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Sclerostin: A Novel Key to Bone and Dental Treatment

[1,2]Galli Carlo, [1]Lumetti Simone, [1]Manfredi Edoardo, [1]Macaluso Guido and [2]Passeri Giovanni
[1]Department of Biomedical, Biotechnological and Translational Sciences,
Università di Parma Via Gramsci, 14 43100 Parma, Italy
[2]Department of Internal Medicine, Università di Parma, Italy

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

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-Odendaal *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).

Corresponding Author: Macaluso Guido, Department of Biomedical, Biotechnological and Translational Sciences, Università di Parma Via Gramsci 14 43100 Parma, Italy Tel: +39 0521 986722 Fax: +39 0521 292955

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 11β-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 OG2-11β-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 kB 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 (Huelsken *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/β-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 β-catenin, a protein of the Armadillo family. Although β-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 β-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 β-catenin, which translocates to the nucleus. Once inside the nucleus, β-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 β-catenin-mediated signaling plays a relevant role in bone and cartilage metabolism (Krishnan *et al*., 2006; Williams and Insogna, 2009). Conditional ablation of β-catenin from osteochondral progenitors delayed fracture healing in mice (Huang *et al*., 2009) and blocking β-catenin and TCF binding in osteoblasts through conditional expression of ICAT modulator impaired skeletal growth (Chen *et al*., 2008). Consistently, conditional deletion of β-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). β-catenin can also act on mature bone and by controlling thee 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 (Battaglino *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/β-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; Hoeppner *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 glucorticoid-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 of 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 the mechanisms that underlie tooth-loss induced alveolar remodelind 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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REFERENCES

Aguirre, J.I., L.I. Plotkin, S.A. Stewart, R.S. Weinstein and A.M. Parfitt *et al*., 2006. Osteocyte apoptosis is induced by weightlessness in mice and precedes osteoclast recruitment and bone loss. J. Bone Miner. Res., 21: 605-615. PMID: 16598381

Amler, M.H., 1969. The time sequence of tissue regeneration in human extraction wounds. Oral Surg. Oral Med. Oral Pathol., 27: 309-318. DOI: 10.1016/0030-4220(69)90357-0

Amrein, K., S. Amrein, C. Drexler, H.P. Dimai and H. Dobnig *et al*., 2012. Sclerostin and its association with physical activity, age, gender, body composition and bone mineral content in healthy adults. J. Clin. Endocrinol. Metab., 97: 148-154. PMID: 21994959

Angers, S. and R.T. Moon, 2009. Proximal events in WNT signal transduction. Nat. Rev. Mol. Cell. Biol., 10: 468-477. PMID: 19536106

Arasu, A., P.M. Cawthon, L.Y. Lui, T.P. Do and P.S. Arora *et al*., 2012. Serum sclerostin and risk of hip fracture in older caucasian women. J. Clin. Endocrinol. Metab., 97: 2027-2032. PMID: 2466341

Babij, P., W. Zhao, C. Small, Y. Kharode and P.J. Yaworsky *et al*., 2003. High bone mass in mice expressing a mutant LRP5 gene. J. Bone Miner Res., 18: 960-974. PMID: 12817748

Balemans, W., E. Piters, E. Cleiren, M. Ai and V.L. Wesenbeeck *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. PMID: 18521528

Baron, R. and G. Rawadi, 2007. Targeting the WNT/beta-catenin pathway to regulate bone formation in the adult skeleton. Endocrinology, 148: 2635-2643. PMID: 17395698

Battaglino, R.A., S. Sudhakar, A.A. Lazzari, E. Garshick and R. Zafonte *et al*., 2012. Circulating sclerostin is elevated in short-term and reduced in long-term SCI. Bone, 51: 600-605. PMID: 22575440

Bellido, T., A.A. Ali, I. Gubrij, L.I. Plotkin and Q. Fu *et al*., 2005. Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: A novel mechanism for hormonal control of osteoblastogenesis. Endocrinology, 146: 4577-4583. DOI: 10.1210/en.2005-0239

Bikle, D.D. and B.P. Halloran, 1999. The response of bone to unloading. J. Bone Miner. Metab., 17: 233-244. PMID: 10575587

Bikle, D.D., T. Sakata and B.P. Halloran, 2003. The impact of skeletal unloading on bone formation. Gravit. Space Biol. Bull., 16: 45-54. PMID: 12959131

Boyden, L.M., J. Mao, J. Belsky, L. Mitzner and A. Farhi *et al*., 2002. High bone density due to a mutation in LDL-receptor-related protein 5. N Engl. J. Med., 346: 1513-1521. PMID: 12015390

Bratu, E.A., M. Tandlich and L. Shapira, 2009. A rough surface implant neck with microthreads reduces the amount of marginal bone loss: A prospective clinical study. Clin. Oral. Implants Res., 20: 827-832. PMID: 19508341

Britz, H.M., C.D. Thomas, J.G. Clement and D.M. Cooper, 2009. The relation of femoral osteon geometry to age, sex, height and weight. Bone, 45: 77-83. PMID: 19303955

Cardaropoli, G., M. Araujo and J. Lindhe, 2003. Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. J. Clin. Periodontol., 30: 809-818. PMID: 12956657

Chang, P.C., Y.J. Seol, J.A. Cirelli, G. Pellegrini and Q. Jin *et al*., 2009. PDGF-B gene therapy accelerates bone engineering and oral implant osseointegration. Gene Ther., 17: 95-104. PMID: 19741730

Chen, M., M. Zhu, H. Awad, T.F. Li and T.J. Sheu *et al*., 2008. Inhibition of beta-catenin signaling causes defects in postnatal cartilage development. J. Cell Sci., 121: 1455-1465. PMID: 18397998

Clevers, H., 2006. WNT/beta-catenin signaling in development and disease. Cell, 127: 469-480. PMID: 17081971

Cortellini, P., M. Nieri, G.P. Prato and M.S. Tonetti, 2008. Single minimally invasive surgical technique with an enamel matrix derivative to treat multiple adjacent intra-bony defects: Clinical outcomes and patient morbidity. J. Clin. Periodontol., 35: 605-613. PMID: 18476997

Deal, C., 2009. Potential new drug targets for osteoporosis. Nat. Clin. Pract. Rheumatol., 5: 20-27. PMID: 19098925

Duyck, J., H.J. Ronold, V.H. Oosterwyck, I. Naert and J.V.D. Sloten *et al*., 2001. The influence of static and dynamic loading on marginal bone reactions around osseointegrated implants: An animal experimental study. Clin. Oral. Implants Res., 12: 207-218. PMID: 1135947

Eddleston, A., M. Marenzana, A.R. Moore, P. Stephens and M. Muzylak *et al*., 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. PMID: 19419292

Eraslan, O. and O. Inan, 2010. The effect of thread design on stress distribution in a solid screw implant: A 3D finite element analysis. Clin. Oral Invest., 14: 411-416. PMID: 19543925

Esposito, M., M.G. Grusovin, P. Felice, G. Karatzopoulos and H.V. Worthington *et al*., 2009. Interventions for replacing missing teeth: Horizontal and vertical bone augmentation techniques for dental implant treatment. Cochrane Database Syst. Rev., 7: CD003607-CD003607. PMID: 19821311

Fernandez, J.R., J.M. Garcia-Aznar and R. Martinez, 2012. Piezoelectricity could predict sites of formation/resorption in bone remodelling and modelling. J. Theor. Biol., 292: 86-92. PMID: 22001080

Forwood, M.R. and C.H. Turner, 1995. Skeletal adaptations to mechanical usage: Results from tibial loading studies in rats. Bone, 17: 197S-205S. PMID: 8579917

Franz-Odendaal, T.A., B.K. Hall and P.E. Witten, 2006. Buried alive: How osteoblasts become osteocytes. Dev. Dyn., 235: 176-190. PMID: 16258960

Fu, D., Z. Hou, Q.H. Qin, L. Xu and Y. Zeng, 2011. Influence of shear stress on behaviors of piezoelectric voltages in bone. J. Applied Biomech., 28: 387-393. PMID: 22084058

Garcia-Martin, A., P. Rozas-Moreno, R. Reyes-Garcia, S. Morales-Santana and B. Garcia-Fontana *et al*., 2012. Circulating levels of sclerostin are increased in patients with type 2 diabetes mellitus. J. Clin. Endocrinol. Metab., 97: 234-241. PMID: 22031520

Garnero, P., E. Sornay-Rendu, F. Munoz, O. Borel and R.D. Chapurlat, 2012. Association of serum sclerostin with bone mineral density, bone turnover, steroid and parathyroid hormones and fracture risk in postmenopausal women: The OFELY study. Osteoporos. Int. PMID: 22525978

Gaudio, A., P. Pennisi, C. Bratengeier, V. Torrisi and B. Lindner *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: 2248-2253. PMID: 20305005

Gennari, L., D. Merlotti, R. Valenti, E. Ceccarelli and M. Ruvio *et al*., 2012. Circulating sclerostin levels and bone turnover in type 1 and type 2 diabetes. J. Clin. Endocrinol. Metab., 97: 1737-1744. PMID: 22399511

Giannobile, W.V. and M.J. Somerman, 2003. Growth and amelogenin-like factors in periodontal wound healing. A systematic review. Ann. Periodontol., 8: 193-204. PMID: 14971254

Glass, D.A., P. Bialek, J.D. Ahn, M. Starbuck and M.S. Patel *et al*., 2005. Canonical WNT signaling in differentiated osteoblasts controls osteoclast differentiation. Dev. Cell, 8: 751-764. PMID: 15866165

Gong, Y., R.B. Slee, N. Fukai, G. Rawadi and S. Roman-Roman *et al*., 2001. LDL Receptor-Related Protein 5 (LRP5) affects bone accrual and eye development. Cell, 107: 513-523. PMID: 11719191

Gordon, M.D. and R. Nusse, 2006. WNT signaling: Multiple pathways, multiple receptors and multiple transcription factors. J. Biol. Chem., 281: 22429-22433. PMID: 16793760

Guadalupe-Grau, A., T. Fuentes, B. Guerra and J.A. Calbet, 2009. Exercise and bone mass in adults. Sports Med., 39: 439-468. PMID: 19453205

Hamilton, C.J., V.J. Swan and S.A. Jamal, 2010. The effects of exercise and physical activity participation on bone mass and geometry in postmenopausal women: A systematic review of pQCT studies. Osteoporos. Int., 21: 11-23. PMID: 19504035

Hartmann, C., 2006. A WNT canon orchestrating osteoblastogenesis. Trends Cell Biol., 16: 151-158. PMID: 16466918

Hirao, M., J. Hashimoto, N. Yamasaki, W. Ando and H. Tsuboi *et al*., 2007. Oxygen tension is an important mediator of the transformation of osteoblasts to osteocytes. J. Bone. Miner. Metab., 25: 266-276. PMID: 17704991

Hoeppner, L.H., F.J. Secreto and J.J. Westendorf, 2009. WNT signaling as a therapeutic target for bone diseases. Expert Opin. Ther. Targets, 13: 485-496. PMID: 19335070

Holdsworth, G., P. Slocombe, C. Doyle, B. Sweeney and V. Veverka *et al*., 2012. Characterization of the interaction of sclerostin with the low density Lipoprotein Receptor-Related Protein (LRP) family of WNT co-receptors. J. Biol. Chem., 3: 26464-26477. PMID: 22696217

Holmen, S.L., T.A. Giambernardi, C.R. Zylstra, B.D. Buckner-Berghuis and J.H. Resau *et al*., 2004. Decreased BMD and limb deformities in mice carrying mutations in both Lrp5 and Lrp6. J. Bone Miner Res., 19: 2033-2040. PMID: 15537447

Huang, D.W., B.T. Sherman and R.A. Lempicki, 2009. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucl. Acids Res., 37: 1-13. DOI: 10.1093/nar/gkn923

Huelsken, J., R. Vogel, V. Brinkmann, B. Erdmann and C. Birchmeier *et al*., 2000. Requirement for beta-catenin in anterior-posterior axis formation in mice. J. Cell Biol., 148: 567-578. PMID: 10662781

Iizuka, T., S.C. Miller and S.C. Marks, 1992. Alveolar bone remodeling after tooth extraction in normal and osteopetrotic (ia) rats. J. Oral Pathol. Med., 21: 150-155. PMID: 1602406

Isaksson, H., I. Grongroft, W. Wilson, C.C. Donkelaar and B. Rietbergen *et al*., 2009. Remodeling of fracture callus in mice is consistent with mechanical loading and bone remodeling theory. J. Orthop. Res., 27: 664-672. PMID: 18985689

Isidor, F., 2006. Influence of forces on peri-implant bone. Clin. Oral Implants Res., 2: 8-18. PMID: 16968378

Joeng, K.S., C.A. Schumacher, C.R. Zylstra-Diegel, F. Long and B.O. Williams, 2011. Lrp5 and Lrp6 redundantly control skeletal development in the mouse embryo. Dev. Biol., 359: 222-229. PMID: 21924256

Kaigler, D., J.A. Cirelli and W.V. Giannobile, 2006. Growth factor delivery for oral and periodontal tissue engineering. Exp. Opin. Drug Deliv., 3: 647-662. DOI: 10.1517/17425247.3.5.647

Kato, M., M.S. Patel, R. Levasseur, I. Lobov and B.H. Chang *et al*., 2002. Cbfa1-independent decrease in osteoblast proliferation, osteopenia and persistent embryonic eye vascularization in mice deficient in Lrp5, a WNT coreceptor. J. Cell Biol., 157: 303-314. PMID: 11956231

Keller, H. and M. Kneissel, 2005. SOST is a target gene for PTH in bone. Bone, 37: 148-158. PMID: 15946907

Kitamura, E., R. Stegaroiu, S. Nomura and O. Miyakawa, 2004. Biomechanical aspects of marginal bone resorption around osseointegrated implants: Considerations based on a three-dimensional finite element analysis. Clin. Oral Implants Res., 15: 401-412. PMID: 15248874

Kokubu, C., U. Heinzmann, T. Kokubu, N. Sakai and T. Kubota *et al*., 2004. Skeletal defects in ringelschwanz mutant mice reveal that Lrp6 is required for proper somitogenesis and osteogenesis. Development, 131: 5469-5480. PMID: 15469977

Kong, L., K. Hu, D. Li, Y. Song and J. Yang *et al*., 2008. Evaluation of the cylinder implant thread height and width: A 3-dimensional finite element analysis. Int. J. Oral Maxillofac. Implants, 23: 65-74. PMID: 18416414

Kramer, I., G.G. Loots, A. Studer, H. Keller and M. Kneissel, 2010. Parathyroid Hormone (PTH)-induced bone gain is blunted in SOST over expressing and deficient mice. J. Bone Miner. Res., 25: 178-189. PMID: 19594304

Krishnan, V., H.U. Bryant and O.A. Macdougald, 2006. Regulation of bone mass by WNT signaling. J. Clin. Invest., 116: 1202-1209. PMID: 16670761

Leupin, O., I. Kramer, N.M. Collette, G.G. Loots and F. Natt *et al*., 2007. Control of the SOST bone enhancer by PTH using MEF2 transcription factors. J. Bone Miner. Res., 22: 1957-1967. PMID: 17696759

Li, X., K.S. Warmington, Q.T. Niu, F.J. Asuncion and M. Barrero *et al*., 2010. Inhibition of sclerostin by monoclonal antibody increases bone formation, bone mass and bone strength in aged male rats. J. Bone Miner. Res., 25: 2647-2656. PMID: 20641040

Li, X., M.S. Ominsky, K.S. Warmington, Q.T. Niu and F.J. Asuncion *et al*., 2011. Increased bone formation and bone mass induced by sclerostin antibody is not affected by pretreatment or cotreatment with alendronate in osteopenic, ovariectomized rats. Endocrinology, 152: 3312-3322. PMID: 21733832

Li, X., M.S. Ominsky, K.S. Warmington, S. Morony and J. Gong *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. PMID: 19049336

Li, X., M.S. Ominsky, Q.T. Niu, N. Sun and B. Daugherty *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. PMID: 18269310

Li, X., Y. Zhang, H. Kang, W. Liu and P. Liu *et al*., 2005. Sclerostin binds to LRP5/6 and antagonizes canonical WNT signaling. J. Biol. Chem., 280: 19883-19887. PMID: 15778503

Lin, C., X. Jiang, Z. Dai, X. Guo and T. Weng *et al*., 2009. Sclerostin mediates bone response to mechanical unloading through antagonizing WNT/beta-catenin signaling. J. Bone Miner. Res., 24: 1651-1661. PMID: 19419300

Lynch, S.E., L. M. Wisner-Lynch, Nevins and M.L. Nevins, 2006. A new era in periodontal and periimplant regeneration: Use of growth-factor enhanced matrices incorporating rhPDGF. Comp. Contin. Educ. Dent., 27: 672-678. PMID: 17191649

Marenzana, M., K. Greenslade, A. Eddleston, R. Okoye and D. Marshall *et al*., 2011. Sclerostin antibody treatment enhances bone strength but does not prevent growth retardation in young mice treated with dexamethasone. Arthritis Rheum., 63: 2385-2395. PMID: 21484764

Martin, B.L. and D. Kimelman, 2009. WNT signaling and the evolution of embryonic posterior development. Curr. Biol., 19: R215-R219. PMID: 19278640

Martin, T.J. and E. Seeman, 2008. Bone remodelling: Its local regulation and the emergence of bone fragility. Best Pract. Res. Clin. Endocrinol. Metab., 22: 701-722. PMID: 19028353

Matsuo, K. and N. Irie, 2008. Osteoclast-osteoblast communication. Arch. Biochem. Biophys., 473: 201-209. PMID: 18406338

Mellal, A., H.W. Wiskott, J. Botsis, S.S. Scherrer and U.C. Belser, 2004. Stimulating effect of implant loading on surrounding bone. Comparison of three numerical models and validation by *in vivo* data. Clin. Oral Implants Res., 15: 239-248. PMID: 15008937

Mikels, A.J. and R. Nusse, 2006. Purified WNT5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. PLoS Biol., 4: e115-e115. PMID: 16602827

Miki, T., S.Y. Yasuda and M. Kahn, 2011. WNT/β-catenin signaling in embryonic stem cell self-renewal and somatic cell reprogramming. Stem Cell Rev., 7: 836-846. PMID: 21603945

Modder, U.I., K.A. Hoey, S. Amin, L.K. McCready and S.J. Achenbach *et al*., 2011. Relation of age, gender and bone mass to circulating sclerostin levels in women and men. J. Bone Miner. Res., 26: 373-379. PMID: 20721932

Morse, L.R., S. Sudhakar, V. Danilack, C. Tun and A. Lazzari *et al*., 2012. Association between sclerostin and bone density in chronic spinal cord injury. J. Bone Miner. Res., 27: 352-359. PMID: 22006831

Mosimann, C., G. Hausmann and K. Basler, 2009. Beta-catenin hits chromatin: Regulation of WNT target gene activation. Nat. Rev. Mol. Cell Biol., 10: 276-286. PMID: 19305417

Moustafa, A., T. Sugiyama, L.K. Saxon, G. Zaman and A. Sunters *et al*., 2009. The mouse fibula as a suitable bone for the study of functional adaptation to mechanical loading. Bone, 44: 930-935. DOI: 10.1016/j.bone.2008.12.026

Nevins, M., M. Camelo, M.L. Nevins, R.K. Schenk and S.E. Lynch, 2003. Periodontal regeneration in humans using recombinant Human Platelet-Derived Growth Factor-BB (rhPDGF-BB) and allogenic bone. J. Periodontol., 74: 1282-1292. PMID: 14584860

Nkenke, E. and F. Stelzle, 2009. Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: A systematic review. Clin. Oral Implants Res., 20: S124-S133. PMID: 19663959

Nusse, R., 2008. WNT signaling and stem cell control. Cell Res., 18: 523-527. PMID: 19028988

O'Brien, C.A., D. Jia, L.I. Plotkin, T. Bellido and C.C. Powers *et al*., 2004. Glucocorticoids act directly on osteoblasts and osteocytes to induce their apoptosis and reduce bone formation and strength. Endocrinology, 145: 1835-1841. PMID: 14691012

O'Brien, C.A., L.I. Plotkin, C. Galli, J.J. Goellner and A.R. Gortazar *et al*., 2008. Control of bone mass and remodeling by PTH receptor signaling in osteocytes. PLoS ONE, 3: e2942-e2942. DOI: 10.1371/journal.pone.0002942

Orsini, E., G. Giavaresi, A. Trire, V. Ottani and S. Salgarello, 2012. Dental implant thread pitch and its influence on the osseointegration process: An *in vivo* comparison study. Int. J. Oral Maxillofac. Implants, 27: 383-392. PMID: 22442779

Orsini, E., S. Salgarello, M. Bubalo, Z. Lazic and A. Trire *et al*., 2009. Histomorphometric evaluation of implant design as a key factor in peri-implant bone response: A preliminary study in a dog model. Minerva Stomatol., 58: 263-275. PMID: 19516235

Paic, F., J.C. Igwe, N. Ravi, M.S. Kronenberg and T. Franceschetti *et al*., 2009. Identification of differentially expressed genes between osteoblasts and osteocytes. Bone, 45: 682-692. PMID: 19539797

Parfitt, A.M., 2002. Targeted and nontargeted bone remodeling: Relationship to basic multicellular unit origination and progression. Bone, 30: 5-7. PMID: 11792557

Poole, K.E., R.L. Bezooijen, N. Loveridge, H. Hamersma and S.E. Papapoulos *et al*., 2005. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB J., 19: 1842-1844. PMID: 16123173

Rawadi, G. and S. Roman-Roman, 2005. WNT signalling pathway: A new target for the treatment of osteoporosis. Exp. Opin. Ther. Targets, 9: 1063-1077. PMID: 16185158

Robling, A.G., P.J. Niziolek, L.A. Baldridge, K.W. Condon and M.R. Allen *et al*., 2008. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. J. Biol. Chem., 283: 5866-5875. PMID: 18089564

Rodda, S.J. and A.P. McMahon, 2006. Distinct roles for Hedgehog and canonical WNT signaling in specification, differentiation and maintenance of osteoblast progenitors. Development, 133: 3231-3244. PMID: 16854976

Sawakami, K., A.G. Robling, M. Ai, N.D. Pitner and D. Liu *et al*., 2006. The WNT co-receptor LRP5 is essential for skeletal mechanotransduction but not for the anabolic bone response to parathyroid hormone treatment. J. Biol. Chem., 281: 23698-23711. PMID: 16790443

Schipani, E., K. Kruse and H. Juppner, 1995. A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. Science, 268: 98-100. PMID: 7701349

Sculean, A., D. Nikolidakis and F. Schwarz, 2008. Regeneration of periodontal tissues: Combinations of barrier membranes and grafting materials-biological foundation and preclinical evidence: A systematic review. J. Clin. Periodontol., 35: S106-S116. PMID: 18724845

Shahnazari, M., W. Yao, M. Corr and N.E. Lane, 2008. Targeting the WNT signaling pathway to augment bone formation. Curr. Osteoporos. Rep., 6: 142-148. DOI: 10.1007/s11914-008-0025-5

Sokol, S.Y., 2011. Maintaining embryonic stem cell pluripotency with WNT signaling. Development, 138: 4341-4350. PMID: 21903672

Souza, D.R.L., M. Matsuura, F. Eckstein, S.C. Rawlinson and L.E. Lanyon *et al*., 2005. Non-invasive axial loading of mouse tibiae increases cortical bone formation and modifies trabecular organization: A new model to study cortical and cancellous compartments in a single loaded element. Bone, 37: 810-818. PMID: 16198164

Spatz, J.M., E.E. Fields, E.W. Yu, P.D. Pajevic and M.L. Bouxsein *et al*., 2012. Serum sclerostin increases in healthy adult men during bed rest. J. Clin. Endocrinol. Metab., 97: E1736-E1740. PMID: 22767636

Tatsumi, S., K. Ishii, N. Amizuka, M. Li and T. Kobayashi *et al*., 2007. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. Cell Metab., 5: 464-475. PMID: 17550781

Tian, X., R.B. Setterberg, X. Li, C. Paszty and H.Z. Ke *et al*., 2010. Treatment with a sclerostin antibody increases cancellous bone formation and bone mass regardless of marrow composition in adult female rats. Bone, 47: 529-533. PMID: 20561907

Tian, X., W.S. Jee, X. Li, C. Paszty and H.Z. Ke, 2011. Sclerostin antibody increases bone mass by stimulating bone formation and inhibiting bone resorption in a hindlimb-immobilization rat model. Bone, 48: 197-201. PMID: 20850580

Trombelli, L. and R. Farina, 2008. Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. J. Clin. Periodontol., 35: S117-S135. PMID: 18724846

Tu, X., Y. Rhee, K.W. Condon, N. Bivi and M.R. Allen *et al*., 2012. Sost downregulation and local WNT signaling are required for the osteogenic response to mechanical loading. Bone, 50: 209-217. PMID: 22075208

Turner, C.H., S.J. Warden, T. Bellido, L.I. Plotkin and N. Kumar *et al*., 2009. Mechanobiology of the skeleton. Sci. Signal. DOI: 10.1126/scisignal.268pt3

Ulm, C., G. Tepper, R. Blahout, X. Rausch-Fan and S. Hienz *et al*., 2009. Characteristic features of trabecular bone in edentulous mandibles. Clin. Oral Implants Res., 20: 594-600. PMID: 19530316

Vandeweghe, S., J. Cosyn, E. Thevissen, J. Teerlinck and D.H. Bruyn, 2010. The influence of implant design on bone remodeling around surface-modified Southern Implants®. Clin. Implant Dent. Relat. Res., 14: 655-662. PMID: 20977610

Velde, V.D.T., B. Collaert, L. Sennerby and D.H. Bruyn, 2009. Effect of implant design on preservation of marginal bone in the mandible. Clin. Implant Dent. Relat. Res., 12: 134-141. PMID: 19220843

Verheyen, E.M. and C.J. Gottardi, 2010. Regulation of WNT/beta-catenin signaling by protein kinases. Dev. Dyn., 239: 34-44. PMID: 19623618

Veverka, V., A.J. Henry, P.M. Slocombe, A. Ventom and B. Mulloy *et al*., 2009. Characterization of the structural features and interactions of sclerostin: molecular insight into a key regulator of WNT-mediated bone formation. J. Biol. Chem., 284: 10890-10900. PMID: 19208630

Voskaridou, E., D. Christoulas, E. Plata, C. Bratengeier and A. Anastasilakis *et al*., 2012. High circulating sclerostin is present in patients with thalassemia-associated osteoporosis and correlates with bone mineral density. Horm. Metab. Res., 44: 909-913. PMID: 22581647

Wang, R.F., B. Kang, L.A. Lang and M.E. Razzoog, 2009. The dynamic natures of implant loading. J. Prosthet. Dent., 101: 359-371. PMID: 19463663

Wang, Y., C. Wan, L. Deng, X. Liu and X. Cao *et al*., 2007. The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. J. Clin. Invest., 117: 1616-1626. PMID: 17549257

Weidauer, S.E., P. Schmieder, M. Beerbaum, W. Schmitz and H. Oschkinat *et al*., 2009. NMR structure of the WNT modulator protein Sclerostin. Biochem. Biophys. Res. Commun., 380: 160-165. PMID: 19166819

Weinstein, R.S., 2010. Glucocorticoids, osteocytes and skeletal fragility: The role of bone vascularity. Bone, 46: 564-570. PMID: 19591965

Wennstrom, J.L. and J. Lindhe, 2002. Some effects of enamel matrix proteins on wound healing in the dento-gingival region. J. Clin. Periodontol., 29: 9-14. PMID: 11846843

Wikesjo, U.M., M. Qahash, Y.H. Huang, A. Xiropaidis and G. Polimeni *et al*., 2009. Bone morphogenetic proteins for periodontal and alveolar indications; biological observations-clinical implications. Orthod. Craniofac. Res., 12: 263-270. PMID: 19627529

Williams, B.O. and K.L. Insogna, 2009. Where WNTs went: The exploding field of Lrp5 and Lrp6 signaling in bone. J. Bone Miner Res., 24: 171-178. PMID: 19072724

Winkler, D.G., M.K. Sutherland, J.C. Geoghegan, C. Yu and T. Hayes *et al*., 2003. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J., 22: 6267-6276. DOI: 10.1093/emboj/cdg599

Wiskott, H.W. and U.C. Belser, 1999. Lack of integration of smooth titanium surfaces: A working hypothesis based on strains generated in the surrounding bone. Clin. Oral Implants Res., 10: 429-444. PMID: 10740452

Xiong, J., M. Onal, R.L. Jilka, R.S. Weinstein and S.C. Manolagas *et al*., 2011. Matrix-embedded cells control osteoclast formation. Nat. Med., 17: 1235-1241.

Yavropoulou, M.P., A.H. Lierop, N.A. Hamdy, R. Rizzoli and S.E. Papapoulos, 2012. Serum sclerostin levels in Paget's disease and prostate cancer with bone metastases with a wide range of bone turnover. Bone, 51: 153-157. PMID: 22579776