Sclerostin and Dickkopf-1 as Therapeutic Targets in Bone Diseases

Hua Zhu Ke, William G. Richards, Xiaodong Li, and Michael S. Ominsky

Metabolic Disorders Research, Amgen Inc., Thousand Oaks, California 91320

The processes of bone growth, modeling, and remodeling determine the structure, mass, and biomechanical properties of the skeleton. Dysregulated bone resorption or bone formation may lead to metabolic bone diseases. The Wnt pathway plays an important role in bone formation and regeneration, and expression of two Wnt pathway inhibitors, sclerostin and Dickkopf-1 (DKK1), appears to be associated with changes in bone mass. Inactivation of sclerostin leads to substantially increased bone mass in humans and in genetically manipulated animals. Studies in various animal models of bone disease have shown that inhibition of sclerostin using a monoclonal antibody (Scl-Ab) increases bone formation, density, and strength. Additional studies show that Scl-Ab improves bone healing in models of bone repair. Inhibition of DKK1 by monoclonal antibody (DKK1-Ab) stimulates bone formation in younger animals and to a lesser extent in adult animals and enhances fracture healing. Thus, sclerostin and DKK1 are emerging as the leading new targets for anabolic therapies to treat bone diseases such as osteoporosis and for bone repair. Clinical trials are ongoing to evaluate the effects of Scl-Ab and DKK1-Ab in humans for the treatment of bone loss and for bone repair. **(*Endocrine Reviews* 33: 747–783, 2012)**

I. Introduction

Skeletal mass, structure, and biomechanical properties are controlled by the processes of bone growth, modeling, and remodeling (1, 2). Bone growth and modeling

Abbreviations: AAV, Adeno-associated virus; ALN, alendronate; AS, ankylosing spondylitis; BAC, bacterial artificial chromosome; BFR, bone formation rate; BFR/BS, BFR/bone surface; BMD, bone mineral density; BMP, bone morphogenetic protein; CTx-1, C-terminal telopeptide of type 1 collagen; cyno, cynomolgus monkey; DKK1, Dickkopf-related protein 1; DKK1-Ab, DKK1 antibody; DXA, dual-energy x-ray absorptiometry; hPTH, human PTH; hTNFtg, human TNF transgenic; KO, knockout; LRP, low-density lipoprotein receptor-related protein; OA, osteoarthritis; OI, osteogenesis imperfecta; OPG, osteoprotegerin; OVX, ovariectomized, or ovariectomy; P1NP, procollagen type 1 N-terminal propeptide; pQCT, peripheral quantitative computed tomography; RA, rheumatoid arthritis; RANKL, receptor activator of nuclear factor-κB ligand; Scl-Ab, sclerostin antibody; siRNA, small interfering RNA; TNFα-Ab, TNFα antibody; TRACP5b, tartrate-resistant acid phosphatase form 5b; WT, wild-type.

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are dominant during growth and development and are the determinant factors for skeletal size and shape, whereas bone remodeling plays an important role in maintaining skeletal mass and structure during adulthood and aging.

Osteoclasts and osteoblasts are two groups of cells that resorb and form bone, respectively. Osteocytes, a third bone cell type, play an important role in regulating bone resorption and bone formation activities of osteoclasts and osteoblasts (3). Understanding the communications among these cell types in controlling bone resorption and bone formation is the subject of ongoing research. Many biochemical and biomechanical factors play important roles in regulating these processes. Dysregulated bone resorption and/or bone formation may lead to metabolic bone diseases, such as primary osteoporosis, secondary osteoporosis (inflammation-induced bone loss, drug treatment-induced bone loss, and other conditions associated with lower bone mass), and multiple myeloma. In addition, bone resorption and bone formation play important roles in bone repair and regeneration (4, 5). Therefore, alterations in bone resorption and bone formation may also lead to delayed fracture healing or nonunion.

Osteoporosis is a skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture (6). Osteoporosis is a global health problem that is growing in significance as the population of the world both increases and ages. In the United States, approximately 10 million Americans older than 50 yr of age have osteoporosis, and about 1.5 million fragility fractures occur each year. It is estimated that one in two women and one in five men aged 50 yr will have an osteoporotic fracture in their remaining lifetime (7).

Significant advances have been made in discovering and developing effective pharmacological therapies for osteoporosis. Currently, two classes of agents are used for treatment of osteoporosis: antiresorptive agents that target osteoclasts and inhibit bone resorption and destruction, and anabolic agents that target osteoblasts and stimulate bone formation and rebuilding of bone mass and structure. Approved antiresorptive agents include estrogen, bisphosphonates, selective estrogen receptor modulators, a receptor activator of nuclear factor-κB ligand (RANKL) inhibitor, and others (8, 9). Most recently, a cathepsin-K inhibitor, a new antiresorptive, has demonstrated efficacy in reducing bone resorption and increasing bone mineral density (BMD) in a phase 2 clinical trial (10, 11). Clinical trials to evaluate the efficacy of cathepsin-K inhibitors in reducing fracture risk in postmenopausal women are ongoing. Although some of these antiresorptive agents have shown good efficacy in reducing bone resorption, increasing BMD, and decreasing risk for skeletal fragility fractures, their ability to stimulate bone formation and rebuild bone structure and strength to the levels of young, healthy adults remains to be studied.

The only bone anabolic agents approved for the treatment of osteoporosis are human PTH [hPTH (1–84) and hPTH (1–34)]. Results from preclinical and clinical studies showed that PTH stimulates both bone formation and bone resorption, resulting in net gains in BMD and reduced fracture risk [see review by Kawai *et al.* (8)]. Therapeutic PTH is given as a daily sc injection, and its use is limited to 2-yr duration due to observations of induction of osteosarcoma in long-term rat studies. Thus, additional bone anabolic agents are needed to better manage osteoporosis and other metabolic bone diseases. Therefore, a great medical need still exists for bone anabolic agents that are easy to administer and that have minimal safety concerns.

Skeletal fractures may occur as a consequence of trauma as well as fragility and represent a significant public health problem. Over 6 million adults suffer fractures in the Unites States annually. These fractures can be associated with significant morbidity, health care utilization, and costs (12), in particular when fractures are associated with poor outcomes (*e.g.*, nonunion, malunion, revision surgery, reduced function, work absence). The incidence of impaired or nonunion fractures has been reported to be 5–10% of all fractures (13). Biological therapies, such as local application of bone morphogenetic proteins (BMP) (14–16), were developed to accelerate fracture healing and reduce fracture-associated complications. However, to date there are no approved systemic therapies to accelerate fracture healing and reduce fracture-associated complications. Agents that promote bone formation may provide benefits in healing delayed union or nonunion fractures and accelerating normal fracture healing, thus reducing the associated burden.

Recent research has identified key pathways involved in skeletal metabolism. For example, the Wingless-type mouse mammary tumor virus integration site (Wnt) pathway plays an important role in bone formation and regeneration (17–20). Loss-of-function mutations in low-density lipoprotein receptor-related protein (LRP) 5 and LRP6, which function as coreceptors for Wnts, decrease BMD, whereas gain-of-function mutations of LRP5 increase BMD in rodents and humans (21–24). Secreted Wnt inhibitors such as sclerostin and Dickkopf-related protein 1 (DKK1) bind to coreceptors LRP5/6 and inhibit their association with Wnts, whereas secreted Frizzled-related proteins and other Wnt inhibitors, such as Wnt inhibitory factor-1, directly interact with Wnts and Frizzled receptors to interrupt binding of Wnts to LRP5/6.

Increased BMD is observed in individuals with the high bone mass disorder sclerosteosis (OMIM 269500), which results from loss-of-function mutations in the sclerostin gene (*SOST*) (25, 26). Another high bone mass disorder, Van Buchem disease (OMIM 239100), which has a phenotype that is similar in many aspects to sclerosteosis, is caused by a 52-kb deletion of genomic DNA approximately 35 kb downstream of the *SOST* (27). In mice, overexpression of sclerostin or DKK1 resulted in lower BMD due to lower rates of bone formation (28–33), whereas deletion of sclerostin or haploinsufficiency of DKK1 led to increased BMD due to higher rates of bone formation (29, 34–39).

In addition to regulation of bone mass and bone formation, recent evidence has suggested an important role for Wnt signaling in fracture repair. Stimulation of Wnt signaling by LiCl (40) or Wnt3a administration (41) results in enhanced bone healing in mice. Conversely, inhibition of Wnt signaling via knockout (KO) of the LRP5 gene (42), recombinant DKK1 administration, or adenoviral overexpression of DKK1 in mice impaired the healing response (40, 43). Furthermore, increased DKK1 expression was found in stromal cells collected from human nonunion fractures, suggesting that Wnt inhibition may impede complete healing (44).

This evidence supports the conclusion that Wnt pathway activation promotes bone formation and bone healing, making Wnt inhibitors attractive therapeutic targets for the treatment of skeletal disorders such as osteoporosis and bone healing. The goal of this review is to summarize the current literature on the role of sclerostin and DKK1 in skeletal physiology and the potential utility of monoclonal antibodies targeting sclerostin or DKK1 in the treatment of conditions associated with bone loss and bone repair.

II. Mechanism of Action of Sclerostin and Dickkopf-1 (DKK1)

Wnt signaling utilizes canonical and noncanonical pathways to establish the metazoan body plan and regulate postnatal physiology (http://www.stanford.edu/group/nusselab/cgi-bin/Wnt/). Canonical Wnt signaling employs extracellular Wnt ligands that bind Frizzled and LRP5/6 coreceptors at the cell surface to transduce a signal that results in the intracellular activation of β-catenin. Components of the Wnt signaling pathway are evolutionarily well-conserved and are found in primitive metazoans (46–48). The Wnt antagonist DKK1 was expressed by early invertebrates (49, 50), whereas sclerostin did not appear until the emergence of bony vertebrates (http://uswest.ensembl.org/Homo_sapiens/Gene/Compara_Tree?g=ENSG00000167941;r=17:41831103-41836156;t=ENST00000301691), which may suggest a more defined role of sclerostin in the development and maintenance of the skeleton, with a broader role for DKK1.

Sclerostin was initially characterized as a BMP antagonist, based primarily on its homology to the DAN family of cystine knot-containing proteins, although it has now been well-established that sclerostin can modulate Wnt signaling (17, 20, 52). Sclerostin and DKK1 interact with the extracellular domains on LRP5 (www.uniprot.org/uniprot/O75197) and LRP6 (www.uniprot.org/uniprot/O75581) to competitively prevent the binding of various Wnt ligands to these coreceptors. The seminal discoveries that osteoporosis pseudoglioma syndrome (OMIM 259770) and a high bone mass syndrome (OMIM 144750) were caused by loss-of-function and gain-of-function mutations in LRP5, respectively, provided the first insight into the profound role of Wnt signaling in the establishment and maintenance of the human skeleton (21–23). The high bone mass-causing mutation in LRP5 (G171V) residing in blade four of the first β-propeller motif blocked the ability of DKK1 to inhibit Wnt signaling through this receptor. Subsequently, six additional high bone mass-causing mutations in LRP5 were identified, and all localized to the first β-propeller motif (54, 55), demonstrating that these mutations also impaired DKK1 Wnt inhibitory activity and binding to LRP5. In addition, a recent report described the first deletion in LRP5 that resulted in a high bone mass phenotype; this mutation produced an in-frame deletion of two amino acid residues, including glycine 171, within the first β-propeller domain of LRP5 (56).

Recognition of the phenotypic similarities between patients with high bone mass resulting from dominant gain-of-function mutations in LRP5 and patients with sclerosteosis or Van Buchem disease (57) led researchers to ask whether sclerostin functioned within the Wnt signaling pathway and whether it interacted with LRP5 in a manner analogous to DKK1. Initial studies conducted to address this hypothesis failed to demonstrate that sclerostin could directly inhibit Wnt3a activity in C3H10T1/2 cells (58) and instead showed that sclerostin inhibited BMP-6-induced osteogenesis, further supporting the argument that sclerostin was a BMP inhibitor. The authors concluded that sclerostin acted upon BMP induced by Wnt3a to indirectly block Wnt-induced osteogenesis (58). Conversely, another study published later that year showed that sclerostin could directly inhibit Wnt signaling in LRP5-transfected 293 cells and in MC3T3 osteoblastic cells (59). The authors demonstrated that sclerostin bound LRP5/6 and defined the binding site within the first two β-propel-

ler and epidermal growth factor-like domains, the same region identified in the LRP5 high bone mass-causing mutations.

Other groups examined the effect of high bone mass mutations in LRP5 and LRP6 on sclerostin binding. Ellies *et al.* (60) demonstrated that sclerostin could inhibit Wnt8-induced secondary axis formation in *Xenopus* embryos and Wnt activity in mammalian cell lines. Furthermore, they demonstrated that the high bone mass-causing G171V mutation in LRP5 and an engineered mutation, G158V, at the conserved residue in LRP6 prevented sclerostin binding in cells expressing these variants. These findings were subsequently confirmed and extended to show that other high bone mass-causing mutations in LRP5, with the exception of R154M, abolished the ability of sclerostin to physically interact with this receptor (61) and inhibit the Wnt pathway. Balemans *et al.* (62) compared side by side the ability of high bone mass mutations to impair the activity of DKK1 and sclerostin on Wnt pathway inhibition and demonstrated a mutation-dependent decrease in the activity of both of these proteins at LRP5. The preponderance of data suggests that high bone mass syndromes resulting from mutations in LRP5 and diseases resulting from mutations in the *SOST* (sclerosteosis and Van Buchem disease) share a common underlying mechanism.

Structural insight into the regions of the sclerostin protein that are responsible for its Wnt inhibitory activity have come from the identification of the solution structure of the molecule by nuclear magnetic resonance imaging (63, 64) and the generation of antibodies that neutralize the inhibitory activity of sclerostin on Wnt signaling (63, 65). Sclerostin is an atypical member of the cystine-knot containing family of proteins. Unlike other members, there is no evidence that sclerostin forms homo- or heterodimers, and it contains largely disordered amino- and carboxy-termini. The central core of sclerostin comprises the cystine knot and three loop regions. Loops 1 and 3 are twisted antiparallel β-sheets that form finger-like structures; the loop 2 region of sclerostin is without an organized nuclear magnetic resonance structure, but it may adopt a more constrained structure when bound to a ligand or receptor. Antibodies that prevent the ability of sclerostin to inhibit Wnt signaling bind loop 2, implying that loop 2 is critical for the binding of sclerostin to LRP5/6 (63).

Recent data have provided additional information regarding the complexity of Wnt signaling at LRP5/6 (63, 66–68). These studies described distinct ligand binding domains on LRP5/6 receptors that recognize different classes of Wnt proteins and inhibitors. The Wnt1 class, which is composed of Wnt 1, 2, 6, 7a, 7b, 9a, 9b, and 10b, binds β-propeller 1 of LRP5/6 (see illustration in Fig. 1). The Wnt3 class encompasses Wnt3 and 3a, and binds β-propeller 3 of LRP5/6 (Fig. 1). DKK1 was shown to bind independently to LRP5/6 fragments containing both the first and the third β-propeller regions (66), whereas sclerostin bound only the region containing the first β-propeller of LRP6 (67) (Fig. 1). As predicted by the binding studies, DKK1 inhibited both the Wnt1 and Wnt3 classes, whereas sclerostin only inhibited the Wnt1 class and not the Wnt3 class. In fact, an enhancement, as opposed to inhibition, of Wnt3a signaling was observed with sclerostin (67).

The recently described crystal structure of LRP6 confirmed that the third β-propeller motif of LRP6 binds DKK1 (69). An additional crystallography study that further defined the interaction of DKK1 and sclerostin with the first β-propeller of LRP6 identified a conserved amino acid motif, NXI, present in both sclerostin and DKK1 that is responsible for mediating that interaction (70). These findings and those mentioned in the previous paragraph underscore the mechanistic differences between sclerostin and DKK1 and may suggest that sclerostin has evolved as a more refined regulator of Wnt signaling, whereas DKK1, given its more ancient phylogeny and pan-Wnt inhibitory activity, may function more widely as a brake on Wnt signaling. Such findings have potentially important ramifications for bone biology; when the system is constantly responding to stress and mechanistic forces, a molecule like sclerostin, which is more selective in its activity and restricted in its expression (see *Section III*) may function more selectively and significantly in normal skeletal physiology.

In addition to binding LRP5/6, both sclerostin and DKK1 bind other transmembrane molecules to augment their inhibitory activity on the Wnt signaling pathway. Another member of the LRP family, LRP4 (http://www.uniprot.org/uniprot/O75096), also known as Megf7, has recently been described as a binding partner for sclerostin and DKK1 (72). Initial insight into the function of LRP4 in bone biology came from LRP4 KO mice that exhibited limb abnormalities (73). The positional cloning of LRP4 mutations that caused polysyndactyly in mice (74) and syndactyly in cattle (75, 76) confirmed the importance of this molecule in limb formation. The human condition Cenani-Lenz syndactyly syndrome (OMIM 212780) was subsequently found to result from loss-of-function mutations in LRP4 that impaired the ability of this protein to inhibit Wnt signaling (77). More recently, two patients with high bone mass were found to have different mutations in LRP4 (78).

Recent data indicate that LRP4 acts as a negative regulator of LRP5/6 signaling by augmenting the inhibitory activity of sclerostin on this pathway (78). The interaction

Figure 1.

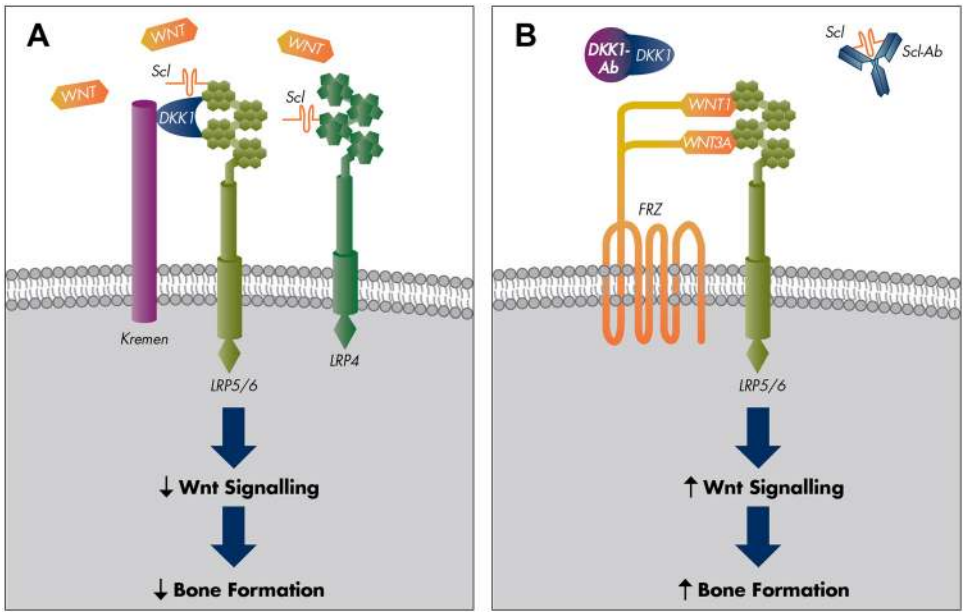

Figure 1. Mechanism of action of sclerostin and DKK1 at the cell surface. A, Sclerostin (Scl) and DKK1 bind Wnt coreceptors LRP5/6 to inhibit Wnt binding and signaling to decrease bone formation. Sclerostin and DKK1 bind the first β-propeller of LRP5 and LRP6 to inhibit Wnt 1 class Wnt signaling. DKK1 also binds the third β-propeller to inhibit Wnt 3a class Wnt signaling. DKK1 and sclerostin can also utilize coreceptors to augment Wnt inhibitory activity. DKK1 forms a ternary complex with LRP5 or LRP6 and Kremen receptors 1 or 2, which results in the internalization of the complex. Sclerostin binds LRP4 to enhance its activity, although the mechanism has not yet been determined, and whether a ternary complex between sclerostin, LRP4, and LRP5 or -6 forms is not known. B, Scl-Ab and DKK1-Ab prevent the interaction of these molecules with LRP5 and LRP6, thereby allowing Wnt 1 and Wnt 3a class Wnts to bind the first and third β-propellers, respectively. Wnts form a complex with Frizzled (FRZ) receptors and LRP5/6 to transduce an intracellular signal leading to increased bone formation. For diagram purposes Wnt1 and 3a are shown binding FRZ, whereas likely only a single Wnt binds a single FRZ.

of sclerostin with LRP4 was confirmed by demonstrating that overexpression of LRP4 greatly enhanced the LRP5/6-inhibitory activity of sclerostin, whereas knockdown of LRP4 impaired the activity of sclerostin on Wnt signaling and mineralization. The activity of DKK1 was not significantly affected by alterations in LRP4 expression.

DKK1 also binds to a two-member family of proteins referred to as Kremen 1 and Kremen 2 (79). Binding of DKK1 to Kremens results in the formation of a DKK1-Kremen-LRP5/6 ternary complex (Fig. 1A) that is internalized by the cell leading to the removal of LRP5/6 from the cell surface. The importance of Kremen molecules and the DKK1-Kremen ternary complex in limb development and bone formation was demonstrated in mice with targeted deletions of these molecules (80). Double homozygous mutant Kremen1/2 animals had subtle patterning defects in the forelimb that were further enhanced by the deletion of a single DKK1 allele, suggesting that DKK1 and Kremen function to regulate bone mass through modulation of LRP5/6 receptors in rodents. Homozygous deletion of either Kremen 1 or 2 (Krm1 KO or Krm2 KO) show normal bone formation and bone mass, whereas double of Kremen 1 and 2 (Krm1/2 KO) show increased bone formation and bone mass in younger (12-wk-old) mice (80). However, it was reported that adult (24-wk-old) mice with a single mutant of Kremen 2 (Krm2 KO) display higher bone formation and bone mass (81). These results may suggest functional redundancy of Kremen1 and Kremen 2 in skeletal growth and development and the importance of Kremen 2 in bone formation during skeletal maintenance/remodeling.

III. Sclerostin and DKK1 Expression

Sclerostin and DKK1 are expressed in the limb bud during embryogenesis, and the involvement of these molecules in the development of the embryonic skeleton is exemplified in the sclerostin LRP6 genetic complementation experiments (82) and by patterning defects observed in the developing limb of DKK1 KO (83) and DKK1 hypomorphic doubleridge mice (84, 85).

Sclerostin and DKK1 expression continue postnatally in the established skeleton where various intrinsic and extrinsic cues regulate the impact of these molecules on the skeleton in normal and pathophysiological states. The ex-

Figure 2.

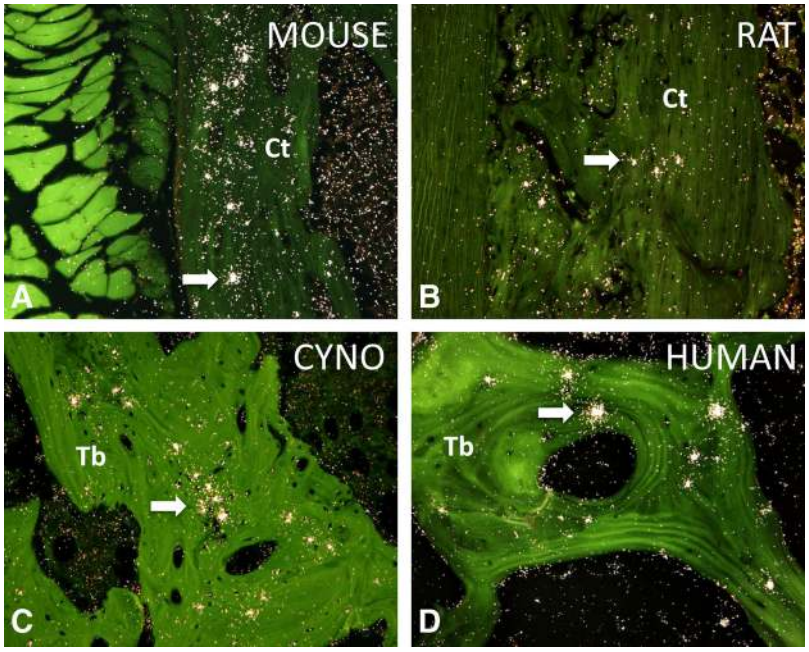

Figure 2. Localization of *SOST* expression in osteocytes. *In situ* hybridization was performed to identify *SOST* expression in samples of cortical bone (Ct) from a 10-wk-old mouse (A), cortical bone from a 20.5-month-old OVX rat (B), trabecular bone (Tb) from a 4-yr-old cyno (C), and trabecular bone from a 76-yr-old osteoporotic woman (D). *White arrows* highlight the *SOST* expression (*white dots*) in the bone osteocytes. The magnification is consistent across images (10× objective).

pression of sclerostin is relatively restricted in the adult skeleton where localized expression was first described in osteocytes (32, 86, 87). Figure 2 highlights the osteocytic expression of sclerostin in mouse, rat, monkey, and human bone. Extraosseous sclerostin expression has been described in specific regions of the developing embryonic and neonatal heart, although sclerostin expression was not detected in adult cardiac tissue (88, 89). Most recently, Zhu *et al.* (90) showed that sclerostin expression increases with calcification of vascular smooth muscle cells and expression of sclerostin in calcified aortas from the Enpp1 KO mice. However, the function of sclerostin in the vasculature has not been determined, and there have been no reports of impaired cardiac or vascular function in patients with sclerosteosis or Van Buchem disease or in *SOST* KO mice. In addition, sclerostin expression has been reported in articular chondrocytes (91). Similarly, weak sclerostin expression has been noted in the kidney (25), but the functional relevance of this expression is unclear.

DKK1 is expressed within osteoblasts and osteocytes (30); expression outside of bone has been shown in skin, placenta, prostate, and platelets with lower levels of expression observed in endothelium and other tissues (92–95). Interestingly, overexpression of DKK1 in cultured endothelial cells promoted endothelial mesenchymal transition and enhanced mineralization in these cultures, highlighting the complexity of this signaling pathway in various tissues and cell types (96).

IV. Regulation of Sclerostin and DKK1 Expression

A. Aging and BMD

Bone loss in aging is associated with decreased wall thickness, a measure of the osteoblasts' ability to refill remodeling space. Some studies indicate that circulating sclerostin levels increase with age in humans as bone mass declines (97–99). However, in iliac crest biopsies from patients with postmenopausal osteoporosis, *SOST* expression levels were 60% lower compared with postmenopausal women with normal BMD (100), possibly suggesting that this decreased expression could be a compensatory response to fragility, or that lower BMD reflects fewer osteocytes in the sample. These results are consistent with the positive association reported between lumbar BMD and sclerostin levels in healthy adults after adjustment for age and sex (99). In rodent studies, DKK1 appears to be more readily detected in young animals than in adults (101). It has been reported that in humans, serum DKK1 levels were negatively correlated with dual-energy x-ray absorptiometry (DXA) BMD at the lumbar spine and femur (102). Overall, regulation of sclerostin and DKK1 expression by aging and BMD has not been well established and requires further investigation.

B. Growth factors and hormones

Candidates for regulators of sclerostin and DKK1 include growth factors and hormones that impact bone formation, including BMP, estrogen, and PTH. Osteogenic BMPs have been tested for the ability to induce *SOST* expression in human osteoblast and mesenchymal stem cell cultures (103). BMP-2, -4, and -6 treatment of these cells resulted in a profound increase in sclerostin transcript expression in both cell types, and the BMP-6-stimulated expression of sclerostin in human mesenchymal stem cells was 1000-fold that observed in nontreated cells. The decreased expression of sclerostin and DKK1 in UMR106

and osteocytic MLO-A5 cells under hypoxic conditions is thought to result from decreased BMP signaling due to increased expression of BMP inhibitors by these cells (104). Additionally, mice with an osteoblast-specific deletion of BMP receptor type 1a have decreased sclerostin and DKK1 levels, increased Wnt signaling, and high bone mass; whereas those expressing a constitutively active BMP receptor type 1a express increased levels of sclerostin and DKK1 (105).

Sex hormones are important regulators of bone metabolism. In the adult and aging skeleton, estrogen deficiency leads to bone loss and contributes significantly to the development of postmenopausal osteoporosis. In a recent study, serum sclerostin levels were measured in a large (n = 767) population of men and women of various ages that included both pre- and postmenopausal women. Sclerostin levels were significantly higher in men than in women and were significantly increased in postmenopausal compared with premenopausal women (98). Increased serum sclerostin was negatively correlated with femoral neck BMD and the free estrogen index in another postmenopausal population (106). Additional studies have examined the impact of estradiol therapy on serum sclerostin levels (107, 108). In postmenopausal women receiving estradiol treatment for 4 wk, a significant 27% decrease in circulating sclerostin was observed compared with a control group. In elderly men, it was observed that estrogen had a greater impact on sclerostin levels than did testosterone. Surprisingly, in both sexes serum sclerostin levels correlated best with a marker of bone resorption rather than with markers of bone formation (108). These findings in humans contrast with a recent report showing decreased sclerostin mRNA in calvaria and femur of ovariectomized (OVX) mice when compared with sham controls (109).

The impact of the PTH analog teriparatide [PTH (1–34)] and PTH receptor signaling on sclerostin and DKK1 expression has been extensively studied in cellular and animal models and in humans treated with PTH. In rodents, PTH (1–34) treatment resulted in a rapid, nearly 2-fold decrease of sclerostin expression in the calvaria and femur that corresponded to increased bone formation and bone mass (110). This group extended these findings to calvarial organ cultures and osteoblastic UMR-106 cells and showed that the PTH receptor used cAMP signaling to inhibit sclerostin expression. Although some discrepancies among studies do exist, other groups have also demonstrated in rodents that intermittent (111) as well as chronically (112) elevated PTH suppresses sclerostin mRNA and protein levels in bone. The importance of this down-regulation in sclerostin for the anabolic action of PTH was further demonstrated in genetic mouse models. Genetic overexpression of *SOST* in transgenic mice resulted in a blunting of the anabolic effects of PTH (29). Sclerostin ablation in *SOST* KO mice also resulted in a reduction in PTH anabolism, reflected in a modest increase in trabecular and cortical bone formation and no increase in BMD (31, 113). These studies suggest that the anabolic effect of PTH on bone is in part due to its impact on sclerostin expression. In humans, a prospective clinical study in postmenopausal women demonstrated that intermittent PTH treatment resulted in a significant (12.7%) decrease in serum sclerostin and a nonsignificant (24%) decrease in bone marrow plasma sclerostin levels compared with controls after 14 d (114). However, teriparatide for 6 months or longer did not alter serum sclerostin levels in a separate clinical study (113). Additional evidence for a PTH-sclerostin relationship comes from patients with renal osteodystrophy who exhibit both elevated serum PTH and reduced sclerostin levels (115).

Similar to the observed PTH-mediated suppression of sclerostin expression in osteoblastic cultures, PTH was shown to decrease DKK1 expression in MC3T3 cells. Furthermore, the addition of PTH to *ex vivo* embryonic tibia and femur cultures led to a readily visualized reduction in DKK1 transcript levels in perichondrial cells and trabecular osteoblasts by 6 h that approached a complete inhibition of DKK1 expression in the tibia by 8 h (28). In contrast, DKK1 levels rose significantly after 12 months of teriparatide treatment in postmenopausal women and were maintained at similar levels through 18 months (113). Importantly, the observed elevation in serum DKK1 corresponded with a waning of the anabolic effect of teriparatide, leading the authors to speculate that the elevation of DKK1 levels after 12 months of treatment contributed to the declining efficacy of teriparatide to increase bone formation markers. These data largely supported findings from a previous study that demonstrated increases in DKK1 levels during teriparatide treatment with a return to control levels by 6 months after treatment discontinuation (116). However, a correlation between elevated DKK1 and decreased teriparatide efficacy was not observed. Whether DKK1 is a direct target of the PTH signaling pathway, or whether the observed increases in serum DKK1 levels are a compensatory effort by the body to decrease PTH-induced increases in Wnt signaling has not yet been described.

C. Glucocorticoids

Certain drugs, such as glucocorticoids, have a detrimental impact on skeletal metabolism. Glucocorticoid-induced osteoporosis is a significant side effect observed in patients taking these antiinflammatory agents over a prolonged period of time and is the most common form of

secondary osteoporosis (117). Glucocorticoids impact bone metabolism by both increasing bone resorption and decreasing bone formation, ultimately resulting in bone loss in trabecular and cortical compartments. RANKL has been identified as a key regulator of the increased bone resorption; however, the specific regulators responsible for the decreased bone formation have not yet been identified. Several studies have demonstrated that glucocorticoids induce DKK1 expression in osteoblasts both *in vitro* (118) and *in vivo* (33), and that inhibition of DKK1 using small interfering RNA (siRNA) prevented the dexamethasone-induced suppression of primary human osteoblast differentiation (119). A significant induction of both DKK1 and sclerostin expression was observed in bones of mice treated with prednisolone for 56 d, which also resulted in significant negative effects on trabecular bone. Interestingly, if mice were treated with hPTH (1–34) in addition to prednisolone from d 28 to 56, the glucocorticoid-induced bone loss was reversed, and expressions of sclerostin and DKK1 were significantly decreased compared with those observed in either prednisolone- or placebo-treated animals (120). In postmenopausal women on glucocorticoid therapy, serum sclerostin was significantly higher, and serum DKK1 was significantly lower compared with non-glucocorticoid-treated patients, suggesting that circulating Wnt inhibitors may be modulated by glucocorticoids in humans (121).

In addition to osteoporosis, glucocorticoid therapy increases the risk of hip osteonecrosis, resulting in significant disability (122). Patients with osteonecrosis of the femur head that were taking corticosteroids had significantly greater serum DKK1 levels and DKK1 mRNA in bone than a control population of patients with femur neck fractures, with serum DKK1 correlating positively with disease progression (123). In bone marrow stromal cells collected from these patients, DKK1 siRNA reduced dexamethasone-induced apoptosis, further supporting the potential positive impact of DKK1 inhibition on glucocorticoid-mediated cell death. Taken together, results from these studies suggest that inhibition of sclerostin and/or DKK1 may provide a therapeutic benefit on bone to patients on glucocorticoid therapy.

D. Inflammation

Sclerostin and DKK1 are also regulated by mediators of inflammation and could thus play a role in promoting inflammatory bone loss. In osteoblastic UMR106.01 cells, prostaglandin E2 down-regulated *SOST* expression and activated Wnt signaling—an effect that was replicated by an agonist to the prostaglandin E2 receptor EP2, or blocked by using a siRNA to the EP2 gene (Ptger2) (124). *SOST* expression was also up-regulated in human osteoblast cells *in vitro* after exposure to TNF-α (125, 126) or polyethylene-wear particles from implant surfaces (127). Sclerostin expression was decreased in bone biopsies from patients with osteoarthritis (OA) or ankylosing spondylitis (AS) (128). No increase in sclerostin expression was seen in bone biopsies from rheumatoid arthritis (RA) patients (128), and sclerostin expression was greater in synovial tissue from RA patients as compared with OA patients (129). Sclerostin and DKK1 may also be potential serum biomarkers of inflammatory disease and its progression. In patients with AS, lower serum sclerostin levels were associated with greater progression of disease (128). Although serum levels of DKK1 did not correlate with the incidence of radiographic hip OA, patients in the highest quartile of serum DKK1 had diminished risk of radiographic hip OA progression compared with patients in the lowest quartile (130). It is unclear whether these serum and tissue level changes in sclerostin and DKK1 are actively modulating these inflammatory diseases or are instead a consequence of the inflammatory process.

DKK1 contributes to the pathology of joint remodeling in RA and other forms of chronic inflammatory-induced bone disease (131–133). Bone erosion is a classical feature observed in the joints of patients with RA, and there is a clear link demonstrating that localized expression inflammatory cytokines stimulate osteoclast activity by inducing molecules such as RANKL. Insight into the potential contribution of DKK1 to this process came from the observation that bone formation was often repressed in RA patients, thereby contributing to the lack of repair of eroded joints (133). Diarra *et al.* (134) demonstrated that DKK1 expression in serum of patients with RA correlated well with disease severity and that DKK1 could be detected in the synovial tissue isolated from patients undergoing joint replacement therapy for RA. These authors went on to demonstrate that TNF receptor-1 and the MKK3/p38MAPK pathway were required for the regulation of DKK1 in arthritis. In addition, the regulation of osteoprotegerin (OPG) by Wnt-signaling was mediated via DKK1 in this model, and it was also demonstrated that OPG regulates DKK1 expression, suggesting the existence of a regulatory feedback loop between DKK1 and OPG.

E. Diabetes

In humans with type II diabetes, serum sclerostin levels were higher than in controls (135). Sclerostin and DKK1 expression in bone were also up-regulated in a diabetic rat model (136). In contrast, sclerostin expression was lower in a mouse model of type I diabetes compared with nondiabetic controls, although this effect was attributed to an increase in osteocyte death (137). The therapeutics used to control diabetes may also affect the sclerostin and DKK1

axis. Use of thiazolidinediones has been associated with bone loss in humans, and a recent study reported an increase in serum sclerostin after 24 wk of treatment with pioglitazone (138). Pioglitazone also increased serum DKK1 levels after 90 d in 11 diabetic patients and rapidly increased DKK1 expression in cultured adipocytes (139). Furthermore, activation of the glucagon-like peptide-1 by exendin-4 administration resulted in reduced serum sclerostin expression and increased plasma glucose and BMD in diabetic rats (140). If these elevations in serum sclerostin and DKK1 are associated with the bone loss and fragility that occurs in patients with diabetes, administration of inhibitory antibodies may directly counteract the diabetes-associated comorbidity.

F. Multiple myeloma

Some of the most compelling data for the role of DKK1 in pathologies of the skeleton come from studies in multiple myeloma, which is a B-cell malignancy characterized by the expansion of an aberrant long-lived plasma cell population in the bone marrow (141, 142). The presence of myeloma plasma cells in the bone marrow compartment often leads to the destructive osteolytic bone disease that is a prominent comorbidity associated with this malignancy. The bone destruction is characterized by extensive bone resorption and repressed bone formation that results in severe osteolysis occurring at multiple sites throughout the skeleton. The mechanisms underlying the increased osteoclast activity that leads to rampant bone resorption and the formation of lytic lesions throughout the skeleton include the increased expression of various molecules, including RANKL and other cytokines involved in the generation and activation of osteoclasts (143, 144).

The mechanism underlying the suppression of bone formation and the uncoupling of osteoblast and osteoclast activity has been clearly demonstrated to involve the overexpression of DKK1 from the myeloma plasma cell. Initial insight into this mechanism came from the analysis of microarray experiments conducted on bone marrow plasma cells isolated from patients newly identified with multiple myeloma or control individuals (145). A number of genes whose expression was altered in patients with lytic bone lesions were identified; DKK1 was the only secreted factor that was up-regulated in the myeloma plasma cell, and hence could be predicted to influence the surrounding microenvironment. The veracity of this finding has been confirmed by a number of independent groups that have demonstrated increased expression of DKK1 in patients with multiple myeloma and have shown a clear correlation with lytic bone destruction (146–151). Tian *et al.* (145) further demonstrated that bone marrow plasma isolated from five myeloma patients was capable of inhibiting osteogenesis *in vitro*. In addition to a direct inhibitory effect on osteoblasts, Qiang *et al.* (152) demonstrated that recombinant DKK1, as well as multiple myeloma-derived DKK1, suppressed Wnt-mediated OPG expression while enhancing RANKL expression from osteoblasts. One would predict that the skewed RANKL:OPG ratio would greatly favor osteoclastogenesis, thereby contributing to the profound bone resorption observed in myeloma-induced bone disease, suggesting that DKK1 overexpression in this setting uncouples bone formation from bone resorption by increasing osteoclast activation while suppressing osteoblast activity.

A recent report demonstrated that sclerostin levels are elevated in multiple myeloma. Patients with active myeloma had significantly higher levels of circulating sclerostin as compared with patients with monoclonal gammopathy of undetermined significance or controls (153). Importantly, elevated sclerostin levels were associated with fractures at diagnosis and with shorter survival times.

G. Matrix mineralization

Because matrix mineralization occurs during the transition from osteoblast to osteocyte, sclerostin expression is activated according to *in vitro* and *in vivo* evidence. In osteoblast cultures, sclerostin mRNA was only apparent after the initiation of mineralization (154). In human iliac biopsies, sclerostin-negative osteocytes were nearer to bone surfaces, suggesting that *SOST* expression was related to osteocyte and/or bone age (86). Impairment of matrix mineralization with a bisphosphonate *in vivo* resulted in osteocytes that did not positively stain for sclerostin expression (155). Furthermore, recombinant sclerostin decreased mineralization in primary human osteoblasts *in vitro*, and elevated sclerostin mRNA levels were found in the femurs of hypophosphatemic mice with mineralization defects (156). Although the signaling mechanisms are currently unknown, the process of an osteocyte embedding in mineralized matrix in bone clearly activates sclerostin expression.

H. Mechanical environment

Bone is a remarkable organ that has the ability to sense and respond to alterations in its loading environment, with disuse resulting in bone loss and overloading resulting in bone augmentation (157). Although the process of bone mechanosensation is not clearly defined, osteocytes and their networked processes within bone are considered the likely sensor (158). After sensation of an altered loading environment, osteocytes respond to affect bone formation on the bone surface. Insight into the mechanisms underlying skeletal mechanotransduction at a molecular

level has come from studies of Wnt signaling and sclerostin expression under various loading conditions in bone (159–163). These data suggested that the anabolic response to exercise and other forms of mechanical stress could be mediated through the regulated expression of ligands for LRP5/6, including sclerostin and DKK1, which are expressed in osteocytes (164), making them obvious candidates as mediators of mechanotransduction.

Sclerostin protein and mRNA expression were decreased under loading conditions in rodent ulna, especially in areas of greatest stress (165), supporting the hypothesis that sclerostin is an osteocyte-derived molecular signal mediating mechanotransduction. DKK1 transcripts were also reduced under loading conditions, albeit to a lesser extent. Importantly, the reduction in sclerostin expression corresponded spatially to increased bone formation in the loaded bone. The observation of reduced sclerostin levels in response to loading is not restricted to long bones because decreased sclerostin expression was also found in osteocytes in high-stress regions of alveolar bone in a rat model of orthodontic tooth movement (166). In addition, sclerostin expression was decreased after exposure of mature osteoblastic cells (UMR 106.01) to fluid shear loading *in vitro* (167).

The effect of unloading or disuse on sclerostin expression is not as established, although limited data suggest that sclerostin expression is up-regulated. After 3 d of unloading (tail suspension) in normal mice, sclerostin mRNA expression increased in the unloaded tibia, which preceded the decrease in bone formation and bone volume (165). However, sclerostin protein appeared unaffected by tail suspension. Subsequent studies demonstrated that *SOST* KO mice did not exhibit the decreased Wnt signaling and bone loss normally associated with disuse, thereby demonstrating the critical involvement of sclerostin in mediating mechanotransduction via the Wnt signaling pathway (35). In addition, serum sclerostin is increased after unloading in tail-suspended rats (168), as well as in immobilized stroke patients (169). These results led to a recently developed theoretical model to try to simulate the adaptation of trabecular structure to different loading conditions using sclerostin as the key regulator (170). The findings that skeletal-loading conditions alter endogenous sclerostin expression suggest that inhibition of sclerostin activity may provide a therapeutic avenue to increase bone mass in a manner similar to that observed in normal physiological conditions such as exercise.

V. Associations between Wnt Signaling and Cancer

The complex relationship between the Wnt-signaling pathway and tumorigenesis has been extensively studied (171–174). In fact, Wnt proteins were first identified in mammals through an oncogene locus called int-1 that was associated with mammary tumorigenesis in mice (175). Constitutive Wnt activation by genetic mutation of intracellular signaling components (*e.g.*, β-catenin, *adenomatous polyposis coli* tumor suppressor) has been shown to cause tumors in several tissues including the liver and colon (171). In addition, the secreted Wnt inhibitor Wnt inhibitory factor 1 has been shown to be down-regulated in various cancers (176), including osteosarcoma (177), and its genetic deletion was associated with increased susceptibility to spontaneous and radiation-induced osteosarcomas in mice (177).

Consistent with a positive association between Wnt activation and cancer, decreased DKK1 expression was observed in several cancers including colon cancer and renal cell carcinoma (178). Conversely, in addition to multiple myeloma, increased levels of DKK1 have been observed in numerous tumor types including hepatocellular carcinoma (179), pancreatic ductal adenocarcinoma (PDAC) (180), as well as other cancers (181). Evidence for a direct role for DKK1 elevation in tumorigenesis was observed in PDAC cell lines, where DKK1 knockdown reduced their growth rate and invasiveness (180). In a mouse model of multiple myeloma, it was recently shown that active vaccination with DKK1 protected against tumor formation as well as having a therapeutic effect on established myeloma (182). These and other data have led to the somewhat surprising suggestion that DKK1 may be a target for cancer immunotherapy (181). The complexity of DKK1 in tumorigenesis has recently been well reviewed by Menezes *et al.* (183). A relationship between sclerostin and tumorigenesis is less established, perhaps based on its narrower scope of expression and Wnts it can inhibit compared with DKK1.

To date, there have been no reported cases of osteosarcoma or increased incidence of tumor formation in individuals with high bone mass mutations in LRP5 or in those with sclerosteosis (lack of sclerostin), although the population size is very small. Ultimately, whether the therapeutic inhibition of DKK1 or sclerostin contributes to carcinogenesis would need to be addressed by formal safety studies.

VI. Sclerostin Mutations and Bone

A. Sclerosteosis and Van Buchem disease

Sclerosteosis is an extremely rare, autosomal recessive genetic disorder characterized by a high bone mass phenotype that has been mainly diagnosed among Afrikaners in South Africa (184, 185) and in a few individuals and

families in other parts of the world (186). Patients with sclerosteosis usually have a tall stature and syndactyly, with increased skeletal density that is easily visualized by x-ray examination and cortical thickening that is present throughout the skeleton (185). It is a progressive process, leading to complications such as increased intracranial pressure, facial palsy, and loss of hearing, vision, or smell (186). Bone overgrowth in the jaw results in complications that include delayed tooth eruption, malocclusion, irregular tooth shape, and difficulty extracting teeth (187). The hyperostotic skeleton is very resistant to trauma, and no affected person is known to have suffered a fracture. Abnormalities unrelated to bone overgrowth have not been reported in sclerosteosis patients.

Analysis of bone turnover in sclerosteosis patients helped to characterize the disorder. An early study revealed that the nonspecific bone formation marker alkaline phosphatase was elevated, particularly in children with sclerosteosis (188), and a more recent study demonstrated that serum levels of the bone formation marker procollagen type 1 N-terminal propeptide (P1NP) were about 3-fold greater than in healthy controls, whereas serum levels of the bone resorption marker C-terminal telopeptide of type 1 collagen (CTx-1) were within the normal range (189). Very limited histomorphometric data reported from a single sclerosteosis patient revealed that the bone formation rate (BFR) was about 4-fold above the upper-normal range and the number of osteoclasts per area of bone tissue was within the low-normal range (190), consistent with the biochemical findings. These data demonstrated that sclerosteosis is caused primarily by excessive osteoblast-mediated bone formation with limited changes in bone resorption.

Two independent genetic mapping studies revealed that sclerosteosis was caused by loss-of-function mutations of *SOST* (25, 26). The *SOST* gene is located on chromosome 17q12–21. Sclerosteosis patients had markedly increased BMD at all skeletal sites including the lumbar spine, total hip, and forearm (191), a finding consistent with x-ray observation. In human heterozygous carriers of the *SOST* mutation, BMD has been reported to be either within (male carriers) or above (female carriers) the high normal BMD reference range of sex- and age-matched healthy subjects (191). Serum P1NP levels were significantly greater in the carriers than in healthy subjects (189). These findings made sclerostin and its pathway an interesting target for the potential anabolic treatment of osteoporosis and other bone-related disorders.

Van Buchem disease is another very rare, recessively inherited, high bone mass disorder, mostly found in the population of a fishing village in The Netherlands. Van Buchem disease is radiologically very similar to sclerosteosis but is less severe. Unlike patients with sclerosteosis, patients with Van Buchem disease have normal stature and no syndactyly. Serum levels of the bone formation markers P1NP and osteocalcin are elevated in Van Buchem disease (192). But, unlike in sclerosteosis, levels of the urinary bone resorption marker cross-linked N-telopeptide have been reported to be higher in patients with Van Buchem disease as compared with heterozygous carriers (192). No mutations of the *SOST* gene were found in patients with Van Buchem disease, but a 52-kb deletion located 35-kb downstream of *SOST* was identified (27, 57), which resulted in an absence of postnatal sclerostin expression (31), further highlighting the key role of sclerostin in regulating bone formation.

Additionally, polymorphisms in *SOST* have been associated with low BMD in older men and women (193–195), supporting a causal relationship between altered *SOST* expression and BMD.

B. Sclerostin KO mice

Similar to sclerostin inactivation in humans, mice with a targeted deletion of the sclerostin gene (*SOST* KO mice) have increased bone density throughout the skeleton (29, 34–36) and a low incidence of syndactyly (196). Significant increases in areal BMD were found as early as 1 month of age in *SOST* KO mice (35). Our longitudinal *in vivo* DXA analysis showed that whole leg BMD increased progressively from 1 to 4 months of age, continuously increased at a slower rate between 4 and 12 months of age, then maintained peak BMD levels up to 18 months of age (end of study) in male *SOST* KO mice (Fig. 3A). These results are generally in agreement with the femoral BMD findings in *SOST* KO mice reported by others (29, 35, 36). Lumbar spine BMD peaked at 4 months of age and was then maintained at the peak level up to 18 months of age in our study (Fig. 3B). The pattern of increasing spine BMD in our study differed from another report by Brommage *et al.* (36) in which lumbar spine BMD peaked at month 24 (end of study) in male *SOST* KO mice as determined by *ex vivo* measurements.

MicroCT or static histomorphometric analysis showed that trabecular bone volume and trabecular thickness at the distal femur were more than doubled in both male and female *SOST* KO mice (29, 34–36). Trabecular number was also significantly increased in some studies (34, 35). Furthermore, cortical area at the femoral shaft of *SOST* KO mice was almost doubled due to the decreased endocortical (internal) perimeter and increased periosteal (external) perimeter (34, 36). Figure 3C illustrates greater trabecular bone volume and cortical thickness in *SOST* KO mice.

Figure 3.

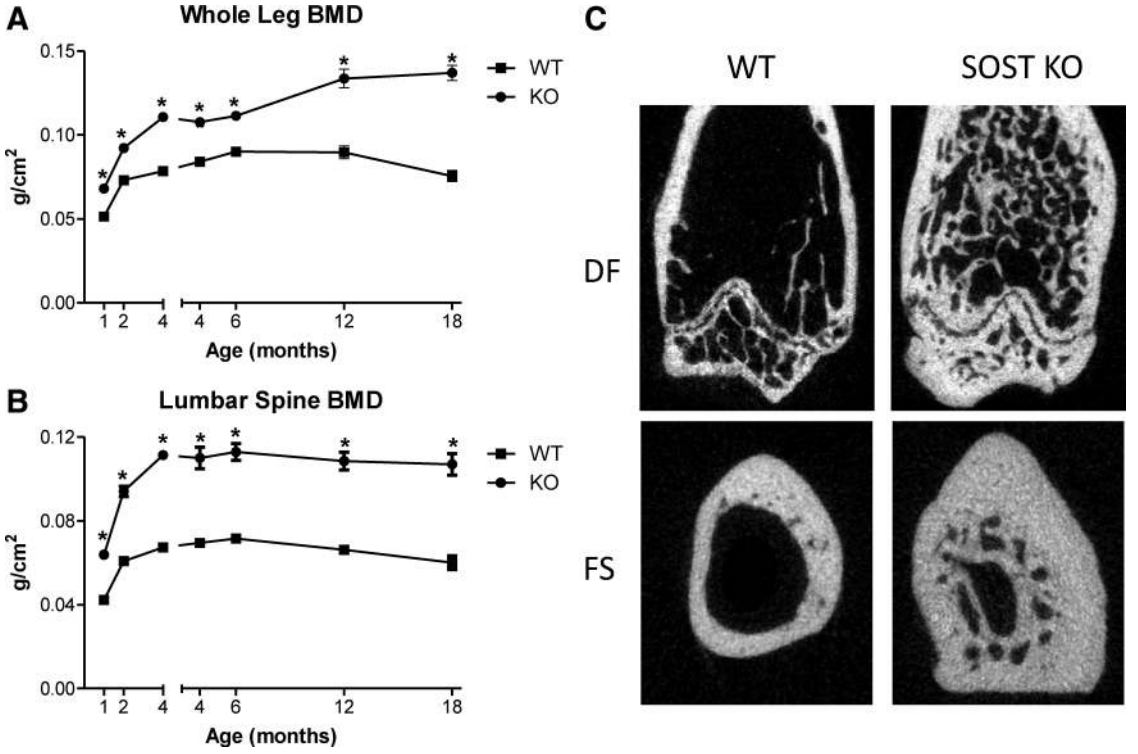

Figure 3. High bone mass in *SOST* KO mice. Areal BMD of whole leg (A) and lumbar spine (B) regions determined in two sets of male WT and *SOST* KO mice at 1, 2, 4, 6, 12, and 18 months of age. Data are expressed as the mean ± SEM; n = 10–12 per group for the first set of mice up to age of 4 months, and n = 6–8 for the second set of mice up to age of 18 months. *, $P < 0.05$ compared with the age-matched WT control. C, MicroCT images of distal femur (DF) and femoral shaft (FS) from 18-month-old male WT and *SOST* KO mice show greater trabecular bone volume and cortical thickness in KO compared with WT mice.

Dynamic histomorphometric analysis in *SOST* KO mice revealed that bone formation was markedly increased on the surfaces of trabecular bone (29, 34, 35) and on the endocortical and periosteal surfaces of cortical bone (34). Serum osteocalcin levels were significantly higher in *SOST* KO mice. The trabecular osteoclast surface in *SOST* KO mice was no different from wild-type (WT) controls (29, 34), and tartrate-resistant acid phosphatase form 5b (TRACP5b), a marker of osteoclast number, was not altered (34). Consistent with the increases in bone formation and bone mass, robust increases in bone strength were also found in both male and female *SOST* KO mice (34) as determined by mechanical testing of long bones and lumbar vertebral bodies. These data from *SOST* KO mice confirmed the critical role of sclerostin as an inhibitor of bone formation.

Consistent with the expected increase in Wnt signaling after sclerostin ablation, a greater percentage of osteocytes in *SOST* KO mice stained positively for β-catenin by immunohistochemistry (197). In addition, significantly fewer of the *SOST* KO osteocytes were apoptotic, perhaps reflecting a role of sclerostin in regulating osteocyte longevity (35, 197).

In mice, as in humans, BMD decreases after estrogen loss. When female *SOST* KO mice were subjected to estrogen deficiency by OVX, bone loss due to increased bone resorption was observed (36, 198). These results suggest that increased bone resorption caused by estrogen deficiency can occur in mice without sclerostin.

C. Sclerostin overexpression in mice

In contrast to the high bone mass phenotype in *SOST* KO mice, transgenic mice overexpressing human *SOST* exhibit a low bone mass phenotype (29, 31, 32). Two different methods were used to generate these transgenic mice. One method used the mouse osteocalcin promoter OG2 to overexpress human sclerostin in bone (32), whereas the other method used bacterial artificial chromosome (BAC) technology to express human sclerostin (29, 31). Both types of *SOST* transgenic mice had reduced amounts of trabecular bone and thinner cortices at lumbar vertebrae and calvaria (29, 31, 32), and dynamic histomorphometric analysis demonstrated a decreased BFR

TABLE 1. Genetic validation of sclerostin and DKK1 in mice

	Bone mass and strength	Bone formation	Bone resorption	Ref.
SOST KO	↑ BMD, TBV, ↑ Tb.Th, Ct.Th ↑ Bone strength	↑ Tb. BFR/BS ↑ Ec. BFR/BS ↑ Ps. BFR/BS ↑ Osteocalcin	⇆ Oc.S/BS ⇆ TRACP5b	29, 34–36
SOST Tg	↓ BMD, TBV ↓ Tb.Th, Ct.Th ↓ Bone strength	↓ Tb. BFR/BS	⇆ Oc.S/BS	29, 31, 32
DKK1$^{+/-}$	↑ TBV, Tb.Th	↑ Tb. BFR/BS	⇆ Oc.N/T Ar	37–39
DKK1$^{d/-}$	Tb.N, ↑ Ct.Th ↑ Bone strength	⇆ Osteocalcin	⇆ Dpyr/Creat	37–39
DKK1 Tg	↓ BMD, TBV, ↓ Tb.N	↓ Tb. BFR/BS ↓ Osteocalcin	⇆ Oc.S/BS ⇆ or ↑ TRACP5b	28, 30, 33
DKK1-AAV	↓ BMD, TBV, Tb.N	⇆ BFR/BS ↓ Tb. BFR/TV	↑ TRACP5b ↑ Oc.S/BS	101, 259

BFR/TV, BFR/total volume; Ct.Th, cortical thickness; DKK1$^{+/-}$, DKK1 heterozygous; DKK1$^{d/-}$, offspring of DKK1$^{+/-}$ and DKK1$^{+/d}$; Dpyr/Creat, deoxypyridinoline/creatinine; Ec., endocortical; Oc.N/T Ar, osteoclast number/total area; Oc.S/BS, osteoclast surface; Ps., periosteal; TBV, trabecular bone volume; Tb., trabecular bone; Tg, transgenic; Tb.N, trabecular number; Tb.Th, trabecular thickness; ↑, greater or increased; ↓, decreased; ⇆, unchanged.

in both types of transgenic mice at up to 5 months of age as compared with respective WT littermates (31, 32). BAC-*SOST* transgenic mice were followed until 8 months of age, at which point the trabecular bone formation rate returned to the level of WT controls, but reduced trabecular and cortical bone mass persisted (29). Bone resorption parameters were similar between the *SOST* transgenic mice and WT littermates in both studies (29, 32). Consistent with the decreases in bone formation and bone mass, *SOST* transgenic mice had more fragile bones as demonstrated by decreased bone strength (32). *Ex vivo* mesenchymal cell culture demonstrated reduced bone alkaline phosphatase activity and mineralization in the *SOST* transgenic mice as compared with WT littermates (32). These findings indicate that overexpression of human sclerostin in mice resulted in marked reduction in osteoblast differentiation, osteoblast activity, and bone formation.

In addition to the low bone mass phenotype, BAC-*SOST* transgenic mice had a wide range of missing digits (31). The severity of digit deformity correlated with embryonic *SOST* expression. Digit deformity was not reported in OG2-*SOST* transgenic mice, likely due to the use of the osteocalcin promoter. However, OG2-*SOST* transgenic mice had increased cartilage area in the growth plate (32), a finding that was not reported in BAC-*SOST* transgenic mice (31).

A brief summary of the effects of sclerostin deletion and overexpression on bone mass, bone formation, bone resorption, and bone strength in mice is listed in Table 1.

VII. Inhibition of Sclerostin by Monoclonal Antibody (Scl-Ab)

Pharmacological inhibition of sclerostin by monoclonal antibody has been explored in various animal models of bone diseases and in humans (65, 199–205), and Scl-Ab has demonstrated efficacy in preclinical models of bone loss conditions, osteogenesis imperfecta (OI), fracture healing, implant fixation, and other bone disorders. Across these models, Scl-Ab has demonstrated a consistent ability to increase bone formation, bone mass, and bone strength at multiple sites. These results are summarized in Tables 2 and 3 and are discussed below.

A. Effects of Scl-Ab on bone mass and strength in animal models

1. Estrogen deficiency-induced bone loss

The first published report of pharmacological inhibition of sclerostin (65) focused on the restorative effects of sclerostin antibody (Scl-Ab) in estrogen-deficient, aged, OVX rats, a widely used rodent model of postmenopausal osteoporosis. In this study, 5-wk treatment with Scl-Ab resulted in complete reversal of OVX-induced bone loss and increased bone mass and bone strength to levels greater than sham controls. The Scl-Ab-mediated increases in BMD and BFR in the OVX-rat model were shown to be dose-dependent (Fig. 4, A and B) (206). At the 25-mg/kg dose, Scl-Ab treatment more than doubled trabecular bone volume and trabecular thickness at multiple sites (Fig. 4, C–F) and increased cortical thickness at the femur shaft after 5 wk (65). Furthermore, Scl-Ab increased both trabecular and cortical bone mass when given to 10-month-old intact female rats for 4 wk (201) (Fig. 4, G–J), or when given immediately after OVX in adult rats (207). The results from these short-term studies indicated that Scl-Ab could increase bone mass and rebuild bone structure in different stages of estrogen deficiency and in gonad-intact adult female rats. Results from a recent 12-wk study

TABLE 2. Effects of Scl-Ab on bone in animal studies

Animals	Models	Length of treatment (wk)	Major findings	Ref.
19,[a] 11.5,[b] and 6[c] month ♀ SD rats	OVX	5[a], 6[b,c]	↑ BMD, TBV, Tb.Th, Ct.Th ↑ Bone strength ↑ BFR/BS, ↓ or ⇆ Oc.S/BS ↑ Osteocalcin, P1NP ⇆ CTx-1, ↓ TRACP5b	65[a], 207[b], 218
10-month ♀ or 16-month ♂ SD rats ≈22 g ♂ Balb/c mice	Gonad-intact	4, 5, 10	↑ BMD, TBV, Tb.Th, Ct.Th ↑ Bone strength ↑ BFR/BS, ⇆ Oc.S/BS, ↓ Er.S/BS ↑ Osteocalcin, ⇆ CTx-1	63, 200, 216
9-month ♂ SD rats	ORX	6[d]	↑ BMD, TBV, Tb.Th, Ct.Th ↑ Bone strength ↑ BFR/BS, ↓ Er.S/BS	208
10-month ♀ SD rats 16-wk ♀ Swiss Webster mice ♀ C57BL/6J mice	Normal- and Under-loaded	3, 4	↑ TBV, Tb.Th, Ct.Th ↑ Bone strength ↑ BFR/BS, ⇆ Oc.S/BS, ↓ Er.S/BS ↑ Osteocalcin, ⇆ TRACP5b	168, 201, 209, 219
7-wk Balb/c mice	Dexamethasone	9	↑ BMD, TBV, Tb.Th, Ct.Th ↑ Bone strength ⇆ Osteocalcin, P1NP, ↓ TRACP5b	202
CB-17 SCID mice	Colitis	3, 12	↑ BMD, TBV, Tb.Th, Ct.Th ↑ Bone strength ↑ Osteocalcin, ↓ TRACP5b	199
9-wk ♀ Zuck$^{fa/fa}$ rats	Diabetes	12	↑ BMD, Ct.Th ↑ Osteocalcin, ⇆ CTx-1	210
3–5 yr ♀ or 4–5 yr ♂ cynos	Gonad-intact	8, 10	↑ BMD, TBV, Tb.Th, Ct.Th ↑ Bone strength ↑ BFR/BS, ↓ Oc.S/BS, Er.S/BS ↑ Osteocalcin, P1NP; ⇆ CTx-1	203, 204
8-wk or 6-month ♂ Brtl/+ mice 8 wk ♂ oim mice	OI	2, 5, 10	↑ TBV, Tb.Th, Tb.N, Ct.Th ↑ Strength, ↓ brittleness and fracture	213–215

Ct.Th, Cortical thickness; Ec., endocortical; Er.S/BS, eroded surface/bone surface; Oc.S/BS, osteoclast surface; ORX, orchiectomy; Ps., periosteal; SD, Sprague Dawley; TBV, trabecular bone volume; Tb., trabecular bone; Tb. N, trabecular number; Tb. Th, trabecular thickness; Zuck$^{fa/fa}$ rats, Zucker diabetic fatty rats; ↑, greater or increased; ↓, decreased; ⇆, unchanged; ♀, female; ♂, male.

[a] Treatment begins 13 months after OVX.

[b] Treatment begins 5 months after OVX.

[c] Treatment begins immediately after OVX.

[d] Treatment begins 3 months after ORX.

in OVX rats with established osteopenia showed that Scl-Ab resulted in further increases in BMD of the lumbar spine, femur metaphysis, and femoral diaphysis (Fig. 5A).

2. Age-related or androgen deficiency-induced bone loss

Osteoporotic fractures occur not only in women but also in men, with the incidence increasing with age or after androgen ablation (7). Therefore, the preclinical evaluation of therapeutic agents to increase BMD and reduce fracture risk in men has used the aged male rat and orchiectomized rat models of male osteoporosis. In 16-month-old gonad-intact male rats, 5 wk of Scl-Ab treatment increased bone mass and bone strength to levels found in younger rats (200), further supporting the rebuilding potential of Scl-Ab. Moreover, in a rodent model of androgen deficiency induced by orchiectomy, 6 wk of Scl-Ab restored bone mass and bone strength to levels similar to sham controls (208).

3. Disuse/immobilization-induced bone loss

As discussed in *Section IV.H.*, sclerostin appears to play a role in the response to mechanical loading. Therefore, it is a reasonable hypothesis that inhibition of sclerostin by monoclonal antibody would protect against bone loss resulting from disuse or immobilization of bones. In rats, Scl-Ab resulted in increased cortical and cancellous bone mass in a hindlimb immobilization model (201). In mice, Scl-Ab was also effective at preventing bone loss caused by tail suspension (168, 209) or botulinum toxin (Botox)-induced muscle paralysis in mice (209). These results suggest that inhibition of sclerostin by treatment with Scl-Ab is able to protect against bone loss in animal models of disuse or immobilization.

4. Glucocorticoid-induced bone loss

In young mice treated with dexamethasone for 6 to 9 wk, Scl-Ab treatment prevented the loss of cortical bone

TABLE 3. Effects of Scl-Ab in animal models of bone repair

Species	Models	Length of treatment (wk)	Major findings	Ref.
Mice	Femur osteotomy	2, 4, 6	↑ Radiographic density, bone mass, and strength	246, 249
Rats	Femur osteotomy	3, 6	↑ Bone mass	248
	Closed femur fracture	2, 4, 7	↑ Radiographic density, bone mass, and strength	204, 246
	Tibia diaphysis core defect	5	↑ Bone mass, ↓ time to bridging	249
	Tibia metaphysis core defect	3	↑ Bone formation and bone mass	250
	Femur diaphysis gap defect	12	↑ Radiographic score, bone mass, bridging	252, 251
	Femur distraction osteogenesis	6	↑ Radiographic union, bone mass, MAR	253
	Tibia metaphysis screw implant	2, 4	↑ Bone mass, strength	254
	Femur IM rod implant	4, 8, 12	↑ Bone mass, strength	71, 255
	Spine fusion	6	↑ Bone mass, ⇆ stability by manual palpation	257
Cynos	Fibular osteotomy	10	↑ Bone mass, bone strength, ↓ soft callus (cartilage/fibrous)	204

MAR, Mineral apposition rate; IM, intramedullary; ↑, greater or increased; ↓, decreased; ⇆, unchanged.

mass and strength and increased trabecular bone volume and thickness to levels greater than baseline (202). Scl-Ab did not prevent the growth retardation induced by glucocorticoid administration in these young mice.

5. Chronic inflammation-induced bone loss

In a mouse colitis model induced with $CD45RB^{high}$ cells, Scl-Ab treatment initiated before the transfer of CD45RB cells effectively prevented bone loss associated with colitis without impacting inflammation (199). In addition, when Scl-Ab was administered after the establishment of disease (50 d after CD45RB cell transfer), further loss of bone mass and bone strength was prevented by Scl-Ab treatment. Scl-Ab treatment has also been reported to prevent systemic bone loss in a mouse model of collagen-induced arthritis (20). These preclinical results suggest that Scl-Ab may represent a potential anabolic therapy for bone loss conditions associated with chronic inflammation.

6. Bone loss associated with type II diabetes mellitus

In Zucker diabetic fatty (ZDF) rats, a model of type II diabetes, Scl-Ab treatment from 9 to 21 wk of age increased trabecular and cortical bone mass at the distal femur and lumbar vertebra without affecting serum glucose or renal function (210).

7. Osteogenesis imperfecta

OI is a group of genetic disorders that affect synthesis of type 1 procollagen in one of every 20,000 live births (211). These mutations lead to bones that are brittle and susceptible to fracture, resulting in reduced stature, impaired mobility, and poorer health in affected patients throughout their life span. Current therapies for OI patients include bisphosphonates, which increase BMD but do not consistently reduce fractures in these patients (212). Although it is unlikely that any therapeutic could fully correct the impaired bone material strength seen in this disorder, an anabolic approach to increase bone volume could result in improved whole bone strength and reduced risk for fractures. To date, the effects of Scl-Ab have been reported in two mouse models of OI. In mice with a milder form of OI (Brtl/+), Scl-Ab increased bone formation, bone mass, and bone strength at both young (213) and adult (214) ages. In young mice with a severe form of OI (oim/oim), 10 wk of Scl-Ab treatment significantly increased cortical and trabecular bone mass and strength, resulting in more than a 50% reduction in skeletal fractures (215). These initial studies support the potential of Scl-Ab as a bone therapeutic in OI patients.

8. Gonad-intact nonhuman primates

Two studies have reported the use of Scl-Ab in adolescent, gonad-intact nonhuman primates (203, 204). The first study was performed in female cynos dosed once monthly with 3, 10, and 30 mg/kg for 2 months (n = 2–4 per group); the second study was performed in male cynos dosed every 2 wk with 30 mg/kg for 10 wk (n = 18–21 per group), beginning 1 d after a fibular osteotomy surgery. Scl-Ab treatment was associated with marked increases in bone mass at cortical and cancellous sites. Figure 5B reports *in vivo* DXA and peripheral quantitative computed tomography (pQCT) results from the male cyno study, with 10 wk of Scl-Ab treatment resulting in significant increases in areal BMD of lumbar spine, volumetric BMD in the proximal tibia metaphysis, and cortical area in the radial diaphysis. The improvements in vertebral bone mass were associated with improvements in trabecular thickness and compressive strength at the vertebra in both studies. These studies demonstrate that Scl-Ab is capable of producing robust increases in bone volume in nonhuman primates after relatively short treatment periods.

Figure 4.

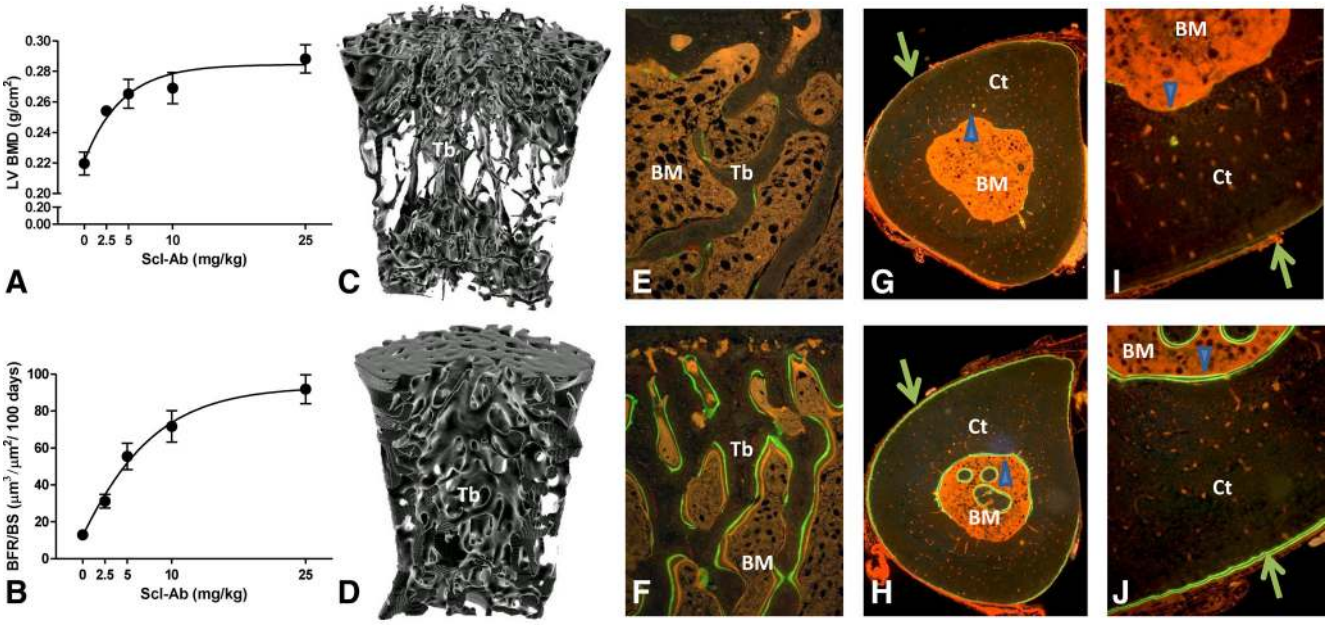

Figure 4. Scl-Ab dose-dependently increased areal BMD and trabecular BFR (BFR/BS). A, Dose-response curve of lumbar vertebral BMD (LV-BMD). B, Dose-response curve of trabecular BFR/BS at second lumbar vertebra. Dose-response curve was generated based on curve fit of nonlinear regression. LV-BMD at wk 5 was used for curve fit. BFR/BS was generated based on the measurements of calcein and tetracycline labels given 12 and 2 d before necropsy. Eleven-month-old female rats (5 months after OVX) received sc injections of Scl-Ab at 0, 2.5, 5, 10, or 25 mg/kg (twice weekly) for 5 wk. [Modified from Ref. 206.] Data are expressed as the mean ± SEM; n = 6–10 per group. Scl-Ab increases trabecular and cortical bone in animal models. C and D, Representative microCT images of cancellous bone from the fourth lumbar vertebral body of 19-month-old female rats (13 months after OVX) treated with vehicle (C) or Scl-Ab (D) at 25 mg/kg, sc injection, twice per week for 5 wk (65). Scl-Ab-treated rat had higher trabecular bone volume and more plate-like and thicker trabeculae than the vehicle-treated rat. E and F, Fluorescent images of lumbar vertebral body trabecular bone from OVX rats treated with vehicle (E) or 25 mg/kg Scl-Ab (F) twice per week for 5 wk showed that Scl-Ab treatment increased bone volume and bone formation on trabecular surface (206). G–J, The effects on cortical bone are represented by fluorescent images of cross-sections of tibial diaphysis from 10-month-old gonad-intact female rats treated with vehicle (G and I) or Scl-Ab at 25 mg/kg, sc injection (H and J), twice per week for 4 wk (216). Panel I is a representation of higher magnification of G, while panel J is a representation of higher magnification of H. Scl-Ab increased bone formation on both periosteal (*arrow*) and endocortical (*arrowhead*) surfaces, leading to the reduction of the marrow cavity area after short-term treatment. BM, Bone marrow; Tb, trabeculae; Ct, cortex.

B. Effect of Scl-Ab on bone formation and resorption in animal models

Consistent with its mechanism of action, Scl-Ab increased bone formation in every one of the animal models described above. In aged OVX rats, BFR/bone surface (BFR/BS) was markedly increased on trabecular, endocortical, and periosteal surfaces (65). These increases in BFR/BS stemmed from increases in both mineral apposition rate and mineralizing surface, indicating that Scl-Ab treatment increases both the number and activity of osteoblasts. Similar increases in bone formation indices were observed in aged male rats, gonad-intact adult female rats, and immobilized female rats (200, 201, 216). Furthermore, the effect of Scl-Ab on BFR/BS appeared to be 2-fold greater on the endocortical surface than on the periosteal surface in these short-term studies. The effect of Scl-Ab on bone formation does not appear to depend upon bone turnover status because Scl-Ab has been shown to increase bone formation in both lower (65, 206, 216) and higher (207) bone turnover status in rodent models. Marrow composition was also not a factor in the anabolic effects because Scl-Ab resulted in significantly greater BFR/BS in both red and yellow (fatty) marrow sites in female rats (216).

In adolescent cynos, Scl-Ab increased the serum bone formation markers P1NP and osteocalcin, which corresponded with increased histological bone formation in vertebral trabecular bone, and on the endocortical and periosteal surfaces of the femur diaphysis (203, 204, 217). The increased bone formation was primarily due to increases in the amount of mineralizing surface, although the rate of mineral apposition on those surfaces was also higher in the treated females.

In contrast to the clear effect of Scl-Ab on bone formation, the effect of Scl-Ab on bone resorption in rodent and nonhuman primate studies appears to be less consistent. TRACP5b, a marker of osteoclast number, is generally

Figure 5.

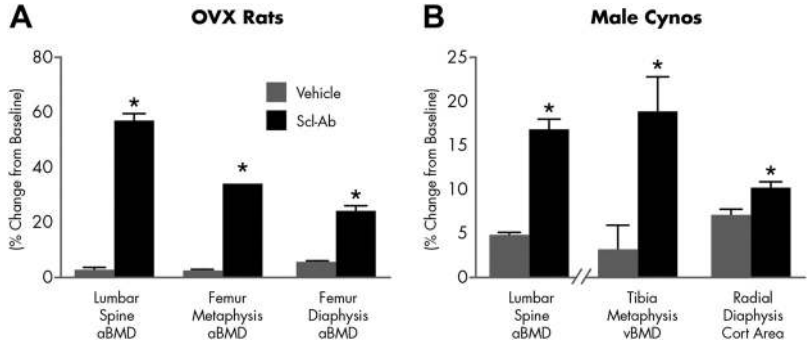

Figure 5. Scl-Ab increases trabecular and cortical bone mass in animal models. *In vivo* densitometry was used to assess the effects of Scl-Ab in OVX rats (A) and adolescent male cynos (B). A, In OVX rats after 12 wk of 15 mg/kg Scl-Ab twice weekly, DXA BMD was markedly increased from baseline at sites containing trabecular bone (lumbar spine and femur metaphysis), as well as purely cortical sites (femur diaphysis). B, In cynos after 10 wk of 30 mg/kg Scl-Ab every 2 wk, increases observed in lumbar spine BMD by DXA, proximal tibia vBMD by pQCT, and radius diaphyseal cortical area by pQCT are shown. Data are expressed as percentage change from baseline, mean ± SE; n = 8–10/group (rats) and 17–20/group (cynos). *, $P < 0.05$ *vs.* vehicle by 2-tailed *t* test. aBMD, Areal BMD; vBMD, volumetric BMD.

reduced or unchanged by Scl-Ab in rodent studies (199, 202, 218, 219) (Table 2). However another bone resorption marker, serum CTx-1, was not affected by Scl-Ab treatment in aged male rats and in OVX rats (200, 206, 218). In cynos treated with Scl-Ab, serum CTx-1 remained relatively unchanged in females (203) and was slightly elevated in males in a model of fracture healing (204). Osteoclast surface, a histological index of bone resorption, was either decreased (65, 204) or unchanged (200, 218) after Scl-Ab treatment. Eroded surface, another histological index of bone resorption, was decreased after Scl-Ab treatment in immobilized rats and normal-loaded rats (201, 216), as well as in male cynos (204). It is unclear whether the reduction in eroded surface after Scl-Ab treatment was a direct effect on the osteoclasts or secondary to the marked increase in bone-forming surface. Cortical porosity was also reduced by Scl-Ab in aged OVX rats (65), but no consistent treatment effects were found in cynos (203, 204). These results differ from the increases in cortical porosity reported in OVX primates after 16 months of treatment with PTH (1–84) or PTH (1–34) (220, 221), therapeutics that clearly stimulate bone resorption as well as bone formation in both monkeys and humans. In addition, transcript levels of TRACP5b and cathepsin K were decreased in rat bones after 5 wk of Scl-Ab treatment (222). Across studies, Scl-Ab consistently reduced tissue level resorption markers in trabecular bone, whereas systemic biomarker responses were less concordant. The effects of longer term Scl-Ab treatment on bone resorption parameters, including cortical porosity, have not yet been reported.

The mechanism by which the sclerostin pathway intersects with bone resorption may involve the OPG-RANKL axis because the osteoclast inhibitor OPG is considered a downstream target of Wnt/β-catenin signaling (17, 223–226). However, *in vivo* data demonstrating an effect of Scl-Ab on serum OPG levels is limited; data from one *in vivo* study showed no effects of Scl-Ab on serum RANKL or OPG levels in a mouse model of colitis (199). Therefore, the effects of Scl-Ab treatment on the OPG-

Figure 6.

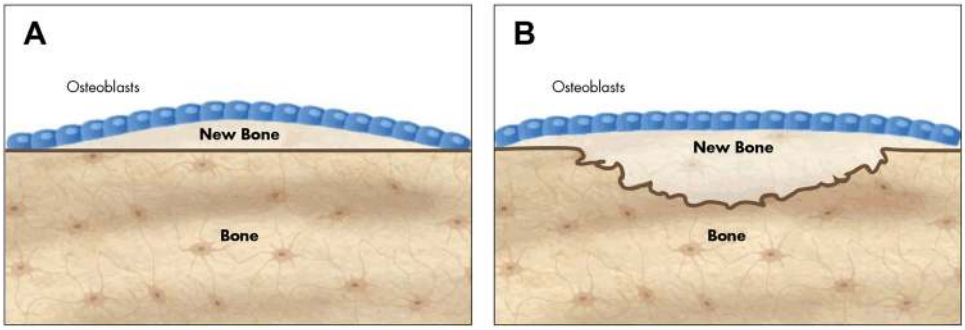

Figure 6. Tissue level mechanism of Scl-Ab-induced bone formation. Bone formation can occur on quiescent surface (modeling; A) or preresorptive surface (remodeling; B). The majority of the new bone formation induced by Scl-Ab in rodent and nonhuman primates occurred on quiescent surface, indicating that Scl-Ab induces modeling-based bone formation (A). On preresorptive surface, new bone formation induced by Scl-Ab is greater than the amount that was resorptive, thus creating positive bone balance (B). Furthermore, Scl-Ab can extend the new bone formation to cover over the quiescent surface near the preresorptive surface (B).

RANKL axis in animal models and in humans requires further investigation.

Bone formation can occur with or without prior activation of bone resorption as part of the remodeling and modeling processes, respectively. In adult rodents (227) and humans (228), the vast majority of bone formation occurs via remodeling, with bone formation following bone resorption on the same bone surface. Scl-Ab treatment in OVX rats (206) and adolescent male cynos (229) was shown to increase trabecular bone formation primarily on quiescent surfaces, indicating that Scl-Ab stimulated modeling-based bone formation without prior activation of bone resorption (as illustrated in Fig. 6A). Scl-Ab increased modeling-based mineralizing surface from 0.4 to 27% of total bone surface after 10 wk, with no significant effect on remodeling-based mineralizing surface (from 17 to 20%) (229). The mechanism of increased modeling-based bone formation induced by Scl-Ab is an area of continuing research. Remodeling sites that were present prior to treatment were likely affected by Scl-Ab as well because a greater percentage of remodeling surfaces with labels spanning an 8-wk period were observed. This extension of bone formation likely resulted in a positive bone balance as reflected in the nonsignificant increase in wall thickness reported in Scl-Ab-treated female cynos (illustrated in Fig. 6B).

C. Effects of Scl-Ab on bone material properties

As a bone therapeutic is developed, it is important to establish that the treatment-related increases in BMD correspond to improvements in bone strength. Because bone mass and strength typically show strong linear correlations in humans (230) and in nonhuman primates (231), therefore these relationships provide an important benchmark for evaluating whether the bone mass accrued during drug treatment has normal biomechanical properties (232). In OVX rats (233) and adolescent female cynos (203) treated with Scl-Ab, the correlation between trabecular and/or cortical bone mass with bone strength remained consistent. At cortical sites, material properties can be calculated from the whole bone strength parameters, after correcting for cortical geometry. At the femur diaphysis, moderate increases in material properties after Scl-Ab treatment have been reported in aged OVX rats (233) and colitic mice (199). Although it is possible that Scl-Ab may have had a positive effect on bone matrix properties in these studies, some of the apparent positive changes may be due to changes in cortical microstructure (*i.e.*, porosity) or as a result of the limitations in accounting for changes in geometry when testing short, thick rodent bones (234). In the more slender femurs from female cynos, 8 wk of Scl-Ab treatment resulted in normal material properties (203).

Sclerostin inhibition could affect material strength by altering matrix mineralization, and as mentioned in *Section IV.G.* bone mineralization and sclerostin may be linked. Bone formed during Scl-Ab treatment in rats and cynos has normal lamellar structure as determined by polarized light microscopy, with no apparent accumulation of unmineralized osteoid (65, 203). In rats treated for up to 8 wk, Scl-Ab did not impact tissue mineralization by ash analysis (200) or quantitative backscatter electron imaging (235), despite large increases in bone volume. Similarly, in WT and Brtl/+ OI mice, 2 wk of Scl-Ab treatment did not affect tissue mineralization or the stiffness of the newly formed bone as measured by nanoindentation. Although these results are encouraging, osteoid mineralization occurs quickly in rodents, and the effects of Scl-Ab on bone matrix mineralization in primates and humans have not yet been reported.

D. Scl-Ab in sequence or combination with antiresorptive agents

Antiresorptive agents are commonly used to manage patients with osteoporosis. Bone anabolic agents may be considered in patients at high risk for fracture or when additional increases in bone mass are required to further reduce fracture risk in these patients. Human studies in which antiresorptive therapy is concurrently used with or followed by PTH (1–34) or PTH (1–84) have in some cases shown a blunting of the anabolic effects of PTH (236–239). These results can be explained, at least in part, by the majority of increased bone formation by PTH treatment occurring at the remodeling surface (228), although it was reported that PTH can activate bone-lining cells to become mature osteoblasts in mice (240) and in rats (241). Therefore, reduced remodeling by antiresorptive therapies may reduce the anabolic effects of PTH. Importantly, Scl-Ab stimulated the majority of bone formation on quiescent surfaces of trabecular bone in OVX rats and in nonhuman primates (206, 229), suggesting that much of the bone anabolic activities of Scl-Ab are independent of bone resorption. Thus, it would be expected that pretreatment or combined treatment with antiresorptive therapies would not block the anabolic activity of Scl-Ab.

In a sequential alendronate (ALN) to Scl-Ab transition study in OVX rats, Scl-Ab treatment had similar anabolic effects in increasing bone formation, bone mass, and bone strength when administered to OVX rats pretreated with either vehicle or ALN for 6 wk (218). Specifically, serum osteocalcin and the BFR on trabecular, endocortical, and periosteal surfaces responded similarly to Scl-Ab in OVX rats pretreated with vehicle or ALN. ALN alone decreased

serum bone resorption and formation markers and trabecular BFR at the lumbar vertebrae, consistent with its mechanism of action as an antiresorptive, and prevented further bone loss. Furthermore, cotreatment of OVX rats with ALN and Scl-Ab did not blunt the anabolic effects of Scl-Ab on bone formation, bone mass, and bone strength (218).

A discontinuation study in OVX rats treated with Scl-Ab showed that the increased BMD levels achieved over 8 wk of Scl-Ab treatment were maintained for a period after discontinuation followed by a gradual decline (Fig. 7, A and B) (242). The decline in BMD was more apparent in the lumbar vertebral region (Fig. 7A) compared with the femur-tibia region (Fig. 7B) in OVX rats. In addition, the slope of decline was similar between rats treated with Scl-Ab at 12.5 or 25 mg/kg in the lumbar vertebral region. The declines in BMD were associated with increased serum TRACP5b and decreased serum osteocalcin (our unpublished data), indicating that increased bone resorption and decreased bone formation contributed to the declines in BMD after discontinuation of Scl-Ab treatment.

E. Effects of Scl-Ab on growth plate cartilage, subchondral bone, and articular cartilage

The effects of Scl-Ab on growth plate and articular cartilage have been examined in rats and mice. In aged male rats treated with Scl-Ab for 5 wk, longitudinal growth rate, a dynamic measurement of long bone growth, was similar between the Scl-Ab-treated group and vehicle controls (200). Tibial length and growth plate thickness were also similar between treated and control rats in this study. As noted earlier, Scl-Ab treatment did not prevent the growth retardation induced by dexamethasone in young mice (202). Collectively, these results illustrate that inhibition of sclerostin by treatment with Scl-Ab has no effect on growth plate cartilage and long bone growth in rats and mice. Similar to effects of sclerostin inhibition at other skeletal sites, *SOST* KO mice and Scl-Ab-treated rats have significantly increased bone formation and bone mass in the subchondral bone of the distal femur epiphysis (243). Although it has been reported that sclerostin may play a role in cartilage degradation in OA models (91), examination of the articular cartilage in aged sclerostin KO mice and rats treated with Scl-Ab did not reveal any histological change in articular surface and articular thickness (243).

F. Effects of Scl-Ab on animal models of bone repair

Fracture healing is a complex temporal process that involves many tissue types and signaling pathways to reconstitute the broken bone (4). Proteins important to this process such as the BMP have been developed as therapeutics (4, 5, 16) because they stimulate bone formation to enhance healing.

Inhibition of sclerostin may be an anabolic approach to enhance fracture healing. In the absence of sclerostin in

Figure 7.

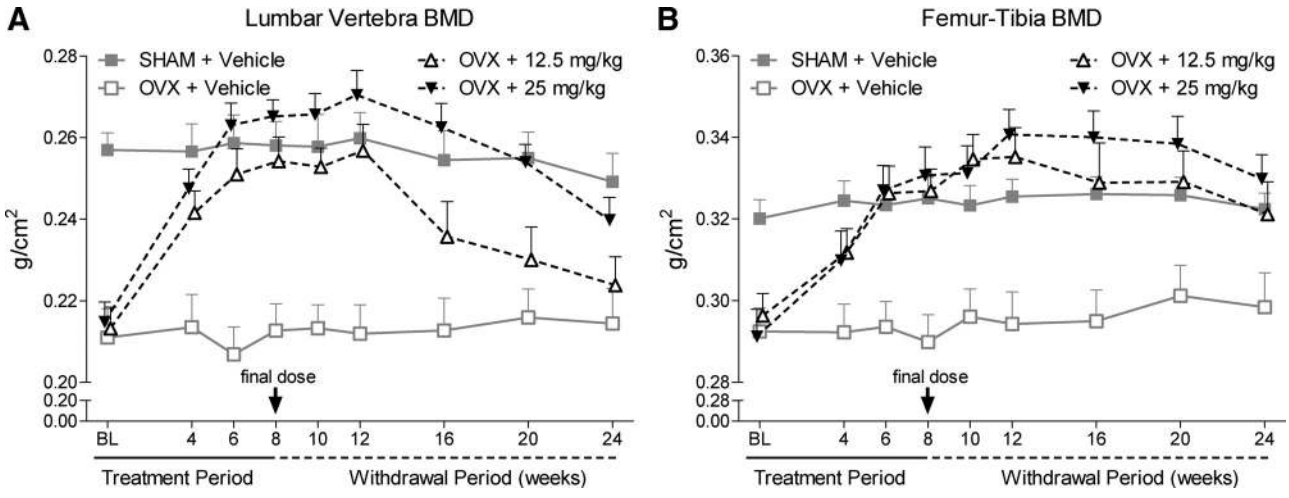

Figure 7. Changes in BMD after discontinuation of Scl-Ab treatment in OVX rats. A, Lumbar vertebral BMD (LV-BMD). B, Femur-tibia BMD. Thirteen-month-old female rats (7.5 months after OVX) received nine sc injections of Scl-Ab at 12.5 or 25 mg/kg (once a week). Thereafter, treatment was discontinued, and BMD was followed up to wk 24. Data are expressed as mean + SEM; n = 8–10 per group. Lumbar vertebral BMD in 12.5 mg/kg group was significantly greater than the vehicle-treated OVX group at wk 4, 6, 8, 10, and 12. Lumbar vertebral BMD in 25 mg/kg group was significantly greater than the vehicle-treated OVX group at wk 4, 6, 8, 10, 12, 16, and 20. Femur-tibia BMD in 12.5 mg/kg group was significantly greater than the vehicle-treated OVX group at wk 6, 8, 10, 12, 16, and 20. Femur-tibia BMD in 25 mg/kg group was significantly greater than the vehicle-treated OVX group at wk 6, 8, 10, 12, 16, 20, and 24.

Figure 8.

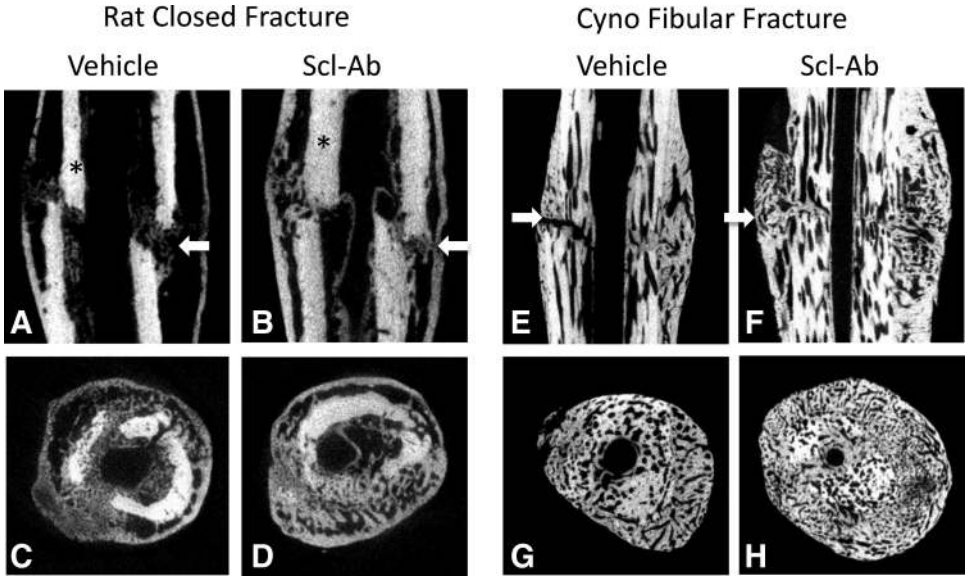

Figure 8. Representative microCT images of healed fractures in Scl-Ab fracture models. Seven-month-old male rats underwent closed femur fracture and were treated with vehicle (A and C) or Scl-Ab (B and D) for 7 wk. Four- to 5-yr-old male cynos underwent fibular osteotomy surgery and were treated with vehicle (E and G) or Scl-Ab (F and H) for 10 wk. *Top*, Longitudinal images; *bottom*, cross-sectional images. The experimental details were described in Ref. 204.

SOST KO mice, fracture healing is enhanced, resulting in a more mature callus in the early phase and a stronger callus in the later phase of healing after closed femur fracture or tibial osteotomy (244, 245). To date, Scl-Ab has demonstrated positive effects in several models of fracture healing in rodents and nonhuman primates, as summarized in Table 3. Scl-Ab enhanced fracture healing in femur osteotomy models in mice (246, 247) and rats (248), in a closed femur fracture model in rats (204, 246), and in a fibular osteotomy model in male cynos (204). In each study, Scl-Ab significantly increased the bone mass at the fracture site and the strength of the fracture union. Figure 8 illustrates the increase in bone volume observed by microCT after Scl-Ab treatment in the rat closed fracture and cyno fibular osteotomy models. Although it is not yet clear how Scl-Ab affected the histological callus composition throughout the healing process, Scl-Ab-treated cyno fibulae contained less cartilage and fibrous tissue compared with controls at the end of the study. Future work will demonstrate whether Scl-Ab affects the progression of non-bone components (fibrous, vascular, and cartilaginous) during fracture healing.

In long bone fracture models, bone formation occurs via two processes, endochondral and intramembranous ossification. Endochondral ossification begins with a cartilaginous callus that is replaced by bone, whereas intramembranous ossification is a result of direct bone apposition. Core defect models heal purely by intramembranous ossification, and Scl-Ab accelerated the rate of bone healing in core defects in the tibia diaphysis (249) and metaphysis (250) of rats. Large gap defects also primarily heal by direct bone apposition, and Scl-Ab enhanced healing in critical-sized defects in the rat femur shaft, resulting in increased bridging and mineralized bone mass in the gap in Scl-Ab-treated rats (251, 252).

Scl-Ab treatment improved fracture healing in several additional orthopedic models in rats. Distraction osteogenesis is a process used to lengthen limbs in which a segment of new bone is formed as the limb is slowly lengthened. In a rat model of distraction osteogenesis, Scl-Ab treatment resulted in earlier bony bridging of the consolidating defect (253). Scl-Ab also enhanced healing in animal models of implant fixation in the rat tibial metaphysis (254) and the rat femur shaft (255, 256). In a posterolateral spinal fusion model in rats, Scl-Ab increased bone mass in the fusion (257). In sum, the data from animal models of bone repair suggest that Scl-Ab may have therapeutic applications in orthopedic settings to accelerate healing or prevent complications of delayed healing.

G. Effects of Scl-Ab in humans

Inhibition of sclerostin by treatment with Scl-Ab increased bone formation, bone mass, and bone strength in animal models of bone loss and bone repair. To confirm the anabolic effects of Scl-Ab in humans and further explore its potential utility to treat human bone diseases, a

Figure 9.

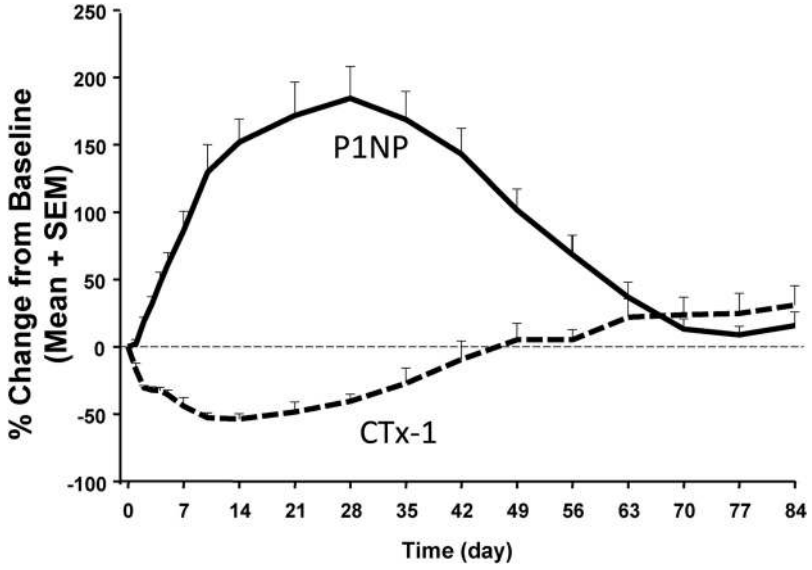

Figure 9. Early response of serum bone formation (P1NP) and bone resorption (CTx-1) markers to a single dose of Scl-Ab administration in human (10 mg/kg, sc injection). Scl-Ab increases bone formation and decreases bone resorption simultaneously, thus creating a larger anabolic window in early stage of treatment. The data for longer-term and multiple-dose effects of Scl-Ab on bone formation and bone resorption in human are an area of continuous investigation. [Modified from Ref. 205 with permission from Journal of Bone and Mineral Research.]

randomized, double-blind, placebo-controlled, single-dose study was performed in healthy men or postmenopausal women between 45 and 59 yr of age (205). Results from this study indicated that a single administration of Scl-Ab dose-dependently increased the serum bone formation markers P1NP, osteocalcin, bone-specific alkaline phosphatase, with maximum increases observed approximately 1 month after dosing. Scl-Ab also dose-dependently decreased the serum bone resorption marker C-telopeptide. These changes in bone turnover markers corresponded to significant increases in BMD of the lumbar spine and total hip in both men and women, with the largest increases observed at d 85, the last time point they were measured. The combination of increased bone formation and decreased bone resorption simultaneously in the early response to Scl-Ab creates a larger window for bone accrual as shown in Fig. 9. The ability of Scl-Ab to simultaneously stimulate bone formation and inhibit bone resorption represents the first agent to clearly demonstrate these effects in human subjects. These biomarker profiles are consistent with the histomorphometric data in animal models that demonstrated the ability of Scl-Ab to increase bone formation while decreasing tissue-level bone resorption (206, 229). The early response to Scl-Ab differs from that of daily teriparatide (PTH 1–34) treatment, which increases both serum bone formation and resorption markers and has been shown to primarily increase remodeling-based bone formation in human biopsies (228, 258).

Longer-term effects of Scl-Ab on bone formation, bone resorption, BMD, reducing fracture risk, and enhancing fracture healing in humans are the subjects of ongoing clinical trials.

VIII. DKK1 Mutations and Bone

The discovery of low and high bone mass phenotypes due to loss- or gain-of-function mutations in LRP5 facilitated the investigation of DKK1 as a regulator of bone mass. DKK1 homozygous KO mice display severe defects in head formation that result in anterior truncations and death at birth (83). Additionally, homozygous DKK1 KO mice exhibit limb abnormalities, further highlighting the requirement of this molecule for pattern formation in the developing embryo. DKK1 heterozygous ($DKK1^{+/-}$) mice and mice with a hypomorphic $DKK1^{d}$ (doubleridge) allele that express a low amount of DKK1 are viable, which has allowed the use of these mice to examine the physiological role of DKK1 in bone (37, 38). Moreover, mice overexpressing DKK1 were generated using either a type I collagen promoter (28, 30, 33) or an adeno-associated virus (AAV) (101). The phenotypes of these mice are summarized in Table 1 and discussed below.

A. Reduced DKK1 expression in mice

$DKK1^{+/-}$ and heterozygous $DKK1^{d}$ ($DKK1^{+/d}$) mice were mated to produce offspring of the following genotypes: $DKK1^{+/+}$, $DKK1^{+/d}$, $DKK1^{+/-}$, and $DKK1^{d/-}$ (38, 39). $DKK1^{+/d}$ mice had a 25% reduction in calvarial DKK1 expression, with greater trabecular bone volume in the distal femur and no significant changes in the femur cortex as compared with WT controls. $DKK1^{+/-}$ mice had a 41% reduction in calvarial DKK1 mRNA, with greater bone volume in the distal femur and cortical midshaft compared with controls. Similar increases in trabecular bone volume were observed in another line of $DKK1^{+/-}$ mice (37). These observed changes associated with increased bone formation were not accompanied by changes in bone resorption (37). Significant increases in bone strength were found in proximal tibia, but not at femoral shaft of $DKK1^{+/-}$ mice. $DKK1^{d/-}$ mice had the lowest

DKK1 expression in the calvaria (75% less than WT control) (38), which corresponded to the most dramatic increases in both trabecular and cortical bone volume. Femur diaphyseal bone strength was significantly greater in $DKK1^{d/-}$ mice (39). Together these results indicate that the progressive reduction in DKK1 increased trabecular and cortical bone mass in mice.

B. DKK1 overexpression in mice

In contrast to the high bone mass phenotype seen in mice with reduced DKK1 expression, transgenic mice overexpressing DKK1 via the Col1A1 3.6- or 2.3-kb promoter exhibit a low bone mass phenotype (28, 30, 33). DKK1 transgenic mice had a thinner calvarial cortex, less trabecular bone volume, and reduced trabecular number in the distal femur (30), vertebrae, and proximal tibia (28, 33). Bone formation was reduced in DKK1 transgenic mice, as reflected in lower osteoblast surface, BFR, and/or serum osteocalcin levels (28, 30, 33). Bone resorption parameters at the serum and tissue level were either unchanged (30, 33) or increased (28) in DKK1 transgenic mice.

Transgenic mouse models are valuable to understanding the function of a targeted gene as expressed within its target tissue. However, from the previously mentioned transgenic studies, it was not clear whether increased systemic DKK1 levels in adult mice could affect bone similarly. To study this, AAV was used to drive DKK1 overexpression in and subsequent secretion from the liver of adult mice. DKK1 overexpression using AAV in adult mice caused significant decreases in lumbar BMD within 2 wk, confirming the negative impact of increased circulating DKK1 on bone (101). Bone resorption was increased in AAV-DKK1 mice, as reflected in significant increases in serum TRACP5b at wk 2 and osteoclast surface at wk 8 (259). If this effect was simply an increase in remodeling rate, the increased bone resorption should have been followed by increased bone formation. However, bone formation parameters were not increased in AAV-DKK1 mice (101). The neutral effect on bone formation in this model likely reflected the combined direct negative effect of DKK1 on osteoblastic bone formation and a positive secondary effect due to increased bone resorption. These results demonstrated that increased DKK1 levels in the circulation or within bone were capable of regulating bone formation and/or resorption and led to decreased bone mass in adult mice.

IX. Inhibition of DKK1 by Monoclonal Antibody (DKK1-Ab)

The confirmation in genetic models that DKK1 expression levels were associated with changes in bone mass led to studies of pharmacological inhibition of DKK1 by monoclonal antibody (DKK1-Ab) in animal models of diseases. Similar to Scl-Ab, DKK1-Ab has demonstrated efficacy in gonad-intact rodents (260) and rodent models of fracture healing (101) and implant fixation (261). Unlike Scl-Ab, DKK1-Ab did not demonstrate efficacy in adult OVX rats (260). In nonhuman primates, Scl-Ab had robust effects after 2 months in adolescent cynos, whereas DKK1-Ab modestly improved bone mass in aged OVX rhesus monkeys after 9 months (101, 262). In addition, DKK1-Ab demonstrated efficacy in rodent models of RA (134), AS (263), and multiple myeloma (264). Table 4 summarizes the results from these DKK1-Ab studies.

A. Effects of DKK1-Ab on bone metabolism in animal models

1. Gonad-intact rodents

In our study using both young (6-wk-old) and mature (34-wk-old) male mice, 3 wk of DKK1-Ab treatment at 20 mg/kg three times per week resulted in significant increases in trabecular volumetric BMD and trabecular number at the distal femur as compared with vehicle controls (101, 260). Compared with controls, trabecular thickness was slightly greater in young mice after DKK1-Ab treatment, whereas no increase in trabecular thickness was seen in the adult mice. Cortical thickness was not significantly affected by DKK1-Ab treatment in the same study. Similar findings were reported in gonad-intact young (6-wk-old) female mice treated with rising doses of a fully human DKK1-Ab for 4 wk (265). Significantly greater trabecular thickness (+10%) was reported in young male mice treated with DKK1-Ab for 4 wk (266). In gonad-intact young female rats, we observed increases in trabecular number after DKK1-Ab treatment for 3 wk (101, 260). In addition, cortical thickness was increased and accompanied by significantly greater periosteal bone formation.

2. Estrogen deficiency-induced bone loss

In adult OVX mice, a single administration of fully human DKK1-Ab increased BMD at the lumbar spine and whole leg in a pharmacokinetic study (262). Furthermore, in adult estrogen-deficient mice with established osteopenia, weekly treatment with fully human DKK1-Ab at 2 or 20 mg/kg for 8 wk restored BMD at the lumbar spine and whole femur back to the levels of sham controls (262). In this study, after 24 d of 2 mg/kg DKK1-Ab treatment, the endocortical BFR was increased by 86%, primarily due to increased mineralizing surface. Mineral apposition rate remained unchanged in the DKK1-Ab-treated group. After 56 d of treatment, endocortical BFR was no longer significantly greater in the 2 mg/kg DKK1-Ab group but

TABLE 4. Effects of DKK1-Ab on bone and fracture healing in animal studies

Animals	Models	Length of treatment (wk)	Major findings	Ref.
Mice	Intact	3–4	↑ Bone mass	101, 260, 265, 266
Mice	hTNFtg	4	↓ Bone resorption and erosion	134, 269
			↑ Bone formation and mass	263
			↑ Fusion of sacroiliac joints	
Mice	Multiple myeloma	4	↓ Lytic lesion	276
			↑ Bone formation and mass	
Mice	OVX[a]	8	↑ Bone formation and mass	262
Rats (young)	Intact	3	↑ Bone formation and mass	101
Rats (adult)	OVX[b]	4	⇆ Bone formation, resorption and mass	101, 267
Nonhuam primates (adult)	OVX[c]	10	↑ Bone formation and mass, ⇆ bone resorption	262
Mice	Closed femur fracture	4	↑ BMD and bone strength at fracture site[d]	42
Rats	Proximal tibia screw implant	4	↑ Pull-out strength	261
Rats	Closed femur fracture	7	↑ BMD and bone strength at fracture site	101

↑, Greater or increased; ↓, decreased; ⇆, unchanged.

[a] Treatment begins 12 wk after OVX.

[b] Treatment begins 2 or 3 months after OVX.

[c] Treatment begins more than 8 yr after OVX.

[d] Increased bone strength was not observed in the group where treatment started at d 4.

was significantly greater in the 20 mg/kg DKK1-Ab group as compared with OVX controls. No significant changes were observed on periosteal BFR.

In contrast to the positive findings with DKK1-Ab in gonad-intact young rats and estrogen-deficient mice, 4 wk of DKK1-Ab treatment did not increase BMD and bone formation on trabecular, endocortical, and periosteal surfaces in adult OVX rats with established osteopenia (101, 267). No DKK1 mRNA expression was detected in bone of 10-month-old OVX rats by *in situ* hybridization, whereas its expression was readily detectable in young growing rats (101). Both circulating and bone DKK1 protein levels were higher in young rats than those in adult rats (101) and in adult mice compared with adult rats. The different expression profile of DKK1 in older rats may explain the discrepancy in efficacy of the DKK1-Ab between this and other studies.

Circulating levels of DKK1 and DKK1 expression in bone have not been reported in nonhuman primates. DKK1-Ab treatment modestly increased bone formation markers and BMD in aged OVX rhesus macaques (262). Serum levels of the bone formation marker P1NP increased after 10 d of treatment and remained elevated for up to 70 d of treatment, with no significant changes in bone resorption markers. DXA analysis showed that 9 months of DKK1-Ab treatment was associated with about a 5% increase in areal BMD at the lumbar spine and whole body regions compared with baseline, whereas the vehicle control group remained unchanged. pQCT analysis showed roughly 11 and 5% increases in trabecular volumetric BMD at the lumbar spine and in integral volumetric BMD at the distal radius, respectively, in the DKK1-Ab group.

3. Glucocorticoid-induced bone loss

DKK1 mRNA and serum DKK1 have been reported to increase in glucocorticoid-treated mice with suppressed bone formation (120, 266). However, a preliminary study showed that DKK1-Ab treatment did not prevent the reduction in serum P1NP in response to dexamethasone in mice (266). In contrast, treatment with DKK1 antisense oligonucleotides prevented bone loss and increased bone formation in a rodent model of glucocorticoid-induced bone loss (268). Therefore, the role of DKK1 inhibition in glucocorticoid-induced bone loss warrants further investigation.

4. RA-induced bone loss

A direct causative role for DKK1 in the suppression of bone formation in inflammatory arthritis was tested by treating TNFα-overexpressing transgenic (hTNFtg) mice with a DKK1-Ab (134). These mice were treated with various doses of DKK1-Ab either alone or in combination with a TNFα antibody (TNFα-Ab) for 4 wk. The DKK1-Ab did not affect paw swelling or grip strength in these mice, indicating that DKK1 does not contribute to the inflammatory process in these animals. Importantly, DKK1 inhibition blocked bone erosion even in the presence of a profound ongoing inflammatory response, suggesting that DKK1 was a critical molecule involved in coupling inflammation to bone loss. Inhibition of DKK1 activity within the inflamed joint resulted in osteophyte

formation at sites most subject to inflammation-induced bone erosion, further indicating that DKK1 blocked bone formation at these sites. Analysis of histomorphometry and bone biomarkers in DKK1-Ab-treated hTNFtg mice clearly demonstrated that in addition to promoting bone formation in this model, DKK1-Ab also inhibited osteoclast activity and bone resorption at the joint. This latter effect on bone resorption was secondary to increased OPG expression stimulated by DKK1 inhibition. In addition to severe joint erosion, the hTNFtg mouse model also develops a TNFα-driven osteopenic phenotype at skeletal sites that are not affected by obvious inflammation. DKK1-Ab treatment prevented the systemic bone loss in this model, again by promoting bone formation and inhibiting bone resorption (269). An interesting finding in this study was that TNFα also induced the expression of sclerostin and that anti-DKK1-Ab reduced TNFα-driven sclerostin expression both *in vitro* and *in vivo*. The potential relevance of elevated DKK1 levels in mediating bone erosion in chronic inflammatory conditions has been extended to studies in models of systemic lupus erythematosus (270), psoriatic arthritis (271), and additional studies in RA (272–274).

5. AS-induced bone loss

AS is a TNFα-mediated rheumatic condition of the axial-skeleton characterized by inflammatory pain and early bone erosions. As the disease progresses, new bone formation occurs at the eroded surfaces, resulting in the formation of bony spurs (syndesmophytes). Syndesmophyte formation ultimately restricts the joint spaces and may result in fusion of adjacent joints or vertebrae, leading to ankylosis that is a hallmark of AS. Nonsteroidal anti-inflammatory drugs and TNFα inhibitors are capable of modulating inflammation and pain associated with the disease, yet they are unable to control the bone remodeling ongoing within the affected joints.

Based on observations of osteophyte formation at the eroded surface of peripheral joints in the hTNFtg model, Uderhardt *et al.* (263) hypothesized that increased Wnt signaling is potentially mediated by decreased DKK1 levels and contributes to joint ankylosis. hTNFtg mice were treated with a TNFα-Ab, DKK1-Ab, or a combination of both antibodies. TNFα-Ab and the combination of TNFα-Ab and DKK1-Ab controlled inflammation at the sacroiliac joints, whereas DKK1-Ab alone did not affect inflammation. Each antibody effectively diminished bone erosions, and the combination of both antibodies completely inhibited bone erosions within the joint. DKK1-Ab, either alone or in combination with TNFα-Ab, led to a significant increase in bone formation within the sacroiliac joint that progressed to ankylosis of the joints. Because bony proliferation and progressive ankylosis do not normally occur in this model and were only observed upon DKK1-Ab treatment, the authors concluded that activated Wnt signaling leads to sacroiliac joint fusion in this model. Whether decreased DKK1 levels or activated Wnt signaling directly contributes to syndesmophyte formation in AS remains to be clinically demonstrated, but the data suggest that inhibition of Wnt signaling, perhaps by utilizing DKK1 protein, may inhibit this process.

6. Multiple myeloma-induced bone loss

The direct involvement of DKK1 in the generation of osteolytic lesions has been demonstrated by the use of DKK1-Ab in different models of myeloma-induced bone disease. *In vitro*, the ability of bone marrow plasma with high DKK1 levels isolated from myeloma patients to inhibit osteoblast differentiation *in vitro* was reversed by the inclusion of DKK1-Ab (145). Yaccoby *et al.* (275) used the SCID-rab model in which SCID mice were implanted with a small piece of rabbit bone that was allowed to engraft and then were injected with bone marrow cells isolated from patients with multiple myeloma. DKK1-Ab treatment increased BMD of the implanted myelomatous bone, which was accompanied by increased osteoblast number and decreased osteoclast number. Importantly, biomarkers of tumor burden were also reduced in this model; however, whether this was a direct effect of DKK1-Ab on the myeloma itself, or a secondary effect resulting from inhibition of osteolytic bone resorption was not determined.

Heath *et al.* (276) used the well-characterized syngeneic 5T2MM mouse myeloma model to explore the effect of DKK1 expression on osteolytic bone disease. In addition to several primary human myeloma cell lines, murine 5T2MM myeloma cells were shown to express DKK1, in addition to other components of the Wnt signaling pathway. 5T2MM cells injected into the tail vein of C57BL/KaLwRij mice home to bone marrow and contribute to the formation of osteolytic lesions. DKK1-Ab prevented the 5T2MM-mediated loss of osteoblasts and preserved osteoblast function, which prevented the formation of osteolytic bone disease in this model as was evident by a significant reduction in lesion number and by the preservation of cortical and trabecular bone volume. Unlike the data obtained in the SCID-rab model, DKK1-Ab treatment did not prevent the 5T2MM-induced increase in osteoclast numbers, nor was tumor burden affected. Whether the differences observed between the two models reflect intrinsic differences in the models or the specific DKK1-Ab used is presently unclear. Nonetheless, others have demonstrated in yet another murine model of multiple myeloma that activation of Wnt signaling with LiCl reduced osteoclast numbers and tumor burden within the

bone microenvironment, but not at extraosseous sites (277).

That activation of Wnt signaling in multiple myeloma decreases tumor burden may seem counterintuitive, given the prevailing dogma that an activated canonical Wnt signal is tumorigenic. However, accumulating evidence suggests that activation of canonical Wnt signaling and its impact on tumor formation and progression is a complex process that is dependent not only on cellular context, but also on the microenvironment surrounding the tumor. In the case of multiple myeloma, it is likely that the impact of DKK1 inhibition and subsequent activation of Wnt signaling on tumor burden is at least in part secondary to inhibition of the ongoing osteolysis. Impacting this process is likely to interrupt the complex interplay between tumor and bone that results in the vicious cycle that ensues in this disease (278). The data generated using DKK1-Ab in preclinical models that were predicated on the initial findings of increased DKK1 expression in patients with multiple myeloma have provided the rationale for the evaluation of DKK1-Ab in human clinical trials for multiple myeloma-induced bone disease (264). Early results from a phase I/II study in patients with relapsed or refractory multiple myeloma treated with DKK1-Ab demonstrated increased BMD in the first two patients treated with the 10 mg/kg dose. Additionally, elevations in P1NP and osteocalcin were observed in one patient receiving the 10 mg/kg dose, and no DKK1-Ab-related adverse events were reported for any dose (279). Additional clinical trials are currently under way (45, 51, 53).

B. Effects of DKK1-Ab in animal models of bone repair

As described in the Introduction, Wnt signaling may play an important role in bone healing, and DKK1 is a potential therapeutic target. Recently, two independent studies demonstrated that adenoviral-mediated DKK1 overexpression impaired the bone regeneration process in mouse models of bone repair (40, 43). In TOPgal mice with systemic administration of a virus expressing DKK1, very little new bone was formed in the skeletal injury site as compared with the controls (43). Furthermore, adenoviral-mediated DKK1 delivery to the fracture site delayed fracture healing in a mouse tibia fracture model (40). These results imply that DKK1 overexpression is capable of negatively impacting the healing process, suggesting that DKK1 inhibition may have beneficial effects during the fracture repair process.

Indeed, sc administration of DKK1-Ab has been reported to enhance fracture healing in a mouse closed femur fracture model (42). After 4 wk, the callus area and strength were significantly greater in mice treated with DKK1-Ab immediately after surgery as compared with vehicle controls. We demonstrated that bone mass and strength at the fracture site were significantly greater after 7 wk of DKK1-Ab treatment in skeletally mature male rats with closed femur fracture (101). In normally loaded tibia with an implanted screw, DKK1-Ab increased pull-out strength, indicating DKK1-Ab enhanced bone healing (261). However, in unloaded tibia after Botox immobilization in the same model, DKK1-Ab was unable to improve fixation strength. DKK1-Ab also partially prevented the trabecular and cortical bone loss in the region adjacent to the screw of unloaded tibia, suggesting a potential effect of DKK1-Ab in disuse-induced bone loss. These results together suggest that systemic DKK1 inhibition enhanced the bone healing process in animal models of bone repair.

X. Conclusion

The initial observations stemming from human genetics have led to a significant understanding of the relationship between Wnt signaling molecules and bone formation and have engendered an exciting new era for potential development of novel anabolic therapies for skeletal disorders. There have been numerous recent advances in delineating receptor-ligand interactions, inhibitor-ligand interactions, and downstream signaling of this pathway in the regulation of bone metabolism, particularly in bone formation. Insight into the basic mechanisms of action has provided opportunities to seek better anabolic therapies for bone diseases such as osteoporosis and bone repair. As reviewed in this article, sclerostin and DKK1 are emerging as the leading new targets for anabolic therapies. Sclerostin expression is limited primarily to the skeleton and is maintained during aging, whereas expression of DKK1 is more widespread and decreases in bone with age. Inhibition of sclerostin or DKK1 by monoclonal antibodies has demonstrated efficacy in increasing bone formation, bone mass, and bone strength in various animal models of bone diseases. The current literature has emphasized the shorter-term effects of Scl-Ab and DKK1-Ab; if these molecules are to be developed as longer-term clinical therapeutics, then studies of greater duration will be required to demonstrate the long-term efficacy and safety of these molecules, including assessments of bone quality and cancer risk.

It appears that sclerostin antibody can stimulate bone formation, increase bone mass and bone strength, and enhance bone healing at any age in animal models of bone loss and bone healing. However, DKK1-Ab appears to consistently stimulate bone formation and increase bone mass in younger animal models of bone loss and less so in

adult animal models of bone loss, but it enhances bone healing in both young and adult animal models. As discussed, this latter finding may be due to the low level of DKK1 expression in adult or aged uninjured skeleton, which increases upon skeletal injury or fracture.

Importantly, Scl-Ab has been tested in men and postmenopausal women and has been shown to significantly increase serum bone formation markers and significantly decrease serum bone resorption markers, leading to significant increases in BMD after a single administration. To our knowledge, this is the first such molecule that has been shown to simultaneously increase bone formation markers and decrease bone resorption markers, although the mechanism of decreasing bone resorption with sclerostin antibody is not yet clear. DKK1-Ab is currently being tested in patients with multiple myeloma-induced bone loss, with the hope that inhibition of DKK1 activity will impair the destructive bone loss associated with this disease. The data summarized and reviewed in this article clearly demonstrate the great potential for antibodies to sclerostin or DKK1 in managing skeletal disorders such as osteoporosis and fracture healing, inflammatory bone diseases, and multiple myeloma. The ability of Scl-Ab and DKK1-Ab to reduce fracture risk and enhance fracture healing or to impair inflammation-induced bone loss in human subjects remains to be investigated. For a list of clinical trials with Scl-Ab or DKK1-Ab that are currently ongoing for the treatment of skeletal diseases, please see www.clinicaltrials.gov. The results from these clinical trials will provide important information regarding the potential benefits of Scl-Ab and DKK1-Ab in the management of skeletal disorders.

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Address all correspondence and requests for reprints to: Hua Zhu Ke, Ph.D., One Amgen Center Drive, MS 29-M-B, Thousand Oaks, California 91320. E-mail: hke@amgen.com.

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