

Review

The BMP signaling and in vivo bone formation

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Abstract

Bone morphogenetic proteins (BMPs) are multi-functional growth factors that belong to the transforming growth factor β (TGF β) superfamily. The roles of BMPs in embryonic development and cellular functions in postnatal and adult animals have been extensively studied in recent years. Signal transduction studies have revealed that Smads 1, 5 and 8 are the immediate downstream molecules of BMP receptors and play a central role in BMP signal transduction. Studies from transgenic and knockout mice and from animals and humans with naturally occurring mutations in BMPs and their signaling molecules have shown that BMP signaling plays critical roles in bone and cartilage development and postnatal bone formation. BMP activities are regulated at different molecular levels. Tissue-specific knockout of a specific BMP ligand, a subtype of BMP receptors or a specific signaling molecule is required to further determine the specific role of a BMP ligand, receptor or signaling molecule in a particular tissue.

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1. Introduction

BMPs belong to the members of the TGF β superfamily. The activity of BMPs was discovered in the 1960s (Urist, 1965), but the BMP proteins were purified and sequenced in late 1980s (Luyten et al., 1989; Wozney et al., 1988). After that, recombinant BMP proteins were expressed (Wozney et al., 1988; Wozney, 1992). To date, over 20 BMP family members have been identified and characterized. BMP signals are mediated by type II and type I serine/threonine kinase receptors. Three type I receptors have been shown to bind BMP ligands, type IA and IB BMP receptors (BMPR-IA or ALK-3 and BMPR-IB or ALK-6) and type IA activin receptor (ActR-IA or

ALK-2) (Koenig et al., 1994; ten Dijke et al., 1994; Macias-Silva et al., 1998). Three type II receptors for BMPs have also been identified and they are type II BMP receptor (BMPR-II) and type II and IIB activin receptors (ActR-II and ActR-IIB) (Yamashita et al., 1995; Nohno et al., 1995; Rosenzweig et al., 1995; Kawabata et al., 1995). Whereas BMPR-IA, IB, and II are specific to BMPs, ActR-IA, II, and IIB are also signaling receptors for activins. These receptors are expressed differentially in various tissues. Type I and type II BMP receptors are both indispensable for signal transduction. After ligand binding they form a heterotetrameric-activated receptor complex consisting of two pairs of a type I and type II receptor complex (Moustakas and Heldi, 2002). The type I BMP receptor substrates include a protein family, the Smad proteins, that play a central role in relaying the BMP signal from the receptor to target genes in the nucleus. A significant advancement has been achieved in recent years on understanding of BMP signaling mechanism and in vivo functions of BMP ligands, receptors and signaling molecules.

Abbreviations: BMP, bone morphogenetic protein; TGF β , transforming growth factor β ; BMPR, BMP receptor; *Runx2*, Runt-related gene 2; Smad, *Sma/Mad*; GDF, growth/differentiation factor; CKO, conditional knockout; *Smurf1*, Smad ubiquitin regulatory factor 1.

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2. BMP signal transduction

Smad proteins play a central role in BMP signaling. Smads 1, 5 and 8 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; Chen et al., 1997a). After release 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. Smads 1 and 5 directly binds to DNA; however, the affinity is relatively low and interaction with sequence-specific DNA binding proteins is critical for the formation of a stable DNA-binding complex (Derynck et al., 1998). The first demonstration that Smads can directly bind to DNA was reported in *Drosophila* (Kim et al., 1997). Vestigial, labial and ultrabithorax (*Ubx*) are decapentaplegic (*dpp*)-responsive genes. *Mad*, a *Drosophila* homologue of Smad, was shown to directly bind to the enhancer of these genes and GCCGnCGC (GCCG motif) was identified as the consensus binding site. It has been reported that Smads 1 and 5 interact with bone-specific transcription factor *Runx2* (Hanai et al., 1999; Lee et al., 2000; Zhao et al., 2003) and activate the transcription of target genes such as *COX-2* and type X collagen (Col-X) in osteoblasts or in chondrocytes (Chikazu et al., 2002; Leboy et al., 2001). The association of *Smad1* with homeodomain-containing proteins suggests another important mechanism of BMP signaling in osteoblasts and in chondrocytes. The Hox homeodomain proteins play important roles in controlling pattern formation of the vertebrate skeleton. It has been shown that *Smad1* directly interacts with *Hoxc8* to activate the transcription of osteopontin gene (Shi et al., 1999; Yang et al., 2000; Liu et al., 2004), which is a marker gene for osteoblast and chondrocyte differentiation. In contrast, *Smad6* heterodimerizes with *Hoxc8* and represses BMP-2-induced gene transcription (Bai et al., 2000). Smads 1 and 5 are two Smad proteins which have been shown to play an important role in osteoblast differentiation in C2C12 myoblast/osteoblast precursor cells and other osteoblastic cell lines (Yamamoto et al., 1997; Fujii et al., 1999). In addition to Smads, BMPs also activate non-Smad signaling pathways such as mitogen-activated protein kinase (MAPK) family of molecules including ERK1/2 and p38 (Guicheux et al., 2003; Reilly et al., 2005).

3. Biological functions of BMPs

Physiological roles of BMPs and BMP receptor signaling in normal bone formation have been investigated. Injection of BMP-2 locally over the surface of calvariae of mice induces periosteal bone formation without a prior cartilage

phase (Chen et al., 1997b). *Bmpr1a* is widely expressed in a variety of tissues during development and in multiple adult tissues (Dewulf et al., 1995). In contrast, expression of *Bmpr1b* is restricted in early mesenchymal cells and differentiated chondrocytes (Ashique et al., 2002). Over-expression of a dominant-negative *Bmpr1b* but not *Bmpr1a* inhibits chondrocyte differentiation (Enomoto-Iwamoto et al., 1998) and over-expression of a constitutively active *Alk-2* promotes chondrocyte maturation (Zhang et al., 2003a) in chicken sternal chondrocytes. Over-expression of a dominant-negative *Bmpr1b* also inhibits osteoblast differentiation in osteoblast precursor cells (Chen et al., 1998). In the transgenic mice in which expression of a dominant-negative *Bmpr1b* transgene is targeted to the osteoblast lineage using the osteoblast-specific type I collagen promoter, the postnatal bone formation is reduced (Zhao et al., 2002). These findings demonstrate that BMP receptor signaling plays a necessary role in normal chondrocyte and osteoblast differentiation and postnatal bone formation.

4. Naturally occurring mutations in BMPs and BMP receptors

Studies of naturally occurring mutations of BMPs and BMP receptors have shown that BMPs play important roles in several inherited diseases. In mice with short ear mutations *Bmp5* gene was disrupted. Mutations in the *Bmp5* gene are associated with a wide range of skeletal defects, including reductions in long bone width and the size of several vertebral processes and an overall lower body mass (Kingsley et al., 1992; Mikic et al., 1995). Mutations in growth/differentiation factor-5 (*Gdf5* and CDMP-1) gene result in brachypodism in mice (Storm et al., 1994) and chondrodysplasia in humans (Thomas et al., 1996, 1997). Both *Bmp5* and *Gdf5* genes are localized on chromosome 2 in mice and on chromosome 20 in humans (Storm et al., 1994). GDF5 has been shown to bind BMPR-IB specifically (Nishitoh et al., 1996) and null mutations in the *Bmpr1b* gene causes a similar skeletal phenotype as that observed in *Gdf5* mutant mice (Yi et al., 2000). Heterozygous missense mutations in *Bmpr1b* gene in humans cause brachydactyly type A2 through a dominant-negative effect. The skeletal phenotype of patients is similar to that of acromesomelic chondrodysplasias of Grebe, Hunter–Thompson, and DuPan types caused by homozygous mutations in the gene coding for GDF5 (Lehmann et al., 2003). In contrast, homozygous mutations in *Bmpr1b* gene in humans show a severe defect in limb formation, including aplasia of the fibula, severe brachydactyly, ulnar deviation of the hands, and fusion of carpal/tarsal bones (Demirhan et al., 2005). Fibrodysplasia ossificans progressiva (FOP) is an extremely rare and disabling genetic disorder characterized by congenital malformations of the great toes and by progressive heterotopic endochondral ossification in predict-

able anatomical patterns. Ectopic expression of *Bmp4* was found in FOP patients (Gannon et al., 1997; Xu et al., 2000).

5. Knockout of *Bmp*, *Bmpr* and *Smad* genes

To determine the roles of BMP ligands, receptors and signaling proteins in embryonic development and in postnatal life, null mutations of BMP ligands, receptors and *Smad* genes have been created and phenotypic changes in these animals have been extensively studied. Mice deficient for *Bmp2* and *Bmp4* are nonviable. Homozygous *Bmp2* mutant embryos die between embryonic day 7.5 (E7.5) and 10.5 (E10.5) and have defects in cardiac development, manifested by the abnormal development of the heart in the exocoelomic cavity (Zhang and Bradley, 1996). Homozygous *Bmp4* mutant embryos die between E6.5 and E9.5 and show little or no mesodermal differentiation (Winnier et al., 1995). *Bmp2/4* conditional knockout (CKO) mice in which *Bmp2* and *Bmp4-loxP* mice were crossed with *Prx1-Cre* transgenic mice show severe defects in bone development (Cox et al., 2004). Similarly *Bmp4* CKO mice (*Coll-Cre* mice were used) also showed defects in bone development and postnatal bone formation (Guo et al., 2004). In contrast, *Bmp3* KO mice show increased bone mass, suggesting that endogenous BMP3 antagonizes BMP signaling in vivo (Daluiski et al., 2001). *Bmp6*-deficient mice are viable and fertile and show no overt defects in tissues known to express *Bmp6* mRNA (Solloway et al., 1998). *Bmp6* is mainly expressed in hypertrophic cartilage. Since *Bmp2* and *6* are co-expressed in this tissue, BMP-2 may functionally compensate the loss of BMP-6 in *Bmp6* null mutant mice. Conditional knockout of *Bmp2* gene in cartilage in the background *Bmp6* null mutation is required to answer this question. *Bmp7*-deficient mice die shortly after birth because of poor kidney development. Histological analysis of mutant embryos at several stages of development reveals that metanephric mesenchymal cells fail to differentiate, resulting in a virtual absence of glomerulus in newborn kidneys. In addition, *Bmp7*-deficient mice have eye defects that appear to originate during lens induction. *Bmp7*-deficient mice have minor defects in the skeleton (Luo et al., 1995; Dudley et al., 1995).

Null mutation of the *Bmpr1a* gene causes embryonic lethality in mice. Animals die at E9.5. Homozygous mutants with morphological defects are first detected at E7.5. No mesoderm forms in the mutant embryos, suggesting that *Bmpr1a* is essential for the inductive events that lead to the formation of mesoderm during gastrulation (Mishina et al., 1995). Using *Cre-loxP* CKO approach, the *Bmpr1a* gene has been deleted in different tissues to investigate its specific functions. Using *Mx1-Cre* to specifically delete the *Bmpr1a* gene in bone marrow, a recent report shows that a parallel increase in trabecular osteoblasts and neighboring haematopoietic stem cells (HSCs) is observed in these CKO mice, suggesting that

osteoblasts are a major component of the HSC niche within the bone marrow (Zhang et al., 2003b). Conditional deletion of β -catenin and *Bmpr1a* gene by *Brn4-Cre* (*Cre* activity is restricted to limb ectoderm) shows the interaction between *Wnt*/ β -catenin and BMP signaling during limb development (Soshnikova et al., 2003). β -catenin acts downstream of the BMP receptor IA in apical ectodermal ridge (AER) induction. β -catenin controls *Bmp4* expression in the ectoderm which is responsible for the formation of positive feedback loop. In contrast, β -catenin acts upstream or in parallel to the BMP receptor IA during dorsal–ventral patterning (Soshnikova et al., 2003).

Mice lacking *Bmpr1b* are viable and exhibit defects in the appendicular skeleton. In *Bmpr1b*-deficient mice, proliferation of prechondrogenic cells and chondrocyte differentiation in the phalangeal region are markedly reduced. In adult mutant mice, the proximal interphalangeal joint is absent and the phalanges are replaced by a single rudimentary element, while the distal phalanges are not affected. The metacarpal and metatarsal bones are reduced although the lengths of the radius, ulna and tibia are normal (Yi et al., 2000). The appendicular defects in *Bmpr1b* mutant mice resemble those seen in mice homozygous for the *Gdf5* null mice (*Gdf5^{bp-j}*). Since *Gdf5* has been shown to play a critical role in cartilage formation and binds *Bmpr1b* with high affinity (Nishitoh et al., 1996); these findings suggest that *Bmpr1b* plays a non-redundant role in cartilage formation in vivo. BMP ligands may utilize multiple type I BMP receptors to mediate their signaling during cartilage and bone formation. In *Bmpr1b* and *Bmp7* double mutant mice, severe appendicular skeletal defects have been observed in the forelimbs and hind limbs. The ulna and radius are severely affected (absent or shortened) (Yi et al., 2000). Since BMP-7 binds efficiently to both *Bmpr1b* and ActR-IA (*Alk2*) (Macias-Silva et al., 1998), it is conceivable that *Bmpr1b* and ActR-IA (*Alk2*) play important synergistic or overlapping roles in cartilage and bone formation in vivo.

Smad1 null mutant mice die at E10.5 because the mutant embryos fail to connect to the placenta. *Smad1* mutant embryos show overgrowth of the posterior visceral endoderm as well as extra-embryonic ectoderm and mesoderm of the chorion. The overgrowth effect on the allantois in *Smad1* mutant embryos leads to a dramatic reduction in the size and patterning of this tissue and concomitant failure to form the umbilical connection to the placenta (Tremblay et al., 2001). Deleting the *Smad1* gene specifically in osteoblasts (using *Coll-Cre* mice) causes reduction in bone mass, demonstrating that endogenous BMPs regulate bone mass through *Smad1* signaling (Chen et al., 2003). Homozygous *Smad5* null mutant mice die between E10.5–11.5 due to defects in angiogenesis. The mutant yolk sacs lack normal vasculature and had irregularly distributed blood cells. The mutant embryos also have enlarged blood vessels surrounded by decreased numbers of vascular smooth muscle cells (Yang et al., 1999). The results

suggest that *Smad5* may regulate endothelium–mesenchyme interactions during angiogenesis.

6. Negative regulation of BMP signaling

BMPs are potent stimulators on bone formation and on other cellular functions. The activity of BMPs is controlled at different molecular levels: 1) a series of BMP antagonists bind BMP ligands and inhibit BMP functions; 2) *Smad6* is a member of the Smad family. It binds type I BMP receptors and prevents the binding and phosphorylation of Smads 1, 5 and 8; 3) Tob is an anti-proliferative protein. It selectively binds Smads 1 and 5 and inhibits BMP signaling in osteoblasts; and 4) Smad ubiquitin regulatory factor 1 (*Smurf1*) is an E3 ubiquitin ligase. It interacts with *Smad1* and 5 and mediates the degradation of these Smad proteins.

Mutations in genes of BMP antagonists have shown the importance of BMP activity in a given system. For example, proximal symphalangism is an autosomal-dominant disorder with ankylosis of the proximal interphalangeal joints, carpal and tarsal bone fusion, and conductive deafness. These symptoms are shared by another disorder of joint morphogenesis, multiple synostoses syndrome. Recently, it was reported that both disorders were caused by heterozygous mutations of the human noggin gene. To date, seven mutations of noggin gene have been identified from unrelated families affected with joint morphogenesis (Gong et al., 1999; Takahashi et al., 2001). Noggin is a secreted polypeptide, which binds and inactivates BMPs 2, 4 and 7. Co-crystal structures of noggin and BMP-7 show that noggin inhibits BMP signaling by blocking the molecular interfaces of the binding epitopes for both type I and type II BMP receptors (Groppe et al., 2002), thus preventing BMP-7 to bind with BMP receptors. This 3D crystal structure clearly shows how noggin specifically inhibits BMP-7 and other BMPs such as BMPs 2 and 4. A transgenic mouse model has recently been established using the osteocalcin promoter (OG2) to drive the noggin transgene. The animals develop osteopenia/osteoporosis. Significant reductions in bone mineral density, bone volume and bone formation rates are observed in adult transgenic mice (Devlin et al., 2003; Wu et al., 2003).

Sclerostosis is a recessive inherited osteosclerotic disorder caused by mutations in the protein sclerostin. Mutations in the *SOST* gene (encoding sclerostin) in humans cause high bone mass phenotype (Balemans et al., 2001). Recently it was found that sclerostin is related in sequence to the family of secreted BMP antagonists, which includes Noggin, Chordin, Gremlin and Dan. Sclerostin is expressed in osteoblasts and osteocytes and binds BMPs 5, 6 and 7 with high affinity. Expression of sclerostin in multipotent fibroblast C3H10T1/2 cells blocks osteoblast differentiation and over-expression of sclerostin in osteoblasts under the control of the osteocalcin promoter in transgenic mice causes osteoporosis (Winkler et al., 2003).

Taken together, these findings provide evidences that activation of endogenous BMP signaling enhances bone formation and regulation of the BMP activity in postnatal stage is required for normal bone formation. In addition to its inhibitory effect of sclerostin on BMP ligands, a recent report demonstrates that sclerostin also blocks Wnt signaling by binding to *Lrp5/6* proteins (Li et al., 2005).

Smad6 is another member of Smad family which plays a negative regulatory role in BMP signaling by stably binding to type I BMP receptors. *Smad6* interrupts the phosphorylation of Smads 1 and 5 proteins and the subsequent heteromerization with *Smad4* (Imamura et al., 1997). Expression of *Smad6* is regulated by BMPs. In mouse *Smad6* promoter, four overlapping copies of the GCCGnCGC-like motif, which is the binding element for Smads 1 and 5, have been identified (Ishida et al., 2000). These findings establish a negative feedback regulation mechanism for BMP signaling. *Smad6* knock-in mice show that expression of *Smad6* is largely restricted to the heart and blood vessels. *Smad6* mutant mice have multiple cardiovascular abnormalities. Hyperplasia of the cardiac valves and outflow tract separation defects indicate that *Smad6* plays an important function in the regulation of endocardial cushion transformation. The development of aortic ossification and elevated blood pressure in *Smad6* mutant mice demonstrate that *Smad6* also plays a role in homeostasis of adult cardiovascular system (Galvin et al., 2000).

Tob is a member of a novel anti-proliferative protein family. Tob inhibits BMP-induced, Smad-dependent transcription in osteoblasts through its association with Smads 1 and 5 proteins (Yoshida et al., 2000). In Tob knockout mice, BMP-2 signaling is enhanced and the effects of BMP-2 on osteoblast proliferation and differentiation are increased. In long bones, bone volume and bone formation rates are increased (Yoshida et al., 2000). BMP-2-induced local bone formation is also enhanced in Tob knockout mice (Usui et al., 2002).

7. Ubiquitin–proteasome degradation of BMP signaling proteins

Another important regulatory mechanism by which the activity of BMP signaling proteins is modulated involves ubiquitin-mediated proteasomal degradation. The ubiquitin–proteasome proteolytic pathway is essential for various important biological processes including cell-cycle progression, gene transcription, and signal transduction (Hershko and Ciechanover, 1998; Weissman, 2001). The formation of ubiquitin–protein conjugates requires three enzymes that participate in a cascade of ubiquitin transfer reactions: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3). The specificity of protein ubiquitination is determined by E3 ubiquitin ligases, which play a crucial role in defining substrate specificity

and subsequent protein degradation by 26S proteasomes (Hershko, 1983; Ciechanover et al., 2000).

Smurf1 was identified by the yeast two-hybrid assay by its ability to interact with Smads 1 and 5 (Zhu et al., 1999) and later immunoprecipitation studies show that *Smurf1* has much higher affinity to bind Smads 6 and 7 (Ebisawa et al., 2001). *Smurf1* is a member of the Hect domain family of E3 ubiquitin ligases. Hect domain proteins represent a major subclass of E3 ligases and contain a conserved cysteine, located at the carboxyl terminal of the Hect domain, which is capable of forming a thioester bond with ubiquitin (Hochtrasser, 1996; Zhu et al., 1999). Mutation of this conserved cysteine residue to an alanine (C710A) abolished the ubiquitination and degradation activity of *Smurf1* (Zhu et al., 1999). Another motif often found in the Hect domain family of E3 ligase is the WW domain, which contains two highly conserved tryptophans and a conserved proline in an approximately 30-amino acid region (Rotin, 1998; Zhu et al., 1999). The WW domains have a preference for binding to small proline-rich sequences, PPXY motifs, and different WW domains possess different substrate specificity. Mutations of the PY motif of Smads 1 and 5 proteins prevent them to interact with *Smurf1* and inhibit the degradation of these Smad proteins (Zhu et al., 1999). *Smurf1* is located in the nucleus and it is exported to the cell membrane and cytoplasm when it binds *Smad6* or 7 and induces the degradation of type I TGF β and BMP receptors and Smads 1 and 5 (Ebisawa et al., 2001; Suzuki et al., 2002; Murakami et al., 2003). Several lines of evidence suggest that *Smurf1* may have synergistic effect with *Smad6* and inhibit BMP signaling (Murakami et al., 2003; Horiki et al., 2004). Recent report showed that the E3 ligase complex SCF $^{\beta}$ -TrCP1 is responsible for *Smad4* ubiquitination and degradation (Wan et al., 2004).

Since bone-specific transcription factor *Runx2* interacts with *Smad1* protein (Hanai et al., 1999; Lee et al., 2000)

whose degradation is mediated by *Smurf1* (Zhu et al., 1999), the effect of *Smurf1* on *Runx2* degradation was examined in myoblast/osteoblast precursor C2C12 cells. *Smurf1* induces *Runx2* degradation in a ubiquitin–proteasome-dependent manner (Zhao et al., 2003). Similar findings are also reported when *Smurf1* is transfected into osteoblastic OB-6 cells (Bellido et al., 2003). *Smurf1* may interact with *Runx2* in a direct or indirect mechanism. The conserved PY motif has been identified in all Runx family members. It has been reported that *Smurf1* mediates *Runx3* degradation in 293 cells and *Runx3* with the PY motif mutation is resistant to *Smurf1*-induced degradation (Jin et al., 2004).

The role of *Smurf1* in bone formation in vivo was examined by generating *Coll1* (2.3 kb)–*Smurf1* transgenic mice in which expression of a *Smurf1* transgene is targeted to osteoblasts. In *Coll1*–*Smurf1* transgenic mice, trabecular bone volume and bone formation rates are decreased and osteoblast proliferation and differentiation are inhibited (Zhao et al., 2004). Recent studies also demonstrate that bone mass is increased in *Smurf1* null mutant mice (Yamashita et al., 2005). These findings demonstrate that regulation of BMP signaling proteins may also play an important physiological role in bone formation in vivo.

Although a significant achievement has been made in recent years in understanding the role of BMP signaling in vivo (Table 1), tissue-specific knockouts of the individual BMP ligands, receptors and signaling molecules are required to further determine the specific roles of BMP signaling in a particular tissue since null mutations of most of BMP ligands, receptors and signaling molecules produce lethal phenotype perinatally. Generation of tissue-specific and inducible conditional knockout alleles for BMP ligands, receptors and signaling molecules would allow us to gain further information about physiological functions of BMP signaling in postnatal and adult animals.

Table 1
Bone phenotypes of mice with alterations in BMP signaling genes

| Genes | Mutations | Promoters | Phenotypes | References |
|-------------------|-----------|----------------|-------------------------------|---|
| <i>Bmp3</i> | KO | – | Increased bone mass | Daluiski et al., 2001 |
| <i>Bmp4</i> | CKO | <i>Coll1a1</i> | Defects in bone development | Guo et al., 2004 |
| <i>Bmp2/4</i> | CKO | <i>Prx-1</i> | Defects in bone development | Cox et al., 2004 |
| <i>Bmp5</i> | Mutation | – | Low bone mass | Kingsley et al., 1992; Mikic et al., 1995 |
| <i>Bmp7</i> | KO | – | Minor defects in bone | Luo et al., 1995; Dudley et al., 1995 |
| <i>Gdf5</i> | Mutation | – | Brachypodism/chondrodysplasia | Storm et al., 1994; Thomas et al., 1996, 1997 |
| Noggin | Tg mice | OG2 | Osteopenia/osteoporosis | Devlin et al., 2003; Wu et al., 2003 |
| SOST ^a | Mutation | – | High bone mass | Balemans et al., 2001 |
| SOST ^a | Tg mice | OG2 | Osteopenia | Winkler et al., 2003 |
| <i>Bmpr1a</i> | CKO | Mx1 | Increased bone mass | Zhang et al., 2003b |
| <i>Bmpr1b</i> | KO | – | Brachypodism | Yi et al., 2000 |
| <i>Bmpr1b</i> | Tg mice | <i>Coll1a1</i> | Osteopenia | Zhao et al., 2002 |
| <i>Bmpr1b</i> | Mutation | – | Brachypodism/chondrodysplasia | Lehmann et al., 2003; Demirhan et al., 2005 |
| <i>Smad1</i> | CKO | – | Osteopenia | Chen et al., 2003 |
| <i>Tob1</i> | KO | – | Increased bone mass | Yoshida et al., 2000; Usui et al., 2002 |
| <i>Smurf1</i> | Tg mice | <i>Coll1a1</i> | Osteopenia | Zhao et al., 2004 |
| <i>Smurf1</i> | KO | – | Increased bone mass | Yamashita et al., 2005 |

^a Recent report showed that sclerostin (SOST-encoded protein) also blocks Wnt/Lrp5 signaling (Li et al., 2005).

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References

- Ashique, A.M., Fu, K., Richman, J.M., 2002. Signalling via type IA and type IB bone morphogenetic protein receptors (BMPR) regulates intramembranous bone formation, chondrogenesis and feather formation in the chicken embryo. *Int. J. Dev. Biol.* 46, 243–253.
- Bai, S., Shi, X., Yang, X., Cao, X., 2000. *Smad6* as a transcriptional corepressor. *J. Biol. Chem.* 275, 8267–8270.
- Balemans, W., et al., 2001. Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). *Hum. Mol. Genet.* 10, 537–543.
- Bellido, T., et al., 2003. Proteasomal degradation of *Runx2* shortens parathyroid hormone-induced anti-apoptotic signaling in osteoblasts: a putative explanation for why intermittent administration is needed for bone anabolism. *J. Biol. Chem.* 278, 50259–50272.
- Chen, Y., Bhushan, A., Vale, W., 1997a. *Smad8* mediates the signaling of the receptor serine kinase. *Proc. Natl. Acad. Sci. U. S. A.* 94, 12938–12943.
- Chen, D., et al., 1997b. Bone morphogenetic protein 2 (BMP-2) enhances BMP-3, 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., et al., 1998. Differential roles for BMP receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. *J. Cell Biol.* 142, 295–305.
- Chen, D., et al., 2003. BMP signaling through the *Smad1* pathway is required for normal postnatal bone formation. *J. Bone Miner. Res.* 18, S6.
- Chikazu, D., et al., 2002. Bone morphogenetic protein 2 induces cyclooxygenase 2 in osteoblasts via a Cbfa1 binding site: role in effects of bone morphogenetic protein 2 in vitro and in vivo. *J. Bone Miner. Res.* 17, 1430–1440.
- Ciechanover, A., Orian, A., Schwartz, A.L., 2000. The ubiquitin-mediated proteolytic pathway: mode of action and clinical implications. *J. Cell. Biochem.* 77, 40–51.
- Cox, K., Harfe, B., Tabin, C.J., Rosen, V., 2004. Absence of both *Bmp2* and *Bmp4* during skeletal development results in severe defects in osteoblasts but not in chondrocytes. *J. Bone Miner. Res.* 19, S11.
- Daluisi, A., et al., 2001. Bone morphogenetic protein-3 is a negative regulator of bone density. *Nat. Genet.* 27, 84–88.
- Demirhan, O., et al., 2005. A homozygous BMPRI1B mutation causes a new subtype of acromesomelic chondrodysplasia with genital anomalies. *J. Med. Genet.* 42, 314–317.
- Derynck, R., Zhang, Y., Feng, X.-H., 1998. Smads: transcriptional activator of TGF β responses. *Cell* 95, 737–740.
- Devlin, R.D., et al., 2003. Skeletal over-expression of noggin results in osteopenia and reduced bone formation. *Endocrinology* 144, 1972–1978.
- Dewulf, N., et al., 1995. Distinct spatial and temporal expression patterns of two type I receptors for bone morphogenetic proteins during mouse embryogenesis. *Endocrinology* 136, 2652–2663.
- Dudley, A.T., Lyons, K.M., Robertson, E.J., 1995. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev.* 9, 2795–2807.
- Ebisawa, T., et al., 2001. *Smurf1* interacts with transforming growth factor-beta type I receptor through *Smad7* and induces receptor degradation. *J. Biol. Chem.* 276, 12477–12480.
- Enomoto-Iwamoto, M., et al., 1998. Bone morphogenetic protein signaling is required for maintenance of differentiated phenotype, control of proliferation, and hypertrophy in chondrocytes. *J. Cell Biol.* 140, 409–418.
- Fujii, M., et al., 1999. Roles of bone morphogenetic protein type I receptors and Smad proteins in osteoblast and chondroblast differentiation. *Mol. Biol. Cell* 10, 3801–3813.
- Galvin, K.M., et al., 2000. A role for smad6 in development and homeostasis of the cardiovascular system. *Nat. Genet.* 24, 171–174.
- Gannon, F.H., Kaplan, F.S., Olmsted, E., Finkel, G.C., Zasloff, M.A., Shore, E., 1997. Bone morphogenetic protein 2/4 in early fibromatous lesions of fibrodysplasia ossificans progressiva. *Hum. Pathol.* 28, 339–343.
- Gong, Y., et al., 1999. Heterozygous mutations in the gene encoding noggin affect human joint morphogenesis. *Nat. Genet.* 21, 302–304.
- Groppe, J., et al., 2002. Structural basis of BMP signaling inhibition by the cystine knot protein noggin. *Nature* 420, 636–642.
- Guicheux, J., Lemonnier, J., Ghayor, C., Suzuki, A., Palmer, G., Caverzasio, J., 2003. Activation of p38 mitogen-activated protein kinase and c-Jun-NH₂-terminal kinase by BMP-2 and their implication in the stimulation of osteoblastic cell differentiation. *J. Bone Miner. Res.* 18, 2060–2068.
- Guo, D., et al., 2004. *Bmp4* is necessary for bone formation: conditional *Bmp4* knock-out using the 3.6 kb and 2.3 kb collagen 1a1 promoter-Cre and *Bmp4*-floxed mice. *J. Bone Miner. Res.* 19, S14.
- Hanai, J.I., et al., 1999. Interaction and functional cooperation of PEBP2/CBF with Smads. Synergistic induction of the immunoglobulin germline C alpha promoter. *J. Biol. Chem.* 274, 31577–31582.
- Hershko, A., 1983. Ubiquitin: roles in protein modification and breakdown. *Cell* 34, 11–12.
- Hershko, A., Ciechanover, A., 1998. The ubiquitin system. *Annu. Rev. Biochem.* 67, 425–479 (Review).
- Hochtrasser, M., 1996. Ubiquitin-dependent protein degradation. *Annu. Rev. Genet.* 30, 405–439.
- Hoodless, P.A., et al., 1996. MADR1, a MAD-related protein that functions in BMP2 signaling pathways. *Cell* 85, 489–500.
- Horiki, M., et al., 2004. *Smad6/Smurf1* overexpression in cartilage delays chondrocyte hypertrophy and causes dwarfism with osteopenia. *J. Cell Biol.* 165, 433–445.
- Imamura, T., et al., 1997. *Smad6* inhibits signalling by the TGF-beta superfamily. *Nature* 389, 622–626.
- Ishida, W., et al., 2000. *Smad6* is a *Smad1/5*-induced smad inhibitor. Characterization of bone morphogenetic protein-responsive element in the mouse *Smad6* promoter. *J. Biol. Chem.* 275, 6075–6079.
- Jin, Y.H., et al., 2004. Transforming growth factor-beta stimulates p300-dependent Runx3 acetylation, which inhibits ubiquitination-mediated degradation. *J. Biol. Chem.* 279, 29409–29417.
- Kawabata, M., Chytil, A., Moses, H.L., 1995. Cloning of a novel type II serine/threonine kinase receptor through interaction with the type I transforming growth factor-beta receptor. *J. Biol. Chem.* 270, 5625–5630.
- Kim, J., Johnson, K., Chen, H.J., Carroll, S., Laughon, A., 1997. *Drosophila* Mad binds to DNA and directly mediates activation of vestigial by decapentaplegic. *Nature* 388, 304–308.
- Kingsley, D.M., et al., 1992. The mouse short ear skeletal morphogenesis is associated with defects in a bone morphogenetic member of the TGF β superfamily. *Cell* 71, 399–410.
- Koenig, B.B., et al., 1994. Characterization and cloning of a receptor for BMP-2 and BMP-4 from NIH 3T3 cells. *Mol. Cell. Biol.* 14, 5961–5974.
- Leboy, P., et al., 2001. Smad-Runx interactions during chondrocyte maturation. *J. Bone Jt. Surg., Am. Vol.* 83-A, S15–S22.
- Lee, K.S., et al., 2000. *Runx2* is a common target of transforming growth factor-beta1 and bone morphogenetic protein 2, and cooperation between *Runx2* and *Smad5* induces osteoblast-specific gene expression in the pluripotent mesenchymal precursor cell line C2C12. *Mol. Cell. Biol.* 20, 8783–8792.

- Lehmann, K., et al., 2003. Mutations in bone morphogenetic protein receptor 1B cause brachydactyly type A2. *Proc. Natl. Acad. Sci. U. S. A.* 100, 12277–12282.
- Li, X., et al., 2005. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. *J. Biol. Chem.* 280, 19883–19887.
- Liu, Z., et al., 2004. Molecules mimicking *Smad1* interacting with Hox stimulate bone formation. *J. Biol. Chem.* 279, 11313–11319.
- Luo, G., Hofmann, C., Bronchers, A.J., Sohocki, M., Bradley, A., Karsenty, G., 1995. BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. *Genes Dev.* 9, 2808–2820.
- Luyten, F.P., et al., 1989. Purification and partial amino acid sequence of osteogenin, a protein initiating bone differentiation. *J. Biol. Chem.* 264, 13377–13380.
- Macias-Silva, M., Hoodless, P.A., Tang, S.J., Buchwald, M., Wrana, J.L., 1998. Specific activation of *Smad1* signaling pathways by the BMP7 type I receptor, ALK2. *J. Biol. Chem.* 273, 25628–25636.
- Mikic, B., van der Meulen, M.C., Kingsley, D.M., Carter, D.R., 1995. Long bone geometry and strength in adult BMP-5 deficient mice. *Bone* 16, 445–454.
- Mishina, Y., Suzuki, A., Ueno, N., Behringer, R.B., 1995. *Bmpr* encodes a type I bone morphogenetic protein receptor that is essential for gastrulation during mouse embryogenesis. *Genes Dev.* 9, 3027–3037.
- Moustakas, A., Heldt, C.H., 2002. From mono- to oligo-Smads: the heart of the matter in TGF-beta signal transduction. *Genes Dev.* 16, 1867–1871.
- Murakami, G., Watabe, T., Takaoka, K., Miyazono, K., Imamura, T., 2003. Cooperative inhibition of bone morphogenetic protein signaling by *Smurf1* and inhibitory Smads. *Mol. Biol. Cell* 14, 2809–2817.
- Nishimura, R., Kato, Y., Chen, D., Harris, S.E., Mundy, G.R., Yoneda, T., 1998. *Smad5* 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.
- Nishitoh, H., et al., 1996. Identification of type I and type II serine/threonine kinase receptors for growth/differentiation factor-5. *J. Biol. Chem.* 271, 21345–21352.
- Nohno, T., et al., 1995. Identification of a human type II receptor for bone morphogenetic protein-4 that forms differential heteromeric complexes with bone morphogenetic protein type I receptors. *J. Biol. Chem.* 270, 22522–22526.
- Reilly, G.C., Golden, E.B., Grasso-Knight, G., Leboy, P.S., 2005. Differential effects of ERK and p38 signaling in BMP-2 stimulated hypertrophy of cultured chick sternal chondrocytes. *Cell Commun. Signal.* 3, 3.
- Rosenzweig, B.L., et al., 1995. Cloning and characterization of a human type II receptor for bone morphogenetic proteins. *Proc. Natl. Acad. Sci. U. S. A.* 92, 7632–7636.
- Rotin, D., 1998. WW (WWP) domains: from structure to function. *Curr. Top. Microbiol. Immunol.* 228, 115–133 (Review).
- Shi, X., Yang, X., Chen, D., Chang, Z., Cao, X., 1999. *Smad1* interacts with homeobox DNA-binding proteins in bone morphogenetic protein signaling. *J. Biol. Chem.* 274, 13711–13717.
- Solloway, M.J., Dudley, A.T., Bikoff, E.K., Lyons, K.M., Hogan, B.L., Robertson, E.J., 1998. Mice lacking *Bmp6* function. *Dev. Genet.* 22, 321–339.
- Soshnikova, N., et al., 2003. Genetic interaction between Wnt/beta-catenin and BMP receptor signaling during formation of the AER and the dorsal–ventral axis in the limb. *Genes Dev.* 17, 1963–1968.
- Storm, E.E., Huynh, T.V., Copeland, N.G., Jenkins, N.A., Kingsley, D.M., Lee, S.J., 1994. Limb alterations in *brachypodism* mice due to mutations in a new member of the TGFβ superfamily. *Nature* 368, 639–643.
- Suzuki, C., et al., 2002. *Smurf1* regulates the inhibitory activity of *Smad7* by targeting *Smad7* to the plasma membrane. *J. Biol. Chem.* 277, 39919–39925.
- Takahashi, T., et al., 2001. Mutations of the NOG gene in individuals with proximal symphalangism and multiple synostosis syndrome. *Clin. Genet.* 60, 447–451.
- ten Dijke, P., et al., 1994. Identification of type I receptors for osteogenic protein-1 and bone morphogenetic protein-4. *J. Biol. Chem.* 269, 16985–16988.
- Thomas, J.T., Lin, K., Nandedkar, M., Camargo, M., Cervenka, J., Luyten, F.P., 1996. A human chondrodysplasia due to a mutation in a TGFβ superfamily member. *Nat. Genet.* 12, 315–317.
- Thomas, J.T., et al., 1997. Disruption of human limb morphogenesis by a dominant negative mutation in CDMP1. *Nat. Genet.* 17, 58–64.
- Tremblay, K.D., Dunn, N.R., Robertson, E., 2001. Mouse embryos lacking *Smad1* signals display defects in extra-embryonic tissues and germ cell formation. *Development* 128, 3609–3621.
- Urist, M.R., 1965. Bone formation by autoinduction. *Science* 150, 893–899.
- Usui, M., et al., 2002. Enhancing effect of Tob deficiency on bone formation is specific to bone morphogenetic protein-induced osteogenesis. *J. Bone Miner. Res.* 17, 1026–1033.
- Wan, M., Tang, Y., Tytler, E.M., Vickers, S., Lu, S., Cao, X., 2004. *Smad4* protein stability is regulated by ubiquitin ligase SCF^{β-TrCP1}. *J. Biol. Chem.* 279, 14484–14487.
- Weissman, A.M., 2001. Themes and variations on ubiquitylation. *Nat. Rev., Mol. Cell Biol.* 2, 169–178 (Review).
- Winkler, D.G., et al., 2003. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. *EMBO J.* 22, 6267–6276.
- Winnier, G., Blessing, M., Labosky, P.A., Hogan, B.L.M., 1995. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev.* 9, 2105–2116.
- Wozney, J.M., 1992. The bone morphogenetic protein family and osteogenesis. *Mol. Reprod. Dev.* 32, 160–167.
- Wozney, J.M., et al., 1988. Novel regulators of bone formation: molecular clones and activities. *Science* 242, 1528–1534.
- Wu, X.B., et al., 2003. Impaired osteoblastic differentiation, reduced bone formation, and severe osteoporosis in noggin-overexpressing mice. *J. Clin. Invest.* 112, 924–934.
- Xu, M.Q., et al., 2000. Linkage exclusion and mutational analysis of the noggin gene in patients with fibrodysplasia ossificans progressiva (FOP). *Clin. Genet.* 58, 291–298.
- Yamamoto, N., Akiyama, S., Katagiri, T., Namiki, M., Kurokawa, T., Suda, T., 1997. *Smad1* and *Smad5* act downstream of intracellular signalings of BMP-2 that inhibits myogenic differentiation and induces osteoblast differentiation in C2C12 myoblasts. *Biochem. Biophys. Res. Commun.* 238, 574–580.
- Yamashita, H., et al., 1995. Osteogenic protein-1 binds to activin type II receptors and induces certain activin-like effects. *J. Cell Biol.* 130, 217–226.
- Yamashita, M., et al., 2005. Ubiquitin ligase *Smurf1* controls osteoblast activity and bone homeostasis by targeting MEKK2 for degradation. *Cell* 121, 101–113.
- Yang, X., et al., 1999. Angiogenesis defects and mesenchymal apoptosis in mice lacking SMAD5. *Development* 126, 1571–1580.
- Yang, X., Ji, X., Shi, X., Cao, X., 2000. *Smad1* domains interacting with Hoxc-8 induce osteoblast differentiation. *J. Biol. Chem.* 275, 1065–1072.
- Yi, S.E., Daluiski, Pederson, A.R., Rosen, V., Lyons, K.M., 2000. The type I BMP receptor BMPRII is required for chondrogenesis in the mouse limb. *Development* 127, 621–630.
- Yoshida, Y., et al., 2000. Negative regulation of BMP/Smad signaling by Tob in osteoblasts. *Cell* 103, 1085–1097.
- Zhang, H., Bradley, A., 1996. Mice deficient for BMP-2 are nonviable and have defects in amnion/chorion and cardiac development. *Development* 122, 2977–2986.
- Zhang, D., Schwarz, E.M., Rosier, R.N., Zuscik, M.J., Puzas, J.E., O’Keefe, R.J., 2003. ALK2 functions as a BMP type I receptor and induces Indian hedgehog in chondrocytes during skeletal development. *J. Bone Miner. Res.* 18, 1593–1604.
- Zhang, J., et al., 2003. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* 425, 836–841.

- Zhao, M., et al., 2002. Bone morphogenetic protein receptor signaling is necessary for normal murine postnatal bone formation. *J. Cell Biol.* 157, 1049–1060.
- Zhao, M., Qiao, M., Oyajobi, B., Mundy, G.R., Chen, D., 2003. E3 ubiquitin ligase *Smurf1* mediates core-binding factor $\alpha 1/Runx2$ degradation and plays a specific role in osteoblast differentiation. *J. Biol. Chem.* 278, 27939–27944.
- Zhao, M., Qiao, M., Harris, S.E., Oyajobi, B., Mundy, G.R., Chen, D., 2004. *Smurf1* inhibits osteoblast differentiation and bone formation in vitro and in vivo. *J. Biol. Chem.* 279, 12854–12859.
- Zhu, H., Kavsak, P., Abdollah, S., Wrana, J., Thomsen, G.H.A., 1999. SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. *Nature* 400, 687–693.