Sclerostin, an emerging therapeutic target for treating osteoporosis and osteoporotic fracture: A general review

Pui Kit Suen*, 1, Ling Qin*

Musculoskeletal Research Laboratory, Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong SAR, China

Received 12 March 2015; received in revised form 2 August 2015; accepted 12 August 2015
Available online 12 September 2015

Summary Osteoporosis and its associated fracture risk has become one of the major health burdens in our aging population. Currently, bisphosphonate, one of the most popular antiresorptive drugs, is used widely to treat osteoporosis but so far still no consensus has been reached for its application in treatment of osteoporotic fractures. However, in old patients, boosting new bone formation and its remodelling is essential for bone healing in age-related osteoporosis and osteoporotic fractures. Sclerostin, an inhibitor of the Wnt/β-catenin signalling pathway that regulates bone growth, has become an attractive therapeutic target for treating osteoporosis. In this review, we summarize the recent findings of sclerostin and its potential as an effective drug target for treating both osteoporosis and osteoporotic fractures.

Copyright © 2015, The Authors. Published by Elsevier (Singapore) Pte Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Osteoporosis is becoming a major health burden that mainly occurs in the aging population, especially in postmenopausal women [1]. Osteoporosis is characterized by the imbalance in bone formation and resorption. Excessive bone resorption leads to low bone mineral density (BMD) and the deterioration of bone microarchitecture, which results in a loss of bone strength and an increase in fracture risks [2]. According to the World Health Organization, osteoporosis is defined as BMD with > 2.5 standard...
deviations below the young adult average BMD, calculated as a T-score of $-2.5$ [3,4]. Lifetime risk of osteoporotic fracture at age 50 years is estimated to range from 13–22% in men and 40–53% in women in western countries [2]. In Hong Kong, the incident rates (per 100,000 population) of hip fracture at age $>65$ years are 342 and 703 for men and women, respectively [5], while the prevalence of radiographic vertebral fracture (mean age 72 years) are 15% and 17% for men and women, respectively [6]. The prevalence of osteoporosis and osteoporotic fracture has generated huge social and economic impact.

Drug therapy is an effective way to prevent osteoporosis. Bisphosphonates are a class of antiresorptive drugs that inhibit bone resorption and have been widely used to treat osteoporosis. Bisphosphonates have a high binding affinity to bone minerals and induce osteoclasts apoptosis during bone resorption [7]. Denosumab is another antiresorptive drug, a humanized monoclonal antibody that inhibits the binding of receptor activator of nuclear factor κB ligand (RANKL) to its receptor, leading to inhibition of osteoclast development, formation and survival [8]. Although antiresorptive drugs are effective against osteoporosis, their adverse effects include atypical bone fractures after long-term treatment and an increase in the risk of jaw osteonecrosis at higher doses [9–11]. In fracture healing, bisphosphonate treatment does not impair fracture union in healthy rats [12,13], but the callus remodelling is delayed in ovariectomised (OVX) rats [14], suggesting that the role of bisphosphonates in improving fracture healing remains controversial [15,16]. Furthermore, antiresorptive drugs, such as bisphosphonates and denosumab, are unable to rebuild bone that has been lost, so there is a need for an anabolic drug to promote bone building. Teriparatide [recombinant human parathyroid hormone (1–34), rhPTH1-34] was the first Food and Drug Administration (FDA) approved anabolic drug against osteoporosis [17]. Teriparatide exerts its anabolic activity through increased osteoblasts number and activity, increasing the osteogenic differentiation of mesenchymal stem cells (MSCs), and decreasing osteoclasts apoptosis [18–20]. However, teriparatide delivery is inconvenient as it requires daily subcutaneous injections, when compared with bisphosphonates, which are taken orally. In addition, due to the potential risk of osteosarcoma, the use of teriparatide is limited to a maximum treatment of 2 years [21,22]. In light of these achievements, developing new drugs for treating osteoporosis continues to be a necessity.

The recent discoveries and advances in the molecular pathophysiology of osteoporosis have led to the development of new therapeutic drugs for osteoporosis treatment, such as the sclerostin monoclonal antibody as a new anabolic drug for osteoporosis [23,24]. Sclerostin, an inhibitor of the Wnt/β-catenin signalling pathway that regulates bone growth, has become an attractive therapeutic target for treating osteoporosis. In this review, recent findings of sclerostin, and its potential as an effective drug target for treating osteoporosis and osteoporotic fractures, are summarized, together with the findings of preclinical experiments.

**Cellular events of bone remodelling and osteoporosis**

Our skeleton is constantly undergoing bone remodelling in response to mechanical loading, microdamage repair, and mineral homeostasis. The activation of the bone remodelling process is mediated by osteocytes through osteocyte apoptosis, which recruits osteoclasts precursor cells for bone resorption [25]. The osteoclasts precursor cells, originated from the hematopoietic lineage, then differentiate into osteoclasts to resorb the bone matrix [26,27]. Besides osteocytes, osteoblasts also regulate bone resorption though the expression of the RANKL and osteoprotegerin [28,29]. The resorption of bone matrix by osteoclasts results in the release of growth factors to recruit osteoblasts for bone formation [30]. Therefore, the bone remodelling process is tightly regulated by the balanced activity of bone formation by osteoblasts and bone resorption by osteoclasts, through extensive crosstalk between the osteocytes, osteoblasts, and osteoclasts. Osteoporosis is caused by the imbalance of the bone formation and bone resorption, with the rate of bone resorption exceeding the rate of bone formation [31]. Peak bone mass is achieved at around age 30 years, and, after that, bone is lost with increasing age [32,33]. Bone loss is accelerated in postmenopausal women due to the absence of oestrogen, which stimulates osteoblast apoptosis and osteoclast activity, resulting in osteoporosis [34].

**Sclerostin and skeletal mass**

Sclerosteosis is a rare autosomal recessive gene disorder characterized by its high bone mass phenotype in radiographs and an increased BMD in lumbar spine, hip and forearm [35–37]. Patients affected by sclerosteosis carry a loss of functional mutation of the sclerostin gene, which is caused by either nonsense mutations near the 5′ region of the sclerostin mRNA transcript or mutations that affect the correct splicing and stability of the transcript [35,36]. In a similar high bone mass disorder, van Buchem disease, the sclerostin gene is not mutated, but the 52-kb region downstream of the sclerostin gene is deleted [38]. This 52-kb region contains an enhancer element (termed ECR5) for proper sclerostin gene expression, thus result in the loss of sclerostin gene expression in the van Buchem disease [39]. Sclerostin is a secreted glycoprotein that is mainly expressed in bone matrix and cartilage matrix and has been reported to suppress the mineralization of osteoblasts in cell culture environment [36,40]. The function of sclerostin as an inhibitor of bone formation has been further demonstrated in transgenic mice. Sclerostin knock out (SOST-KO) mice displays a high bone mass with increased bone formation and bone strength [41,42], whereas overexpression of sclerostin in mice results in low bone mass with decreased bone formation and bone strength [40,43]. Sclerostin inhibits bone formation through inhibiting the Wnt/β-catenin signalling [44], and its expression is regulated by mechanical unloading and
oestrogen deficiency in osteocytes \[42,45–48\]. Wnt/β-catenin signalling is important for osteoblast differentiation and proliferation \[49\]. Sclerostin has been found to be responsible for both inhibition of the osteoblastogenesis and preosteocyte differentiation of osteoblasts \[50,51\]. Sclerostin also stimulates RANKL secretion from osteocytes to induce osteoclastogenesis \[52,53\], leading to an increase in bone resorption. In addition, osteoclasts induce osteoblasts for bone formation through the Wnt signalling pathway \[54,55\]. Inhibitors of the Wnt signalling pathway (such as Dickkopf-related protein 1, DKK1) suppress the osteoclasts-mediated osteoblast bone formation \[54\]. This suggests that sclerostin may also suppress the osteoclast-mediated osteoblast bone formation. It is also demonstrated that sclerostin is expressed in osteoclasts from aged mice, indicating that sclerostin contributes to the age-related decoupling of bone turnover \[56\]. Taken together, these data show that sclerostin plays an important role in modulating bone formation and bone turnover, through antagonizing the Wnt/β-catenin signalling pathway in osteoblasts and modulating RANKL level that act on osteoclasts (Figure 1).

**Molecular biology of sclerostin and Wnt/β-catenin signalling pathway**

Sclerostin is an antagonist of the Wnt/β-catenin signalling pathway

Sclerostin is a secreted glycoprotein that is mainly expressed by osteocytes \[57\]. The serum level of sclerostin increases with age, in both men and women \[58\]. Although serum sclerostin is positively correlated with BMD in postmenopausal women, it is negatively correlated with bone turnover markers. This suggests that the serum sclerostin level is generally reflected by the number of osteocytes where osteocytes number is higher in high BMD individuals \[59\]. In fact, sclerostin may act as a paracrine rather than endocrine signalling molecule for osteoblasts functions, as its target cells, the osteoblasts, are in close proximity with osteocytes.

The Wnt/β-catenin signalling pathway plays an important role in regulating cell proliferation and differentiation, including osteoblasts and its differentiation from MSCs \[55,60\]. The Wnt proteins are secreted glycoproteins that

---

**Figure 1** The molecular mechanisms of sclerostin in modulating bone turnover. Sclerostin is secreted by osteocytes to modulate bone turnover via responding to mechanical unloading or oestrogen deficiency. Sclerostin inhibits bone formation by inhibiting the osteogenic differentiation of mesenchymal stem cells or osteoprogenitor cells and the proliferation of osteoblasts. Sclerostin also stimulates RANKL secretion from osteocytes, where RANKL is essential for osteoclasts formation and activity. A recent study shows that sclerostin is also expressed in osteoclasts in aged mice \[56\].
stimulate the signalling pathway through binding to the receptors low-density lipoprotein receptor-related protein 5/6 (LRP5/6) and co-receptor Frizzled. The receptor complex prevents the phosphorylation of β-catenin by inhibiting the glycogen synthase kinase 3 (GSK3) activity involving the protein complex with Disheveled (Dsh), Axin and adeno-matous polyposis coli (APC) [61]. This results in cytoplasmic accumulation and subsequent nuclear translocation of β-catenin [60]. Beta-catenin is a transcriptional coactivator that interacts with other transcription factors to mediate downstream gene expression, leading to cell differentiation and proliferation [55] (Figure 2).

Wnt signalling is also tightly regulated by several types of secreted antagonists, such as sclerostin and DKK1 [62].

Similar to the role of sclerostin in bone formation in mice, DKK1 also negatively regulates bone formation. Bone-specific DKK1 overexpression in transgenic mice resulted in osteopenia [63]. Deleting both DKK1 alleles was lethal, while deleting a single allele of DKK1 gene increased bone mass [64]. It is further demonstrated that DKK1 expression level is inversely proportional to the bone mass in mice [65]. Sclerostin and DKK1 inhibit the Wnt signalling pathway through binding to LRP5/6, displacing the Wnt proteins, leading to the dissociation of the LRP5/6 and Frizzled receptor complex [66–68]. This results in phosphorylation of β-catenin and eventually degraded by proteasome (Figure 2). In addition, DKK1 was also found to mediate the recruitment of Kremen co-receptor to LRP5/6 and induced

![Figure 2](image-url)  
**Figure 2** Sclerostin and the Wnt/β-catenin signalling pathway. (A) Sclerostin is an antagonist of the Wnt/β-catenin signalling pathway. Sclerostin binds to LRP5/6 receptors, replaces the Wnt proteins and disrupts the Wnt-LRP5/6-Frizzled interaction. The GSK3/Axin/APC protein complex is released to the cytosol and phosphorylates the β-catenin. The phosphorylated β-catenin is then degraded by proteasome. In addition, the sclerostin-LRP5/6 interaction leads to internalization into the endosome and subsequent degradation. (B) The Wnt/β-catenin signaling is activated through the interaction of Wnt proteins with the receptor LRP5/6 and co-receptor Frizzled. The receptor complex prevents the phosphorylation of β-catenin by GSK3/Dsh/Axin/APC complex that results in cytosolic accumulation of β-catenin. The β-catenin then translocates to the nucleus and activates transcription of target genes. Injection of Scl-Ab eliminates the inhibitory effects of sclerostin and activates the Wnt/β-catenin signaling. APC = adenomatosis polyposis coli; Dsh = Dishevelled; GSK3 = glycogen synthase kinase-3; LRP5/6 = low-density lipoprotein receptor-related protein 5/6; Scl-Ab = sclerostin monoclonal antibody; SOST = sclerostin.
the endocytosis of LRP5/6, leading to a reduction in LRP5/6 mediated signalling [66,67]. Recently, sclerostin has been found to mediate the endocytosis of LRP6 upon its binding to LRP6 [68]. The clathrin-dependent endocytosis of sclerostin-LRP6 complex is degraded in a proteasome-dependent manner [68], possibly within the endosome [69].

Mutations in the N-terminal human LRP5 protein that reduce its affinity to sclerostin and result in a high bone mass disorder further demonstrate the importance of sclerostin-LRP5 interaction in regulating bone mass [70,71]. Meanwhile, β-catenin activity is required for osteoblast differentiation of MSCs. Activation of Wnt signalling pathway in mouse MSCs culture upregulated Runx2 expression [49], while inactivation of β-catenin in MSCs in transgenic mice resulted in the loss of factors required for osteoblast differentiation, such as Runx2 and Osx, and disrupted osteoblast differentiation [72]. It has also been discovered that β-catenin activity in MSCs promotes osteoblastogenesis and inhibits chondrogenesis [72].

**Molecular structure of sclerostin and its interaction with LRP5/6**

Structural analysis of sclerostin and LRP5/6 provides more detailed information on how they interact. Sclerostin contains a cystine-knot motif with a flexible loop domain flanked by two fixed-structure finger domains [73]. Later, it is demonstrated that the PNAIG motif presence in the flexible loop domain of sclerostin is responsible for its interaction with the LRP6 receptor [74]. It was also shown that effective sclerostin monoclonal antibody binds to this region to interfere with the sclerostin-LRP interaction [75]. The LRP5/6 is a transmembrane protein. The extracellular domain of LRP5/6 consists of four β-propeller domains separated by four EGF domains, followed by LDLA repeats, transmembrane domains and a cytoplasmic domain [76]. The cytoplasmic domain is involved in recruiting the GSK3/Dsh/Axin/APC complex, whereas the extracellular domain is responsible for Wnt ligand binding and subjected to antagonist inhibition. Structural analysis reveals that the first two propeller domain (E1E2) of LRP6 are necessary for both sclerostin and Wnt ligand binding, suggesting that sclerostin and Wnt1 compete for the same binding site of LRP6 [76]. Conformational changes in the LRP6 propeller domains and/or steric hindrance effects of sclerostin may also be responsible for sclerostin inhibition of other Wnt ligands binding to LRP6 [76].

**Regulation of sclerostin expression**

Given that sclerostin is important for regulating bone formation, it is not surprising that the expression of sclerostin is tightly regulated by various factors, including mechanical stimuli, oestrogen deficiency, vitamin D, Prostaglandin E2, parathyroid hormone (PTH), and transforming growth factor (TGF)-β. The expression of sclerostin is upregulated in tibia in a hindlimb unloading model (tail suspension) in mice and downregulated in ulna in loading model in both rat and mice [45]. It is further demonstrated that sclerostin downregulation in osteocytes is required for osteogenesis in mechanical loading condition in transgenic mice [46], and SOST-KO mice are resistant to bone loss due to mechanical unloading [42]. Oestrogen deficiency also stimulates sclerostin expression. Sclerostin expression is upregulated in the femora of OVX mice, which is reversed by β-oestradiol injection [47]. In postmenopausal women, oestrogen treatment significantly reduces the sclerostin level in bone and serum [48]. These data indicate that sclerostin plays an important role in bone loss during oestrogen deficiency in postmenopausal osteoporosis and mechanical unloading conditions in immobilization after osteoporotic fracture.

Vitamin D (1,25-dihydroxyvitamin D3) is an important regulator of bone mass and sclerostin. It was shown that vitamin D stimulates sclerostin gene expression through the vitamin D response element presence in the upstream promoter region of human sclerostin gene [80]. In addition, in SOST-KO mice, the serum vitamin D and phosphorus is increased and renal excretion of calcium is decreased, indicating a positive mineral balance in the deletion of sclerostin [81]. Taken together, these reports suggest that the vitamin D and sclerostin control of calcium homeostasis and bone growth is complex [82].

Prostaglandin E2 is a lipid-derived hormone that is found to be involved in activation of β-catenin signalling in response to mechanical loading [83,84]. Mechanical loading induces the synthesis and release of prostaglandin E2, upon binding to its receptor, and suppression of sclerostin expression [85,86]. In a β-catenin reporter transgenic mouse model, it shows rapid response of osteocytes to mechanical loading [84]. As early as 1 hour after in vivo loading of ulna, β-catenin expression is upregulated, and pretreatment of inhibitor of prostaglandin E2 synthesis diminishes the β-catenin expression during mechanical loading [84]. In cell culture models, inhibition of prostaglandin E2 synthesis prevents the sclerostin downregulation in response to mechanical stimulation [85,86].

TGF-β is an important regulator of stem cell differentiation and proliferation. During osteogenic differentiation, TGF-β regulates β-catenin signalling, and can either promote or inhibit osteogenic differentiation depending on environmental clues [87]. TGF-β enhanced sclerostin expression in mature osteoblasts and regulated sclerostin expression through the ECR5 enhancer element on the downstream enhancer region of sclerostin [88].

The expression of sclerostin is regulated at transcriptional level by PTH. Decreased sclerostin expression is observed in PTH-treated mice and transgenic mice overexpressing PTH receptor in osteocytes [89,90]. Similar results are observed in clinical studies, where the serum sclerostin level is negatively correlated with PTH level, especially for postmenopausal women [91,92], and PTH administration reduces the serum sclerostin level [93,94]. These results indicate that the anabolic effects of PTH act through inhibiting the sclerostin expression.
The 52-kb downstream enhancer region of sclerostin gene plays an important role in regulating sclerostin gene expression. This 52-kb region contains an ECR5 enhancer element, and the transcriptional activity of ECR5 is controlled by the myocyte enhancer factor-2c (MEF2C) transcription factor [95]. Mice lacking ECR5 or bone-specific expression of MEF2C display a high bone mass phenotype with low sclerostin expression, indicating the importance of MEF2C and its binding to ECR5 enhancer for controlling sclerostin transcription [95,96]. Several factors are responsible for regulating the binding of MEF2C to its responsive sequence, including PTH and TGF-β [88,97]. Histone deacetylases (HDACs) are a class of histone-modifying enzymes that remove the acetyl group from histone proteins and result in stronger histone–DNA interaction and suppress gene expression. In bone, HDAC5 is a negative regulator of sclerostin expression through blocking the binding of MEF2C to the sclerostin enhancer region [98]. Overexpression of HDAC5 in osteocyte cell cultures suppresses sclerostin expression [99], while knockout of HDAC5 in osteocyte cell cultures or transgenic mice results in increased sclerostin expression [98].

Taken together, these data show that the regulation of sclerostin expression is complex and controlled by various factors. Mechanical signals and hormonal signals are major players in regulating sclerostin expression in osteocytes. The binding of MEF2C to its binding sites on the 52-kb downstream enhancer region of sclerostin gene are subjected to the control of various factors, including PTH, TGF-β, and HDAC5, which in turn, is crucial for sclerostin expression. In addition, the roles of MEF2C (and HDAC5) in regulating sclerostin expression in other signalling pathways, such as mechanical signals or oestrogen deficiency remain largely unknown and require future research. However, it is possible that MEF2C is the major player in regulating sclerostin expression, as the 52-kb enhancer region controls sclerostin expression.

Other drug treatments for osteoporosis treatment

Besides bisphosphonates, odanacatib and denosumab are two antiresorptive drugs that are recently under clinical trials or have been approved by the FDA to treat postmenopausal osteoporosis. Denosumab was approved by FDA in 2010 [100], and Odanacatib has just finished Phase 3 clinical trials [101].

Denosumab is a fully humanized anti-RANKL antibody that suppresses the development and activity of osteoclasts and results in decreasing bone resorption and increasing BMD [102]. In a Phase 2 clinical study on postmenopausal women, it was reported that denosumab treatment significantly increased the BMD at lumbar spine, total hip, and radius [103]. Denosumab treatment has also been found to be able to reduce the level of serum bone resorption marker and increase the level of serum bone formation marker [103]. Although the reduced BMD at lumbar spine and hip back to baseline level is observed in discontinued denosumab treatment, the BMD increased again when the treatment restarts [103]. In a large-scale Phase 3 clinical study, it was reported that denosumab treatment significantly reduced the fracture risk of postmenopausal women [104].

Odanacatib is a small molecule inhibitor of cathepsin K activities. Cathepsin K is a lysosomal protease that mainly expresses in osteoclasts to degrade collagen I during bone resorption [105]. Odanacatib treatment reduces bone resorption activity of osteoclasts, but has no effect on osteoclast formation and survival, suggesting that these osteoclasts are able to stimulate or maintain osteoblast activity for bone formation [105]. In fact, elevated bone formation rate has been observed in cathepsin K-deficient mice and Odanacatib-treated rabbits [106,107], indicating both antiresorptive and anabolic effects of Odanacatib treatment. In a Phase 2 clinical trial on postmenopausal osteoporotic women with low bone mass, Odanacatib treatment significantly increased the BMD at the spine and hip in a dose-dependent manner [108]. The serum bone resorption markers significantly decreased in the Odanacatib treatment while the bone formation markers transiently decreased initially, and returned to the baseline level at the end of the 2-year study [108]. In the extended 3-year study, a progressive increase in BMD in spine, femoral neck, and hip were observed in continuous Odanacatib treatment [109], while discontinued Odanacatib treatment resulted in a gradual decline of BMD to the baseline level [109]. The bone resorption markers continued to decline with continuous Odanacatib treatment, while the bone formation markers remained at baseline level [109]. A large-scale Phase 3 clinical trial has been designed to assess the fracture prevention efficacy and safety of Odanacatib in postmenopausal women with osteoporosis [101].

Sclerostin monoclonal antibody for treatment of osteoporosis

Scl-Ab treatment in animal models

Inhibiting antagonists of the Wnt signalling pathway provides an attractive strategy for treatment of osteoporosis. Sclerostin monoclonal antibody (Scl-Ab) inhibits the function of sclerostin, thus re-activating the Wnt signalling pathway in osteoblasts has provided an excellent therapeutical approach to enhance bone formation (Figure 2). As sclerostin is mainly expressed in osteocytes [40,57], it is expected that Scl-Ab treatment is bone specific with minor side effects.

In normal rodents and nonhuman primates, Scl-Ab treatment has been reported to increase bone formation, bone mass and bone strength in a dose-dependent manner [110,111]. In a study of animal model of osteoporosis, Ovx rats, Scl-Ab treatment also significantly increased bone formation, bone mass, and bone strength of the animals [112]. The increase in bone formation is illustrated by the increase in osteoblasts number and serum bone formation markers, and a decrease in bone resorption as illustrated by decrease in osteoclast number [110–112]. Similar bone anabolic effects of Scl-Ab treatment have also been observed in male animal models. Scl-Ab treatments in 6-month-old male rats, aged (16-month-old) male rats, and male cynomolgus monkeys have displayed improvement of
bone formation, bone mass, and bone strength at spine and femur [113,114]. The effects of Scl-Ab on vertebral body with different bone marrow composition are studied in more details in aged female rats. Scl-Ab treatment is able to increase bone volume fraction and trabecular thickness in both yellow (fat) marrow rich fifth caudal vertebral body and red marrow rich fourth lumbar vertebral body, indicating that Scl-Ab is effective in increasing bone formation regardless of bone marrow composition [115]. An increase in the mineralization surface, mineral apposition rate and bone formation rate, as well as decrease in the eroded surface, have also been observed in both caudal and lumbar vertebral bodies in a dose dependent manner [115]. Taken together, Scl-Ab treatment increases the serum bone formation markers and decreases the bone resorption markers, which indicates that bone formation and bone resorption are uncoupled in Scl-Ab treatment, thus creating a large therapeutic window for osteoporosis treatment [116].

Besides OVX-induced osteoporosis, the effects of Scl-Ab treatment in other animal models have also been reported. In an animal study of hindlimb unloading, Scl-Ab treatment increases the bone formation, bone mass, and bone strength through increasing bone formation and inhibiting bone resorption [111,117]. Scl-Ab treatment is also shown to have enhanced fracture healing and bone repair, with increased bone formation, accelerated fracture repair and fracture callus strength in rat studies [118–120] as well as bilateral fibular osteotomy in cynomolgus monkeys [114].

Clinical trials of Scl-Ab for osteoporosis treatment

Supported by the encouraging results in the animal studies, clinical trials were conducted to investigate the effects and efficacy in humans, including those with normal bone mass or low bone mass.

A Phase 1 clinical trial of Scl-Ab has been conducted on postmenopausal women and healthy men with normal bone mass. Scl-Ab is proven to improve significantly the BMD in lumbar spine and hip, which is possibly due to the increased bone formation and decreased bone resorption as in the serum markers [121]. In the next study on postmenopausal women and healthy men with low bone mass (BMD T-score between −2.5 and −1.0), Scl-Ab treatment improved the BMD in a dose-dependent manner with minor adverse effects [23]. Minor adverse effects include headaches, upper respiratory tract infections, arthralgia, back pain, abdominal pain, and pain in the hands and feet; the percentages of participants reporting adverse events are similar in the placebo group and pooled Scl-Ab treatment groups [23]. Scl-Ab treatment-specific adverse effects—reaction site pain—are also reported [23]. Scl-Ab treatment increases the serum bone formation markers and decreases bone resorption markers as early as 1 week after the first injection [23]. Scl-Ab also significantly increases the BMD at lumbar spine and hip as early as 3 months after the first injection [23].

In a Phase 2 clinical trial study, the efficacy of various doses of Scl-Ab is compared with placebo or other osteoporotic drug treatments (alendronate and teriparatide) in postmenopausal women with low BMD (T-score < −2.0 at the lumbar spine) over a 12-month treatment period [24]. Similar to the Phase 1 clinical study, Scl-Ab was found to have efficiently improved the BMD at the lumbar spine, hip, and femoral neck as early as 3 months after the first injection compared with placebo controls in a dose-dependent manner with minor adverse effects [24]. The adverse effects are similar to those reported in the Phase 1 clinical trial [24]. Additional adverse effects are gastrointestinal, constipation, bronchitis, urinary tract infection, musculoskeletal pain, and fatigue [24]. The percentages of adverse effects are similar in both pooled placebo group and Scl-Ab treatment group [24]. The increased bone formation markers and decreased bone resorption markers are observed as early as 1 week after the first injection [24]. Compared with alendronate and teriparatide, a 210 mg monthly dose of Scl-Ab treatment provides the best improvement in BMD, starting as early as 6 months after the first injection [24]. Currently, Scl-Ab is in Phase 3 clinical trials to evaluate its effects in postmenopausal women with low BMD (T-score < −2.5).

Given the large improvement of BMD in clinical trials of Scl-Ab treatment in osteoporosis, we can expect the fracture risk to be lowered with Scl-Ab treatment, provided that Scl-Ab treatment improves the bone strength in the preclinical studies. It is thus worth tracing the fracture rates of the patients after Scl-Ab treatment in future studies.

Future perspectives of Scl-Ab in osteoporosis treatment

Long-term safety of Scl-Ab treatment

The effects of long-term Scl-Ab treatment are still under investigation. Currently, there are two studies focusing on the bone formation and bone strength of long-term effects (26 weeks) of Scl-Ab on OVX rats. Long-term Scl-Ab treatment progressively increases the bone mass and bone strength in OVX rats, which is explained by an increase in cortical and trabecular bone formation and reduced osteoclast activity [122]. In addition, Scl-Ab treatment shows a remarkable increase in bone formation rate in early stage (6 weeks) but such increase diminishes at the later stage (26 weeks), which is characterized by a significant increase in serum bone formation markers and decrease in bone resorption markers [122]. However, as Wnt/β-catenin signalling pathway is elevated in osteosarcoma cells [123], the relationship between long-term sclerostin inhibition and the risk of bone cancer shall be investigated with regard to the long-term safety of Scl-Ab administration.

The combined effects of Scl-Ab and other osteoporotic drugs

An animal study has demonstrated that Scl-Ab treatment has similar results in increase of bone formation, bone mass, and bone strength with or without pretreatment of alendronate, a widely used bisphosphonate, in OVX rats [124]. Besides, cotreatment of Scl-Ab and alendronate have
similar efficacies in bone formation, bone mass, and bone strength to Scl-Ab alone or alendronate-pretreated OVX rats [124]. These data suggest that Scl-Ab treatment does not affect the previous alendronate treatment.

There is clinical interest in using monoclonal antibody against DKK1 (DKK1-Ab) to treat multiple myeloma, as DKK1 produced by multiple myeloma cells is a key factor responsible for bone loss in multiple myeloma [125]. In a transgenic mouse model, it is clearly shown that the reduced DKK1 expression results in an increase in bone mass [63–65], thus proposing the use of DKK1-Ab for treating osteoporosis. In OVX animals, DKK1-Ab treatment improves bone formation and bone mass [126]. As both Scl-Ab and DKK1-Ab are targeted to upregulate the Wnt signalling pathway, the combined effects of both antibodies may provide better improvement in bone formation than single treatments.

The potential of drug–drug interaction of Scl-Ab and other osteoporotic drugs have not been fully explored. Future studies may also investigate the effectiveness of combined treatment, especially in patients with severe osteoporosis.

**Scl-Ab treatment in male osteoporosis**

Male osteoporosis is becoming a more important issue as the population ages. Although research related to osteoporosis treatment mainly focuses on women, in fact, about 30% of hip fractures and 20% of vertebral fractures occur in men [127,128] and the mortality associated with hip fractures in elderly men is significantly higher than that in women [129]. Scl-Ab treatment improves the bone formation, bone mass, and bone strength in aged male rats and male cynomolgus monkeys [113,114]. Similarly, Scl-Ab has been discovered to display anabolic effects in adult male rats through increasing bone formation and bone mass, especially in trabecular bone, as early as 6 weeks after the first injection (Figure 3). These data support the use of pharmacological drugs for osteoporosis treatment in men, and currently several clinical trials have been undertaken to investigate the potential use of current osteoporosis drugs in men [130]. A recent study analysed the effects of teriparatide for osteoporosis treatment in men. The changes of BMD and bone turnover makers are directly compared in men and women in the same study [131]. Differences in BMD of the lumbar spine and femoral neck, and the serum P1NP biomarkers are found to be similar between male and female groups [131]. Similar findings are also observed in the clinical trials of Scl-Ab in healthy men with low bone mass [23]. These findings suggest that the osteoporosis drug treatments have similar bone anabolic effects on both men and women. Further studies are required to establish the efficacy in preventing bone fractures and the safety of these treatments.

**Sclerostin monoclonal antibody for potential healing enhancement of osteoporotic fracture**

The effects of Scl-Ab treatment on fracture healing have been investigated in various animal models. Scl-Ab treatment increases bone mass and strength at the fracture site in rats using either a closed femoral fracture model [114] or a femoral osteotomy fracture model [119,132]. More bony tissue and less cartilage tissue have been observed at the fracture site in the rat femoral osteotomy fracture model [119,132] and cynomolgus monkey bilateral fibular osteotomy model [114], indicating that Scl-Ab treatment is able to enhance endochondral ossification during fracture healing. Similarly, the bone mass and strength is increased during fracture healing in SOST-KO mice, in both a femoral closed fracture model [133] and a tibial closed fracture model with external fixation [134]. In both models, the endochondral ossification is hastened as evidenced by increased cartilage removal [133,134]. These studies indicate that downregulation of sclerostin expression enhanced fracture healing through faster endochondral ossification. As the downstream target of sclerostin, the Wnt/β-catenin signalling pathway is well-known to be an important factor to facilitate proper fracture healing [135]. Beta-catenin signalling is activated in the fracture callus throughout the entire period of fracture healing [136], and precise regulation of β-catenin expression in the fracture site is required for fracture healing [136]. Condition knock down of the β-catenin gene expression in the fracture callus impairs fracture healing [136]. The β-catenin level has also been shown to elevate during the femoral bone defect healing in SOST-KO mice [137], as well as open tibial fracture healing in mice treated with DKK1-Ab treatment [138], indicating that the inhibition of sclerostin or DKK1 promotes...
bone healing though activating the Wnt/β-catenin signalling pathway. Although no investigations have been reported that Scl-Ab treatment will activate the Wnt/β-catenin signalling during fracture healing, results from these two related studies [137,138] support that Scl-Ab treatment shall also act through the same signalling pathway to promote fracture healing.

Scl-Ab treatment also enhances bone repair in osteoporotic condition. In a tibial drill-hole defect model in O VX rat, Scl-Ab treatment accelerates the intramembranous bone repair in both the trabecular bone and cortical bone of the defect region [120]. This indicates that Scl-Ab treatment also enhances bone formation and bone healing in O VX conditions. In addition, based on our previous study in rat femoral osteotomy healing, Scl-Ab treatment has proven to enhance fracture healing through the hastened endochondral ossification and improved angiogenesis [119] (Figure 3). Angiogenesis is essential for bone healing, in both normal and osteoporotic fracture healing [139,140]. Given that Scl-Ab improves fracture healing in a rat long-bone closed fracture model [114], rat femoral osteotomy [119] or cynomolgus monkey bilateral fibular osteotomy model [114], Scl-Ab is also expected to be able to improve osteoporotic fracture healing. Clinical trials are essential to support the potential routine applications of Scl-Ab for fracture healing enhancement in osteoporotic patients, apart from the known effects of Scl-Ab in the prevention of secondary osteoporosis in this high-risk group.

Concluding remarks

The current understanding of Wnt signalling pathway in regulating bone formation and remodelling provides attractive strategies for developing new treatments for osteoporosis and osteoporotic fracture repair. Sclerostin, as an antagonist of the Wnt signalling pathway, is an effective therapeutic target for osteoporosis treatment but its potential for osteoporotic fracture repair still requires long-term and perspective clinical trial studies for conformation. As sclerostin is mainly expressed in bone cells, treatments that inhibit sclerostin function should have minimal adverse effects. Clinical trial studies have shown that the Scl-Ab treatment has strong bone anabolic effects, and is effective in improvement of osteoporosis as compared with treatments using other drugs. Future studies of Scl-Ab on its long-term safety, drug–drug interaction, or synergistic effects and male osteoporosis of Scl-Ab treatment may provide new horizons for the osteoporosis as well as osteoporotic fracture treatment.

Conflicts of interest

All the authors declare that they have no conflicts of interest.

Funding/support

This study was supported by SMART Program, Lui Che Woo Institute of Innovative Medicine, Faculty of Medicine, The Chinese University of Hong Kong.

References

[1] Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. J Bone Miner Res 2007;22:465–75.
[2] Johnell O, Kanis J. Epidemiology of osteoporotic fractures. Osteoporos Int 2005;16(Suppl. 2):S3–7.
[3] Kanis JA, McCloskey EV, Johansson H, Oden A, Melton Jr L, Khaltaev N. A reference standard for the description of osteoporosis. Bone 2008;42:467–75.
[4] Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO study group. World Health Organ Tech Rep Ser 1994;843:1–129.
[5] Chau PH, Wong M, Lee A, Ling M, Woo J. Trends in hip fracture incidence and mortality in Chinese population from Hong Kong 2001–09. Age Ageing 2013;42:229–33.
[6] Kwok AW, Gong JS, Wang YX, Leung JC, Kwok T, Griffith JH, et al. Prevalence and risk factors of radiographic vertebral fractures in elderly Chinese men and women: results of Mr. OS (Hong Kong) and Ms. OS (Hong Kong) studies. Osteoporos Int 2013;24:877–85.
[7] Russell RG, Watts NB, Ebetino FH, Roger MJ. Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. Osteoporos Int 2008;19:733–59.
[8] Baron R, Ferrari S, Russell RG. Denosumab and bisphosphonates: different mechanisms of action and effects. Bone 2011;48:677–92.
[9] Suresh E, Pazianas M, Abrahamsen B. Safety issues with bisphosphonate therapy for osteoporosis. Rheumatology 2014;53:19–31.
[10] Kharazmi M, Hallberg P, Warfvinge K, Michaelsson K. Risk of atypical femoral fractures and osteonecrosis of the jaw associated with alendronate use compared with other oral bisphosphonates. Rheumatology 2014;53:1911–3.
[11] Rasmusson L, Abtahi J. Bisphosphonate associated osteonecrosis of the jaw: an update on pathophysiology, risk factors, and treatment. Int J Dent 2014;2014:471035.
[12] McDonald MM, Dulai S, Godfrey C, Amanat N, Sztynda T, Little DG. Bolus or weekly zoledronic acid administration does not delay endochondral fracture repair but weekly dosing enhances delays in hard callus remodeling. Bone 2008;42:653–62.
[13] McDonald MM, Morse A, Mikulec K, Peacock L, Baldock PA, Kostenuik PJ, et al. Matrix metalloproteinase-driven endochondral fracture union proceeds independently of osteoclast activity. J Bone Miner Res 2013;28:1550–60.
[14] Fu LJ, Tang TT, Hao YQ, Dai KR. Long-term effects of alendronate on fracture healing and bone remodeling of femoral shaft in ovariectomized rats. Acta Pharmacol Sinica 2013;34:387–92.
[15] Savardias T, Wallace RJ, Salter DM, Simpson AH. Do bisphosphonates inhibit direct fracture healing?: a laboratory investigation using an animal model. Bone Joint J 2013;95-B:1263–8.
[16] Xue D, Li F, Chen G, Yan S, Pan Z. Do bisphosphonates affect bone healing? A meta-analysis of randomized controlled trials. J Orthop Surg Res 2014;9:45.
[17] Uhlehn AV, Leder BZ. Anabolic therapies for osteoporosis. Endocrinol Metab Clin North Am 2012;41:507–25.
[18] Yu B, Zhao X, Yang C, Crane J, Xian L, Lu W, et al. Parathyroid hormone induces differentiation of mesenchymal stromal/stem cells by enhancing bone morphogenetic protein signaling. J Bone Miner Res 2012;27:2001–14.
[19] Jilka RL, Weinstein RS, Bellido T, Roberson P, Parfitt AM, Manolagas SC. Increased bone formation by prevention of
osteoblast apoptosis with parathyroid hormone. J Clin Invest 1999;104:439–46.

[20] Canalis E, Giustina A, Bilezikian JP. Mechanisms of anabolic therapies for osteoporosis. N Engl J Med 2007;357:905–16.

[21] Vahle JL, Long GG, Sandusky G, Westmore M, Ma YL, Sato M. Bone neoplasms in F344 rats given teriparatide [rhPTH(1-34)] are dependent on duration of treatment and dose. Toxicol Pathol 2004;32:426–38.

[22] Watanabe A, Yoneyama S, Nakajima M, Sato N, Takao-Kawabata R, Isogai Y, et al. Osteosarcoma in Sprague–Dawley rats after long-term treatment with teriparitide (human parathyroid hormone 1-34). J Toxicol Sci 2012;37:617–29.

[23] Padhi D, Allison M, Kivitz AJ, Gutierrez MJ, Stouch B, Wang C, et al. Multiple doses of sclerostin antibody romosozumab in healthy women and postmenopausal women with low bone mass: a randomized, double-blind, placebo-controlled study. J Clin Pharmacol 2014;54:168–78.

[24] McClung MR, Grauer A, Boonen S, Bolognese MA, Brown JP, Seeman E, Delmas PD. Bone quality: the material and structural basis of bone strength and fragility. N Engl J Med 2014;370:412–20.

[25] Seeman E, Delmas PD. Bone quality—the material and structural basis of bone strength and fragility. N Engl J Med 2006;354:2250–61.

[26] Katagiri T, Takahashi N. Regulatory mechanisms of osteoblast and osteoclast differentiation. Oral Dis 2002;8:147–59.

[27] Feng X, McDonald JM. Disorders of bone remodeling. Annu Rev Med 2003;54:435–53.

[28] Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Katagiri T, Takahashi N. Regulatory mechanisms of osteoblast and osteoclast differentiation. Oral Dis 2002;8:147–59.

[29] Watanabe A, Yoneyama S, Nakajima M, Sato N, Takao-Kawabata R, Isogai Y, et al. Osteosarcoma in Sprague–Dawley rats after long-term treatment with teriparitide (human parathyroid hormone 1-34). J Toxicol Sci 2012;37:617–29.

[30] Martin TJ, Sims NA. Osteoclast-derived activity in the human parathyroid hormone 1-34). J Toxicol Sci 2012;37:617–29.
Sclerostin in osteoporosis and treatment

Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. Proc Natl Acad Sci U. S. A 2008;105:20764–9.

[56] Ota K, Quint P, Ruan M, Pederson L, Westendorf JJ, Khosla S, et al. Sclerostin is expressed in osteoclasts from aged mice and reduces osteoclast-mediated stimulation of mineralization. J Cell Biochem 2013;114:1901–7.

[57] van Bezoelen RL, Roelen BA, Visser A, van der Wee-Pals L, de Will T, Kerperien M, et al. Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. J Exp Med 2004;199:805–14.

[58] Modder UI, Hoey KA, Amin S, McCready LK, Achenbach SJ, Riggs BL, et al. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res 2011;26:373–9.

[59] Garnero P, Sornay-Rendu E, Munoz F, Borel O, Chapurlat RD. 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 2013;24:489–94.

[60] Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. J Clin Invest 2006;116:1202–9.

[61] Morvan F, Boulukos K, Clement-Lacroix P, Roman Roman S, Agholme F, Aspenberg P. Wnt signaling and orthopedics, an overview. Acta Orthop 2011;82:125–30.

[62] Cidem M, Karacan I, Arat NB, Ozkaya O, Guzel SP, et al. Serum sclerostin is decreased following vitamin D treatment in young vitamin D-deficient female adults. Rheumatol Int 2015;35:1991–7.

[63] Wijenayaka AR, Yang D, Prideaux M, Ito N, Kogawa M, Anderson PH, et al. 1α,25-dihydroxyvitamin D stimulates human SOST gene expression and sclerostin secretion. Mol Cell Endocrinol 2015;413:157–67.

[64] Ryan ZC, Ketha H, McNulty MS, McGee-Lawrence M, Craig TA, Grande JP, et al. Sclerostin alters serum vitamin D metabolite and fibroblast growth factor 23 concentrations and the urinary excretion of calcium. Proc Natl Acad Sci U. S. A 2013;110:6199–204.

[65] Gottlman D. Inferences from genetically modified mouse models on the skeletal actions of vitamin D. J Steroid Biochem Mol Biol 2015;148:219–24.

[66] Boneviald LF, Johnson ML. Osteocytes, mechanosensing and Wnt signaling. Bone 2008;42:606–15.

[67] Ara-Castillo N, Kim-Weroha NA, Kamel MA, Javaheri B, Holst C, Krumlauf RE, et al. Identification of the first deletion in the LRP5 gene in a patient with autosomal dominant osteopetrosis. J Bone Miner Res 2011;26:2812

[68] O'Brien CA, Plotkin LI, Galli C, Goellner JJ, Gortazar AR, Rooney DC, Yellowley CE, Loots GG. Prostaglandin E2 signals through PTGER2 to regulate sclerostin expression. PLoS One 2011;6:e17772.

[69] Galea GL, Sunters A, Meakin LB, Zaman G, Sugiyama T, Lanyon LE, et al. Sost down-regulation by mechanical strain in human osteoblast cells involves PGE2 signaling via EP4. FEBS Lett 2011;585:2450–4.

[70] Zhou S. TGF-beta regulates beta-catenin signaling and osteoblast differentiation in human mesenchymal stem cells. J Cell Biochem 2011;112:1651–60.

[71] Genetos DC, Yellowley CE, Loo CG. Prostaglandin E2 (PGE2) regulates sclerostin expression. PLoS One 2011;6:e17772.

[72] O'Brien CA, Plotkin LI, Galli C, Goellner JJ, Gortazar AR, Rooney DC, Yellowley CE, Loots GG. Prostaglandin E2 signals through PTGER2 to regulate sclerostin expression. PLoS One 2011;6:e17772.

[73] Galea GL, Sunters A, Meakin LB, Zaman G, Sugiyama T, Lanyon LE, et al. Sost down-regulation by mechanical strain in human osteoblast cells involves PGE2 signaling via EP4. FEBS Lett 2011;585:2450–4.

[74] Zhou S. TGF-beta regulates beta-catenin signaling and osteoblast differentiation in human mesenchymal stem cells. J Cell Biochem 2011;112:1651–60.

[75] Otal P, Martin M, Lebleu B, Sakane H, Yamamoto H, Kikuchi A. LRP6 is internalized by endocytic recycling. Nat Rev Mol Cell Biol 2009;10:597–608.

[76] Holdsworth G, Slocombe P, Doyle C, Sweeney B, Veverka V, Le Riche K, et al. Characterization of the interaction of sclerostin with the low density lipoprotein receptor-related protein (LRP) family of Wnt co-receptors. J Cell Biochem 2012;112:26464–77.

[77] Dawson-Hughes B, Harris SS, Ceglia L, Palermo NJ. Effect of supplemental vitamin D and calcium on serum sclerostin levels. Eur J Endocrinol 2014;170:645–50.

[78] Zanetti M, Marhold J, et al. Characterization of the interaction of sclerostin with the low density lipoprotein receptor-related protein (LRP) family of Wnt co-receptors. J Cell Biochem 2012;112:26464–77.

[79] Dawson-Hughes B, Harris SS, Ceglia L, Palermo NJ. Effect of supplemental vitamin D and calcium on serum sclerostin levels. Eur J Endocrinol 2014;170:645–50.

[80] Nakamura H, Yamamoto H, Sakane H, Yamamoto H, Kikuchi A. LRP6 is internalized by endocytic recycling. Nat Rev Mol Cell Biol 2009;10:597–608.

[81] Quimon J, et al. Anti-Sclerostin antibody inhibits internalization of Dkk1 to suppress its phosphorylation in the lipid raft and is important for the formation of Dkk1 oligomers containing its receptors, Frizzled and LRP. Development 2004;131:503–15.

[82] Alaswad MS, Al-Kadi HA, Rouzi AA, Qari MH. Determinants of serum sclerostin levels in healthy pre- and postmenopausal women. J Bone Miner Res 2011;26:2812–22.
[93] Drake MT, Srinivasan B, Modder UL, Peterson JM, McCready LK, Riggs BL, et al. Effects of parathyroid hormone treatment on circulating sclerostin levels in postmenopausal women. J Clin Endocrinol Metab 2010;95:5056–62.

[94] Piemonte S, Romagnoli E, Bratengeier C, Woloszczuk W, Tancredi A, Pepe J, et al. Serum sclerostin levels decline in post-menopausal women with osteoporosis following treatment with intermittent parathyroid hormone. J Endocrinol Invest 2012;35:866–8.

[95] Collette NM, Genetos DC, Economides AN, Xie L, Shahnazari M, Yao W, et al. Targeted deletion of Sost distal enhancer increases bone formation and bone mass. Proc Natl Acad Sci U. S. A. 2012;109:14092–7.

[96] Kramer I, Baertschi S, Halleux C, Keller H, Knessel M. Mef2c deletion in osteocytes results in increased bone mass. J Bone Miner Res 2012;27:360–73.

[97] Leupin O, Kramer I, Collette NM, Loots GG, Natt F, Knessel M, et al. Control of the SOST bone enhancer by PTH using MEF2 transcription factors. J Bone Miner Res 2007;22:1957–67.

[98] Wein MN, Spatz J, Nishimori S, Doench J, Root D, Babij P, et al. HDAC5 controls MEF2C-driven sclerostin expression in osteocytes. J Bone Miner Res 2015;30:400–11.

[99] Baertschi S, Baur N, Luenders-Lefevre V, Voshol J, Keller H. Class I and IIa histone deacetylases have opposite effects on sclerostin gene regulation. J Biol Chem 2014;289:24995–5009.

[100] Charopoulos I, Orme S, Giannoudis PV. The role and efficacy of denosumab in the treatment of osteoporosis: an update. Expert Opin Drug Saf 2011;10:205–17.

[101] Bone HG, Dempster DW, Eisman JA, Greenspan SL, McClung MR, Nakamura T, et al. Odanacatib for the treatment of postmenopausal osteoporosis: development history and design and participant characteristics of LOFT, the Long-Term Odanacatib Fracture Trial. Osteoporos Int 2015;26:699–712.

[102] Dempster DW, Lambing CL, Kostenuik PJ, Grauer A. Role of RANK ligand and denosumab, a targeted RANK ligand inhibitor, in bone health and osteoporosis: a review of preclinical and clinical data. Clin Ther 2012;34:521–36.

[103] Miller PD, Wagman RB, Peacock M, Lewiecki EM, Bolognese MA, Weinstein RL, et al. Effect of denosumab on bone mineral density and biochemical markers of bone turnover: six-year results of a phase 2 clinical trial. J Clin Endocrinol Metab 2011;96:394–402.

[104] Cummings SR, San Martin J, McClung MR, Siris ES, Eastell R, Reed IR, et al. Denosumab for prevention of fractures in women. J Clin Endocrinol Metab 2010;95:5056–62.

[105] Leung P, Pickarski M, Zhuo Y, Masarachia PJ, Duong LT. The effects of the cathepsin K inhibitor odanacatib on osteoclastic bone resorption and vesicular trafficking. Bone 2011;49:623–35.

[106] Pennypacker B, Shea M, Liu Q, Masarachia P, Saftig P, Rodan S, et al. Bone density, strength, and formation in adult cathepsin K (−/−) mice. Bone 2009;44:199–207.

[107] Pennypacker BL, Duong le T, Cusick TE, Masarachia PJ, Gentile MA, Gauthier JY, et al. Cathepsin K inhibitors prevent bone loss in estrogen-deficient rabbits. J Bone Miner Res 2011;26:252–62.

[108] Bone HG, McClung MR, Roux C, Recker RR, Eisman JA, Verbruggen N, et al. Odanacatib, a cathepsin-K inhibitor for osteoporosis: a two-year study in postmenopausal women with low bone density. J Bone Miner Res 2010;25:937–47.

[109] Langdahl B, Binkley N, Bone H, Gilchrist N, Resch H, Rodriguez Portales J, et al. Odanacatib in the treatment of postmenopausal women with low bone mineral density: five years of continued therapy in a phase 2 study. J Bone Miner Res 2012;27:2251–8.

[110] Ominsky MS, Vlasseros F, Jolette J, Smith SY, Stouch B, Doelgast G, et al. Two doses of sclerostin antibody in cynomolgus monkeys increases bone formation, bone mineral density, and bone strength. J Bone Miner Res 2010;25:948–59.

[111] Tian X, Lee WS, Li X, Paszty C, Ke HZ. Sclerostin antibody increases bone mass by stimulating bone formation and inhibiting bone resorption in a hindlimb-immobilization rat model. Bone 2011;48:197–201.

[112] Li X, Ominsky MS, Warnington KS, Morony S, Gong J, Cao J, et al. Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. J Bone Miner Res 2009;24:578–88.

[113] Li X, Warnington KS, Niu QT, Asuncion FJ, Barrero M, Grisanti M, et al. Inhibition of sclerostin by monoclonal antibody increases bone formation, bone mass, and bone strength in aged male rats. J Bone Miner Res 2010;25:2647–56.

[114] Ominsky MS, Li C, Li X, Tan HL, Lee E, Barrero M, et al. Inhibition of sclerostin by monoclonal antibody enhances bone healing and improves bone density and strength of non-fractured bones. J Bone Miner Res 2011;26:1012–21.

[115] Tian X, Setterberg RB, Li X, Paszty C, Ke HZ, Jee WS. Treatment with a sclerostin antibody increases cancellous bone formation and bone mass regardless of marrow composition in adult female rats. Bone 2010;47:529–33.

[116] Rossini M, Gatti D, Adami S. Involvement of WNT/beta-catenin signaling in the treatment of osteoporosis. Calcif Tissue Int 2013;93:121–32.

[117] Spatz JM, Eilman R, Cloutier AM, Louis L, van Vliet M, Suva LJ, et al. Sclerostin antibody inhibits skeletal deterioration due to reduced mechanical loading. J Bone Miner Res 2013;28:865–74.

[118] Agholme F, Li X, Isaksson H, Ke HZ, Aspenberg P. Sclerostin antibody treatment enhances metaphyseal bone healing in rats. J Bone Miner Res 2010;25:2412–8.

[119] Suen PK, He YX, Chow DH, Huang L, Li C, Ke HZ, et al. Sclerostin monoclonal antibody enhanced bone fracture healing in an open osteotomy model in rats. J Orthop Res 2014;32:997–1005.

[120] McDonald MM, Morse A, Mikulec K, Peacock L, Yu N, Baldock PA, et al. Inhibition of sclerostin by systemic treatment with sclerostin antibody enhances healing of proximal tibial defects in ovariectomized rats. J Orthop Res 2012;30:1541–8.

[121] Padhi D, Jang G, Stouch B, Fang L, Posvar E. Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody for treatment of osteoporosis: an update. J Cell Biochem 2014;115:625–36.

[122] Stolina M, Dwyer D, Niu QT, Villasenor KS, Kurimoto P, Grisanti M, et al. Temporal changes in systemic and local expression of bone turnover markers during six months of sclerostin antibody administration to ovariectomized rats. J Bone Miner Res 2011;26:19–26.

[123] Stolina M, Dwyer D, Niu QT, Villasenor KS, Kurimoto P, Grisanti M, et al. Developmental changes in systemic and local expression of bone turnover markers during six months of sclerostin antibody administration to ovariectomized rats. J Bone Miner Res 2012;27:2251–8.

[124] Cai Y, Cai T, Chen Y. Wnt pathway in osteosarcoma, from oncogenic to therapeutic. J Cell Biochem 2014;115:625–31.

[125] Li X, Ominsky MS, Warnington KS, Niu QT, Asuncion FJ, Barrero M, et al. Increased bone formation and bone mass induced by sclerostin antibody is not affected by pretreatment or cotreatment with alendronate in osteopenic, ovariectomized rats. Endocrinology 2011;152:3312–20.

[126] Qiang YW, Barlogie B, Rudikoff S, Shaughnessy Jr JD. Dkk1-induced inhibition of Wnt signaling in osteoblast differentiation is an underlying mechanism of bone loss in multiple myeloma. Bone 2008;42:669–80.
Glantschnig H, Scott K, Hampton R, Wei N, McCracken P, Nantermet P, et al. A rate-limiting role for Dickkopf-1 in bone formation and the remediation of bone loss in mouse and primate models of postmenopausal osteoporosis by an experimental therapeutic antibody. J Pharmacol Exp Ther 2011;338:568–78.

Eastell R, Boyle IT, Compston J, Cooper C, Fogelman I, Francis RM, et al. Management of male osteoporosis: report of the UK Consensus Group. QJM 1998;91:71–92.

Legrand E, Chappard D, Pascaretti C, Duquenne M, Rondeau C, Simon Y, et al. Bone mineral density and vertebral fractures in men. Osteoporos Int 1999;10:265–70.

Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int 2006;17:1726–33.

Giusti A, Bianchi G. Treatment of primary osteoporosis in men. Clin Interv Aging 2015;10:105–15.

Niimi R, Kono T, Nishihara A, Hasegawa M, Matsumine A, Sudo A. Analysis of daily teriparatide treatment for osteoporosis in men. Osteoporos Int 2015.

Feng G, Chang-Qing Z, Yi-Min C, Xiao-Lin L. Systemic administration of sclerostin monoclonal antibody accelerates fracture healing in the femoral osteotomy model of young rats. Int Immunopharmacol 2015;24:7–13.

Li C, Ominsky MS, Tan HL, Barrero M, Niu QT, Asuncion FJ, et al. Increased callus mass and enhanced strength during fracture healing in mice lacking the sclerostin gene. Bone 2011;49:1178–85.

Morse A, Yu NY, Peacock L, Mikulec K, Kramer I, Kneissel M, et al. Endochondral fracture healing with external fixation in the Sost knockout mouse results in earlier fibrocartilage callus removal and increased bone volume fraction and strength. Bone 2015;71:155–63.

Silkstone D, Hong H, Alman BA. Beta-catenin in the race to fracture repair: in it to Wnt. Nat Clin Pract Rheumatol 2008;4:413–9.

Chen Y, Whetstone HC, Lin AC, Nadesan P, Wei Q, Poon R, et al. Beta-catenin signaling plays a disparate role in different phases of fracture repair: implications for therapy to improve bone healing. PLoS Med 2007;4:e249.

McGee-Lawrence ME, Ryan ZC, Carpio LR, Kakar S, Westendorf JJ, Kumar R. Sclerostin deficient mice rapidly heal bone defects by activating beta-catenin and increasing intramembranous ossification. Biochem Biophys Res Commun 2013;441:886–90.

Jin H, Wang B, Li J, Xie W, Mao Q, Li S, et al. Anti-DKK1 antibody promotes bone fracture healing through activation of beta-catenin signaling. Bone 2015;71:63–75.

Hankenson KD, Dishowitz M, Gray C, Schenker M. Angiogenesis in bone regeneration. Injury 2011;42:556–61.

Gruber R, Koch H, Doll BA, Tegtmeyer F, Einhorn TA, Hollinger JO. Fracture healing in the elderly patient. Exp Gerontol 2006;41:1080–93.