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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 (TGFβ) 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.

1. Introduction

BMPs belong to the members of the TGFβ 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; TGFb, transforming growth factor b; 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 constrast, 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-