Control of Bone Homeostasis by the Wnt Inhibitor Sclerostin

Meghan E. McGee-Lawrence¹ · Mark W. Hamrick¹

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Abstract Wnt signaling is an important osteogenic pathway regulating skeletal development and maintenance, and sclerostin is a potent extracellular inhibitor of this process. New anabolic skeletal therapies are needed to treat osteoporosis in the aging population, and pre-clinical and clinical studies demonstrate that targeting sclerostin with neutralizing antibodies releases an inhibitory brake on osteogenic Wnt signaling, promoting new bone formation and suppressing bone resorption to ultimately increase net bone mass. In this article, we review recent evidence regarding the regulation of sclerostin production in vivo under normal and disease states and summarize recent findings regarding the efficacy, mechanism of action, and potential complications of sclerostin-targeting therapies (i.e., sclerostin-neutralizing antibodies) in the treatment of skeletal disorders. While recent studies have revealed a great deal of information regarding sclerostin's biological effects and regulatory patterns, much remains to be learned about the role of this molecule in the skeleton and other body systems.

Keywords Sost · Wnt · Romosozumab · Osteogenesis · Osteoporosis · Bone formation

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✉ Meghan E. McGee-Lawrence
mmcgeelawrence@augusta.edu

¹ Department of Cellular Biology & Anatomy, Medical College of Georgia, Augusta University, Augusta, GA 30912, USA

Introduction

Osteoporosis is the most common bone disease in humans; 54 million Americans either presented with or were at risk for this disorder in 2010 [1, 2], and these numbers will grow exponentially with the aging American population [3]. The most widely used osteoporosis treatments (e.g., anti-resorptive bisphosphonates or RANKL-inhibiting denosumab) slow bone loss, but they do not build new bone or counteract age-induced loss of osteoblastic differentiation potential from progenitor cells [4]. Parathyroid hormone (PTH) therapy, in the form of teriparatide, is the only FDA-approved agent that stimulates new bone formation, and its clinical usage is limited to an 18-month “anabolic window” of efficacy [5].

Wnt signaling has long been recognized as an important osteogenic pathway in the regulation of skeletal development and regeneration. In canonical Wnt signaling, binding of Wnt ligands to Frizzled and Lrp5/6 co-receptors stabilizes β -catenin, allowing it to accumulate in the cytosol, translocate to the nucleus, and upregulate expression of Wnt target genes, resulting in stimulation of osteoblast progenitor proliferation and differentiation. Sclerostin, encoded by the *SOST* gene, is an extracellular inhibitor of the canonical Wnt pathway. This predominant osteocyte-produced glycoprotein was identified through genetic screening of patients with the sclerosing bone disorders sclerosteosis and van Buchem's disease, locating genetic mutations in the *SOST* gene or its regulatory elements that produced a high bone mass phenotype in affected patients [6–8]. Sclerostin antagonizes Wnt signaling by binding to Lrp4, 5, and 6 co-receptors [9, 10], blocking the ability of Wnt ligands to bind and stimulate downstream signaling. Although primarily produced by osteocytes, sclerostin expression has also been detected in other cells like aged osteoclasts [11], calcifying vascular tissue [12, 13], and synoviocytes [14•]. As an inhibitor of Wnt signaling, sclerostin suppresses

both osteoblastic differentiation and activity [15, 16]. Additionally, sclerostin stimulates RANKL expression in osteocytes, promoting osteoclastic bone resorption [17]. Accordingly, sclerostin-deficient mice develop high bone mass resulting from an immense increase in osteoblastic bone formation and smaller but significant decreases in osteoclastic bone resorption [18, 19].

In this article, we review recent evidence regarding the regulation of sclerostin production *in vivo* under normal and disease states and summarize recent findings regarding the efficacy of sclerostin-targeting therapies (i.e., sclerostin-neutralizing antibodies) in the treatment of skeletal disorders.

Regulation of Sclerostin Production

As a powerful inhibitor of osteoblastic bone formation, sclerostin levels must be tightly regulated in the body. Recent findings regarding physiological regulators of Sost and sclerostin expression are summarized below. Although excellent earlier literature exists on many of these topics, we have made an effort to highlight the results of recent studies (published within the past five years).

Parathyroid Hormone

It has been known for over a decade that PTH treatment suppresses sclerostin production by human and murine osteocytes [16, 20, 21]. Several recent reports have confirmed this phenomenon *in vivo*, demonstrating that serum sclerostin levels are negatively correlated with PTH levels in non-diabetic patients [22] and decline in response to an acute infusion of PTH [23]. Additionally, Sost overexpression abrogates increases in cortical and cancellous bone mass caused by constitutively active PTH1R signaling [24]. Suppression of sclerostin by PTH requires the presence of the Wnt receptor LRP6 [25] and occurs, at least in part, through the nuclear accumulation of histone deacetylase 5 (Hdac5). Hdac5, in turn, subsequently inhibits myocyte enhancer factor 2 (MEF2)-dependent stimulation of the Sost bone enhancer [26•, 27] (discussed in detail below).

Sex Steroids (Estrogen, Testosterone)

In general, the sex steroids appear to inhibit sclerostin production, best evidenced by the fact that sclerostin levels increase in the case of sex steroid deficiency [28, 29]. Estrogen treatment of postmenopausal women decreases circulating sclerostin [28, 29], but the role of testosterone is more controversial. One study demonstrated that treatment with estrogen, but not testosterone, prevents increases in sclerostin levels following induction of acute sex steroid deficiency [28]. However,

serum sclerostin levels are negatively correlated with testosterone in male human patients, and treatment of human osteocytes with dihydrotestosterone (DHT) reduces Sost expression in these cells, suggesting a potentially important role for testosterone in the biology of sclerostin regulation [20].

Vitamin D

The Sost gene is positively regulated by $1\alpha,25$ -dihydroxyvitamin D ($1,25D$), as both Sost messenger RNA (mRNA) and sclerostin protein are rapidly increased in response to $1,25D$ treatment in osteocyte-differentiated human SaOS2 cells [30, 31]. While sclerostin expression is not necessarily altered by vitamin D deficiency in adults, sclerostin levels are suppressed in babies born to vitamin D deficient as compared to vitamin D-sufficient mothers [32]. However, the role of vitamin D in Sost regulation is not completely clear, as conflicting evidence exists. For example, murine Sost gene expression is suppressed by $1,25D$ treatment in IDG-SW3 cells near the end of the osteoblast-to-osteocyte transition period (i.e., near the time when the cells are considered mature osteocytes) [33]. Interestingly, Sost-deficient mice demonstrate increased serum levels of $1,25D$, resulting, in part, from enhanced expression of 25-hydroxyvitamin D 1α -hydroxylase cytochrome P450 (cyp27B1) in the kidney [18]; this could represent a compensatory mechanism driven by the absence of circulating sclerostin in the knockout animals but more likely reflects the drive for a positive mineral balance to support increased bone formation in the sclerostin-deficient animals.

Mechanical Loading

Bone is a mechanoresponsive tissue, demonstrating hypertrophy in response to increased mechanical loading and atrophy in response to disuse. Mechanical loading suppresses sclerostin production both *in vitro* (e.g., fluid flow loading of osteocytes) [34] and *in vivo* (e.g., ulnar axial loading) [35], whereas disuse increases sclerostin expression *in vitro* [36•] and *in vivo* [37]. Loading-induced sclerostin suppression requires production of nitric oxide, as inhibition of nitric oxide synthase prevents the fluid flow-induced suppression of Sost expression [38]. Administration of sclerostin-neutralizing antibodies abrogates bone loss caused by disuse from hindlimb suspension [39], spinal cord injury [40•], and limb immobilization [41], even when disuse is superimposed over a concurrent effect of estrogen depletion from ovariectomy [42].

Epigenetic Mechanisms

Surprisingly, both circulating and bone mRNA expression of *Sost* are lower in osteoporotic as compared to healthy women [43], despite the fact that sclerostin suppresses bone formation. Subsequent analysis of this phenomenon revealed that the *Sost* promoter is more methylated in patients with established osteoporosis as compared to healthy controls, suggesting that methylation to reduce *Sost* gene expression could be a protective mechanism triggered to help reduce inhibition of Wnt signaling and promote bone formation in osteoporotic patients [43].

Beyond methylation, other epigenetic mechanisms may also influence *Sost* production. Knockdown of *Hdac5* increases *Sost* expression, and overexpression of *Hdac5* suppresses *Sost* expression in osteocytes, and *Hdac5*-deficient mice show increased expression of *Sost* and lower Wnt activity. This effect occurs through modulation of the *Mef2c* transcription factor. *Sost* expression is increased via binding of the transcription factor *Mef2c* to a distal enhancer region (*ECR5*) [44], and *Mef2C* binding at this location is enhanced in the case of *Hdac5* deficiency [26•].

Sclerostin Levels in Human Disease

Many diseases are intertwined with skeletal biology, causing loss of bone mass secondary to organ dysfunction elsewhere in the body. Recently, it has emerged that some of these skeletal phenotypes may be linked with altered circulating levels of sclerostin, as discussed below.

Diabetes

Diabetes mellitus is associated with increased risk of bone fracture [45, 46], and patients may present with reduced bone turnover, suggesting a potential regulatory role for sclerostin in this phenotype [22]. Recent reports indicate that circulating sclerostin levels are increased in both type 1 and type 2 diabetic patients as compared to healthy controls [22, 47], although levels are not different between type 1 and type 2 diabetic patients [48]. This increase likely begins in the pre-diabetic phase when insulin resistance develops, as sclerostin levels are increased in patients with impaired glucose regulation (prior to onset of overt diabetes) as compared to normoglycemic controls and are positively correlated with homeostatic model assessment of insulin resistance (HOMA-IR) [49••]. In patients with type 2 diabetes, sclerostin levels are elevated in patients presenting with fragility fractures compared with non-fractured diabetic controls [50], and higher sclerostin levels are associated with an increased risk of vertebral fractures independent of BMD [51], supporting a role for sclerostin in the fracture-prone phenotype of diabetic patients.

In contrast to this finding, one study revealed that type 1 diabetes patients with the highest tertile of circulating sclerostin levels had decreased risk of fracture; the biological explanation for this confounding observation is not yet known [52].

Kidney Disease

Renal osteodystrophy is a common complication of chronic kidney disease, and the most common type of osteodystrophy in end-stage renal disease is one with low turnover [53]. Sclerostin levels are increased in patients with chronic kidney disease (CKD) both pre- and post-dialysis [12, 54, 55] as compared to healthy controls, with the highest expression occurring at early stages of the disease [56]. At least one study suggests that these high levels of sclerostin are inversely correlated with bone formation rates, potentially contributing to renal osteodystrophy in this patient population [57]. High circulating sclerostin levels in patients with kidney disease are rescued by renal transplantation [58]. Interestingly, circulating sclerostin levels are increased in dialysis patients that develop vascular calcifications as compared to dialysis patients free from calcification complications [12]. Although sclerostin is predominantly produced by osteocytes *in vivo*, its expression is detected by immunohistochemistry and real-time PCR in aortic valves developing calcification as compared to non-calcified valves [12], suggesting that sclerostin could play a role in the biomineralization of extraskelatal tissues.

Liver Disease

Serum sclerostin levels appear to increase the case of liver dysfunction. Several recent reports indicate that sclerostin levels are elevated in patients with cirrhosis as compared to healthy controls [59–61]. The mechanism for the increased sclerostin in cirrhotic patients is not yet known, but proposed contributing factors have included increased retention secondary to liver dysfunction and altered expression of sex steroids [59].

Human Immunodeficiency Virus

Patients infected with human immunodeficiency virus (HIV) often develop low bone mass and subsequent fragility fractures [62]. It was recently reported that sclerostin levels are suppressed in HIV infected as compared to healthy controls for a small cohort of adult patients (33 HIV, 63 controls) [63]. These findings are supported by a second study suggesting reduced sclerostin levels in HIV-infected youths and adolescents as compared to healthy controls [64]. As sclerostin levels were not correlated with bone mineral density in this population, these results raise the possibility that immune function or systemic inflammation could regulate serum sclerostin in HIV-affected patients [63].

Sclerostin Inhibition as an Anabolic Skeletal Therapy

Given the paucity of FDA-approved anabolic skeletal therapies presently on the market, inhibition of sclerostin activity is an attractive target for new osteogenic drug development. The application of sclerostin-neutralizing antibodies (Scl-Abs) for increasing bone mass has been under investigation at least since 2009, when short-term administration of Scl-Ab therapy was shown to improve bone formation, mass, and strength in an ovariectomized rat model of osteoporosis [65]. Below, we summarize recent findings from animal and human studies continuing to pursue this goal and discuss some potential complications that could arise from use of Scl-Ab treatments.

Osteoporosis

Many recent animal studies have focused on better understanding Scl-Ab's mechanism of action, revealing novel effects on bone formation and bone resorption. Whereas bisphosphonate therapies primarily decrease bone resorption and teriparatide administration stimulates increased bone remodeling, Scl-Ab treatments increase bone mass by promoting modeling-based bone formation and simultaneously reducing bone resorption while extending the bone formation period at modeling and remodeling sites [66]. In cortical bone, Scl-Ab promotes both periosteal and endocortical bone formations, leading to a net increase in bone mass [67, 68]. Although Scl-Ab's ability to stimulate bone formation *in vivo* is limited to the early phases of treatment, its ability to suppress bone resorption is sustained for a much greater length of time [67, 69, 70]. Osteoblasts, bone lining cells, and osteocytes collected by laser capture microdissection from Scl-Ab-treated ovariectomized rats show upregulated expression of canonical Wnt targets and genes related to matrix synthesis and mineralization, as would be expected, but surprisingly show no alteration in expression of genes related to osteoclastogenesis (e.g., RANKL) [71], even though sclerostin itself promotes RANKL expression in osteocytes [17].

In humans, two Scl-Ab therapies are at various stages of clinical development as treatments for post-menopausal osteoporosis: romosozumab, developed by Amgen/UCB, and blosozumab, developed by Eli Lilly. Results from phase II trials of romosozumab were published in 2014, demonstrating that all tested dose levels of romosozumab increase bone mineral density at the lumbar spine, total hip, and femoral neck as compared against placebo treatment [72••]. Moreover, high doses of romosozumab (210 mg, delivered monthly) increase bone mineral density at each of these sites to a greater degree than treatment with the anti-resorptive bisphosphonate alendronate or the anabolic agent teriparatide [72••]. Preliminary results from a much larger phase III trial of romosozumab were presented in April of 2016, demonstrating

that 12 months of treatment with romosozumab increases bone mineral density at the total hip and lumbar spine and estimated bone strength at the hip by a greater amount than teriparatide in osteoporotic postmenopausal women [73]. With regard to fracture prevention, recent reports summarizing phase III results indicate that romosozumab reduces the incidence of new vertebral fractures and clinical fractures in postmenopausal women with osteoporosis.

Phase II trials of blosozumab were published in 2015, demonstrating that 12 months of treatment with blosozumab dose-dependently increases bone mineral density at the lumbar spine, femoral neck, and total hip as compared with placebo treatment [74•]. Bone mass gained with blosozumab treatment appears to be maintained, as bone mineral density at the lumbar spine and total hip remains greater in blosozumab treated as compared to placebo-treated postmenopausal women [75•].

Regarding other forms of osteoporosis, clinical trials to investigate the efficacy of romosozumab to combat age-related osteoporosis in men are ongoing [76•, 77••] but have not yet been reported for blosozumab. Notably, circulating sclerostin levels increase with age in humans, although bone mRNA levels of *SOST* are not increased with age, suggesting that extraskeletal production of sclerostin may contribute to these aging-related changes [78]. Scl-Ab therapies have also proved efficacious for abrogating bone loss in other osteoporosis models including glucocorticoid- and disuse-induced osteoporosis. For example, administration of Scl-Ab therapy prevents prednisolone-induced bone loss in mice [79] and blocks disuse-induced bone loss after spinal cord injury in animal models [40•, 80].

Osteogenesis Imperfecta

Osteogenesis imperfecta (OI) is a genetic disorder caused by mutation in type 1 collagen, the major organic component of bone, resulting in a fragile skeleton. Anti-resorptive bisphosphonates have shown some efficacy at preventing fractures in this patient population [81, 82], but Scl-Ab therapies are being considered as new therapeutic approaches to increase bone mass and prevent fractures in OI patients. Although sclerostin antibody treatment cannot rescue the genetic defect in collagen structure, recent evidence from murine studies indicates that Scl-Ab treatments increase whole bone mechanical strength in the skeleton of OI mouse models [83], through an increase in overall bone mass and altered bone matrix chemistry (e.g., mineral to matrix ratio) [84]. This rescue appears to be effective with models of both dominant mild OI (*Brtl*^{+/+} mice) [83, 85] and recessive OI (*Crap*^{-/-} mice) [86]. However, Scl-Ab treatment was unable to improve bone strength in a mouse model of dominant severe OI (*Col1a1*^{Jrt/+} mice) [87], suggesting that efficacy of this therapy may be limited to particular OI patient populations.

Fracture Healing

Sclerostin-deficient mice demonstrate enhanced skeletal healing as compared to wild-type mice [88]. From a translational perspective, it is more interesting to ask whether systemic administration of a Scl-Ab would enhance fracture repair. Animal studies seem promising, as both stabilized bone defects [89–92] and closed fractures [93, 94] heal more rapidly with Scl-Ab administration. Despite success in these animal models, manufacturers of romosozumab abandoned efforts to develop this treatment as a fracture-healing therapeutic agent; preliminary data from phase II studies suggest that treated patients do not demonstrate enhanced time to radiological healing [95]. Thus, sclerostin antibody therapy may be more effective at preventing the onset of new fractures as compared to promoting enhanced healing of existing fractures.

Potential Issues with Anti-Sclerostin Therapies

Despite widespread enthusiasm for developing Scl-Ab therapies as osteogenic agents, concerns have emerged regarding potential side effects. In a recent study, researchers identified that sclerostin is specifically expressed in synovial tissues of patients with rheumatoid arthritis (RA), and subsequent animal studies suggested that expression plays a protective role in the body. Specifically, when mice lacking sclerostin are crossed onto the background of a TNF-dependent RA model (human TNF- α transgenic mice), the double-mutant animals demonstrate enhanced joint inflammation and damage to the cartilage and bone [14•]. This suggests that Scl-Ab treatments could be contraindicated in patients with TNF-dependent arthritis. However, these negative effects were not seen in non-TNF-dependent RA models, and it has also been recently shown that Scl-Ab treatment of human TNF transgenic mice prevents bone and cartilage erosion associated with RA without exacerbating inflammatory metrics like paw swelling and grip strength [96]. Conflicting conclusions between these studies have not yet been reconciled.

Conclusion

New anabolic skeletal therapies are needed to treat osteoporosis in the aging population. Pre-clinical and clinical studies demonstrate that targeting sclerostin with neutralizing antibodies releases an inhibitory brake on osteogenic Wnt signaling, promoting new bone formation and suppressing bone resorption to ultimately increase net bone mass. While recent studies have revealed a great deal of information regarding sclerostin's biological effects and regulatory patterns, much remains to be learned about the role of this molecule in the skeleton and other body systems.

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Compliance with Ethical Standards

Conflict of Interest Meghan E. McGee-Lawrence and Mark W. Hamrick declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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