

BMPs and Wnts in Bone and Cartilage Regeneration

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Abstract Bone morphogenetic proteins (BMPs) play an important role in osteoblast and chondrocyte differentiation and canonical Wnt signaling regulates bone mass. BMP-2 is approved for use in spinal fusions due to degenerative disk disease, and in the treatment of acute open fractures of the tibial shaft. BMP-7 is approved for lumbar spinal fusion and in the treatment of long bone nonunion fractures. Sclerostin monoclonal antibodies are currently under clinical trials for their application in treating patients with osteoporosis and bone fractures. The roles of BMPs and Wnts in bone and cartilage regeneration have been extensively studied in recent years and the progress in this research area is summarized in this chapter.

1 BMP Signaling in Bone and Cartilage Regeneration

Bone morphogenetic proteins (BMPs) are a group of growth factors in the transforming growth factor- β (TGF- β) superfamily (Chen et al. 2004; Cao and Chen 2005). BMPs were originally isolated from bone matrix (Urist 1965; Wozney et al. 1988). However, we now know that BMPs exist in connective tissues of many other organs in the body. For example, BMP-7 is mainly produced in kidney (Ozkaynak et al. 1991; Alper 1994) and BMP-9 is mainly expressed in liver (Song

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et al. 1995). Recombinant BMPs have now been used clinically to treat different types of orthopedic diseases, such as segmental bone defects, nonunion fracture, and for spinal fusion (Gupta and Khan 2005; Garrison et al. 2007).

BMP signaling is a complex process. Smad proteins play a central role in BMP signaling. Smad1/5 transiently and directly interact with activated type I BMP receptors, which phosphorylate the C-terminal SSXS motif of Smad in a ligand-dependent manner (Hoodless et al. 1996; Nishimura et al. 1998). After releasing from the receptor, the phosphorylated Smad proteins form heteromeric complexes with the related protein Smad4, which acts as a shared partner. This complex translocates into the nucleus and participates in gene transcription with other transcription factors (Cao and Chen 2005). Chondrocyte-specific *Smad1/5* double knockout (KO) mice (*Smad1/5^{Col2}*) showed a severe chondrodysplasia phenotype and are embryonic lethal (Retting et al. 2009), suggesting that Smad1/5 signaling is absolutely required for endochondral skeletal development. Since the nuclear translocation of Smad1/5 requires Smad4 binding, the prediction originally was that the chondrocyte-specific deletion of *Smad4* (*Smad4^{Col2}*) will produce similar defects in skeletal development. However, this is not the case. Although *Smad4^{Col2}* mice displayed growth retardation, the skeletal defects of these mice are less severe than those of *Smad1/5^{Col2}* double KO mice and *Smad4^{Col2}* mice survive into adulthood without problems (Zhang et al. 2005a). These findings suggest that, in addition to the Smad4 binding and nuclear translocation, Smad1/5 may be able to use other signaling pathways in chondrocytes.

To better understand bone induction activity among different members of the BMP family, the relative potency of bone formation activity among 14 BMP family members has been compared using an adenovirus gene delivery approach by intramuscular injection of BMP-expressing adenovirus-transduced C2C12 cells into the right quadriceps of nude mice. Radiographic and histological evaluations demonstrated that, in addition to BMP-2 and BMP-7, the well known bone induction agents, BMP-6, and BMP-9 effectively induced ectopic ossification when either AdBMP-transduced osteoblast progenitor cells or the viral vectors were injected into the quadriceps of athymic nude mice (Kang et al. 2004). This study suggests that, in addition to extensively studied BMP-2 and BMP-7, BMP-6, and BMP-9 may also be used clinically for bone and cartilage regeneration approaches.

1.1 *Bmp-2*

BMP-2 is the most studied BMP family member. BMP-2 is approved for use in spinal fusion due to degenerative disk disease and in treatment of acute open fracture of the tibial shaft (Gupta and Khan 2005; Garrison et al. 2007). The utilization of BMP-2 in segmental bone defects, nonunion fracture, spinal fusion, and other orthopedic diseases has been well documented in recent years (Gautschi et al. 2007; McKay et al. 2007; Khosla et al. 2008; Tumialan et al. 2008; Rosen 2009; Lo et al. 2012; Wei et al. 2012).

Although *Bmp2* has an expression pattern similar to other members of the *Bmp* family, such as *Bmp4*, it seems that *Bmp2* plays a unique role in skeletal

development and fracture healing. The chondrocyte-specific deletion of *Bmp2* (targeted by *Col2-CreER* transgenic mice) showed a severe chondrodysplasia phenotype. In contrast, deletion of *Bmp4* in chondrocytes produced minor changes in skeletal development (Shu et al. 2011). Similarly, deletion of *Bmp2* in limb mesenchymal progenitor cells (targeted by *Prx1-Cre* transgenic mice) led to defects in fracture healing (Tsuji et al. 2006). In contrast, BMP-4 is dispensable for skeletogenesis and fracture healing in the limb tissue, since deletion of *Bmp4* in the mesenchymal progenitor cells using *Prx1-Cre* transgenic mice had minor effects on skeletal development and fracture healing (Tsuji et al. 2008). BMP-2 has been demonstrated to regulate expression of other BMP family members in a paracrine regulation manner (Harris et al. 1994; Ghosh-Choudhury et al. 1994; Chen et al. 1997; Edgar et al. 2007). This may explain why *Bmp2*, but not *Bmp4*, is absolutely required for skeletal development and fracture healing.

Although we know that BMP-2 accelerates fracture healing in different animal models, we do not know on which cell population BMP-2 plays a specific role during the fracture healing process. Using chondrocyte- or osteoblast-specific *Bmp2* conditional KO mice (*Bmp2^{Col2}* and *Bmp2^{Col1}*), we demonstrated that the fracture healing process was delayed in chondrocyte-specific, but not osteoblast-specific, *Bmp2* conditional KO mice (Mi et al. 2013). This study has provided important information about the time frame for BMP-2 administration when it is used to promote fracture healing.

Bone fracture healing resembles the endochondral skeletal development process and periosteal tissue plays a critical role during fracture healing. The periosteum, which is the membrane that covers the outer surface of long bones, is divided into an outer fibrous layer and inner osteogenic layer. The fibrous layer contains fibroblasts, while the osteogenic layer contains mesenchymal progenitor cells that are able to differentiate into chondrocytes and osteoblasts after a bone fracture (Colnot et al. 2012). Transplantation of a live bone graft harvested from Rosa 26A mice showed that about 70 % of osteogenesis in the graft was attributed to the expansion and differentiation of donor periosteal progenitor cells. Furthermore, engraftment of BMP-2-producing bone marrow stromal cells on non-vital allografts showed marked increases in cortical graft incorporation and neovascularization, suggesting that BMP-2-induced tissue engineered functional periosteum may improve allograft incorporation and repair (Zhang et al. 2005b). This study indicates that periosteal tissue plays a critical role in bone fracture healing and that BMP-2 promotes periosteal progenitor cells to differentiate into chondrocytes and osteoblasts, leading to endochondral bone formation in the fracture callus.

Although BMP-2 has been used successfully to treat different orthopedic diseases, concerns have also been raised. Recent studies suggest that BMP-2 enhances bone resorption in vitro and in vivo. Treatment with BMP-2 in bone grafts might cause a higher nonunion rate compared to nontreatment group, which was attributed to an aggressive bone resorptive phase prior to osteoinduction (Pradhan et al. 2006). In addition, reports also showed that BMP-2-treated bone grafts for spinal fusion lost their original height and structure, probably due to activated bone resorption (Vaidya et al. 2007). It has been reported that treatment

with BMP-2 in a primate bone defect model increased the size of the defect and the number of osteoclasts by inducing bone resorption followed by bone formation (Seeherman et al. 2010). These reports suggest complications in clinical settings where anabolic effects of BMP-2 are expected, but catabolic effects may occur prior to anabolic effects. To prevent catabolic effects of BMPs, several studies of combining BMP therapy with anti-resorptive drugs, such as bisphosphonates, have been conducted. The addition of zoledronic acid to BMP-7 increased a bone volume significantly compared to BMP-7 alone in bone defect and bone graft models in rats (Little et al. 2005; Harding et al. 2008). These reports suggest that combining BMP and bisphosphonate treatments may have synergistic effects on bone regeneration. Randomized controlled clinical trials are required in order to further investigate the efficacy of this combination treatment in patients.

1.2 *Bmp-4*

1.2.1 Cartilage Repair

The effect of BMP-4 on adult cells is different from those on embryonic stem cells. Muscle derived-stem cells stably expressing *Bmp4* exhibited the chondrocytic phenotype, including *Col2* gene expression. *Bmp4* stably transfected progenitor cells were mixed with fibrin glue and transplanted into cartilage defects in the femoral groves of nude mice. Histological analysis showed that 8 weeks after transplantation, cartilage defects treated with the stem cells overexpressing *Bmp4* were filled with white glossy tissue that was well integrated with the surrounding articular cartilage. The results demonstrated that the transplanted cells became chondrocyte-like cells stained with Safranin O. In contrast, the defects filled with cells stably transfected with LacZ cDNA only contained the fibroblast-like cells (Kuroda et al. 2006).

An important consideration for cartilage repair is possible angiogenesis and osteophyte formation. Muscle-derived stem cells were infected with retroviruses expressing *Bmp4* and soluble *Flt-1* (blocking the VEGF effect). An arthritis model in rats was then established by the intra-articular injection of mono-iodoacetate and the rats were then treated with the cells expressing *Bmp4* and *Flt-1*. The results show that this therapy induced maximal chondrogenesis with undetectable angiogenesis, thus leading to persistent cartilage repair (Matsumoto et al. 2009).

1.2.2 Bone-Tendon-Muscle Interaction

Recent studies suggest that BMP-4 is critical for embryonic development of bone ridges/eminences. Such ridges are the insertion sites of muscles and tendons to bones in embryonic stages and are pivotal for normal biomechanics and the motion

of limbs in adults. Blitz et al. 2009 used the deltoid tuberosity to investigate embryonic bone ridge formation in mice and demonstrated that this process was similar to that of the epiphyseal growth plate. Signals from tendons adjacent to bones initiate the ridge formation and the process was supported and enhanced by the signaling from adjacent muscles. Tendon-specific transcription factor scleraxis (SCX) upregulates BMP-4 expression at the insertion site. The tissue-specific deletion of *Bmp4* in tendons of *Bmp4*^{Scx} mice resulted in aberrant formation of bone ridges in the axial and appendicular skeletons, indicating that normal *Bmp4* expression in tendons is indispensable for the formation of bone ridges (Blitz et al. 2009). The progenitor cells forming bone ridges are not descendent of chondrocytes; instead, they are the Sox9 and SCX double positive cells regulated by TGF- β in the initial process of bone ridge formation. The subsequent differentiation of such cells is regulated by BMP-4 signaling (Blitz et al. 2013). These observations help us understand the mechanism of the bone-tendon interaction and unravel the pathogenesis of some pediatric orthopedic diseases, such as Osgood-Schlatter syndrome, a disease commonly seen in children about 8 years-old with a major clinical manifestation being pain in the insertion site of the patellar tendon in the tibia (Gholive et al. 2007).

1.3 *Bmp-6*

BMP-6 null mutant mice show delayed ossification of developing sterna. The observations made by in situ hybridization revealed that *Bmp6* was specifically expressed in the hypertrophic zone of epiphyseal growth plates, implying that BMP-6 can be used as a marker for chondrocyte hypertrophy (Solloway et al. 1998). In *Bmp6* null mutant mice, the diameters of long bones were smaller than their wild-type (WT) littermates, suggesting that BMP-6 may play a role in appositional bone growth. In addition, the longitudinal bone growth was also affected, suggesting that BMP-6 is also important for the normal function of growth plate chondrocytes (Perry et al. 2008). BMP-6 was also expressed in human cartilage and may play a role in maintenance of the homeostasis of articular cartilage (Bobacz et al. 2003).

1.3.1 Cartilage Repair

BMP-6 has been shown to induce the differentiation of adipose tissue-derived stem cells toward chondrocytes with robust expression of *Col2* and *aggrecan* (Estes et al. 2006). In a recent study, adipose tissue-derived stem cells were genetically modified with a baculovirus system for prolonged and sustained production of BMP-6 and TGF- β 3. Such cells were cultured in porous scaffolds and transplanted to rabbit knee joints to repair cartilage defects. The induced new cartilage-like tissue exhibited a zonal structure typical of normal articular cartilage. No chondrocyte hypertrophy or joint degeneration was observed.

However, these results were not observed in the rabbits transplanted with the stem cells that transiently expressed BMP-6 and TGF- β 3. These findings suggest that prolonged production of these two growth factors and an appropriate scaffold are critical for chondrogenesis and successful cartilage repair (Lu et al. 2014). Consistent with these findings, the injection of adenovirus expressing either BMP-2 or BMP-6 to the knee joint cavity of a pony with large osteochondral defects resulted in the enhanced regeneration of cartilage and subchondral bone, but the long-term effect of such repair was not satisfactory (Menendez et al. 2011).

1.3.2 Bone Regeneration

To investigate the effect of endogenous BMPs, compound deficient mice (*Bmp2*^{+/-}; *Bmp6*^{-/-}) were generated. Such mice exhibited a reduced bone volume, a phenomenon not seen in single KO mice. Impaired endochondral bone formation, but not intra-membranous growth, was detected in fracture calluses of compound deficient mice, suggesting a synergistic effect of endogenous BMP-2 and BMP-6 in normal bone metabolism and bone repair (Kugimiya et al. 2005). Adenovirus expressing *Bmp6* was injected locally after osteotomy surgery in rabbits. The results demonstrated that BMP-6 is potent for osteoinduction and skeletal repair (Bertone et al. 2004). Non-viral delivery of BMPs holds great promise for skeletal repair. Adipose-derived and bone marrow-derived stem cells were nucleofected with *Bmp2* or *Bmp6* and these cells were mixed with fibrin gel and injected to thigh muscles of mice. Local osteogenesis was monitored by μ CT. The results demonstrated that bone marrow-derived cells are superior to the cells from adipose tissue in their potential for osteogenesis and that BMP-6 is a more potent inducer for osteogenesis than BMP-2 (Mizrahi et al. 2013).

1.4 *Bmp-7*

1.4.1 Cartilage Repair and Arthritis

It has been shown that BMP-7 is expressed in human articular cartilage and BMP-7 increased the synthesis of proteoglycans and collagen type 2 (Col2) in human articular chondrocytes (Huch et al. 1997). The addition of BMP-7 upregulated important molecules for cartilage homeostasis, including hyaluronan and CD44 (Chubinskaya et al. 2000; Nishida et al. 2000). A recent report demonstrated that hyaluronan-CD44 signaling potentiated BMP-7-Smad1 signaling, and loss of CD44 caused partial loss of BMP-7 signaling mediating aggrecan production (Luo et al. 2014).

A model for impact injury in articular cartilage was established in sheep by applying contusive forces to the medial femoral condyles, causing injury to the superficial and middle zones of articular cartilage. The sheep were treated with BMP-7 for different time periods. The results showed that treatment with BMP-7

effectively prevented the progression of joint destruction caused by injury, and that BMP-7 may have a chondro-protective effect on patients with articular injury (Hurtig et al. 2009). Similarly, BMP-7 injection into rat knee joints delayed the cartilage degradation caused by excessive running (Sekiya et al. 2009).

Consistent with these findings, BMP-7 enhanced proteoglycan synthesis in the chondrocytes isolated from donors with osteoarthritis. BMP-7 has a synergistic effect with IGF-1. In normal and osteoarthritic chondrocytes, BMP-7 enhanced proteoglycan synthesis, especially when BMP-7 was added with IGF-1 (Loeser et al. 2003; Chubinskaya et al. 2000). Aging is a significant contributor to OA development and BMP-7 and IGF-1 increased proteoglycan synthesis in chondrocytes derived from either young or aged donors. Aging causes partial inhibition of the chondrogenetic response to IGF-1, or BMP-7 plus IGF-1 in proteoglycan synthesis. Aging-related oxidative stress suppressed the effect of BMP-7 through a p38-Smad1 non-canonical pathway (Loeser et al. 2014).

1.4.2 Meniscus Repair

In a recent study, the effect of BMP-7 on *in vivo* induction of fibrocartilage was investigated. BMP-7 at different doses was injected directly into the Achilles tendon of adult Lewis rats and the tendon samples were examined at different time points after injection. The results showed that 4-weeks after surgery, fibrocartilage-like tissue were successfully induced from the tendon following BMP-7 injection. The transformed tendon was sutured to repair meniscus defects. Histological and immunohistochemical analysis of the ‘tendon-meniscus’ samples showed that BMP-7 induced tendon cell transformation to fibrocartilage with enhanced expression of Col2, leading to the regeneration of meniscus and alleviation of articular cartilage degeneration (Ozeki et al. 2013).

1.4.3 Fracture and Spinal Fusion

rhBMP-7 was approved by the FDA in 2001 for the treatment of fracture patients, especially nonunion fractures. BMP-7 has a satisfactory efficacy and an excellent safety profile. Trials have been conducted using BMP-7 with a collagen carrier for revision surgery due to fracture nonunions in different bones, including the tibia and femur. Over 80 % of patients so treated achieved clinical healing. rhBMP-7 and collagen putty have been developed and used for fusion of the cervical and lumbar spine. The outcomes of this treatment are promising despite the common complications, such as soft tissue swelling. Comparative studies of the relative potencies of rhBMP-2 and rh-BMP-7 have been contradictory; one plausible explanation for the discrepancies being the difference in scaffolds. Other factors include the rate of tissue clearance and the numbers of the responding cells near the fracture sites. An important factor that may limit the widespread clinical use of BMP-7 is the cost of the treatment (Lo et al. 2012; Ronga et al. 2013).

1.5 *Bmp-9*

BMP-9 strongly promoted osteoblast differentiation from mesenchymal stem cells (MSCs) both in vitro and in vivo (Kang et al. 2004; Cheng et al. 2003; Luo et al. 2004; Luu et al. 2007; Peng et al. 2003, 2004). Studies from He's laboratory demonstrated that BMP-9 regulated a distinct set of downstream targets that probably play a role in osteoinduction. Unlike other TGF- β superfamily members, the mature BMP-9 protein retains the N-terminal pro-region that is generally cleaved in other BMPs prior to secretion. Retention of the pro-region did not result in functional inhibition of BMP-9 and may in fact stabilize the mature protein after secretion (Brown et al. 2005). Also, unlike other BMPs, BMP-9 has poor affinity for ALK3 (BMPRI-IA), a receptor that generally transduces BMP signaling (Brown et al. 2005). Using dominant-negative mutants of the seven type I receptors, Luo et al. demonstrated that only ALK1 and ALK2 mutants effectively inhibited BMP-9-induced osteogenic differentiation in vitro and in ectopic bone formation assays (Luo et al. 2010). These findings suggest that the mechanisms governing BMP-9-mediated osteoinduction of MSCs may differ from other BMPs (Lamplot et al. 2013).

1.6 *Cross-Talk Between BMP and Wnt Signaling*

The role of BMPs in skeletal development and pattern formation are well documented, however, the role and mechanism of BMPs in bone formation remain unclear. To investigate the interaction between BMP and Wnt signaling, several in vitro studies using mesenchymal progenitor cell lines or primary osteoblasts have been conducted. Differing results have been found.

Several recent studies show that BMP-2 has a synergistic effect with Wnt ligands and β -catenin. β -catenin was required for BMP-2-induced osteoblast differentiation (Mbalaviele et al. 2005; Chen et al. 2007; Zhang et al., 2009). In vivo studies also demonstrated that BMP-2 induced expression of several Wnt ligands and their receptors, and activated β -catenin-mediated T cell factor (TCF)-dependent transcriptional activity. Mice expressing conditional β -catenin null alleles displayed inhibition of BMP-induced chondrogenesis and osteogenesis (Chen et al. 2007). These findings suggest that BMP-2-induced bone formation may be mediated by canonical Wnt/ β -catenin signaling.

In contrast, other reports showed that BMPs induced *Sost* expression in Saos-2 osteosarcoma cells (Yu et al. 2011). Similarly, treatment of cultured calvarial bone with BMP antagonist Noggin increased canonical Wnt signaling (Kamiya et al. 2008). In vivo studies demonstrated that osteoblast-specific conditional KO of BMP receptor type IA (*Bmpr1a^{Coll}*) had increased bone mass during weanling stages. *Bmpr1a^{Coll}* mice show diminished expression of *Sost* and increased Wnt/ β -catenin signaling as assessed by Wnt reporter TOPGAL mice and TOP-flash luciferase reporter. Consistent with the negative regulation of the Wnt pathway by

BMPRIA signaling, treatment of osteoblasts with dorsomorphin, an inhibitor of the Smad-dependent BMP pathway, enhanced Wnt signaling. In addition to *Sost*, *Dkk1* was also down-regulated in bone tissue of *Bmpr1a^{Coll}* mice. Expression levels of *Dkk1* and *Sost* were up-regulated by the treatment with BMP-2 and down-regulated by Noggin. Moreover, mice expressing a constitutively active *Bmpr1a* transgene show up-regulation of both *Dkk1* and *Sost* and partially restored the high bone mass phenotype when crossed with *Bmpr1a^{Coll}* KO mice (Kamiya et al. 2010). These results suggest that BMPRIA in osteoblasts negatively regulates bone mass and Wnt/ β -catenin signaling. BMPRIA-mediated negative regulation of bone mass may be through promoting *Sost* and *Dkk1* expression in osteoblasts. The discrepancy observed in these studies may be due to stage differences of the target cells.

2 Wnt/ β -Catenin Signaling in Bone and Cartilage Regeneration

After more than 10 years research, we now understand that canonical Wnt/ β -catenin signaling controls bone mass. Disruption of any molecule in this signaling pathway in genetic mouse models caused significant changes in bone mass (Gong et al. 2001; Babij et al. 2003; Day et al. 2005; Glass et al. 2005; Hill et al. 2005). Human genetic studies also demonstrated that High Bone Mass (HBM) diseases were observed in patients with *Lrp5* gain-of-function mutations or *Sost* loss-of-function mutations (Gong et al. 2001; Boyden et al. 2002; Little et al. 2002; Van Wesenbeeck et al. 2003; Beighton 1976; Beighton et al. 1976; Balemans et al. 2001; Brunkow et al. 2001; Wergedal et al. 2003). LRP5 is a co-receptor of Wnt/ β -catenin signaling and sclerostin is a negative regulator of LRP5 signaling (Ke et al. 2012). A recombinant form of parathyroid hormone (PTH), designated Teriparatide or Forteo, is an FDA approved anabolic agent which promotes bone formation in patients with osteoporosis (Tsai et al. 2013). Recent studies suggest that the molecular mechanism of PTH action in bone formation may be through inhibition of *Sost* and *Dkk1* expression in osteocytes and osteoblasts (Keller and Kneissel 2005; Bellido et al. 2005; Silvestrini et al. 2007; Leupin et al. 2007; Guo et al. 2010). Therapeutic PTH is given as a daily subcutaneous injection, and its use is limited to 2 years duration due to observations of induction of osteosarcoma and chondrosarcoma in long-term rodent studies. To better manage osteoporosis and other bone loss-associated diseases, additional bone anabolic agents are needed. Two humanized monoclonal antibodies targeting the Wnt/ β -catenin signaling pathway, sclerostin, and *Dkk1* antibodies (Scl-Ab and *Dkk1*-Ab), have been developed in recent years. Preclinical and clinical studies found that these agents have potent anabolic effects on bone formation and fracture healing (Rossini et al. 2013; Weivoda and Oursler 2014).

Sclerostin (Scl) and *Dkk1* bind Wnt co-receptors LRP5/6 to inhibit Wnt binding and signaling, leading to a reduction in bone formation. Sclerostin and *Dkk1* bind the first β -propeller of LRP5 and LRP6 to inhibit Wnt1 class Wnt signaling (Ettenberg et al. 2010; Bourhis et al. 2010). *Dkk1* also binds the third β -propeller to

inhibit Wnt3a class Wnt signaling (Ke et al. 2012). Dkk1 and sclerostin also utilize co-receptors to enhance their inhibitory activity. Dkk1 forms a ternary complex with LRP5 or LRP6 and Kremen receptors 1 or 2, which results in internalization of the complex (Ellwanger et al. 2008; Ke et al. 2012). Scl-Ab and Dkk1-Ab prevent the interaction of these molecules with LRP5 and LRP6, allowing Wnt ligands to bind the LRP5 or LRP6 co-receptor and activate β -catenin signaling.

2.1 Scl-Ab

2.1.1 Scl-Ab in Ovariectomy-Induced Bone Loss

Osteoporosis is a metabolic bone disease characterized by low bone mass and micro-architectural deterioration of bone tissue leading to increased bone fragility. In the United States, approximately 10 million Americans older than 50 years 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 years will have an osteoporotic fracture in their remaining lifetime (Harvey et al. 2008).

Sclerostin antibodies (Scl-Abs) have been reported to have significant bone anabolic activity in various animal models. Treatment with Scl-Ab increased bone mineral density and improved cortical and trabecular architecture at the lumbar vertebrae and femur in aged male rats (Li et al. 2010). Treatment with Scl-Ab was associated with marked increases in bone mass at cortical and trabecular sites in gonad-intact primates (Ominsky et al. 2010). Scl-Ab was also found to increase trabecular thickness and bone strength of lumbar vertebrae and the proximal femur (Ominsky et al. 2011). Moreover, increasing bone formation on remodeling surfaces and along quiescent surfaces (modeling surfaces) was found in Scl-Ab treated animals (Ominsky et al. 2014). This implies that treatment with Scl-Ab might exert a modeling effect. The ovariectomized (OVX) rat model is a widely used animal model for hypogonadal estrogen deficiency induced bone loss. Li et al. reported the effect of Scl-Ab on OVX rats (Li et al. 2009). In OVX rats treated with Scl-Ab, trabecular thickness, trabecular BMD and bone volume in distal femur were restored to levels similar to sham controls. In addition, bone formation at the proximal tibia and lumbar vertebrae was significantly increased in Scl-Ab treated rats. Furthermore, treatment with Scl-Ab resulted in increased osteoblast surface and decreased osteoclast surface. Therefore, treatment with Scl-Ab has robust anabolic effects with marked increases in bone formation, and reverses OVX-induced bone loss.

2.1.2 Scl-Ab in Bone Mechanical Strength

In addition to its efficacy in promoting bone formation and increasing bone mass, Scl-Ab also increased mechanical strength of rat bone. Bone strength

parameters, such as peak load, stiffness, and energy to failure were increased in lumbar vertebrae and femoral diaphysis after treatment with Scl-Ab in OVX animals and aged male rats (Li et al. 2010; Ominsky et al. 2010; Li et al. 2009). Scl-Ab also increased bone strength at the femoral neck, the principal site for osteoporotic fracture in humans (Li et al. 2010). These preclinical studies demonstrate that treatment with Scl-Ab promotes bone formation, increases bone mass and bone strength, and reduces the risk of a secondary osteoporotic fracture.

2.1.3 Scl-Ab in Bone Fracture Healing

Skeletal fractures may occur as a consequence of trauma as well as fragility and represent a significant public health problem. Biological therapies, such as local application of BMPs, 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. It has been shown that Scl-Ab is a potent agent for enhancing fracture healing (Ominsky et al. 2011).

Fracture healing is a complex biologic process, which involves granulation, callus formation, and bone modeling and remodeling. Application of Scl-Ab to enhance fracture healing is an anabolic approach in several bone fracture models. Scl-Ab significantly increased bone mass and bone strength at the site of fracture in a fibular osteotomy model (Ominsky et al. 2011). The fractures in the Scl-Ab group had less callus cartilage with smaller fracture gaps containing more bone and less fibrovascular tissue than the control group. The most recent study has investigated effects on the healing of defects in proximal tibiae of OVX rats (McDonald et al. 2012). Scl-Ab significantly improved repair outcomes, augmenting both intramembranous and endochondral bone formation and enhancing bone formation and bone volume. Diabetes mellitus is recognized as a high-risk factor for fracture incidence and fracture healing delay. ZDF fa/fa rats are an established model of type 2 diabetes mellitus with low bone mass and delayed bone fracture healing. Scl-Ab reversed diabetes-associated low bone density and impaired osteoblast function, improved bone mass and strength, and improved bone defect regeneration in diabetic ZDF rats (Hamann et al. 2013).

2.1.4 Scl-Ab in Osteogenesis Imperfecta

Osteogenesis Imperfecta (OI) is a genetic disorder with the skeletal fragility as the hallmark feature (Cundy 2012). Most patients with OI have mutations in genes encoding type I collagen, *Colla1* and *Colla2*, or in genes encoding proteins that participate in the assembly, modification, and/or secretion of type I collagen (Byers and Pyott 2012). LRP5 is a Wnt co-receptor and regulates

bone mass and bone strength in human. Specific missense mutations in *Lrp5* cause an autosomal dominant phenotype characterized by HBM and increased bone strength (Boyden et al. 2002; Little et al. 2002). The HBM-causing missense mutations make LRP5 resistant to its endogenous inhibitors Dkk1 and sclerostin (Boyden et al. 2002; Semenov and He 2006; Balemans et al. 2008; Ellies et al. 2006). To determine if Scl-Ab has potential for use in treatment of OI disease, Jacobsen et al. have performed two proof-of-principle experiments. They showed that increasing bone anabolism via the LRP5 pathway significantly improved bone mass and bone strength in the *Colla2*^{+p.G610C} mouse model of OI. *Colla2*^{+p.G610C} mice have a missense mutation in the $\alpha 2$ chain of type I collagen, which is identical to that found in a large kindred affected with a moderate form of OI (Daley et al. 2010). The *Colla2*^{+p.G610C} mice have lower bone density and bone strength than their WT littermates (Daley et al. 2010). In the first experiment, the authors crossed *Lrp5*^{+p.A214V} mice with *Colla2*^{+p.G610C} mice and determined the effect of the LRP5 HBM allele on bone properties in the offspring. In the second experiment, they administered Scl-Ab (Li et al. 2009) or vehicle alone to WT and to *Colla2*^{+p.G610C} mice. They found that *Colla2*^{+p.G610C}; *Lrp5*^{+p.A214V} offspring had significantly increased bone mass and strength compared to *Colla2*^{+p.G610C}; *Lrp5*^{+/+} controls. The improved bone properties were not due to altered mRNA expression of type I collagen or its chaperones, nor were they due to changes in mutant type I collagen secretion. In the second experiment they treated *Colla2*^{+p.G610C} mice with Scl-Ab. They found that antibody treated mice had significantly increased bone mass and strength compared to vehicle treated control mice (Jacobsen et al. 2014). These findings indicate increasing bone formation, even without altering bone collagen composition, may benefit patients with OI and that Scl-Ab is a potential treatment for OI disease.

2.1.5 Potential Side Effect

Sclerostin KO (*Sost*^{-/-}) mice have HBM with small bone marrow cavities. Hematopoietic cell fate decisions are dependent on the local microenvironment. Osteoblasts and stromal cells support hematopoietic stem cell quiescence as well as facilitate B-cell development. Recent studies demonstrated that the bone marrow of *Sost*^{-/-} mice is specifically depleted of B cells because of elevated apoptosis at all B-cell developmental stages. In contrast, B-cell function in the spleen was normal. Further analysis confirmed that *Sost* is mainly expressed in osteocytes but not in hematopoietic lineage cells, suggesting that the B-cell defects in *Sost*^{-/-} mice are noncell autonomous. This finding was further confirmed by transplantation of WT bone marrow into lethally irradiated *Sost*^{-/-} recipients. WT \rightarrow *Sost*^{-/-} chimeras displayed a reduction in B cells, whereas reciprocal *Sost*^{-/-} \rightarrow WT chimeras did not, supporting the idea that the *Sost*^{-/-} bone environment cannot fully support normal B-cell development (Cain et al. 2012). These

results demonstrate a novel role for *Sost* in the regulation of bone marrow environments and B cell development and also suggest that another potential side effect for Scl-Ab is affecting bone marrow B-cell survival.

2.2 *Dkk1-Ab*

Based on the same principles applied in the development of Scl-Ab, the scientists at the company, Amgen, further developed Dkk1-Ab as an alternative anabolic agent for the treatment of osteoporosis and fracture healing. As predicted, the administration of Dkk1-Ab indeed increased bone formation, reversed ovariectomy-induced bone loss and accelerated fracture healing in animal studies (Li et al. 2011; Agholme et al. 2011). To determine if Dkk1-Ab promotes bone fracture healing through activation of β -catenin signaling, we treated β -catenin conditional KO mice (β -catenin^{Prx1ER}) with Dkk1-Ab and found that the Dkk1-Ab-induced fracture healing was significantly delayed in β -catenin^{Prx1ER} mice (Jin et al. 2015). It will be interesting to learn if Scl-Ab and Dkk1-Ab activate β -catenin signaling in different populations of cells during fracture healing. Since sclerostin and Dkk1 have very different expression patterns (Atkins et al. 2011; Moustafa et al. 2012; Guo et al. 2010; Hardy et al. 2012), the prediction is that these two antibodies will act on different populations of cells in periosteum tissue during bone callus formation. Mechanisms of actions of Scl-Ab and Dkk1-Ab on bone require further investigation.

Although Scl-Ab and Dkk1-Ab show promising activities in the treatment of osteoporosis and promoting fracture healing, several issues must be considered, such as the potential role of long-term usage of these antibodies in promoting tumorigenesis, development of osteoarthritis, and other side effects. Although patients with osteoporosis are often elderly and no cancer incidence has been reported in patients with *Lrp5* gain-of-function mutations or *Sost* loss-of-function mutations, long-term monitoring for patients prescribed with these humanized antibodies is necessary. Activation of β -catenin signaling could lead to an osteoarthritis-like phenotype and defects in disk degeneration in mice (Zhu et al. 2009; Wang et al. 2012). Potential side effects, such as osteoarthritis and disk degeneration, require consideration. Recent data suggest that sclerostin is expressed in articular cartilage tissue; however, animals with *Sost* deletion or receiving Scl-Ab do not develop osteoarthritis during aging or following mechanical injury (Roudier et al. 2013). In fact, recent findings demonstrated that systemic bone loss in the spine and periarticular bone loss in the proximal tibia were completely blocked and partially reversed by administration of Scl-Ab, but not by inhibition of tumor necrosis factor (TNF) in hTNF-tg mice. Moreover, Scl-Ab completely arrested the progression of bone erosion in hTNF-tg mice and led to significant regression of cortical bone erosions when Scl-Ab was used in combination with TNF inhibitors (Chen et al. 2013).

References

- Agholme F, Isaksson H, Kuhstoss S, Aspenberg P (2011) The effects of Dickkopf-1 antibody on metaphyseal bone and implant fixation under different loading conditions. *Bone* 48(5):988–996. doi:[10.1016/j.bone.2011.02.008](https://doi.org/10.1016/j.bone.2011.02.008)
- Alper J (1994) Boning up: newly isolated proteins heal bad breaks. *Science* 263(5145):324–325
- Atkins GJ, Rowe PS, Lim HP, Welldon KJ, Ormsby R, Wijenayaka AR, Zelenchuk L, Evdokiou A, Findlay DM (2011) Sclerostin is a locally acting regulator of late-osteoblast/preosteocyte differentiation and regulates mineralization through a MEPE-ASARM-dependent mechanism. *J Bone Miner Res* 26(7):1425–1436. doi:[10.1002/jbmr.345](https://doi.org/10.1002/jbmr.345)
- Babji P, Zhao W, Small C, Kharode Y, Yaworsky PJ, Boussein ML, Reddy PS, Bodine PV, Robinson JA, Bhat B, Marzolf J, Moran RA, Bex F (2003) High bone mass in mice expressing a mutant LRP5 gene. *J Bone Miner Res* 18(6):960–974. doi:[10.1359/jbmr.2003.18.6.960](https://doi.org/10.1359/jbmr.2003.18.6.960)
- Balemans W, Ebeling M, Patel N, Van Hul E, Olson P, Dioszegi M, Lacza C, Wuyts W, Van Den Ende J, Willems P, Paes-Alves AF, Hill S, Bueno M, Ramos FJ, Tacconi P, Dikkers FG, Stratakis C, Lindpaintner K, Vickery B, Foernzler D, Van Hul W (2001) Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). *Hum Mol Genet* 10(5):537–543
- Balemans W, Piters E, Cleiren E, Ai M, Van Wesenbeeck L, Warman ML, Van Hul W (2008) The binding between sclerostin and LRP5 is altered by DKK1 and by high-bone mass LRP5 mutations. *Calcif Tissue Int* 82(6):445–453. doi:[10.1007/s00223-008-9130-9](https://doi.org/10.1007/s00223-008-9130-9)
- Beighton P (1976) Genetic disorders in Southern Africa. *South African Medical Journal = Suid-Afrikaanse Tydskrif Vir Geneeskunde* 50(29):1125–1128
- Beighton P, Durr L, Hamersma H (1976) The clinical features of sclerosteosis. A review of the manifestations in twenty-five affected individuals. *Ann Intern Med* 84(4):393–397
- Bellido T, Ali AA, Gubrij I, Plotkin LI, Fu Q, O'Brien CA, Manolagas SC, Jilka RL (2005) Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. *Endocrinology* 146(11):4577–4583. doi:[10.1210/en.2005-0239](https://doi.org/10.1210/en.2005-0239)
- Bertone AL, Pittman DD, Boussein ML, Li J, Clancy B, Seeherman HJ (2004) Adenoviral-mediated transfer of human BMP-6 gene accelerates healing in a rabbit ulnar osteotomy model. *J Orthop Res : Off Publ Orthop Res Soc* 22(6):1261–1270. doi:[10.1016/j.orthres.2004.03.014](https://doi.org/10.1016/j.orthres.2004.03.014)
- Blitz E, Sharir A, Akiyama H, Zelzer E (2013) Tendon-bone attachment unit is formed modularly by a distinct pool of Scx- and Sox9-positive progenitors. *Development* 140(13):2680–2690. doi:[10.1242/dev.093906](https://doi.org/10.1242/dev.093906)
- Blitz E, Viukov S, Sharir A, Shwartz Y, Galloway JL, Pryce BA, Johnson RL, Tabin CJ, Schweitzer R, Zelzer E (2009) Bone ridge patterning during musculoskeletal assembly is mediated through SCX regulation of Bmp4 at the tendon-skeleton junction. *Dev Cell* 17(6):861–873. doi:[10.1016/j.devcel.2009.10.010](https://doi.org/10.1016/j.devcel.2009.10.010)
- Bobacz K, Gruber R, Soleiman A, Erlacher L, Smolen JS, Graninger WB (2003) Expression of bone morphogenetic protein 6 in healthy and osteoarthritic human articular chondrocytes and stimulation of matrix synthesis in vitro. *Arthritis Rheum* 48(9):2501–2508. doi:[10.1002/art.11248](https://doi.org/10.1002/art.11248)
- Bourhis E, Tam C, Franke Y, Bazan JF, Ernst J, Hwang J, Costa M, Cochran AG, Hannoush RN (2010) Reconstitution of a frizzled8.Wnt3a.LRP6 signaling complex reveals multiple Wnt and Dkk1 binding sites on LRP6. *J Biol Chem* 285(12):9172–9179. doi:[10.1074/jbc.M109.092130](https://doi.org/10.1074/jbc.M109.092130)
- Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP (2002) High bone density due to a mutation in LDL-receptor-related protein 5. *New England J Med* 346(20):1513–1521. doi:[10.1056/NEJMoa013444](https://doi.org/10.1056/NEJMoa013444)
- Brown MA, Zhao Q, Baker KA, Naik C, Chen C, Pukac L, Singh M, Tsareva T, Parice Y, Mahoney A, Roschke V, Sanyal I, Choe S (2005) Crystal structure of BMP-9 and functional interactions with pro-region and receptors. *J Biol Chem* 280(26):25111–25118. doi:[10.1074/jbc.M503328200](https://doi.org/10.1074/jbc.M503328200)

- Brunkow ME, Gardner JC, Van Ness J, Paeper BW, Kovacevich BR, Proll S, Skonier JE, Zhao L, Sabo PJ, Fu Y, Alisch RS, Gillett L, Colbert T, Tacconi P, Galas D, Hamersma H, Beighton P, Mulligan J (2001) Bone dysplasia sclerosteosis results from loss of the SOST gene product, a novel cysteine knot-containing protein. *Am J Hum Genet* 68(3):577–589
- Byers PH, Pyott SM (2012) Recessively inherited forms of osteogenesis imperfecta. *Annu Rev Genet* 46:475–497. doi:[10.1146/annurev-genet-110711-155608](https://doi.org/10.1146/annurev-genet-110711-155608)
- Cain CJ, Rueda R, McLelland B, Collette NM, Loots GG, Manilay JO (2012) Absence of sclerostin adversely affects B-cell survival. *J Bone Miner Res* 27(7):1451–1461. doi:[10.1002/jbmr.1608](https://doi.org/10.1002/jbmr.1608)
- Cao X, Chen D (2005) The BMP signaling and in vivo bone formation. *Gene* 357(1):1–8. doi:[10.1016/j.gene.2005.06.017](https://doi.org/10.1016/j.gene.2005.06.017)
- Chen D, Harris MA, Rossini G, Dunstan CR, Dallas SL, Feng JQ, Mundy GR, Harris SE (1997) Bone morphogenetic protein 2 (BMP-2) enhances BMP-3, BMP-4 and bone cell differentiation marker gene expression during the induction of mineralized bone matrix formation in cultures of fetal rat calvarial osteoblasts. *Calcif Tissue Int* 60:283–290
- Chen D, Zhao M, Harris SE, Mi Z (2004) Signal transduction and biological functions of bone morphogenetic proteins. *Front Biosci: J virtual libr* 9:349–358
- Chen M, Lichtler AC, Sheu T, Xie C, Zhang X, O’Keefe RJ, Chen D (2007) Generation of a transgenic mouse model with chondrocyte-specific and tamoxifen-inducible expression of Cre recombinase. *Genesis* 45:44–50
- Chen XX, Baum W, Dwyer D, Stock M, Schwabe K, Ke HZ, Stolina M, Schett G, Bozec A (2013) Sclerostin inhibition reverses systemic, periarticular and local bone loss in arthritis. *Ann Rheum Dis* 72(10):1732–1736. doi:[10.1136/annrheumdis-2013-203345](https://doi.org/10.1136/annrheumdis-2013-203345)
- Cheng H, Jiang W, Phillips FM, Haydon RC, Peng Y, Zhou L, Luu HH, An N, Breyer B, Vanichakarn P, Szatkowski JP, Park JY, He TC (2003) Osteogenic activity of the four-teen types of human bone morphogenetic proteins (BMPs). *J bone joint surg Am* 85-A (8):1544–1552
- Chubinskaya S, Merrihew C, Cs-Szabo G, Mollenhauer J, McCartney J, Rueger DC, Kuettner KE (2000) Human articular chondrocytes express osteogenic protein-1. *J Histochem Cytochem: Off J Histochem Soc* 48(2):239–250
- Colnot C, Zhang X, Knothe Tate ML (2012) Current insights on the regenerative potential of the periosteum: molecular, cellular, and endogenous engineering approaches. *J Orthop Res: Off publ Orthop Res Soc* 30(12):1869–1878. doi:[10.1002/jor.22181](https://doi.org/10.1002/jor.22181)
- Cundy T (2012) Recent advances in osteogenesis imperfecta. *Calcif Tissue Int* 90(6):439–449. doi:[10.1007/s00223-012-9588-3](https://doi.org/10.1007/s00223-012-9588-3)
- Daley E, Streeten EA, Sorkin JD, Kuznetsova N, Shapses SA, Carleton SM, Shuldiner AR, Marini JC, Phillips CL, Goldstein SA, Leikin S, McBride DJ Jr (2010) Variable bone fragility associated with an Amish COL1A2 variant and a knock-in mouse model. *J Bone Miner Res* 25(2):247–261. doi:[10.1359/jbmr.090720](https://doi.org/10.1359/jbmr.090720)
- Day TF, Guo X, Garrett-Beal L, Yang Y (2005) Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Dev Cell* 8(5):739–750. doi:[10.1016/j.devcel.2005.03.016](https://doi.org/10.1016/j.devcel.2005.03.016)
- Edgar CM, Chakravarthy V, Barnes G, Kakar S, Gerstenfeld LC, Einhorn TA (2007) Autogenous regulation of a network of bone morphogenetic proteins (BMPs) mediates the osteogenic differentiation in murine marrow stromal cells. *Bone* 40(5):1389–1398. doi:[10.1016/j.bone.2007.01.001](https://doi.org/10.1016/j.bone.2007.01.001)
- Ellies DL, Viviano B, McCarthy J, Rey JP, Itasaki N, Saunders S, Krumlauf R (2006) Bone density ligand, Sclerostin, directly interacts with LRP5 but not LRP5G171 V to modulate Wnt activity. *J Bone Miner Res* 21(11):1738–1749. doi:[10.1359/jbmr.060810](https://doi.org/10.1359/jbmr.060810)
- Ellwanger K, Saito H, Clement-Lacroix P, Maltry N, Niedermeyer J, Lee WK, Baron R, Rawadi G, Westphal H, Niehrs C (2008) Targeted disruption of the Wnt regulator Kremen induces limb defects and high bone density. *Mol Cell Biol* 28(15):4875–4882. doi:[10.1128/MCB.00222-08](https://doi.org/10.1128/MCB.00222-08)
- Estes BT, Wu AW, Guilak F (2006) Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6. *Arthritis Rheum* 54(4):1222–1232. doi:[10.1002/art.21779](https://doi.org/10.1002/art.21779)

- Ettenberg SA, Charlat O, Daley MP, Liu S, Vincent KJ, Stuart DD, Schuller AG, Yuan J, Ospina B, Green J, Yu Q, Walsh R, Li S, Schmitz R, Heine H, Bilic S, Ostrom L, Mosher R, Hartlepp KF, Zhu Z, Fawell S, Yao YM, Stover D, Finan PM, Porter JA, Sellers WR, Klagge IM, Cong F (2010) Inhibition of tumorigenesis driven by different Wnt proteins requires blockade of distinct ligand-binding regions by LRP6 antibodies. *Proc Natl Acad Sci USA* 107(35):15473–15478. doi:[10.1073/pnas.1007428107](https://doi.org/10.1073/pnas.1007428107)
- Garrison KR, Donell S, Ryder J, Shemilt I, Mugford M, Harvey I, Song F (2007) Clinical effectiveness and cost-effectiveness of bone morphogenetic proteins in the non-healing of fractures and spinal fusion: a systematic review. *Health Technol Assess* 11(30):1–150 iii-iv
- Gautschi OP, Frey SP, Zellweger R (2007) Bone morphogenetic proteins in clinical applications. *ANZ J surg* 77(8):626–631. doi:[10.1111/j.1445-2197.2007.04175.x](https://doi.org/10.1111/j.1445-2197.2007.04175.x)
- Gholive PA, Scher DM, Khakharia S, Widmann RF, Green DW (2007) Osgood Schlatter syndrome. *Curr Opin Pediatr* 19(1):44–50. doi:[10.1097/MOP.0b013e328013dbea](https://doi.org/10.1097/MOP.0b013e328013dbea)
- Ghosh-Choudhury N, Harris MA, Feng JQ, Mundy GR, Harris SE (1994) Expression of the BMP 2 gene during bone cell differentiation. *Crit Rev Eukaryot Gene Expr* 4(2–3):345–355
- Glass DA 2nd, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, Taketo MM, Long F, McMahon AP, Lang RA, Karsenty G (2005) Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell* 8(5):751–764. doi:[10.1016/j.devcel.2005.02.017](https://doi.org/10.1016/j.devcel.2005.02.017)
- Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML (2001) LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* 107(4):513–523 Osteoporosis-Pseudoglioma Syndrome Collaborative G
- Guo J, Liu M, Yang D, Boussein ML, Saito H, Galvin RJ, Kuhstoss SA, Thomas CC, Schipani E, Baron R, Bringham FR, Kronenberg HM (2010) Suppression of Wnt signaling by Dkk1 attenuates PTH-mediated stromal cell response and new bone formation. *Cell Metab* 11(2):161–171. doi:[10.1016/j.cmet.2009.12.007](https://doi.org/10.1016/j.cmet.2009.12.007)
- Gupta MC, Khan SN (2005) Application of bone morphogenetic proteins in spinal fusion. *Cytokine Growth Factor Rev* 16(3):347–355. doi:[10.1016/j.cytogfr.2005.02.004](https://doi.org/10.1016/j.cytogfr.2005.02.004)
- Hamann C, Rauner M, Hohna Y, Bernhardt R, Mettelsiefen J, Goettsch C, Gunther KP, Stolina M, Han CY, Asuncion FJ, Ominsky MS, Hofbauer LC (2013) Sclerostin antibody treatment improves bone mass, bone strength, and bone defect regeneration in rats with type 2 diabetes mellitus. *J Bone Miner Res* 28(3):627–638. doi:[10.1002/jbmr.1803](https://doi.org/10.1002/jbmr.1803)
- Harding AK, Aspenberg P, Kataoka M, Bylski D, Tagil M (2008) Manipulating the anabolic and catabolic response in bone graft remodeling: synergism by a combination of local BMP-7 and a single systemic dosis of zoledronate. *J Orthop Res: Off Publ Orthop Res Soc* 26(9):1245–1249. doi:[10.1002/jor.20625](https://doi.org/10.1002/jor.20625)
- Hardy R, Juarez M, Naylor A, Tu J, Rabbitt EH, Filer A, Stewart PM, Buckley CD, Raza K, Cooper MS (2012) Synovial DKK1 expression is regulated by local glucocorticoid metabolism in inflammatory arthritis. *Arthritis Res Ther* 14(5):R226. doi:[10.1186/ar4065](https://doi.org/10.1186/ar4065)
- Harris SE, Sabatini M, Harris MA, Feng JQ, Wozney J, Mundy GR (1994) Expression of bone morphogenetic protein messenger RNA in prolonged cultures of fetal rat calvarial cells. *J Bone Miner Res* 9(3):389–394. doi:[10.1002/jbmr.5650090314](https://doi.org/10.1002/jbmr.5650090314)
- Harvey N, Dennison E, Cooper C (2008) Epidemiology of osteoporotic fractures. In: Rosen CJ (ed) *Primer on the metabolic bone diseases and disorders of mineral metabolism*, 7th edn. The American Society for Bone and Mineral Research, Washington, DC, pp 198–202
- Hill TP, Spater D, Taketo MM, Birchmeier W, Hartmann C (2005) Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. *Dev Cell* 8(5):727–738. doi:[10.1016/j.devcel.2005.02.013](https://doi.org/10.1016/j.devcel.2005.02.013)

- Hoodless PA, Haerry T, Abdollah S, Stapleton M, O'Connor MB, Attisano L, Wrana JL (1996) MADR1, a MAD-related protein that functions in BMP2 signaling pathways. *Cell* 85(4):489–500
- Huch K, Wilbrink B, Flechtenmacher J, Koepp HE, Aydelotte MB, Sampath TK, Kuettner KE, Mollenhauer J, Thonar EJ (1997) Effects of recombinant human osteogenic protein 1 on the production of proteoglycan, prostaglandin E2, and interleukin-1 receptor antagonist by human articular chondrocytes cultured in the presence of interleukin-1 β . *Arthritis Rheum* 40(12):2157–2161. doi:[10.1002/1529-0131\(199712\)40:12<2157::AID-ART8>3.0.CO;2-J](https://doi.org/10.1002/1529-0131(199712)40:12<2157::AID-ART8>3.0.CO;2-J)
- Hurtig M, Chubinskaya S, Dickey J, Rueger D (2009) BMP-7 protects against progression of cartilage degeneration after impact injury. *J Orthop Res: Off pub Orthop Res Soc* 27(5):602–611. doi:[10.1002/jor.20787](https://doi.org/10.1002/jor.20787)
- Jacobsen CM, Barber LA, Ayturk UM, Roberts HJ, Deal LE, Schwartz MA, Weis M, Eyre D, Zurakowski D, Robling AG, Warman ML (2014) Targeting the LRP5 Pathway Improves Bone Properties in a Mouse Model of Osteogenesis Imperfecta. *J Bone Miner Res* 2014 Feb 12. doi: [10.1002/jbmr.2198](https://doi.org/10.1002/jbmr.2198). [Epub ahead of print]
- Jin H, Wang B, Li J, Xie W, Mao Q, Li S, Dong F, Sun Y, Ke H-Z, Babij P, Tong P, Chen D (2015) Anti-DKK1 antibody promotes bone fracture healing through activation of β -catenin signaling. *Bone* 71:63–75
- Kamiya N, Kobayashi T, Mochida Y, Yu PB, Yamauchi M, Kronenberg HM, Mishina Y (2010) Wnt inhibitors Dkk1 and Sost are downstream targets of BMP signaling through the type IA receptor (BMPRIA) in osteoblasts. *J Bone Miner Res* 25(2):200–210. doi:[10.1359/jbmr.090806](https://doi.org/10.1359/jbmr.090806)
- Kamiya N, Ye L, Kobayashi T, Mochida Y, Yamauchi M, Kronenberg HM, Feng JQ, Mishina Y (2008) BMP signaling negatively regulates bone mass through sclerostin by inhibiting the canonical Wnt pathway. *Development* 135(22):3801–3811. doi:[10.1242/dev.025825](https://doi.org/10.1242/dev.025825)
- Kang Q, Sun MH, Cheng H, Peng Y, Montag AG, Deyrup AT, Jiang W, Luu HH, Luo J, Szatkowski JP, Vanichakarn P, Park JY, Li Y, Haydon RC, He TC (2004) Characterization of the distinct orthotopic bone-forming activity of 14 BMPs using recombinant adenovirus-mediated gene delivery. *Gene Ther* 11(17):1312–1320. doi:[10.1038/sj.gt.3302298](https://doi.org/10.1038/sj.gt.3302298)
- Ke HZ, Richards WG, Li X, Ominsky MS (2012) Sclerostin and Dickkopf-1 as therapeutic targets in bone diseases. *Endocr Rev* 33(5):747–783. doi:[10.1210/er.2011-1060](https://doi.org/10.1210/er.2011-1060)
- Keller H, Kneissel M (2005) SOST is a target gene for PTH in bone. *Bone* 37(2):148–158. doi:[10.1016/j.bone.2005.03.018](https://doi.org/10.1016/j.bone.2005.03.018)
- Khosla S, Westendorf JJ, Oursler MJ (2008) Building bone to reverse osteoporosis and repair fractures. *J Clin Invest* 118(2):421–428. doi:[10.1172/JCI33612](https://doi.org/10.1172/JCI33612)
- Kugimiya F, Kawaguchi H, Kamekura S, Chikuda H, Ohba S, Yano F, Ogata N, Katagiri T, Harada Y, Azuma Y, Nakamura K, Chung UI (2005) Involvement of endogenous bone morphogenetic protein (BMP) 2 and BMP6 in bone formation. *J Biol Chem* 280(42):35704–35712. doi:[10.1074/jbc.M505166200](https://doi.org/10.1074/jbc.M505166200)
- Kuroda R, Usas A, Kubo S, Corsi K, Peng H, Rose T, Cummins J, Fu FH, Huard J (2006) Cartilage repair using bone morphogenetic protein 4 and muscle-derived stem cells. *Arthritis Rheum* 54(2):433–442. doi:[10.1002/art.21632](https://doi.org/10.1002/art.21632)
- Lamplot JD, Qin J, Nan G, Wang J, Liu X, Yin L, Tomal J, Li R, Shui W, Zhang H, Kim SH, Zhang W, Zhang J, Kong Y, Denduluri S, Rogers MR, Pratt A, Haydon RC, Luu HH, Angeles J, Shi LL, He TC (2013) BMP9 signaling in stem cell differentiation and osteogenesis. *Am J Stem Cells* 2(1):1–21. Print 2013
- Leupin O, Kramer I, Collette NM, Loots GG, Natt F, Kneissel M, Keller H (2007) Control of the SOST bone enhancer by PTH using MEF2 transcription factors. *J Bone Miner Res* 22(12):1957–1967. doi:[10.1359/jbmr.070804](https://doi.org/10.1359/jbmr.070804)
- Li X, Grisanti M, Fan W, Asuncion FJ, Tan HL, Dwyer D, Han CY, Yu L, Lee J, Lee E, Barrero M, Kurimoto P, Niu QT, Geng Z, Winters A, Horan T, Stevenson S, Jacobsen F, Chen Q, Haldankar R, Lavalley J, Tipton B, Daris M, Sheng J, Lu HS, Daris K, Deshpande R, Valente EG, Salimi-Moosavi H, Kostenuik PJ, Li J, Liu M, Li C, Lacey DL, Simonet WS, Ke HZ, Babij P, Stolina M, Ominsky MS, Richards WG (2011) Dickkopf-1 regulates bone formation in young growing rodents and upon traumatic injury. *J Bone Miner Res* 26(11):2610–2621. doi:[10.1002/jbmr.472](https://doi.org/10.1002/jbmr.472)

- Li X, Ominsky MS, Warmington KS, Morony S, Gong J, Cao J, Gao Y, Shalhoub V, Tipton B, Haldankar R, Chen Q, Winters A, Boone T, Geng Z, Niu QT, Ke HZ, Kostenuik PJ, Simonet WS, Lacey DL, Paszty C (2009) Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. *J Bone Miner Res* 24(4):578–588. doi:[10.1359/jbmr.081206](https://doi.org/10.1359/jbmr.081206)
- Li X, Warmington KS, Niu QT, Asuncion FJ, Barrero M, Grisanti M, Dwyer D, Stouch B, Thway TM, Stolina M, Ominsky MS, Kostenuik PJ, Simonet WS, Paszty C, Ke HZ (2010) Inhibition of sclerostin by monoclonal antibody increases bone formation, bone mass, and bone strength in aged male rats. *J Bone Miner Res* 25(12):2647–2656. doi:[10.1002/jbmr.182](https://doi.org/10.1002/jbmr.182)
- Little DG, McDonald M, Bransford R, Godfrey CB, Amanat N (2005) Manipulation of the anabolic and catabolic responses with OP-1 and zoledronic acid in a rat critical defect model. *J Bone Miner Res* 20(11):2044–2052. doi:[10.1359/JBMR.050712](https://doi.org/10.1359/JBMR.050712)
- Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, Manning SP, Swain PM, Zhao SC, Eustace B, Lappe MM, Spitzer L, Zweier S, Braunschweiger K, Benchekroun Y, Hu X, Adair R, Chee L, Fitzgerald MG, Tulig C, Caruso A, Tzellas N, Bawa A, Franklin B, McGuire S, Nogue X, Gong G, Allen KM, Anisowicz A, Morales AJ, Lomedico PT, Recker SM, Van Eerdewegh P, Recker RR, Johnson ML (2002) A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. *Am J Hum Genet* 70(1):11–19. doi:[10.1086/338450](https://doi.org/10.1086/338450)
- Lo KW, Ulery BD, Ashe KM, Laurencin CT (2012) Studies of bone morphogenetic protein-based surgical repair. *Adv Drug Deliv Rev* 64(12):1277–1291. doi:[10.1016/j.addr.2012.03.014](https://doi.org/10.1016/j.addr.2012.03.014)
- Loeser RF, Gandhi U, Long DL, Yin W, Chubinskaya S (2014) Aging and oxidative stress reduce the response of human articular chondrocytes to insulin-like growth factor-1 and osteogenic protein-1. *Arthritis Rheumatol*. doi:[10.1002/art.38641](https://doi.org/10.1002/art.38641)
- Loeser RF, Pacione CA, Chubinskaya S (2003) The combination of insulin-like growth factor 1 and osteogenic protein 1 promotes increased survival of and matrix synthesis by normal and osteoarthritic human articular chondrocytes. *Arthritis Rheum* 48(8):2188–2196. doi:[10.1002/art.11209](https://doi.org/10.1002/art.11209)
- Lu CH, Yeh TS, Yeh CL, Fang YH, Sung LY, Lin SY, Yen TC, Chang YH, Hu YC (2014) Regenerating cartilages by engineered ASCs: prolonged TGF-beta3/BMP-6 expression improved articular cartilage formation and restored zonal structure. *Mol Ther* 22(1):186–195. doi:[10.1038/mt.2013.165](https://doi.org/10.1038/mt.2013.165)
- Luo J, Tang M, Huang J, He BC, Gao JL, Chen L, Zuo GW, Zhang W, Luo Q, Shi Q, Zhang BQ, Bi Y, Luo X, Jiang W, Su Y, Shen J, Kim SH, Huang E, Gao Y, Zhou JZ, Yang K, Luu HH, Pan X, Haydon RC, Deng ZL (2010) He TC (2010) TGFbeta/BMP type I receptors ALK1 and ALK2 are essential for BMP9-induced osteogenic signaling in mesenchymal stem cells. *J Biol Chem* 285(38):29588–29598. doi:[10.1074/jbc.M110.130518](https://doi.org/10.1074/jbc.M110.130518). Epub
- Luo N, Knudson W, Askew EB, Veluci R, Knudson CB (2014) CD44 and hyaluronan promote the BMP7 signaling response in chondrocytes. *Arthritis Rheumatol*. doi:[10.1002/art.38388](https://doi.org/10.1002/art.38388)
- Luo Q, Kang Q, Si W, Jiang W, Park JK, Peng Y, Li X, Luu HH, Luo J, Montag AG, Haydon RC, He TC (2004) Connective tissue growth factor (CTGF) is regulated by Wnt and bone morphogenetic proteins signaling in osteoblast differentiation of mesenchymal stem cells. *J Biol Chem* 279(53):55958–55968. doi:[10.1074/jbc.M407810200](https://doi.org/10.1074/jbc.M407810200)
- Luu HH, Song WX, Luo X, Manning D, Luo J, Deng ZL, Sharff KA, Montag AG, Haydon RC, He TC (2007) Distinct roles of bone morphogenetic proteins in osteogenic differentiation of mesenchymal stem cells. *J Orthop Res: Off Publ Orthop Res Soc* 25(5):665–677. doi:[10.1002/jor.20359](https://doi.org/10.1002/jor.20359)
- Matsumoto T, Cooper GM, Gharaibeh B, Meszaros LB, Li G, Usas A, Fu FH, Huard J (2009) Cartilage repair in a rat model of osteoarthritis through intraarticular transplantation of muscle-derived stem cells expressing bone morphogenetic protein 4 and soluble Flt-1. *Arthritis Rheum* 60(5):1390–1405. doi:[10.1002/art.24443](https://doi.org/10.1002/art.24443)
- Mbalaviele G, Sheikh S, Stains JP, Salazar VS, Cheng SL, Chen D, Civitelli R (2005) Beta-catenin and BMP-2 synergize to promote osteoblast differentiation and new bone formation. *J Cell Biochem* 94(2):403–418. doi:[10.1002/jcb.20253](https://doi.org/10.1002/jcb.20253)

- McDonald MM, Morse A, Mikulec K, Peacock L, Yu N, Baldock PA, Birke O, Liu M, Ke HZ, Little DG (2012) Inhibition of sclerostin by systemic treatment with sclerostin antibody enhances healing of proximal tibial defects in ovariectomized rats. *J Orthop Res: Off Publ Orthop Res Soc* 30(10):1541–1548. doi:[10.1002/jor.22109](https://doi.org/10.1002/jor.22109)
- McKay WF, Peckham SM, Badura JM (2007) A comprehensive clinical review of recombinant human bone morphogenetic protein-2 (INFUSE Bone Graft). *Int Orthop* 31(6):729–734. doi:[10.1007/s00264-007-0418-6](https://doi.org/10.1007/s00264-007-0418-6)
- Menendez MI, Clark DJ, Carlton M, Flanigan DC, Jia G, Sammet S, Weisbrode SE, Knopp MV, Bertone AL (2011) Direct delayed human adenoviral BMP-2 or BMP-6 gene therapy for bone and cartilage regeneration in a pony osteochondral model. *Osteoarthritis cartilage/OARS, Osteoarthritis Res Soc* 19(8):1066–1075. doi:[10.1016/j.joca.2011.05.007](https://doi.org/10.1016/j.joca.2011.05.007)
- Mi M, Jin H, Wang B, Yukata K, Sheu TJ, Ke QH, Tong P, Im HJ, Xiao G, Chen D (2013) Chondrocyte BMP2 signaling plays an essential role in bone fracture healing. *Gene* 512(2):211–218. doi:[10.1016/j.gene.2012.09.130](https://doi.org/10.1016/j.gene.2012.09.130)
- Mizrahi O, Sheyn D, Tawackoli W, Kallai I, Oh A, Su S, Da X, Zarrini P, Cook-Wiens G, Gazit D, Gazit Z (2013) BMP-6 is more efficient in bone formation than BMP-2 when overexpressed in mesenchymal stem cells. *Gene Ther* 20(4):370–377. doi:[10.1038/gt.2012.45](https://doi.org/10.1038/gt.2012.45)
- Moustafa A, Sugiyama T, Prasad J, Zaman G, Gross TS, Lanyon LE, Price JS (2012) Mechanical loading-related changes in osteocyte sclerostin expression in mice are more closely associated with the subsequent osteogenic response than the peak strains engendered. *Osteoporos Int (a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA)* 23(4):1225–1234. doi:[10.1007/s00198-011-1656-4](https://doi.org/10.1007/s00198-011-1656-4)
- Nishida Y, Knudson CB, Eger W, Kuettner KE, Knudson W (2000) Osteogenic protein 1 stimulates cells-associated matrix assembly by normal human articular chondrocytes: up-regulation of hyaluronan synthase, CD44, and aggrecan. *Arthritis Rheum* 43(1):206–214. doi:[10.1002/1529-0131\(200001\)43:1<206::AID-ANR25>3.0.CO;2-1](https://doi.org/10.1002/1529-0131(200001)43:1<206::AID-ANR25>3.0.CO;2-1)
- Nishimura R, Kato Y, Chen D, Harris SE, Mundy GR, Yoneda T (1998) Samd5 and DPC4 are key molecules in mediating BMP-2-induced osteoblastic differentiation of the pluripotent mesenchymal precursor cell line C2C12. *J Biol Chem* 273:1872–1879
- Ominsky MS, Li C, Li X, Tan HL, Lee E, Barrero M, Asuncion FJ, Dwyer D, Han CY, Vlasseros F, Samadfam R, Jolette J, Smith SY, Stolina M, Lacey DL, Simonet WS, Paszty C, Li G, Ke HZ (2011) Inhibition of sclerostin by monoclonal antibody enhances bone healing and improves bone density and strength of nonfractured bones. *J Bone Miner Res* 26(5):1012–1021. doi:[10.1002/jbmr.307](https://doi.org/10.1002/jbmr.307)
- Ominsky MS, Niu QT, Li C, Li X, Ke HZ (2014) Tissue level mechanisms responsible for the increase in bone formation and bone volume by sclerostin antibody. *J Bone Miner Res* 29(6):1424–1430
- Ominsky MS, Vlasseros F, Jolette J, Smith SY, Stouch B, Doellgast G, Gong J, Gao Y, Cao J, Graham K, Tipton B, Cai J, Deshpande R, Zhou L, Hale MD, Lightwood DJ, Henry AJ, Popplewell AG, Moore AR, Robinson MK, Lacey DL, Simonet WS, Paszty C (2010) Two doses of sclerostin antibody in cynomolgus monkeys increases bone formation, bone mineral density, and bone strength. *J Bone Miner Res* 25(5):948–959. doi:[10.1002/jbmr.14](https://doi.org/10.1002/jbmr.14)
- Ozeki N, Muneta T, Koga H, Katagiri H, Otabe K, Okuno M, Tsuji K, Kobayashi E, Matsumoto K, Saito H, Saito T, Sekiya I (2013) Transplantation of Achilles tendon treated with bone morphogenetic protein 7 promotes meniscus regeneration in a rat model of massive meniscal defect. *Arthritis Rheum* 65(11):2876–2886. doi:[10.1002/art.38099](https://doi.org/10.1002/art.38099)
- Ozkaynak E, Schnegelsberg PN, Oppermann H (1991) Murine osteogenic protein (OP-1): high levels of mRNA in kidney. *Biochem Biophys Res Commun* 179(1):116–123
- Peng Y, Kang Q, Cheng H, Li X, Sun MH, Jiang W, Luu HH, Park JY, Haydon RC, He TC (2003) Transcriptional characterization of bone morphogenetic proteins (BMPs)-mediated osteogenic signaling. *J Cell Biochem* 90(6):1149–1165. doi:[10.1002/jcb.10744](https://doi.org/10.1002/jcb.10744)
- Peng Y, Kang Q, Luo Q, Jiang W, Si W, Liu BA, Luu HH, Park JK, Li X, Luo J, Montag AG, Haydon RC, He TC (2004) Inhibitor of DNA binding/differentiation helix-loop-helix proteins mediate bone morphogenetic protein-induced osteoblast differentiation of mesenchymal stem cells. *J Biol Chem* 279(31):32941–32949. doi:[10.1074/jbc.M403344200](https://doi.org/10.1074/jbc.M403344200)

- Perry MJ, McDougall KE, Hou SC, Tobias JH (2008) Impaired growth plate function in bmp-6 null mice. *Bone* 42(1):216–225. doi:[10.1016/j.bone.2007.09.053](https://doi.org/10.1016/j.bone.2007.09.053)
- Pradhan BB, Bae HW, Dawson EG, Patel VV, Delamarter RB (2006) Graft resorption with the use of bone morphogenetic protein: lessons from anterior lumbar interbody fusion using femoral ring allografts and recombinant human bone morphogenetic protein-2. *Spine (Phila Pa 1976)* 31 (10):E277–284. doi:[10.1097/01.brs.0000216442.12092.01](https://doi.org/10.1097/01.brs.0000216442.12092.01)
- Retting KN, Song B, Yoon BS, Lyons KM (2009) BMP canonical Smad signaling through Smad1 and Smad5 is required for endochondral bone formation. *Development* 136(7):1093–1104. doi:[10.1242/dev.029926](https://doi.org/10.1242/dev.029926)
- Ronga M, Fagetti A, Canton G, Paiusco E, Surace MF, Cherubino P (2013) Clinical applications of growth factors in bone injuries: experience with BMPs. *Injury* 44(Suppl 1):S34–S39. doi:[10.1016/S0020-1383\(13\)70008-1](https://doi.org/10.1016/S0020-1383(13)70008-1)
- Rosen V (2009) BMP2 signaling in bone development and repair. *Cytokine Growth Factor Rev* 20(5–6):475–480. doi:[10.1016/j.cytogfr.2009.10.018](https://doi.org/10.1016/j.cytogfr.2009.10.018)
- Rossini M, Gatti D, Adami S (2013) Involvement of WNT/beta-catenin signaling in the treatment of osteoporosis. *Calcif Tissue Int* 93(2):121–132. doi:[10.1007/s00223-013-9749-z](https://doi.org/10.1007/s00223-013-9749-z)
- Roudier M, Li X, Niu QT, Pacheco E, Pretorius JK, Graham K, Yoon BR, Gong J, Warmington K, Ke HZ, Black RA, Hulme J, Babij P (2013) Sclerostin is expressed in articular cartilage but loss or inhibition does not affect cartilage remodeling during aging or following mechanical injury. *Arthritis Rheum* 65(3):721–731. doi:[10.1002/art.37802](https://doi.org/10.1002/art.37802)
- Seeherman HJ, Li XJ, Bouxsein ML, Wozney JM (2010) rhBMP-2 induces transient bone resorption followed by bone formation in a nonhuman primate core-defect model. *J bone joint surg Am* 92(2):411–426. doi:[10.2106/JBJS.H.01732](https://doi.org/10.2106/JBJS.H.01732)
- Sekiya I, Tang T, Hayashi M, Morito T, Ju YJ, Mochizuki T, Muneta T (2009) Periodic knee injections of BMP-7 delay cartilage degeneration induced by excessive running in rats. *J Orthop Res: Off publ Orthop Res Soc* 27(8):1088–1092. doi:[10.1002/jor.20840](https://doi.org/10.1002/jor.20840)
- Semenov MV, He X (2006) LRP5 mutations linked to high bone mass diseases cause reduced LRP5 binding and inhibition by SOST. *J Biol Chem* 281(50):38276–38284. doi:[10.1074/jbc.M609509200](https://doi.org/10.1074/jbc.M609509200)
- Shu B, Zhang M, Xie R, Wang M, Jin H, Hou W, Tang D, Harris SE, Mishina Y, O'Keefe RJ, Hilton MJ, Wang Y, Chen D (2011) BMP2, but not BMP4, is crucial for chondrocyte proliferation and maturation during endochondral bone development. *J Cell Sci* 124(Pt 20):3428–3440. doi:[10.1242/jcs.083659](https://doi.org/10.1242/jcs.083659)
- Silvestrini G, Ballanti P, Leopizzi M, Sebastiani M, Berni S, Di Vito M, Bonucci E (2007) Effects of intermittent parathyroid hormone (PTH) administration on SOST mRNA and protein in rat bone. *J Mol Histol* 38(4):261–269. doi:[10.1007/s10735-007-9096-3](https://doi.org/10.1007/s10735-007-9096-3)
- Solloway MJ, Dudley AT, Bikoff EK, Lyons KM, Hogan BL, Robertson EJ (1998) Mice lacking Bmp6 function. *Dev Genet* 22(4):321–339. doi:[10.1002/\(SICI\)1520-6408\(1998\)22:4<321::AID-DVG3>3.0.CO;2-8](https://doi.org/10.1002/(SICI)1520-6408(1998)22:4<321::AID-DVG3>3.0.CO;2-8)
- Song JJ, Celeste AJ, Kong FM, Jirtle RL, Rosen V, Thies RS (1995) Bone morphogenetic protein-9 binds to liver cells and stimulates proliferation. *Endocrinology* 136(10):4293–4297. doi:[10.1210/endo.136.10.7664647](https://doi.org/10.1210/endo.136.10.7664647)
- Tsai JN, Uihlein AV, Lee H, Kumbhani R, Siwila-Sackman E, McKay EA, Burnett-Bowie SA, Neer RM, Leder BZ (2013) Teriparatide and denosumab, alone or combined, in women with postmenopausal osteoporosis: the DATA study randomised trial. *Lancet* 382(9886):50–56. doi:[10.1016/S0140-6736\(13\)60856-9](https://doi.org/10.1016/S0140-6736(13)60856-9)
- Tsuiji K, Bandyopadhyay A, Harfe BD, Cox K, Kakar S, Gerstenfeld L, Einhorn T, Tabin CJ, Rosen V (2006) BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. *Nat Genet* 38(12):1424–1429 Epub 2006 Nov 12
- Tsuiji K, Cox K, Bandyopadhyay A, Harfe BD, Tabin CJ, Rosen V (2008) *J Bone Joint Surg Am* 90(Suppl 1):14–18. doi:[10.2106/JBJS.G.01109](https://doi.org/10.2106/JBJS.G.01109)
- Tumialan LM, Pan J, Rodts GE, Mummaneni PV (2008) The safety and efficacy of anterior cervical discectomy and fusion with polyetheretherketone spacer and recombinant human bone morphogenetic protein-2: a review of 200 patients. *Journal of neurosurgery Spine* 8(6):529–535. doi:[10.3171/SPI/2008/8/6/529](https://doi.org/10.3171/SPI/2008/8/6/529)

- Urist MR (1965) Bone: formation by autoinduction. *Science* 150(3698):893–899
- Vaidya R, Weir R, Sethi A, Meisterling S, Hakeos W, Wybo CD (2007) Interbody fusion with allograft and rhBMP-2 leads to consistent fusion but early subsidence. *J bone joint surg Br* 89(3):342–345. doi:[10.1302/0301-620X.89B3.18270](https://doi.org/10.1302/0301-620X.89B3.18270)
- Van Wesenbeeck L, Cleiren E, Gram J, Beals RK, Benichou O, Scopelliti D, Key L, Renton T, Bartels C, Gong Y, Warman ML, De Vernejoul MC, Bollerslev J, Van Hul W (2003) Six novel missense mutations in the LDL receptor-related protein 5 (LRP5) gene in different conditions with an increased bone density. *Am J Hum Genet* 72(3):763–771. doi:[10.1086/368277](https://doi.org/10.1086/368277)
- Wang M, Tang D, Shu B, Wang B, Jin H, Hao S, Dresser KA, Shen J, Im HJ, Sampson ER, Rubery PT, Zuscik MJ, Schwarz EM, O'Keefe RJ, Wang Y, Chen D (2012) Conditional activation of beta-catenin signaling in mice leads to severe defects in intervertebral disc tissue. *Arthritis Rheum* 64(8):2611–2623. doi:[10.1002/art.34469](https://doi.org/10.1002/art.34469)
- Wei S, Cai X, Huang J, Xu F, Liu X, Wang Q (2012) Recombinant human BMP-2 for the treatment of open tibial fractures. *Orthopedics* 35(6):e847–e854. doi:[10.3928/01477447-20120525-23](https://doi.org/10.3928/01477447-20120525-23)
- Weivoda MM, Oursler MJ (2014) Developments in sclerostin biology: regulation of gene expression, mechanisms of action, and physiological functions. *Current Osteoporosis Rep* 12(1):107–114. doi:[10.1007/s11914-014-0188-1](https://doi.org/10.1007/s11914-014-0188-1)
- Wergedal JE, Veskovic K, Hellan M, Nyght C, Balemans W, Libanati C, Vanhoenacker FM, Tan J, Baylink DJ, Van Hul W (2003) Patients with Van Buchem disease, an osteosclerotic genetic disease, have elevated bone formation markers, higher bone density, and greater derived polar moment of inertia than normal. *J Clin Endocrinol Metab* 88(12):5778–5783. doi:[10.1210/jc.2003-030201](https://doi.org/10.1210/jc.2003-030201)
- Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM, Wang EA (1988) Novel regulators of bone formation: molecular clones and activities. *Science* 242(4885):1528–1534
- Yu L, van der Valk M, Cao J, Han CY, Juan T, Bass MB, Deshpande C, Damore MA, Stanton R, Babij P (2011) Sclerostin expression is induced by BMPs in human Saos-2 osteosarcoma cells but not via direct effects on the sclerostin gene promoter or ECR5 element. *Bone* 49(6):1131–1140. doi:[10.1016/j.bone.2011.08.016](https://doi.org/10.1016/j.bone.2011.08.016)
- Zhang J, Tan X, Li W, Wang Y, Wang J, Cheng X, Yang X (2005a) Smad4 is required for the normal organization of the cartilage growth plate. *Dev Biol* 284(2):311–322. doi:[10.1016/j.ydbio.2005.05.036](https://doi.org/10.1016/j.ydbio.2005.05.036)
- Zhang M, Yan Y, Lim YB, Tang D, Xie R, Chen A, Tai P, Harris SE, Xing L, Qin YX, Chen D (2009) BMP-2 modulates beta-catenin signaling through stimulation of Lrp5 expression and inhibition of beta-TrCP expression in osteoblasts. *J Cell Biochem* 108(4):896–905. doi:[10.1002/jcb.22319](https://doi.org/10.1002/jcb.22319)
- Zhang X, Xie C, Lin AS, Ito H, Awad H, Lieberman JR, Rubery PT, Schwarz EM, O'Keefe RJ, Guldberg RE (2005b) Periosteal progenitor cell fate in segmental cortical bone graft transplantations: implications for functional tissue engineering. *J Bone Miner Res* 20(12):2124–2137. doi:[10.1359/JBMR.050806](https://doi.org/10.1359/JBMR.050806)
- Zhu M, Tang D, Wu Q, Hao S, Chen M, Xie C, Rosier RN, O'Keefe RJ, Zuscik M, Chen D (2009) Activation of beta-catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult beta-catenin conditional activation mice. *J Bone Miner Res* 24(1):12–21. doi:[10.1359/jbmr.080901](https://doi.org/10.1359/jbmr.080901)

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