

## Sclerostin: A Novel Key to Bone and Dental Treatment

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**Abstract: Problem statement:** Tooth loss can induce dramatical remodeling of alveolar bone because of mechanical loading impairment and strategies to help maintain adequate bone levels for subsequent therapies are sorely needed. Recent discoveries have shown that osteocytes, matrix-embedded cells in bone, respond to mechanical stimulation by modulating the expression of the *Sost* gene, which encodes for the protein sclerostin. This protein can oppose the WNT canonical pathway, a signaling cascade which regulates osteoblastic differentiation and bone homeostasis and thus orchestrate bone turn-over according to the skeleton's mechanical needs.

**Conclusion:** Experimental attempts at Sclerostin inhibition have provided interesting data on a novel approach to decrease bone resorption and promote bone formation, with important implications for the orthopedic and dental field.

**Key words:** *Sost* protein, osteocyte, alveolar bone loss

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### INTRODUCTION

Bone is remodeled by teams of bone forming cells, the osteoblasts and bone resorbing cells, the osteoclasts, which cooperate within Bone Multicellular Units (BMU). These structures remodel and renovate the skeleton by gradually replacing old bone with new tissue (Martin and Seeman, 2008; Matsuo and Irie, 2008; Parfitt, 2002). As new matrix is deposited osteoblasts can die by apoptosis or terminally differentiate into lining cells, which form a layer that covers the whole bone surface. Some cells however can become progressively encased within the matrix they contributed to build and be left behind as the BMU progresses, while maintaining their intercellular junctions with the neighboring cells on the surface. As they become trapped within a lacuna in bone these osteoblasts differentiate to a stage known as osteocyte (Franz-Ondendaal *et al.*, 2006; Hirao *et al.*, 2007; Paic *et al.*, 2009). This complex network of cells provides bone with the capability to renovate itself and to respond to outer stimuli, both metabolic and mechanical. The importance of mechanical loading for bone maintenance is now accepted as one of the cornerstones of bone biology (Duyck *et al.*, 2001; Isidor, 2006; Kitamura *et al.*, 2004; Mellal *et al.*, 2004; Wiskott and Belser, 1999). It is known that absence of gravity or prolonged inactivity decrease bone strength and Bone Mineral Density (BMD) in limbs (Bikle and Halloran,

1999; Bikle *et al.*, 2003; Guadalupe-Grau *et al.*, 2009; Hamilton *et al.*, 2010) and alveolar bone too is heavily affected by loss of mechanical stimulation. Tooth loss and thus absence of masticatory loads, induces dramatic remodeling processes that quickly lead to ridge resorption (Amler, 1969; Cardaropoli *et al.*, 2003; Iizuka *et al.*, 1992; Ulm *et al.*, 2009). This can pose significant problems to prosthetics and surgical rehabilitation. Some authors have also shown a relation between the design and shape of endosseous implants, common bone-retained devices used to support dental prosthesis and the preservation of the alveolar ridge, presumably because of alteration in masticatory force loading (Bratu *et al.*, 2009; Eraslan and Inan, 2010; Kong *et al.*, 2008; Orsini *et al.*, 2009; 2012; Velde *et al.*, 2009; Vandeweghe *et al.*, 2010; Wang *et al.*, 2009).

Bone is therefore capable to perceive mechanical forces and respond appropriately to the force vectors that are applied to it, in order to better adapt to the needs and the activity of the organism. Several hypothesis have been formulated to explain this behavior, such as modifications in piezoelectric charges along bone surfaces upon application of mechanical forces (Fernandez *et al.*, 2012; Fu *et al.*, 2011). More recently however, a growing amount of evidence points at the possibility that the osteocytes buried in mineralized matrix can act as mechanosensors (Britz *et al.*, 2009; Souza *et al.*, 2005; Forwood and Turner, 1995; Isaksson *et al.*, 2009).

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**The osteocytes:** Osteocytes and their cellular projections that are contained in bone canaliculi form a wide network in bone, somewhat comparable to the nervous tissue organization. Recent evidence has shown that osteocytes are required for bone maintenance. Osteocyte specific ablation increased bone fragility, intracortical porosity and trabecular bone loss (Tatsumi *et al.*, 2007). Conditional expression of the 11 $\beta$ -HSD2 gene in mouse osteoblasts and osteocytes protected them from glucocorticoid-induced apoptosis (O'Brien *et al.*, 2004). Glucocorticoids induced similar bone loss in both wild-type and OG2-11 $\beta$ -HSD2 transgenic mice but vertebral compression strength was preserved in transgenic mice, where cell apoptosis had been prevented. A possible explanation was that osteocyte loss reduced bone vascularization and canalicular fluid through impaired expression of Vascular Endothelial Growth Factor (VEGF) (Wang *et al.*, 2007), thus reducing bone hydraulic stiffening (Weinstein, 2010). Aguirre *et al.* (2006) showed that mechanical unloading induced osteocyte apoptosis, a phenomenon which promoted osteoclast recruitment (Aguirre *et al.*, 2006) and more recently the expression of Receptor Activator for Nuclear Factor  $\kappa$ B Ligand (RANKL), a molecule that supports osteoclast formation and viability, by osteocytes has been demonstrated to be required for bone remodeling, in contrast to the long-standing idea that osteoblasts are the main source of RANKL (Xiong *et al.*, 2011). Moreover osteocytes appear to be able to act at a distance through a secreted glycoprotein, Sclerostin (Weidauer *et al.*, 2009), which is encoded for by the *Sost* gene (Turner *et al.*, 2009; Winkler *et al.*, 2003).

**Sclerostin and the inhibition of WNT signaling:** Sclerostin, transported through bone canaliculi (Poole *et al.*, 2005) blocks membrane co-receptors LRP4, 5 and 6 (Holdsworth *et al.*, 2012), inhibiting an intracellular signaling cascade known as WNT canonical pathway (Li *et al.*, 2005; Veverka *et al.*, 2009). Several authors have demonstrated that the canonical WNT pathway is required in various events of embryo development (Huelsen *et al.*, 2000; Martin and Kimelman, 2009) and in the control of Embryonic Stem cell (ES) proliferation and differentiation (Miki *et al.*, 2011; Nusse, 2008; Sokol, 2011). The canonical or WNT/ $\beta$ -catenin pathway is activated upon binding of secreted WNT glycoproteins to membrane receptors Frizzled (Fzd) and a member of the low density lipoprotein receptor related protein family, LRP4/5 or 6 (Hartmann, 2006; Mikels and Nusse, 2006). This interaction recruits Disheveled (Dvl) (Gordon and Nusse, 2006), which rescues  $\beta$ -catenin, a

protein of the Armadillo family. Although  $\beta$ -catenin is a normal constituent of cell-to-cell junctions. it can form a cytoplasmic complex with two kinases, Glycogen Synthase Kinase 3 (GSK3) and Casein Kinase 1a (CKI) and two scaffold proteins, Axin and Adenomatous Polyposis Coli (APC) (Angers and Moon, 2009). This molecular complex targets  $\beta$ -catenin for proteosomal degradation through selective phosphorylation (Clevers, 2006; Verheyen and Gottardi, 2010). Activation of Dvl recruits Axin to the cell membrane, disrupting the destruction complex and thus releasing  $\beta$ -catenin, which translocates to the nucleus. Once inside the nucleus,  $\beta$ -catenin can interact with a member of the T Cell Factor/Lymphoid enhancer factor (TCF/Lef1) transcription factor family (Mosimann *et al.*, 2009) and initiate its transcription program. Beside its role in ES cell physiology, the WNT  $\beta$ -catenin-mediated signaling plays a relevant role in bone and cartilage metabolism (Krishnan *et al.*, 2006; Williams and Insogna, 2009). Conditional ablation of  $\beta$ -catenin from osteochondral progenitors delayed fracture healing in mice (Huang *et al.*, 2009) and blocking  $\beta$ -catenin and TCF binding in osteoblasts through conditional expression of ICAT modulator impaired skeletal growth (Chen *et al.*, 2008). Consistently, conditional deletion of  $\beta$ -catenin in murine preosteoblasts disrupted osteoblast differentiation (Rodda and McMahon, 2006). Alterations in bone mass have been observed after LRP5 deletion in rodents or as a consequence of mutations of this gene in humans (Boyden *et al.*, 2002; Gong *et al.*, 2001; Babij *et al.*, 2003; Holmen *et al.*, 2004; Kokubu *et al.*, 2004; Kato *et al.*, 2002; Sawakami *et al.*, 2006; Joeng *et al.*, 2011).  $\beta$ -catenin can also act on mature bone and by controlling the expression of Osteoprotegerin (OPG) (Glass *et al.*, 2005), the decoy receptor of RANKL.

*Sost* deletion increased bone mass and bone formation rate in mice (Li *et al.*, 2008), while *Sost* overexpression induced a low bone mass phenotype (Kramer *et al.*, 2010). Binding of sclerostin to LRP5 is altered in LRP5 mutations with high bone mass phenotype (Balemans *et al.*, 2008) and specific polymorphisms of *Sost* promoter are associated with osteoporosis (Huang *et al.*, 2009). It has been shown that sclerostin levels correlate positively with age (Amrein *et al.*, 2012; Modder *et al.*, 2011) and higher serum levels of sclerostin have been proven to be associated with a higher rate of hip fractures in older women (Arasu *et al.*, 2012). Circulating levels of sclerostin have been demonstrated to correlate with spine and hip BMD in postmenopausal women (Garnero *et al.*, 2012) and to be increased in long term immobilized patients (Gaudio *et al.*, 2010; Spatz *et al.*, 2012), in

Thalassemia-associated Osteoporosis patients (Voskaridou *et al.*, 2012), in Paget's disease and prostate cancer with bone metastasis (Yavropoulou *et al.*, 2012), in type 2 Diabetes (Garcia-Martin *et al.*, 2012; Gennari *et al.*, 2012) and with short-term Spinal Cord Injury (SCI), although sclerostin levels decreased in long-term SCI, reflecting the profound changes that the skeleton undergoes in these patients (Battaglini *et al.*, 2012; Morse *et al.*, 2012). Sost is a PTH target (Bellido *et al.*, 2005; Keller and Kneissel, 2005; Leupin *et al.*, 2007) and the expression of a constitutively active PTH receptor (Schipani *et al.*, 1995) in osteocytes was able to suppress Sost expression in bone and have a dramatic effect on bone mass (O'Brien *et al.*, 2008).

What makes Sost of great importance for the response of bone to loading is that this gene is also controlled by mechanical stimulation. Rodent studies showed that even short mechanical stimulations of long bones in mice are able to reduce the expression of Sost and increase bone formation at the same time (Moustafa *et al.*, 2009; Robling *et al.*, 2008). Conversely, it has been proposed that the negative effects of mechanical unloading on BMD and bone strength are mediated by an antagonistic effect of Sclerostin on WNT/ $\beta$ -catenin signaling (Lin *et al.*, 2009). Consistently with this idea, the ablation of the WNT receptor LRP5 abolished the osteogenic response of ulnae to mechanical loading (Sawakami *et al.*, 2006) and constitutively expression of Sost in osteocytes prevented load anabolic effects on bone (Tu *et al.*, 2012).

**The localization of osteocytes can affect their production of sclerostin:** Osteocytes that are closer to the surface and thus probably undergo a more intense mechanical stimulation, tend to be more negative to sclerostin expression than deeper osteocytes and osteocytes in proximity of an area of bone formation are more often Sclerostin negative (Poole *et al.*, 2005), suggesting that sclerostin distribution could contribute to temporal and spatial regulation of bone remodeling.

**Inhibition of sclerostin and periodontal therapy:**  
**Future perspectives:** On account of the dramatic effects of Sost regulation in animal experimental models, Sost has been proposed as a novel and potent therapeutic target for bone loss (Deal, 2009; Baron and Rawadi, 2007; Hoepfner *et al.*, 2009; Rawadi and Roman-Roman, 2005; Shahnazari *et al.*, 2008). The effect of anti-sclerostin antibodies has been investigated in different experimental settings and it has been convincingly shown that inhibition of sclerostin increases bone mass, bone architectural parameters and bone strength in intact (Li *et al.*, 2010; Tian *et al.*, 2010;

Veverka *et al.*, 2009) and ovariectomized rats (Li *et al.*, 2009; 2011). Similar results were observed in mouse models of colitis-induced- (Eddleston *et al.*, 2009) and glucocorticoid-induced bone loss (Marenzana *et al.*, 2011). These promising results indicate that there could be novel potential applications for the use of this and similar compounds for the treatment of alveolar bone defects. Interestingly, it has been shown that anti-sclerostin antibody prevented unload-induced bone loss in a rat model of hindlimb immobilization (Tian *et al.*, 2011), which suggests that sclerostin is indeed a potential therapeutic target to oppose alveolar bone loss after tooth extraction, a very common clinical situation, which often requires surgery to allow for prosthetic rehabilitation.

A strategy that is being currently developed to target sclerostin in human is the creation of human anti-Sclerostin antibody Amgen, Thousand Oaks, CA; USA and sclerostin small molecule inhibitors Osteogenex, Kansas City, KS; USA. Common therapeutic approaches in dentistry mostly rely on the use of grafts or biomaterials to act as scaffolds to promote the regeneration of endosseous defects (Esposito *et al.*, 2009; Nkenke and Stelzle, 2009; Sculean *et al.*, 2008) with little or no use of growth factors, which have been tested and proposed over the years (Chang *et al.*, 2009; Cortellini *et al.*, 2008; Giannobile and Somerman, 2003; Kaigler *et al.*, 2006; Lynch *et al.*, 2006; Nevins *et al.*, 2003; Trombelli and Farina, 2008; Wennstrom and Lindhe, 2002; Wikesjo *et al.*, 2009). Sclerostin inhibition however seems to act directly on the mechanisms that underlie tooth-loss induced alveolar remodeling and the data that is being gathered on the effects of Sclerostin inhibition in axial bone strongly suggest that this approach should be further investigated in the dental field where it could provide interesting, if not decisive results.

## CONCLUSION

Sclerostin is a novel protein produced by osteocytes which has profound effects on bone remodeling by controlling bone formation and bone resorption and is responsible for coupling hormonal stimuli and mechanical stimulation to bone turnover. As such it is becoming clear that this protein is a useful target for future bone anabolic therapies and possible applications in the dental field can be envisaged to promote the regeneration of alveolar bone.

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