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Bone morphogenetic proteins: from developmental signals to tissue regeneration

Conference on Bone Morphogenetic Proteins

Petra Simic and Slobodan Vukicevic

Laboratory of Mineralized Tissues, School of Medicine, University of Zagreb, Salata 11, 10000 Zagreb, Croatia

Corresponding author: Slobodan Vukicevic
e-mail: vukicev@mef.hr
tel/fax: +38514566812

Poster legend

The 6th International Conference on Bone Morphogenetic Proteins took place between 11 and 15 October 2006, in Cavtat, Croatia, and was organized by S. Vukicevic.

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Introduction

At present, bone is the only human organ that can be fully regenerated by exogenously applied bone morphogenetic proteins (BMPs) when physiological mechanisms of fracture repair fail. Since the identification of BMPs (Urist 1965; Sampath & Reddi 1981; Wozney *et al*, 1988; Ozkaynak *et al*, 1990; Chang et al, 1994), there have been many scientific discoveries and clinical reports on their use (reviewed by Vukicevic & Sampath, 2004). More than 340,000 patients worldwide have been successfully treated with recombinant BMPs for long-bone non-unions, acute fractures and spinal fusions.

At the 6th International Conference on Bone Morphogenetic Proteins, the role of BMPs in signal transduction, developmental biology, metabolic bone diseases, joint and cartilage repair, skeleton reconstruction, kidney regeneration and tumour biology were discussed by leading experts in the field.

Signal transduction

BMPs are dimeric molecules that induce signalling through a heterotetrameric receptor complex composed of two type I and two type II serine-threonine kinase receptors, known as BMP receptors (BMPRs). The mode of BMPR oligomerization determines the resulting BMPR signal. Binding of BMPs to preformed heteromeric receptor complexes activates the nuclear effector proteins known as Smads, whereas binding to the high-affinity type I receptor and recruitment of the low-affinity type II receptor induces a Smad-independent signalling pathway (Miyazono *et al*, 2005). After BMP-induced heterodimeric complex formation, the constitutively active type II receptor kinase phosphorylates the type I receptor and subsequently activates intracellular signalling by phosphorylating downstream components (Fig 1). The BMP ligand connects the receptors—which otherwise have no contact between their ectodomains— and induces allosteric changes. W. Sebald (Würzburg, Germany) showed that BMP2 acts as a rigid clamp, which brings type I and type II receptor chains together for transactivation, rather than changing the affinity of low-affinity receptors. Sebald has also deciphered the binding mode of many BMP-modulator proteins by extensive mutational analyses. H. Y. Lin (Boston, MA, USA) reported that haemojuvelin, which is involved in iron metabolism, is a new BMP co-receptor that regulates hepcidin expression. Haemojuvelin mutants associated with haemochromatosis owing to low levels of hepcidin have impaired BMP signalling ability.

The internalization of BMPRs and its relationship to signalling were largely unexplored prior to the meeting. However, P. Knaus (Berlin, Germany) showed that both types of receptor

undergo constitutive endocytosis through clathrin-coated pits, whereas only BMPRII also undergoes caveolar-like internalization. BMP–Smad signalling is not mediated through caveolae, and Smad phosphorylation at the carboxyl terminus does not require endocytosis and can also take place at the plasma membrane. Smad-dependent signalling is initiated while the BMPRs are still at the plasma membrane. Continuation of this pathway—that is, translocation of activated Smads into the nucleus—requires endocytosis of the receptor complex through clathrin-coated vesicles.

BMP downstream signalling molecules (Smads) can be divided into three distinct subclasses (Fig 1): signal-transducing receptorregulated Smads or R-Smads (Smad1, Smad5 and Smad8); common mediator Smad or Co-Smad (Smad4); and inhibitory Smads or I-Smads (Smad6 and Smad7). K. Miyazono (Tokyo, Japan) showed that Smad6 inhibits the activin receptor-like kinase 3 (ALK3)- mediated BMP signalling pathway more than the ALK2-mediated pathways and mapped four main amino acids in ALK3 that render this receptor highly sensitive to Smad6. Through mutational analyses, Miyazono identified a region at the amino terminus of the kinase of both receptors that is important for this specificity. The R-Smad– Co-Smad complex enters the nucleus and recruits other transcription factors, co-activators and co-repressors to regulate the transcription of target genes by binding to specific DNA sequences, known as BMPresponsive elements (BREs; Fig 1). Y. Ohta (Osaka, Japan) showed that cyclic AMP (cAMP) accelerates BMP signalling by enhancing the transcriptional activity of BREs through a cAMP-responsive element. The activity of BMPs is tightly regulated by gene expression, by protein processing from their precursors and by binding to the naturally occurring secreted soluble BMP antagonists in the extracellular space (Fig 1). P. ten Dijke (Amsterdam, The Netherlands) showed that sclerostin, which is a BMP antagonist, abrogates BMPstimulated bone formation by antagonizing Wnt signalling, rather than by directly antagonizing BMP-induced Smad1–Smad5 phosphorylation and inhibiting direct BMP target genes, such as distalless homeobox 5 (*DLX5*) and muscle segment homeobox-like 2 (*MSX2*). High bone mass in sclerosteosis and van Buchem disease, which is caused by sclerostin deficiency, might therefore result from increased wingless-type (Wnt) signalling.

Developmental biology

The roles of individual BMPs have been studied through the identification of mutated genes in classic mouse mutants, and through conventional gene-targeting approaches, gene disruption and the overexpression of genes encoding BMPs, BMPRs and Smads. Collectively, these studies have confirmed that BMPs have important roles in the development of the

skeleton, nervous system, eye, kidney, heart and primordial germ cells (Vukicevic & Sampath, 2004).

As *BMP2* and *BMP4* homozygous mutant mice show embryonic lethality, S. Harris (San Antonio, TX, USA) and colleagues reported on BMP2 and BMP4 conditional deletions in bone. *BMP4* conditional knockout mice were smaller than wild-type mice by 20–50%, had a bend in the tail and their overall bone mineral density was reduced by 10–15%, with a 30% reduction in the spine density. Deletion of BMP2 in early osteoblasts led to a major decrease in trabecular number in the tibia and spine, with a 30–50% decrease in the cortical thickness. These studies imply that both BMP4 and BMP2 have their own selective functions in osteoblasts. Y. Mishina (Research Triangle Park, NC, USA) showed that the long bones of mice with an osteoblast-specific mutation in BMPR-IA (ALK3) had a reduced bone mass for up to three months after birth. However, by three months of age, the total bone mass in these mice was increased due to reduced bone resorption. Conversely, the mass of the calvarial bones increased from the embryonic period onwards. These results show that the BMP function in osteoblasts alters in an age-dependent manner, and might differ between endochondral and intramembranous bone formation. M. Okamoto (Osaka, Japan) reported that mice overexpressing the BMP4 inhibitor noggin frequently underwent fracture, but showed increased bone volume. This was due to a decreased number of osteoclasts resulting from a decrease in the activation frequency of bone-remodelling units, and from an imbalance between osteoclastic bone resorption and osteoblastic bone formation leading to bone gain. Conversely, transgenic mice overexpressing BMP4 showed severe osteopenia—low bone density—with markedly increased osteoclast number. Tight regulation of BMP expression is therefore required for maintaining the homeostasis between bone formation and resorption.

Y. E. Zhang (Bethesda, MD, USA) reported on the increased bone mass in mice lacking the Smad ubiquitin regulatory factor, Smurf1. Interestingly, this was not caused by alterations in the Smad pathway, but by the accumulation of phosphorylated MAPK/ERK kinase kinase 2 (MEKK2), which physically interacts with Smurf1. Despite the lack of phenotypic developmental abnormalities, *Smurf1–/–* mice display an age-dependent increase in bone mass, which might be due to the enhanced activity of osteoblasts that become more sensitized to BMPs in the absence of Smurf1. Surprisingly, this skeletal abnormality is not caused by an alteration in Smad-mediated BMP signalling, but by an increase in activator protein 1 (AP1) activity, the accumulation of phosphorylated MEKK2 and the activation of the downstream c-Jun amino-terminal kinase (JNK) signalling cascade, which together regulate the biological response to the BMP signal. R. Monteiro (Utrecht, The Netherlands) presented a reportergene

approach to study BMP signalling during vertebrate development. He generated transgenic green fluorescent protein (GFP) reporter mice carrying a Smad1–Smad5-specific response element, which displayed increased sensitivity compared with the previously developed LacZ reporter strain. The GFP approach can also be used to study BMP signalling in adult mice, whereas the LacZ reporter system is not suitable.

BMPs are involved in the development of most organs and tissues, including the somites, nervous system, lung, kidney, skin and gonads, as well as in crucial steps in the establishment of the basic embryonic body plan (Hogan, 1996). Model organisms used for studying the role of BMP pathways in embryogenesis include *Xenopus*, mice, chickens and zebrafish. D. Huylebroeck (Leuven, Belgium) has used a morpholino-based knockdown approach in *Xenopus* to study the function of the Smad-interacting protein Smicl in the regulation of the expression of chordin—a BMP antagonist— in the Spemann organizer. By using a similar approach in zebrafish, he has also shown that Sip Ttrap, which is a novel Alk-interacting and Smad-interacting protein, is essential for gastrulation movement and left–right asymmetry, and functions in an early Nodal–Smad3 pathway and presumably also in later BMP-controlled processes.

A. Zwijsen (Leuven, Belgium) reported BMP gain-of-function defects in Smad5-knockout embryos. In the mouse, Smad5 is essential for primordial germ-cell development, the development of the allantois and closure of the amnion. These defects are consistent with a loss of BMP signalling. By contrast, the appearance of ectopic primordial germ-like cells, and regionalized ectopic vasculogenesis and haematopoiesis in the thickened *Smad5–/–* amnion are defects that have not been reported in other BMP loss-of-function mouse models. Injection of BMP4 into the exocoelom of wild-type embryos can induce thickening of the amnion, mimicking the early amnion pheno type in *Smad5* mutants. Therefore, loss of Smad5 paradoxically results in BMP gain-of-function defects in the amnion.

S. Schulte-Merker (Utrecht, The Netherlands) exploited the fact that in zebrafish, osteoclasts are not active before 12 days postfertilization. Hence, osteo blast function screens can be performed without interference from the osteoclast. He analysed approximately 100 specific mutants, and found that 25% had increased bone mass. In addition, Stocksteif zebrafish mutants show ossification of the entire notochord with the centres of their vertebrae being fused, contrary to the wild type in which only vertebrae ossify. However, the appearance of their osteo blasts did not differ from those of wild-type embryos. This implies that classic osteoblasts might not be responsible for the (over)ossification around the notochord, raising the question of whether other cells could be involved in producing bone matrix in zebrafish.

Skeletal diseases

F. Luyten (Leuven, Belgium) used genetic approaches in mice to show that BMP signalling is crucial in the homeostasis of post natal articular cartilage. Luyten also showed the presence of distinct BMPs, and their antagonists and target cells, in the synovium of arthritic patients. BMPs protect articular cartilage against inflammation-driven destruction in arthritis. In addition, by using a mouse model of ankylosing enthesitis, Luyten provided evidence for the crucial role of BMP signalling in both the initiation and progression of arthritic diseases.

BMP gene mutations show positive linkages to different clinical syndromes (Hartung *et al*, 2006). For example, Luyten showed that mutations in cartilage-derived morphogenetic protein 1 (CDMP1), which is also known as growth-differentiation factor 5 (GDF5), are implicated in two recessive chondrodysplasias: the Hunter–Thompson type and the Grebe type (Thomas *et al*, 1997). A recurrent mutation in the glycine–serine (GS) activation domain of ALK2, which is a BMPR-I, was reported by F. Kaplan and E. Shore (Philadelphia, PA, USA) in all sporadic and familial cases of classic fibrodysplasia ossificans progressiva (FOP). FOP is a serious disorder of heterotopic ossification in humans, which leads to the formation of a second skeleton. New therapies based on inhibiting a crucial nodal point in the BMP signalling pathway will open a new era in treating heterotopic ossifications in humans.

Tumour biology

The role of BMPs in tumour biology is controversial. J. Langenfeld (New Brunswick, NJ, USA) showed that BMP2 and BMP4 have important roles in the regulation of lung and other carcinomas, by promoting tumour invasion and metastasis. BMP2 has also been shown to induce, in a self-autonomous manner, signalling pathways that promote malignant transformation. J. H. Clement (Jena, Germany) reported that high levels of BMP2 at the invasion front enhance the migratory and invasive properties of breast cancer in a xenograft model, as well as promoting vascularization and tumour angiogenesis. A. Bosserhoff (Regensburg, Germany) showed that BMPs promote melanoma cell invasion, angiogenesis and vasculogenic mimicry, and therefore might have important roles in the progression of malignant melanoma. Conversely, C. Löwik (Leiden, The Netherlands) showed, for the first time, that systemically administered BMP7 inhibits breast and prostate cancer growth in the bone marrow, and that BMP7 is strongly downregulated in laser microdissected primary human prostate cancer compared with normal prostate luminal epithelium. BMP7 controls the epithelial homeostasis in the human mammary and prostate gland (Fig 2). Through inhibiting

transforming growth factor-β (TGF-β)-induced activation of Smad2–Smad3 through ALK5, and through inducing E-cadherin expression even in the presence of TGF-β, BMP7 preserves the epithelial phenotype. By counteracting the epithelial-to-mesenchymal transition, BMP7 prevents the acquisition of an invasive metastatic phenotype (Fig 2). However, the exact role of BMPs in the development of tumours might be ambivalent and has yet to be fully explored.

Tissue regeneration

BMPs have been successfully tested in clinical trials and subsequently approved for treating skeletal defects (Vukicevic & Sampath, 2004). Their unique ability to transform muscle into bone (Urist, 1965; Yamaguchi *et al*, 1991) has been used to 'pre-tailor' cranio-facial bones in the back muscles of patients (Fig 3). BMPs inhibit myogenesis and promote the formation of new bone through activating the expression of inhibitor of differentiation–inhibitor of DNA binding (Id) genes. Id proteins then repress transcription by basic helix–loop– helix heterodimers containing myoD/myogenin, which results in the inhibition of myogenesis and leads to the formation of osteoblasts (Fig 3). H. Terheyden (Kiel, Germany) discussed three patients with huge mandibular and maxillary defects. Their bones were regrown in specifically modelled scaffolds loaded with BMP7 and bonemarrow aspirate, which were then placed into the latissimus dorsi muscle for six weeks. Newly formed and properly shaped bone with a new vascular network was then transplanted into the defect in the jaw. A. Westermark (Stockholm, Sweden) showed another example of bone formation from a muscle, in a patient with a defect in the frontal bone of the skull. A section of the latissimus dorsi muscle was dissected, impregnated with BMP2, transferred into the mould and then reinstalled in the patient's back. After four months, newly formed bone was transferred to the frontal defect, which then healed (Fig 3). The efficacy of using BMPs in the regeneration of articular cartilage defects has been previously shown in animal models (Vukicevic & Sampath, 2004). Apart from the local application of BMPs for the regeneration of bone, BMPs have also been systemically used to increase the volume of the skeleton (Simic *et al,* 2006), and to regenerate the kidney following acute and chronic failure in rats (Vukicevic *et al*, 1998; Simic & Vukicevic, 2005). K. Hruska (St Louis, MO, USA) showed diminished vascular calcification following systemic administration of BMP7 in a mouse model of chronic kidney disease induced by renal ablation. BMP2 stimulated the vascular expression of the osteoblast-specific transcription factor Cbfa1 and of the downstream osteoblast programme leading to increased mineralization. A. Celeste (Cambridge, MA, USA) revealed that the livers of mice treated systemically with BMP9 showed microvesicular changes and

necrosis, thereby questioning the potential of BMP9 for the treatment of diabetes, which has been suggested as a therapeutic option (Chen *et al*, 2003).

Future therapeutic perspectives

It is likely that advances made in the identification and characterization of the BMP signalling pathways and promoter sequences, and the regulation of BMP gene expression, will allow the development of small molecules for BMP endogenous upregulation in patients with systemic diseases, such as osteoporosis and chronic kidney failure. K. Nakagawa (Osaka, Japan) reported that the prostaglandin E2 receptor E4 agonist accelerates BMP-dependent osteoblastic differentiation through the protein kinase A signalling pathway. Concurrent local delivery of prostaglandin E2 receptor E4 agonist and BMP2 enhances spinal fusion in a rabbit model, as shown by T. Namikawa (Osaka, Japan). G. Mundy (Nashville, TN, USA) presented a series of chemically unrelated compounds that mimic the effect of BMP2. Statins, which are inhibitors of 3-hydroxy-3-methylglutaryl (HMG) CoA reductase, and proteasome inhibitors, such as bortezomib, are among the most powerful BMP2 mimetics that stimulate bone formation *in vivo*. Bortezomib is used clinically in myeloma patients as an antineoplastic agent, and has been shown to increase BMP2 expression and alkaline phosphatase activity. Statins also increase bone formation systemically when given in large doses transdermally or locally at fractured rat bones. D. Bosukonda (Hopkinton, MA, USA) reported on a novel peptide agonist that binds specifically to BMPRs, thereby inducing Smad signalling and protecting the kidney against cisplatinum-induced nephrotoxicity.

Autologous bone marrow-derived mesenchymal cells could be engineered in the surgical suite to deliver the BMP through *ex vivo* gene therapy with an appropriate carrier matrix to induce new bone formation. A. Hoffmann (Braunschweig, Germany) showed neo tendon formation induced by the manipulation of the Smad8 signalling pathway in mesenchymal stem cells. Adenovirally-modified murine mesenchymal progenitors expressing both Smad8 and BMP2 generate entire ectopic tendon–bone insertions with an osteotendinous junction exhibiting a fibrocartilage enthesis.

Closing remarks

Although diverse actions of BMPs have been extensively investigated, their redundancy and specificity in the development and regeneration of various organs—including bone, kidney, joint cartilage, liver, muscle and brain—remain to be explored. We look forward to hearing more on these themes at the Seventh International Conference on BMPs, which will take

place in Lake Tahoe, California, from 10 to 14 July 2008, and will be organized by A. H. Reddi (Davis, CA, USA), J. Wozney (Boston, MA, USA) and T. K. Sampath (Framingham, MA, USA).

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Figure legends

Figure 1. Bone morphogenetic protein signalling. A member of the BMP family induces heterodimeric complex formation between two BMP receptors (BMPRs). The type II receptor kinase phosphorylates the type I receptor and subsequently activates intracellular signalling. On BMPR activation, Smad1–Smad5–Smad8 forms heterodimeric complexes with Smad4, which then translocate to the nucleus where they act directly and/or cooperate with other molecules to regulate the transcription of target genes. Inhibitory Smad6–Smad7 specifically inhibits BMP signalling. Antagonists, stimulatory and inhibitory co-receptors, and Smurfs are actively involved in maintaining the BMP tissue homeostasis and regulate cross-talk with other signalling pathways. ActR, activin receptor; ALK, activin receptor-like kinase; BAMBI, BMP and activin membrane-bound inhibitor; BMP, bone morphogenetic protein; c-kit, CD117, tyrosine kinase receptor; GPI, glycosylphosphatidyl inositol; HJV, haemojuvelin; P, phosphorylation; Ror2, receptor tyrosine kinase-like orphan receptor 2; Smurf, Smad ubiquitin regulatory factor; Tsg, twisted gastrulation; Tyr, tyrosine; USAG1, uterine sensitization-associated gene 1.

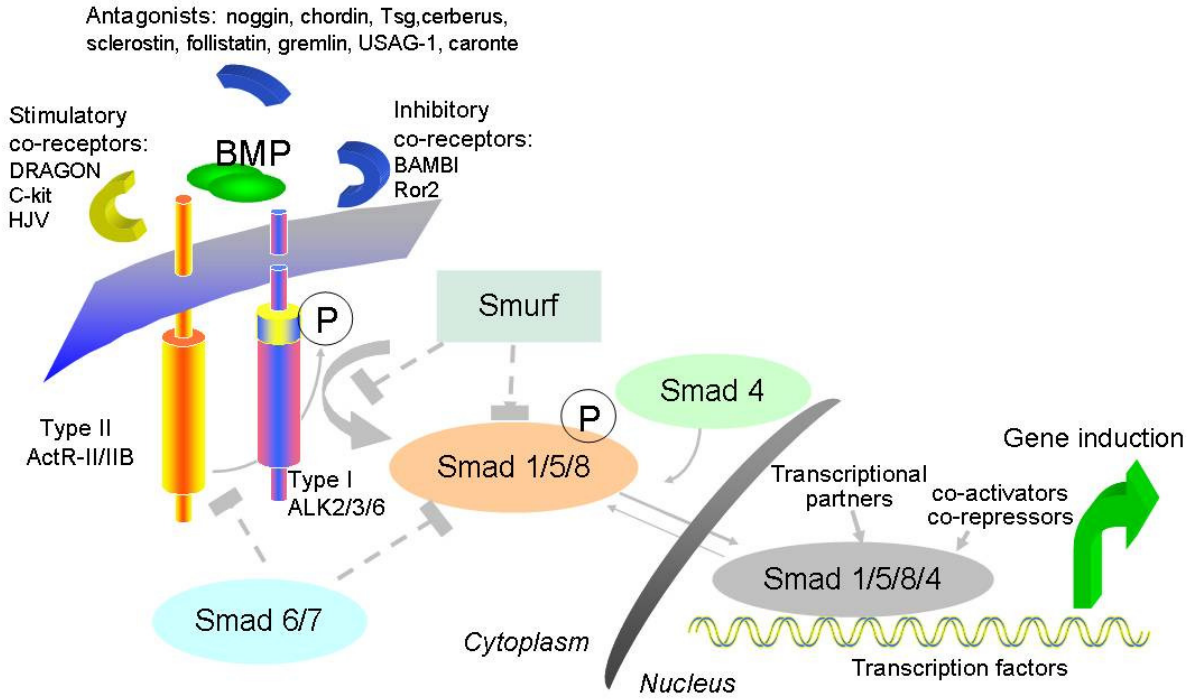

Figure 2. Role of bone morphogenetic proteins in tumour biology. Bone morphogenetic protein (BMP) 7 promotes the mesenchyme-to-epithelial transformation (MET), and specifically inhibits the transforming growth factor-β (TGF-β)-mediated epithelium-to-mesenchyme transformation (EMT), both of which inhibit tumour metastasis and growth. Tumour metastatic cells release growth factors that promote bone resorption by osteoclasts. Osteoclasts then release BMPs from the bone matrix, which in turn inhibit tumour growth.

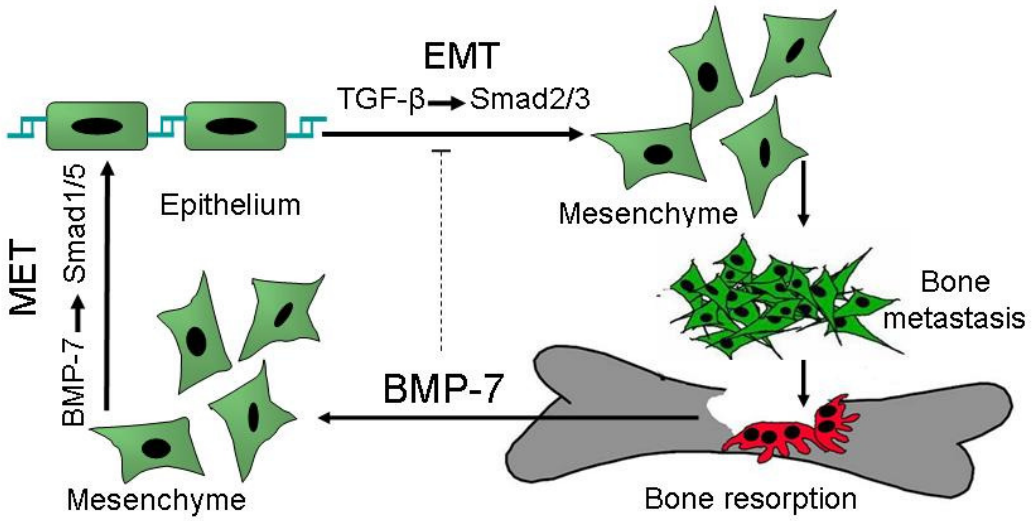

Figure 3. Bone morphogenetic proteins in tissue engineering. Bone morphogenetic proteins (BMPs) inhibit myogenesis, and promote formation of new bones through activating the expression of inhibitor of differentiation (Id) genes, which prevent activity of myoD/myogenin. Scaffolds filled with bone-marrow cells and BMPs are placed into the back muscle of a patient who is in need of a new bone, such as a frontal bone or mandible.