higher expression of SFRP1, another Wnt inhibitor [22]. Then, several local factors regulate the bone formation in addition to sclerostin, the circulating levels being markers of aging rather than a local regulation marker of bone remodeling. Moreover, the expression of sclerostin has been reported in osteoclasts derived from bone marrow of old mice compared to younger mice, indicating that osteoclasts might contribute to the age-related bone loss [23]. However, these results need to be confirmed in a more purified cell population.

In both sexes, serum sclerostin is positively but weakly correlated with BMD, although [24–26] the correlation is higher in postmenopausal women [21, 27]. This positive correlation is a paradigm as high circulating sclerostin is associated with high BMD. The unexpected correlation is surprising with regards to the anti-anabolic effect and is not consistent with age-related bone loss. It has been suggested that sclerostin could be a relevant marker of the pool of mature osteocytes. In addition, sclerostin could be an adaptive mechanism of bone to stimulate bone formation, as shown by the increase in circulating levels with risendronate [28], in contrast to a decrease with raloxifene and estrogen [27, 29]. In addition, serum sclerostin levels predict osteoporotic fractures in the Study of Osteoporosis Fracture cohort independently of BMD [30]. This was not confirmed in other cohorts, likely due to a lower sample size and the use of several assays, which explain a large variability in the concentration [24, 26, 30, 31]. Similar results have been demonstrated in men in whom serum sclerostin levels are positively associated with BMD and microarchitectural parameters [25] along with a reduced risk of fracture [odds ratio: 0.55 (0.31–0.96) in the high quintiles] [32].

Surprisingly, elevated circulating sclerostin is not restricted to bone diseases. Indeed, high levels have been shown in obese patients [21] and in type 2 diabetes, likely in relation with fat mass [33], thus raising the possibility of a key role of sclerostin for insulin resistance [34]. Moreover, high levels have been observed in liver diseases [35], indicating a potential role in regulatory mechanisms in other diseases. Sclerostin is also expressed in hypertrophic chondrocytes during development and contributes to endochondral ossification. Indeed, this process also takes place in osteoarthritis when chondrocyte hypertrophy occurs in articular cartilage [36]. Lack of SOST precipitates the development of osteoarthritis, which suggests a protective role of sclerostin for cartilage. Additional effects beside the skeleton are emerging in chronic kidney diseases [37]. Circulating sclerostin is elevated in relation to the decline of kidney function in parallel with the FGF23 levels, another molecule produced by osteocytes and stimulated by PTH. Sclerostin expression was shown in human biopsies with renal osteodystrophy, in particular at early stages along with Wnt inactivation; both might be contributing to the low bone formation. However, the expression remains high even in the presence of high remodeling, and there is not enough evidence showing a relationship between sclerostin expression and the type of osteodystrophy or the prevalence of fractures [38]. Further studies are warranted to determine the role of sclerostin in the pathogenesis of bone metabolism in chronic kidney diseases. Finally, sclerostin might contribute to the development of vascular calcifications, although the mechanisms are unknown [39].

**Anti-Sclerostin Agents for the Treatment of Bone Fragility**

Based on the mode of action of sclerostin as an inhibitor of bone formation, biotherapies directed against the molecule have been developed with the idea of promoting the anabolic effect. Romosozumab and blosozumab were then tested in animal models. Both biotherapies showed that the inhibition of sclerostin improves bone mass and bone strength. In ovariectomized rats, sclerostin-inhibiting antibodies increased bone formation in the trabecular and cortical compartments [33] and also improved the fracture healing in rodents [40]. Blosozumab prevented both cortical and trabecular bone loss in monkeys [41, 42]. More interestingly, both biotherapies promoted bone formation but did not affect bone resorption [41, 42]. The main interesting and new result is that an anabolic action could be achieved without increasing bone resorption. These results are innovative as they provide here the evidence that a positive balance has been obtained with respect of the coupling between bone formation and resorption.

The unique data of animal models were confirmed in phase II trials in postmenopausal women. Romosozumab induced a significant increase in BMD [43] and a marked rise in circulating anabolic markers without elevation of catabolic parameters [44]. In another phase II trial, romosozumab administrated monthly for 12 months induced a significant increase in BMD at the spine, total hip and femoral neck. The gain in BMD was significantly higher than that observed with alendronate and teriparatide [45]. More interestingly, the bone formation marker P1NP increased during the first 3 months, indicating a rapid and transitory stimulation of bone formation. Of
note, this was associated with a modest decrease in the CTX bone resorption marker. Therefore, the gain in BMD could be explained by the positive bone balance during the bone remodeling cycle. The transient elevation of bone formation restricted to the first 3 months remains to be elucidated, and might involve several regulatory mechanisms in bone remodeling. Phase III trials are underway to assess the efficacy of romosozumab on vertebral and peripheral fractures.

In conclusion, the identification of sclerostin has provided a huge step in the understanding of the regulation of bone remodeling. It has also provided a unique opportunity to develop new anabolic therapies. Anti-sclerostin biotherapies based on the inhibition of sclerostin are under development. These are promising molecules for increasing bone mass and preventing osteoporotic fractures.

References


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