Published in final edited form as:

*J Bone Miner Res*. 2017 May ; 32(5): 889–891. doi:10.1002/jbmr.3141.

# New insights into the local and systemic functions of sclerostin: regulation of quiescent bone lining cells and beige adipogenesis in peripheral fat depots

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## Introduction

The discovery of the association between the Wnt signaling pathway and sclerostin with bone mass has propelled our understanding of skeletal homeostasis over the last decades. Sclerostin is a regulatory secreted glycoprotein that plays a prominent role in skeletal homeostasis (1). The major site of sclerostin production is the mature osteocytes in bone; however, sclerostin is also found in the circulation. Sclerostin is a potent antagonist of Wnt/β catenin signaling. It binds to the Wnt co-receptors LRP4/5/6 and antagonizes downstream signaling (2). By inhibiting canonical Wnt signaling, sclerostin decreases bone formation and osteoblast differentiation and stimulates bone resorption (1;2). Pharmacological inhibition of sclerostin with neutralizing antibodies activates Wnt signaling, robustly increases bone formation, and reduces bone resorption in both animal models and humans (3;4). Thus, sclerostin has quickly become a valuable therapeutic target for the treatment of osteoporosis and other skeletal diseases.

In spite of the rapid advances made over the last years, there are several unresolved enigmas regarding sclerostin action. We know that osteoblasts are the main target cells of sclerostin and that the major actions of the protein are localized in the skeleton. However, the cellular and molecular mechanisms by which monoclonal antibodies to sclerostin increase bone mass are not completely understood; and accumulating evidence supports the notion that sclerostin modulates the activity of other cells in bone besides osteoblasts. In addition, it is not clear why the anabolic effect of pharmacological inhibition of sclerostin wanes with time. Further, although the current knowledge suggests that the effects of sclerostin are circumscribed to the local bone/bone marrow microenvironment, circulating levels of

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**Disclosure**

Authors declare that they have no conflict of interest.

sclerostin not always reflect the actions observed in bone and the protein might also impact cells in non-skeletal tissues.

The current issue of *JBMR* introduces two articles in which Kim, Wein and colleagues examined the effects of pharmacological inhibition of sclerostin on the activity of bone lining cells (5) and Fulzele, Divieti Pajevic, and colleagues investigated the effects of sclerostin on peripheral white adipocyte tissue (WAT) depots (6). These articles describe two previously unknown functions of sclerostin and extend our understanding of the skeletal and non-skeletal functions of this regulatory protein.

## Sclerostin regulates the pool of quiescent bone lining cells

Bone lining cells cover quiescent bone surfaces and are characterized by their flat morphology believed to be indicative of their low matrix biosynthetic activity (7;8). The function of bone lining cells remains unclear, but they may represent a source of osteogenic precursors. Kim et al show that pharmacological inhibition of sclerostin with neutralizing antibodies (Scl-Ab) stimulates the conversion of bone lining cells into active osteoblasts (5). The authors used *in vivo* tamoxifen-inducible cell lineage tracing to genetically label bone lining cells and quantified the effects of the Scl-Ab on their thickness as a surrogate of cellular activity. They found that Scl-ab administration increased the thickness of lining cells covering periosteal and endosteal surfaces of murine cortical bone, while it did not affect proliferation or apoptosis of lining cells. This observation is consistent with the conversion of lining cells into osteoblasts and lead the authors to conclude that sclerostin regulates the pool of quiescent bone lining cells on cortical bone surfaces.

The suggestion that Scl-Ab stimulates conversion of bone lining cells into matrix-producing osteoblasts stems from previous observations by Ominsky and colleagues who reported decreased number of lining cells and increased osteoblast number after Scl-Ab administration in rats (9). These findings are in line with previous studies showing that PTH administration to mice or rats, which markedly decreases Sost/Sclerostin expression (10;11), increased bone lining cell thickness and decreased their number while simultaneously increasing the number of osteoblasts on bone surfaces (12–14). In concert, these findings support the notion that suppression of sclerostin expression or function with PTH or the Scl-Ab promotes lining cell reactivation into osteoblasts and provide a mechanism that may account for the rapid increases in osteoblast number and bone formation after treatment with either therapy. Further, these results infer that exhaustion of the pool of quiescent cells could underlie the declining efficacy of intermittent PTH or Scl-Ab therapies with time.

The assumed consequence of reactivation of bone lining cells with sclerostin inhibition is that the lining cell-derived osteoblasts would be responsible for increasing bone formation. However, one caveat of the study by Kim et at is that no evidence is presented showing that the “converted” osteoblasts indeed are able to form new bone, as bone formation in the areas covered by thicker lining cells was not reported. In addition, if indeed decreased sclerostin with daily injections of PTH mediates activation of lining cells, the contribution of this phenomenon to bone formation by the hormone appears to be minimal, as downregulation of sclerostin has been shown not to be required for the full anabolic effects of PTH (15;16).

Thus, future experiments using fluorochrome labeled bone are required to quantify by dynamic histomorphometry the contribution of lining cell activation to the bone anabolic effects of Scl-ab and/or PTH. Further, lining cell activation was not due to changes in the prevalence of apoptosis or proliferation; therefore, conversion into matrix producing cells might involve changes in cellular metabolism. In addition, it is not known whether stimulation of the biosynthetic capacity of the lining cells is also achieved by other activators of canonical Wnt/β catenin signaling. Future studies are warranted to clarify these unresolved issues.

One intriguing finding of the study of Kim et al is that, besides increasing the thickness of lining cells, Scl-Ab also augmented the total number of genetically labelled lining cells covering bone surfaces (5). This result contrasts with the increase in osteoblast number without changes in the total number of cells on bone surfaces upon PTH administration previously quantified by using a similar approach (12). One potential explanation for this apparent discordancy is that transient versus sustained decreases in sclerostin expression achieved by daily PTH injections or the Scl-Ab, respectively, could result in activation of some versus all lining cells, or in the recruitment of different cell populations by Scl-ab. Indeed, Kim et al found in the marrow OCN-tdTomato positive cells that could contribute to the increased numbers of genetically labeled cells on endocortical bone surfaces (5). The contribution of other cell populations besides lining cells is potentially important, as it is difficult to reconcile that the increased number of osteoblasts could be solely explained by conversion of lining cells considering that the bone surface covered by a lining cell is much greater (> 5 times) than the one covered by an osteoblast (17;18). Further studies combining different methodologies are needed to confirm these findings, and to investigate whether treatment with the Scl-Ab reactivates lining cells also on cancellous bone surfaces, which was not addressed in the current study.

## Sclerostin contributes to beige adipogenesis in peripheral fat depots

The second study by Fulzele et al provides strong evidence for a role of osteocyte-derived sclerostin in a tissue other than bone. Specifically, the authors demonstrate that 3 different genetically modified mouse models with loss of the stimulatory subunit of G-proteins Gsα exhibit increased sclerostin in the circulation and a progressive loss of WAT in gonadal and inguinal stores. The reduced WAT mass was associated with decreased white adipocyte markers, increased beige adipocytes, and reduced canonical Wnt/catenin signaling in these fat depots (6). Beige adipocytes are brown-adipocyte-like cells present in WAT that express the uncoupling protein 1 (UCP1), a mitochondrial protein responsible for dissipating chemical energy to generate heat (19). Fulzele et al also show that conditioned media from a newly generated osteocytic cell line lacking Gsα and secreting high levels of sclerostin increase the expression of UCP1 by primary adipocytes, and this effect was partially reversed by depletion of sclerostin from the conditioned media. Furthermore, Gsα deficient mice treated with Scl-Ab exhibited reduced UCP1 expression in WAT; and, conversely, WT mice treated with mouse recombinant sclerostin displayed increased UCP1 expression in WAT. These findings of Fulzele, Divieti Pajevic, and collaborators demonstrate that bone-derived sclerostin is involved in the genesis of beige adipocytes in peripheral fat depots and add to the increasing list of bone-derived factors with extra-skeletal effects, highlighting the

role of the skeleton as an endocrine organ with the capacity of regulating the function and metabolism of other tissues (20;21)

Sclerostin has been previously shown to positively regulate the differentiation of cells of the adipocyte lineage, as well as marrow adipocytes. An *in vitro* study showed that sclerostin increases the differentiation of preadipocytic 3T3-L1 cells, upregulating adipocyte genes and inducing lipid droplets accumulation (22). Moreover, radiation increases sclerostin expression and the number of bone marrow adipocytes, an effect that was reversed by Scl-Ab or genetic deletion of Sost (23). These findings had suggested a potential role of sclerostin in fat metabolism, in particular in the bone/bone marrow microenvironment. However, the current study emphasizes the ability of sclerostin to negatively regulate adipogenesis in remote organs. Therefore, it highlights the potential biological role of circulating sclerostin and suggests that the mechanisms of sclerostin regulation of fat metabolism differ between bone marrow and peripheral fat depots. Consistent with the Fulzele et al study, Frey et al also showed that manipulation of Sost/sclersotin levels affect peripheral fat; however, they found opposite results, as overexproduction of sclerostin by adenoviral gene transfer increased fat pad weight, whereas Sost knockout mice exhibited decreased peripheral fat weight (24). Overall these findings warrant further investigation.

Fulzele et al propose that the mechanism by which high circulating sclerostin decreases body adiposity involves increased energy expenditure, as mice lacking GSα exhibit decreased food intake and physical activity in the face of normal or slightly elevated oxygen consumption. However, the effect on oxygen consumption is non-significant and no differences were found in the combined food intake, physical activity and oxygen consumption over a 24-h period compared to control mice. Thus, it is not clear whether these modest changes in energy expenditure could fully account for the striking decrease in WAT weight exhibited by the GSα knockout mice. Sclerostin administration to wild type mice also decreased WAT mass; however, it was not shown whether it increased energy expenditure. Nevertheless, these findings are provocative and justify future investigations to further explore the potential role of sclerostin as an endocrine regulator of energy and fat metabolism.

## Conclusions

The two studies published in this *JBMR* issue (5;6) provide new insights into the functions of sclerostin in bone and compelling evidence that the protein also acts in non-skeletal tissues. The results reported by Kim, Wein and colleagues (5) suggest that suppression of sclerostin regulates bone formation at least in some quiescent surfaces by converting inactive lining cells into osteoblasts. These findings identify bone lining cells as cellular targets of Scl-Ab and suggest that this phenomenon could be used therapeutically to treat low bone mass conditions. The findings by Fulzele, Divieti Pajevic and colleagues (6) suggest that sclerostin, similar to other factors secreted by osteocytes/osteoblasts such as FGF-23 or osteocalcin, has an endocrine metabolic action complementary to its function in bone. Further investigations are needed to understand the relevance of circulating sclerostin levels and the effects of this protein in tissues other than bone.

## Acknowledgments

**Grant support:** This manuscript is supported by the National Institutes of Health (R01-AR059357, RO1-AT008754, and AT008754-O2S1) and the Veterans Administration (I01 BX002104-01 ) to T.B.; and a Scholar Award from the American Society of Hematology and a Brian D. Novis Junior Research Grant from the International Myeloma Foundation to J.D.C.

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