Sclerostin: an emerging target for the treatment of cancer-induced bone disease

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Abstract

Purpose of review

This review provides a summary of the current knowledge on Sost/sclerostin in cancers targeting bone, discusses novel observations regarding its potential as a therapeutic approach to treat cancer-induced bone loss, and proposes future research needed to fully understand the potential of therapeutic approaches that modulate sclerostin function.

Recent findings

Accumulating evidence shows that sclerostin expression is dysregulated in a number of cancers that target bone. Further, new findings demonstrate that pharmacological inhibition of sclerostin in preclinical models of multiple myeloma results in a robust prevention of bone loss and preservation of bone strength, without apparent effects on tumor growth. These data raise the possibility of targeting sclerostin for the treatment of cancer patients with bone metastasis.

Summary

Sclerostin is emerging as a valuable target to prevent the bone destruction that accompanies the growth of cancer cells in bone. Further studies will focus on combining anti-sclerostin therapy with tumor targeted agents to achieve both beneficial skeletal outcomes and inhibition of tumor progression.

Keywords (4-6 key words): Sost/sclerostin, cancer, bone, myeloma, osteoblasts, osteoclasts

Introduction

Bone is a frequent and preferred site for cancer metastases and cancer involvement. Up to 70% of patients with advanced breast or prostate cancer present with bone metastasis, and over 90% of patients with multiple myeloma develop osteolytic bone lesions. Upon dissociation from the primary tumor, metastatic cells populate the highly vascularized environment of the bone marrow with a strong affinity. Once bone is colonized, tumor cells in bone may start to grow immediately or survive in a dormant state until activated to initiate tumor formation [1]. The exponential growth of tumor cells in bone disrupts normal bone remodeling driving local bone destruction, induces severe pain, and increases fracture rates dramatically, thereby increasing mortality and reducing the likelihood for disease remission [2].

Current research in bone metastasis aims to develop a greater understanding of the interactions between tumor cells and the cells of the bone marrow environment. The bone microenvironment controls tumor cell dormancy and regulates tumor cell activation through osteoclastic bone resorption during both disease initiation and recurrence [3-6]. In addition, interaction of cancer cells with different cell types in the bone microenvironment induces the secretion of multiple cytokines that stimulate bone resorption and fuel tumor growth [7-9]. Although less well explored, alterations in bone formation act in concert with altered bone resorption in response to the presence of cancer cells in bone, particularly in multiple myeloma [10-12]. Moreover, interactions with stromal cells promote growth, invasion, and metastasis [13]. Hence, we have compelling evidence that interactions between tumor cells and osteoblasts, osteoclasts, and stromal cells present in the bone marrow microenvironment influence tumor behavior and contribute to the development of osteolytic lesions [3;14-16].

Recently, attention has turned to the role of osteocytes in the tumor microenvironment. Osteocytes are the most abundant and long-lived cells in bone and are master regulators of bone remodeling [17-19]. Accumulating data supports that osteocyte function and life span are altered in bones colonized by cancer cells and that osteocytes favor tumor progression and bone destruction [17;20]. Further, the expression of the osteocyte-derived factor sclerostin, a potent inhibitor of bone formation, is increased in a number of cancers that target the skeleton [21-26], suggesting that this protein could play a part in the inhibition of bone formation that accompanies the growth of cancer cells in bone. In this review, we provide a summary of the role of sclerostin in cancer-induced bone disease

and the potential of using neutralizing antibodies against sclerostin as novel therapeutic approach to improve outcomes, quality of life, and survival in cancer patients.

1. Sost/sclerostin: a negative regulator of bone mass and much more

Sclerostin, encoded by the gene Sost, was first identified through genetic linkage analyses in sclerosteosis and van Buchem's disease patients [27;28]. Mutations in the Sost gene observed in these patients result in absence of sclerostin expression or secretion, leading to high bone mass due to exaggerated bone formation [29;30]. Numerous studies followed this discovery, unravelling the numerous mechanisms of action of Sost/sclerostin in regulating bone mass (**Fig. 1**) [31]. In rodents, genetic deletion of Sost/sclerostin results in a progressive and generalized increase in bone mass due to increases in the number and bone-forming activity of osteoblasts [32]. In contrast, sclerostin overexpression reduces bone formation and bone mass in mice [33-35]. Mechanistic studies demonstrated that sclerostin acts as an extracellular inhibitor of canonical Wnt signaling. Assisted by LDL receptor related protein (LRP) 4, sclerostin binds to LRP5 and LRP6, and antagonizes downstream Wnt/β-catenin signaling [36;37], a pathway that stimulates bone formation and negatively regulates bone resorption to enable bone acquisition and maintain bone homeostasis. These findings led to the hypothesis that inhibition of sclerostin might restore bone mass and strength in the osteoporotic skeleton. Amgen (romosozumab), Eli Lilly (blosozumab), and Novartis (BPS804) guided the development of neutralizing antibodies to therapeutically target sclerostin, which resulted in a vast array of animal and human clinical studies demonstrating the ability of anti-sclerostin antibodies to stimulate bone formation and increase bone mass and strength (**discussed in section 4**).

In the last years, there has been an expansion of our knowledge of the actions of sclerostin in both skeletal and non-skeletal tissues. Although osteoblasts are the main target cells for sclerostin in bone, it has become apparent that anti-sclerostin antibodies also decrease bone resorption (**Fig. 1**), suggesting that sclerostin also regulates the differentiation and function of osteoclasts [38]. In this line, sclerostin-mediated inhibition of Wnt/β-catenin signaling leads to a reduction in osteoprotegerin (Opg) expression in osteoblasts and osteocytes, a soluble decoy for the master regulator of osteoclastogensis termed receptor activator of nuclear factor kappa-B ligand (Rankl), with consequent increases in osteoclastic resorption [39-41]. Overexpression of Sost/sclerostin also increases the expression of Rankl in bone [35;42]. Further, constitutive activation of Wnt/β-catenin signaling in osteocytes also increases Rankl expression [42], an effect driven by sclerostin production [42]. Moreover, addition of recombinant

sclerostin to cultures of MLO-Y4 osteocytic cells is sufficient to upregulate Rankl and enhance osteocyte-induced osteoclast formation *in vitro* [43]. Recent findings suggest that sclerostin could also play a part in the regulation of adipocyte differentiation and/or fat production (**Fig. 1**). Sclerostin induces adipogenesis through inhibition of Wnt signaling in pre-adipocytes *in vitro*, and genetic and pharmacological inhibition of sclerostin *in vivo* decreases bone marrow adipose tissue formation [44-48]. Further, increased sclerostin in the circulation results in a progressive loss of white adipose tissue in gonadal and inguinal stores, an effect associated with decreased white adipocyte markers, increased beige adipocytes, and reduced canonical Wnt/β-catenin signaling in these fat depots [49]. Finally, neutralization of sclerostin stimulates the conversion of bone lining cells into active osteoblasts [50], supporting the notion that sclerostin can regulate the pool of quiescent bone lining cells in bone (**Fig. 1**). Altogether, these findings indicate that sclerostin can target different cells in the bone marrow microenvironment. In addition, similar to other factors secreted by osteocytes/osteoblasts, these results suggest that sclerostin has an endocrine metabolic action complementary to its function in bone. Further investigation is warranted to determine the importance of circulating sclerostin levels and their impact in non-skeletal tissues.

Despite our increased knowledge in Sost/sclerostin biology, the specific mechanisms by which this gene is regulated remain as an outstanding unresolved issue. Sclerostin expression is mainly detected in osteocytes, osteocytic lacunae, and along osteocytic canaliculi, indicating that Sost/sclerostin is expressed by mature osteocytes, but not by early osteocytes or osteoblasts [51]. It is important to note that recent evidence suggests that other cells in the bone marrow microenvironment might also express Sost/sclerostin, particularly during development or under pathological conditions [26;52-63]. Yet, whether sclerostin produced by other cells than osteocytes contributes to the skeletal effects attributed to this protein is unclear. The regulation of Sost/sclerostin expression is complex and requires coordination of multiple mechanisms to control sclerostin production in a time and cell-context manner. The first regulatory sequence in the Sost gene was identified in van Buchem patients, which present a homozygous deletion of a 52-kb noncoding located ~35 kb downstream of the Sost transcription start site [34;64]. This sequence contains an enhancer element essential for Sost expression, the evolutionarily conserved region 5 (ECR5). The ECR5 has response elements for the myocyte enhancer factor-2 (Mef2c), a transcription factor that upon binding to ECR5 stimulates Sost mRNA expression [65;66]. Also, epigenetic modifications in the Sost proximal promoter (~1.4Kb upstream the SOST gene), in particular DNA methylation, are responsible for the repression of Sost/Sclerostin expression in osteoblastic cells, and elimination of DNA methyl marks during osteoblast-osteocyte

transition enables the expression of this gene in osteocytes [67;68]. Further, several hormonal stimuli and transcription factors that bind to regulatory regions of the Sost gene and control its transcription have been identified, including parathyroid hormone [65;69-71], transforming growth factor beta and activin-A [72], bone morphogenetic proteins [72-74], tumor necrosis factor alpha and Tnf-related weak inducer of apoptosis [75], and more recently osteoclast-derived leukemia inhibitory factor [76]. Future research efforts should focus on understanding the interplay between the different epigenetic marks and transcription factors, and identifying the specific regulatory mechanisms that lead to the dysregulation of sclerostin expression in bone pathologies, including those caused by cancer.

2. Sost/sclerostin in other bone tumors and cancers that metastasize to bone

The contribution of osteocytes and their derived factors to cancer-induced bone disease is an emerging area of research [77]. Autophagic/apoptotic osteocytes are increased in bone areas infiltrated with myeloma cells [78-80], and reciprocal interactions between myeloma cells and osteocytes alter osteocyte viability and stimulate proliferation in myeloma cells. Also, recent data show that osteocytes can communicate with sensory nerves and contribute to myeloma-induced bone pain [81], one of the most common symptoms of multiple myeloma. In response to increased intraosseous pressure resulting from the growth of prostate cancer cells in the bone marrow, osteocytes produce chemokine (C-C motif) ligand 5, which in turn stimulates the synthesis of matrix metalloproteinases to favor the growth and invasion of prostate cancer cells into bone [20]. Further, recent findings suggest that osteocytes, in addition to osteoblasts, may act as a cell of origin for osteosarcoma [82]. Together, these data support a role for osteocytes in providing a microenvironment that is conducive to tumor growth and the subsequent skeletal destruction and bone pain.

Key to osteocyte regulation of bone response to cancer cell colonization is the osteocyte-derived protein sclerostin. The expression of sclerostin has been assessed both at bone tissue level and systemically in the circulation in a number of cancers that target bone [83], with some conflicting data. In a small cohort of patients, circulating sclerostin levels were higher in postmenopausal women with endocrine-responsive breast cancer compared to those in the premenopausal group, and serum sclerostin increased after treatment with aromatase inhibitors [21;22]. Further, MDA-MB-231 breast cancer cells secrete sclerostin to inhibit osteoblast differentiation through a mechanism dependent on both Runx2 and CBFβ [84]. Circulating sclerostin levels are also significantly

increased in patients with prostate cancer, particularly in those receiving androgen deprivation therapy [23;24]. Sclerostin levels remained unaltered however in renal cell carcinoma patients with osteolytic metastases or patients with indolent systemic mastocytosis [85;86]. It is important to note that sclerostin expression in bone does not always correlate with sclerostin levels in the circulation [87;88]. Indeed, in contrast to clinical studies, preclinical experiments in rodents show that sclerostin has the potential to inhibit prostate cancer invasion and to reduce the incidence of metastases and bone destruction [89;90]. Thus, evaluation of sclerostin at the tissue level is required to determine the levels of this protein in bone and properly assess its potential contribution to the effects of cancer in bone. Nevertheless, these results suggest that the regulation and function of sclerostin may differ among cancers that target bone. Therefore, combinations of clinical measurements of sclerostin with data from animal models are essential to predict the clinical outcome of targeting sclerostin in different bone destructive cancers.

3. Sost/sclerostin dysregulation in multiple myeloma

Elevated sclerostin expression has been implicated in the pathogenesis of bone loss in patients with myeloma, with a number of clinical investigations showing increased serum sclerostin levels in myeloma patient samples compared to healthy subjects or MGUS patients [25;26]. Increased levels of Sost mRNA were found in plasma cells isolated from small cohorts of patients with myeloma [62;63;91], suggesting that myeloma cells are the main source of sclerostin. However, in a more recent study including over 630 myeloma patients, bone marrow plasma cell Sost mRNA was not different to healthy controls, nor was it detected in 56 human or murine myeloma cells lines [92], suggesting elevated serum levels of sclerostin were driven by another source. Indeed when sclerostin plasma concentrations were compared with Sost mRNA and sclerostin protein expression in bone marrow mesenchymal stromal cells and osteocytes, a strong correlation was demonstrated [26], indicating that bone cells are mainly responsible for the increased serum sclerostin levels detected in patients with myeloma. Supporting this notion, *in vitro* experiments show that physical interactions with myeloma cells increase Sost mRNA expression in osteocytes, and the number of osteocytes expressing sclerostin doubled in bones bearing myeloma cells versus healthy controls [79;92;93]. These results led many to hypothesize that elevated sclerostin levels in the bone/bone marrow microenvironment are causal of the suppressed bone formation induced by myeloma, and positioned sclerostin as a bone specific targetable candidate to promote bone formation and overcome bone loss in patients with myeloma.

4. Sost/Sclerostin as a therapeutic tool to prevent cancer induced bone loss

Due to the devastating consequences of tumor cell metastasis and myeloma cell expansion in bone, extensive pre-clinical and clinical research has led to the clinical use of bone targeted therapies, such as the anti-resorptive agents bisphosphonates and denosumab, to prevent bone destruction [94]. Besides stopping tumor-induced skeletal destruction, anti-resorptive therapies can also maintain tumor cells in a dormant state in bone, thus preventing their re-activation and subsequent disease recurrence [3]. Thus, anti-resorptives are considered the mainstay therapy for cancer patients with bone involvement. However, although anti-resorptive therapy is effective at preventing further bone loss, it cannot rebuild lost bone, and therefore fractures still occur in patients with myeloma and metastatic bone disease, even during remission. Thus, anabolic therapies that increase osteoblast activity and stimulate new bone formation are required to help restore bone structure and strength in cancer patients.

Sclerostin is now recognized as a target for the treatment of osteoporotic bone loss. Neutralizing antibodies to sclerostin have shown beneficial outcomes for bone mass and structure in preclinical studies in rodents, and have shown potent skeletal anabolic effects, with increases in bone strength and fracture risk reduction in phase II and III clinical trials for the management of osteoporosis [95-98]. Mechanistically, anti-sclerostin therapy uncouples bone formation from bone resorption, by stimulating osteoblast function while decreasing osteoclast function. These findings have attracted recent attention in the field of cancer, and in particular multiple myeloma [26;92;93], as a potential new avenue for rebuilding lost bone and preventing fractures in patients with bone destructive cancers.

Administration of anti-sclerostin antibodies increased bone volume in immunocompromised mice bearing MM1.S human xenograft cells [26]. Although bone loss compared to naïve control mice in response to MM1.S cells was not demonstrated in this study, anti-sclerostin antibodies increased cancellous bone volume and trabecular thickness in the vertebrae. The circulating levels of the bone formation marker P1NP were elevated by anti-sclerostin treatment, suggesting that stimulation of bone formation was responsible for the increased bone volume. More recently, both genetic deletion of Sost and pharmacologic inhibition with anti-sclerostin antibodies showed significant protection from myeloma-induced bone loss [92;93]. Immunocompetent mice bearing 5TGM1 or 5T2MM cells, and immunocompromised mice bearing human MM1.S cells showed significant trabecular bone loss compared to naïve controls mice. Anti-sclerostin treatment prevented this bone loss, normalizing bone volume to naïve control levels, through increasing bone formation without altering bone resorption parameters [92]. Further,

the development of osteolytic lesions, characteristic of the 5T2MM model, was also prevented with anti-sclerostin treatment, possibly via correcting the myeloma-induced dysregulation of coupling between bone resorption and formation. Importantly, anti-sclerostin-driven protection from bone loss also prevented the decrease in bone strength in mice bearing myeloma, suggesting that anti-sclerostin treatment may prevent myeloma-induced fractures. Noteworthy, anti-sclerostin treatment provided additive protection from 5TGM1-induced bone loss and compromised bone strength when combined with the anti-resorptive agent zoledronic acid [92]. Immunocompromised mice carrying a homozygous deletion of Sost were also protected from bone loss and the development of osteolytic lesions induced by human JJN3 myeloma cells through preservation of bone formation and a modest inhibition of bone resorption [93]. Remarkably, in the same study, administration of anti-sclerostin to mice with established/active myeloma disease, protected from trabecular bone loss and osteolytic lesions and demonstrated that anti-sclerostin stimulates new bone formation and reduces osteoclast numbers in areas colonized by myeloma cells. In concert, these results demonstrate that anti-sclerostin protects from myeloma-induced bone loss by primarily stimulating bone anabolism, with modest or undetectable effects on resorption, and support that combined therapeutic approaches targeting both bone suppression of anabolism and increased catabolism may provide optimal outcomes for myeloma-induced bone disease (**Fig. 2**).

Before clinical applications of anti-sclerostin antibody treatment can be considered in myeloma however, consideration must be given to the possible impact of stimulating Wnt signaling on tumor growth. Inhibiting a soluble Wnt antagonist may increase local Wnt signaling and promote tumor growth [99]. Whilst Wnt signaling stimulation of myeloma cell proliferation has been demonstrated *in vitro* [100], anti-sclerostin antibody treatment had no effect on skeletal and extra-skeletal myeloma burden across all models described above [26;92;93]. Also, anti-sclerostin did not alter the anti-myeloma activity of the proteasome inhibitor carfilzomib *in vivo* [26] or impact myeloma cell proliferation *in vitro*, either in the presence or absence of chemotherapeutic agents [92;93]. These findings suggest that sclerostin does not regulate pathways that control tumor growth, and open the possibility of combining anti-sclerostin therapy with chemotherapeutic drugs to achieve both beneficial skeletal outcomes and inhibition of tumor progression.

It is important to mention that sclerostin is not the only Wnt inhibitor elevated in patients with myeloma or bone metastasis. Suppression of osteoblast differentiation through TGF-β signaling and Dkk-1 has also been

implicated in bone lesions caused by breast cancer bone metastases [101-103]. Also, Dkk1 and SFRP3 mRNA expression was markedly elevated in bone marrow biopsies of 65 patients with myeloma with either absent or overt lytic bone disease [104]. In preclinical models, anti-Dkk1 successfully prevented myeloma-induced bone disease and had variable effects on tumor burden leading to early clinical trials [105-107]. These agents have not however succeeded to reach the clinic, possibly due to an inadequate bone anabolic capacity of the agents or the heterogeneous response of patients [92]. In a recent study, a bispecific antibody inhibiting both sclerostin and Dkk-1 was generated. Dual inhibition of sclerostin and Dkk-1 resulted in greater increases in bone formation and showed superior bone repair activity when compared to inhibition of each of the Wnt antagonists alone in animal models of osteoporosis [108]. Further investigation is needed to determine whether dual inhibition of Dkk-1 and sclerostin is also anabolic in bones colonized by cancer cells, has effects on tumor growth, and prevents fractures to a degree that exceeds that achieved with Wnt antagonist inhibitor monotherapy.

Conclusions and future directions

The discovery of sclerostin and its impact on the skeleton opened a new area in bone therapeutics. Recent results show that sclerostin production is elevated in bones colonized by cancer cells, raising the possibility of targeting sclerostin for the treatment of bone-destructive cancers. Recent animal studies demonstrate that pharmacological inhibition of sclerostin prevents bone loss and stimulates new bone formation in preclinical models of multiple myeloma. Anti-sclerostin antibodies also robustly enhance normal and compromised fracture repair and importantly increase BMP-driven repair of critical size defects [108-114]. Consistent with the anti-fracture properties of anti-sclerostin [96], pharmacological inhibition of sclerostin, either alone or in combination with zolendronic acid, prevents the decline in resistance to fracture in bones bearing myeloma and, in some instances, increased strength above naïve controls [92]. These findings support that anti-sclerostin has the potential to not only prevent bone loss and fractures, but rebuild lost bone and heal osteolytic lesions. Further investigations are required to ascertain whether anti-sclerostin could in fact repair lytic lesions; however, this is an exciting opportunity to dramatically improve outcomes in patients with established disease or those in remission. Future studies should also aim to identify the exact source of sclerostin and the mechanism(s) underlying its aberrant production in the setting of bone cancers. Also, the ability of anti-sclerostin antibodies to promote bone formation in other cancers that target bone warrants further investigation. The recent findings showing that adipocytes appear to affect different aspects of

tumor cell biology [16;115-118], together with the new evidence that anti-sclerostin therapy decreases the number of adipocytes in the marrow [48], raise the possibility that sclerostin could play a part in the increased marrow adiposity observed in myeloma and others cancers in bone, and therefore demand further investigation. Finally, although preliminary, the new evidence showing that anti-sclerostin therapy does not alter tumor growth or the anti-tumor activity of chemotherapeutic drugs provides new avenues to simultaneously improve bone disease and decrease tumor burden in patients with cancer in bone. In conclusion, the current in *vitro* and *in vivo* data support the therapeutic targeting of sclerostin to prevent bone loss, stimulate new bone formation, and reduce the risk of fractures in patients with cancer in bone. These results may lead to the clinical investigation of the effects of anti-sclerostin therapy in cancer-induced bone disease.

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Compliance with Ethical Standards

• **Conflict of interest:** Authors declare that have no conflict of interest.

• **Animal and Human Studies:** This article includes studies with mice performed by the author. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not include any studies with humans performed by the authors.

Figure captions

Fig. 1 Mechanisms of action of Sost/sclerostin in bone. Upon binding to LRP4, 5 and 6, sclerostin inhibits Wnt/βcatenin signaling in mesenchymal stem cells and mature osteoblasts to decrease their proliferation and differentiation and bone forming activity, respectively. Recent findings show that inhibition of Wnt signaling in pre-adipocytes by sclerostin induces adipogenesis and increases bone marrow adipose tissue formation. Further, inhibition of Wnt/βcatenin signaling in osteoblastic cells increases the Rankl/Opg ratio to favor osteoclast differentiation and bone resorption. Thus, neutralization of sclerostin has the potential to stimulate osteoblast differentiation and bone formation, reduce bone resorption, and decrease adipogenesis.

Fig. 2 Actions of Sost/sclerostin in multiple myeloma-induced bone disease. Sclerostin expression is elevated in bone colonized by cancer cells. Based primarily on research done in myeloma, the increase in sclerostin expression favors bone loss and osteolysis by reducing osteoblast numbers and their bone-forming activity and increasing osteoclast differentiation and bone resorption. In addition, it is possible that sclerostin also stimulates the differentiation of mesenchymal precursors towards the adipogenic lineage. Pharmacologic inhibition of sclerostin stimulates new bone formation and modestly reduces bone resorption, thus preventing the bone loss and restoring bone strength. The effects of anti-sclerostin in bone marrow adiposity in bones bearing cancer cells require further investigation.

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