

Sclerostin Mode of Action

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Osteocytes selectively express *SOST*, which encodes the secreted bone formation inhibitor sclerostin [1,2]. Transgenic mice overexpressing *Sost* exhibit reduced bone mass [3,4], while constitutive *Sost* knockout mice have a progressive high bone mass phenotype [5-7]. Sclerosteosis (MIM269500) and van Buchem disease (MIM 239100) patients display lifelong bone overgrowth due to lack of sclerostin [1]. This is in sclerosteosis patients due to mutations in *SOST*, whereas van Buchem disease patients lack a non-coding region downstream of the gene that contains an enhancer element implicated in adult *SOST* bone expression [1,3]. Animal studies, suggest that suppression of sclerostin levels by PTH treatment and by mechanical loading mediates part of bone anabolism induced by either principle [8,9]. Sclerostin was originally believed to act as a bone morphogenetic protein (BMP) antagonist based on its amino acid sequence similarity to members of the DAN/cerberus family of cystine knot-containing secreted glycoproteins, [1,10]. *In vitro* studies revealed however that sclerostin binds to low-density lipoprotein receptor-related protein (LRP) 5 and 6 which are co-receptors for secreted Wnt ligands [11,12]. Currently, it is thought that sclerostin passes through the osteocytic canalicular network to the bone surface where it inhibits osteoblastic canonical Wnt/beta-catenin signaling, which is implicated in bone mass regulation [13]. Consistent with this hypothesis decreased binding of sclerostin to mutated forms of LRP5, which are causative for a high bone mass phenotype, has been demonstrated [14,15]. However, *in vivo* proof for this hypothesis is lacking, especially since recent findings suggest that *Lrp5* does not mediate Wnt signaling in bone but does control pre-osteoblast proliferation indirectly by inhibiting serotonin synthesis in the duodenum [16]. Our recent studies in *Lrp5; Sost* double knockout mice indicate that *Sost* at least requires *Lrp5* dependent pathways, since relative net bone gain is blunted in *Lrp5; Sost* double compared to *Sost* single mutant mice [17]. Future studies will need to address whether the remaining bone gain in the double mutant mice is related to functional compensation by *Lrp6*, which is up-regulated in the bones of these animals. Interestingly, we and others identified recently LRP4 as a novel additional interaction partner for sclerostin [18, 19]. Functional studies indicate that LRP4 acts as a facilitator of sclerostin-mediated inhibition of bone formation [19]. Furthermore, mutations in LRP4 impairing this facilitator function are associated with bone overgrowth in humans [20]. It is currently unknown whether the assumption is correct that sclerostin exerts its action by targeting osteoblasts as a paracrine factor. It may well also affect osteocyte function in an autocrine manner. In line with this possibility it was described that loss of *Sost* function in mice results in decreased osteocyte apoptosis [7]. Moreover, we recently found that osteocytic Wnt/beta-catenin signaling is required for normal bone homeostasis [21] and for full bone anabolism induced by *Sost* loss-of-function [22]. Finally it can currently not be excluded that the sclerostin entering into circulation [23-25] might have additional roles.

While the exact molecular mechanisms by which sclerostin exerts its actions on bone remain to be elucidated, findings to date indicate that sclerostin inhibition might provide an effective osteoporosis therapy. Inhibition of sclerostin by an antibody increases both the extent of bone forming surfaces and osteoblastic bone matrix synthesis at all skeletal envelopes in pre-clinical animal models [26-28]. Consistently, preliminary data from a blinded, placebo-controlled, dose-escalating single-dose study in healthy postmenopausal women revealed that a single anti-sclerostin antibody injection increased bone formation markers and bone mineral density [29]. Notably current data suggest that bone anabolism induced by sclerostin inhibition is not

associated with a concomitant increase in bone resorption and that it is not linked to elevated bone remodeling. In contrast, a reduction in osteoclast number was described at least after short-term treatment [26]. Data obtained from mice with altered *Sost* expression levels and from sclerostin deficient patients have not uncovered yet a role for *Sost* in regulation of osteoclastogenesis or bone resorption [1,4-6]. Therefore, a putative direct or indirect impact of sclerostin on bone resorption requires further investigation. Future studies will reveal whether inhibition of this presumed local Wnt/beta catenin signaling antagonist is safe or whether de-repression of Wnt/beta catenin signaling in bone has oncogenic potential [30]. Finally, while sclerostin function in adult bone mass regulation is established, novel putative roles are currently explored such as involvement in fracture healing [31], in osteoimmunology [32] and in developmental [33], cartilage [34], periodontal [35], kidney [36], and vascular biology [37].

References:

1. van Bezooijen, R.L. et al. (2005) SOST/sclerostin, an osteocyte-derived negative regulator of bone formation. *Cytokine Growth Factor Rev.* 16, 319-327
2. Poole, K.E. et al. (2005) Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. *Faseb J.* 19, 1842-1844
3. Winkler, D.G. et al. (2003) Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. *EMBO J* 22:6267–6276
4. Loots, G.G. et al. (2005) Genomic deletion of a long-range bone enhancer misregulates sclerostin in Van Buchem disease. *Genome Res.* 15, 928-935
5. Li, X. et al. (2008) Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. *J Bone Miner. Res.* 23, 860-869
6. Kramer, I. et al. (2009) PTH Induced bone mass gain is blunted but not abolished in SOST overexpressing and deficient mice. *J. Bone Miner. Res.* 25: 178-189
7. Lin, C. et al. (2009) Sclerostin mediates bone response to mechanical unloading through antagonizing Wnt/beta-catenin signaling. *J. Bone. Miner. Res.* 24, 1651-1661
8. Kramer, I. et al. (2009) Does SOST suppression mediate parathyroid hormone (PTH) bone forming action? *Trends Endocrinol. Metab.* 21(4):237-44
9. Moester, M.J.C. et al. (2010) Sclerostin: current knowledge and future perspective. *Calcif. Tiss. Int.* DOI 10.1007/s00223-010-9372-1
10. ten Dijke, P. et al. (2008) Osteocyte-derived sclerostin inhibits bone formation: its role in bone morphogenetic protein and Wnt signaling. *J. Bone Joint Surg. Am.* 90, 31-35
11. Li, X. et al. (2005) Sclerostin Binds to LRP5/6 and Antagonizes Canonical Wnt Signaling. *J. Biol. Chem.* 280, 19883-19887
12. Semenov, M. et al. (2005) SOST Is a Ligand for LRP5/LRP6 and a Wnt signaling inhibitor. *J. Biol. Chem.* 280, 26770-26775
13. Johnson, M.L. and Kamel, M.A. (2007) The Wnt signaling pathway and bone metabolism. *Curr. Opin. Rheumatol.* 19, 376-382
14. Semenov, M.V. and He, X. (2006) LRP5 Mutations linked to high bone mass diseases cause reduced LRP5 binding and inhibition by SOST. *J. Biol. Chem.* 281, 38276-38284
15. Balemans, W. et al. (2008) The binding between sclerostin and LRP5 is altered by DKK1 and by high-bone mass LRP5 mutations. *Calcif. Tissue Int.* 82, 445-453
16. Yadav, V.K. et al. (2008) Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. *Cell* 135, 825-837
17. Kramer, I. et al. (2009). Sost Exerts its' action via Lrp5 dependent and independent pathways to control bone formation in vivo. *J. Bone. Miner. Res.* 24 Suppl 1
18. Choi, H.Y. et al. (2009) Lrp4, a novel receptor for dickkopf 1 and sclerostin, is expressed by osteoblasts and regulates bone growth and turnover in vivo. *PLOS1* 4, e7930

19. Leupin, O. et al. (2009) LRP4 is a novel osteoblast and osteocyte expressed specific facilitator of sclerostin-mediated inhibition of in vitro bone formation. *J. Bone Miner. Res.* 24 Suppl 1
20. Piters, E. et al. (2010) Identification and characterization of 2 missense mutations in the LRP4 gene causing increased bone mineral density. *Bone* 46, Suppl.1
21. Kramer, I. et al. (2010) Osteocyte Wnt/beta-catenin signaling is required for normal bone homeostasis. *Mol. Cell. Biol.* 30:3071-3085
22. Kramer, I. et al. (2009). Sost deficiency dependent bone gain is blunted in osteocyte specific beta-catenin mutant mice. *J. Bone. Miner. Res.* 24 Suppl 1
23. Mödder, U.I. (2010) Regulation of circulating sclerostin levels by sex steroids in women and in men. *J. Bone Miner. Res.* 2010 May 17. Epub ahead of print
24. Gaudio, A. et al. (2010) Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. *J. Clin. Endocrinol. Metab.* 95:2248-53.
25. Mirza, F.S. (2010) Serum sclerostin levels negatively correlate with parathyroid hormone levels and free estrogen index in postmenopausal women. *J. Clin. Endocrinol. Metab.* 95:1991-1997
26. Li, X. et al. (2009) Sclerostin Antibody Treatment Increases Bone Formation, Bone Mass, and Bone Strength in a Rat Model of Postmenopausal Osteoporosis. *J. Bone. Miner. Res.* 24, 578-588
27. Eddleston, A. (2009) A short treatment with an antibody to sclerostin can inhibit bone loss in an ongoing model of colitis. *J. Bone Miner. Res.* 24:1662-1671
28. Ominsky, M.S. (2010) Two doses of sclerostin antibody in cynomolgus monkeys increases bone formation, bone mineral density, and bone strength. *J. Bone Miner. Res.* 25:948-959.
29. Padhi, D. et al. (2007) Anti-sclerostin antibody increases markers of bone formation in healthy postmenopausal women. *J Bone Miner Res* 21 Suppl 1
30. Kansara, M. et al. (2009) Wnt inhibitory factor 1 is epigenetically silenced in human osteosarcoma, and targeted disruption accelerates osteosarcomagenesis in mice. *J Clin. Invest.* 119:837–851
31. Agholme, F. (2010) Sclerostin antibody treatment enhances metaphyseal bone healing in rats. *J Bone Miner. Res.* May 17. [Epub ahead of print]
32. Schett, G. and Sieper, J. (2009) Inflammation and repair mechanisms. *Clin. Exp. Rheumatol.* 27; 4 Suppl 55:S33-S35.
33. Collette, N.M. (2010) Genetic evidence that SOST inhibits WNT signaling in the limb. *Dev. Biol.* 342:169-179.
34. Van Bezooijen, R.L. (2009) Sclerostin in mineralized matrices and van Buchem disease. *J. Dent. Res.* 88:569-574.
35. Jäger, A. et al. (2010) Localization of SOST/sclerostin in cementocytes in vivo and in mineralizing periodontal ligament cells. *J. Periodontal. Res.* 45:246-254
36. D. Cejka, D. et al. (2010) Sclerostin and dickkopf-1 serum levels in dialysis patients and healthy volunteers. 37th European Calcified Tissue Symposium, June 26 – 30, Glasgow, UK PP408
37. van Bezooijen, R.L. (2007) SOST expression is restricted to the great arteries during embryonic and neonatal cardiovascular development. *Dev. Dyn.* 236:606-612.