Summary
William F. McKay, Steven M. Peckham and Jeffrey M. Badura
Development of a novel compression resistant carrier for recombinant human bone morphogenetic protein-2 (rhBMP-2) and preliminary clinical results

Recombinant human bone morphogenetic protein-2 (rhBMP-2) has been commercially available in the United States since July 2002. It was initially approved for use in anterior lumbar interbody fusions with an interbody cage. It has been further approved for two additional clinical indications: fresh tibial fractures and certain oral maxillofacial procedures. Because of the compression resistance limitations of the ACS carrier, especially in challenging clinical environments where soft tissue compression is unavoidable, a second-generation rhBMP-2 carrier was developed. This carrier was termed the Compression Resistant Matrix (CRM). The CRM carrier is a composite sponge consisting of cross-linked Type I bovine collagen impregnated with biphasic calcium phosphate ceramic granules (15% hydroxyapatite [HA] and 85% β-tricalcium phosphate [β-TCP]). This chapter focuses on the identification, characterization, and pre-clinical testing of the rhBMP-2/CRM bone graft substitute.

Keywords: Recombinant human bone morphogenetic protein-2 (rhBMP-2), absorbable collagen sponge (ACS), compression resistant matrix (CRM), intertransverse posterolateral fusion, interbody fusion, compressive forces, ceramic, hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), rhBMP-2 bulk concentration, rhBMP-2 dose

Michael Suk
Use of recombinant human BMP-2 in orthopedic trauma

Summary
Daniel B. Spagnoli
The application of rhBMP-2/ACS to reconstruction of maxillofacial bone defects

The information provided in this review discusses much, but not all, of the important research that has led to the US FDA approval of the infuse of recombinant human bone morphogenetic protein on an absorbable collagen sponge (rhBMP-2/ACS) for oral, maxillofacial, and dental bone reconstruction. This research has provided compelling evidence that rhBMP-2/ACS is a safe and effective graft substance when used to treat maxillary alveolar ridge buccal wall extraction site defects, and when used as a graft for maxillary sinus lift augmentations. Additional clinical experience in treating congenital cleft defects of the maxillary alveolus, and various other tumors or trauma-related defects are provided as examples of the potential extended application of rhBMP-2/ACS. The graft placement techniques described above,
although useful now, will ultimately be replaced as improved or enhanced matrices become available for combination with rhBMP-2/ACS. Protein-signaled bone grafting with rhBMP-2 has the potential to significantly enhance patient care due to its efficacy and propensity to accelerate wound healing, and by the reduction of donor site morbidity and invasiveness. We have already witnessed significant psychological benefits for patients that fear bone graft procurement.

**Keywords:** rhBMP-2/ACS maxilla, mandible, sinus lift, alveolar ridge, alveolar cleft

**Summary**

**J. Kenneth Burkus**  
**Clinical outcomes using rhBMP-2 in spinal fusion applications**

Bone morphogenetic proteins function as a differentiation factor and act on mesenchymal stem cells to form bone. When used at an optimized concentration and with an appropriate carrier, bone morphogenetic proteins can be successfully used as bone graft replacement in the cervical and lumbar spine. BMP-2 is a naturally occurring protein and is active in normal bone repair. A genetically engineered version of this protein, rhBMP-2, has been extensively evaluated and represents the most studied technology introduced for spine surgery. At a concentration of 1.5 mg/mL, rhBMP-2 applied to an absorbable collagen sponge carrier, trade name INFUSE® Bone Graft, is the first bone morphogenetic protein technology approved in the United States for use in spinal fusions.

**Keywords:** bone morphogenetic protein, rhBMP-2, cervical spine, lumbar spine, degenerative disc disease, bone graft replacement

**Summary**

**Christina Sieber, Gerburg K. Schwaerzer and Petra Knaus**  
**BMP signaling is fine tuned on multiple levels**

Bone Morphogenetic Proteins (BMPs) belong to a large superfamily of growth factors called the Transforming Growth Factor-beta (TGFβ) family. Initially discovered as bone growth factors, many of these proteins play critical and essential roles in the development of most, if not all, organs. BMPs are the key molecules to regulate cell fate decisions via the induction of specific transcription programs, and thus control maintenance of stem cell pluripotency as well as selection and progression of specific differentiation lineages. BMPs signal via two types of transmembrane serine/threonine kinase receptors (BRI and BRII). The activation of the Smad-pathway requires both BRI and BRII preassembled before ligand binding, while non-Smad signaling is initiated via BMP-induced oligomerization of the two receptor types. Both cascades result in transcriptional programs, which affect cell fate characteristic for various cell types.

Here we describe how BMP signaling is fine tuned at multiple levels, beginning with ligand binding to its receptors, activation of the receptors, and downstream when signals enter the nucleus.

**Keywords:** BMP, GDF, BMP-antagonist, BMP receptor, Smad, receptor oligomerization, signal transduction, tissue regeneration
Summary
Daniel Graf and Aris N. Economides
Dissection of BMP signaling using genome engineering tools

The activity of Bone Morphogenetic Proteins is regulated in the extracellular space by a complex network of BMP binding proteins. Functional dissection of this network requires detailed knowledge of their expression in vivo as well as tools for gene ablation. Here, we present how LacZ-mediated gene reporting in combination with immunohistochemistry can be used to identify novel sites of BMP expression and activity. In addition, we describe a general strategy for engineering conditional alleles, using bmp-2 and bmp-7 as examples. Our strategy employs Bacterial Homologous Recombination (BHR) to generate Bacterial Artificial Chromosome (BAC)-based targeting vectors. As this technology allows genome manipulation with base-pair precision, it enables us to consider locus structure and to avoid the generation of hypomorphic alleles. The combination of high resolution expression analysis with high precision genome engineering respectively provide the information and tools necessary to empower functional studies on BMP biology in vivo, and particularly so in adult mice.

Keywords: bmp-2, bmp-4, bmp-7, marker gene, lacZ reporter, gene reporting, conditional allele, Cre-lox, recombineering, BHR, ECR, conserved regions, thymus, histochemistry

Summary
Petra Seemann, Stefan Mundlos and Katarina Lehmann
Alterations of BMP signaling pathway(s) in skeletal diseases

BMPs and GDFs were initially identified because of their potential to induce ectopic cartilage and/or bone when implanted into muscles. Some members of the GDF/BMP family appear to be crucial for endogenous skeletal development and mutations in these genes cause distinct skeletal malformations in humans and mice. In this article we summarize the clinical and molecular findings associated with mutations in signaling components of the BMP pathway, including ligands, receptors and antagonists. Mutations in one of the components can cause specific clinical manifestations as well as overlapping features within a phenotypic spectrum.

Keywords
BMP, GDF5, NOG, BMPR1B, ACVR1, SOST, ROR2, brachydactyly, fibrodysplasia ossificans progressiva, symphalangism, multiple synostosis syndrome, acromesomelic chondrodysplasia, sclerostosis, Robinow syndrome

Summary
Nandini Ghosh-Choudhury and Goutam Ghosh-Choudhury
Signaling crosstalk by bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) are important not only for osteoblast and osteoclast differentiation leading to bone remodeling, but its importance in development of cardiomyocyte and embryogenesis has brought them in the forefront. This review presents an overview of known signaling pathways integrated at various levels of BMP-induced growth and differentiation of various cell types. Upon binding to its receptor complex at the cell
membrane, BMPs activate the conventional Smad signaling pathway, which activates transcription of genes that harbors Smad binding elements (SBE) in the promoter region. The BMP-regulated functions required input from many other signaling pathways some of which are known and are discussed in this review, whereas many of them may still be under investigation. One of the most important mediators of BMP signaling is phosphatidylinositol 3 kinase (PI3K) and its downstream target protein kinase B (also known as Akt kinase). This signal transduction pathway, induced by BMP, integrates with Smads and mitogen activated protein kinase (MAPK) signaling pathways and regulate a host of transcription factors interaction with their respective gene promoters. This complex network not only stimulate gene transcription but also results in feedback mechanisms by inducing expression of critical repressors of gene expression. Included in this review is the emerging role of BMPs in cancer development that implicates the diverse role of this important class of proteins in normal and tumor cell growth.

Summary

Stephen E. Harris, Wuchen Yang, Jelica Gluhak-Heinrich, Dayong Guo, Xou-Dong Chen, Marei A. Harris, Holger Kulessa†, Brigid L.M. Hogan, Alex Lichtler, Barbara Kream, Jianhong Zhang, Jian Q. Feng, Gregory R. Mundy, James Edwards and Yuji Mishina

The role and mechanisms of bone morphogenetic protein 4 and 2 (BMP-4 and BMP-2) in postnatal skeletal development

Using the 3.6 collagen 1a1-Cre mouse model, in which Cre is predominately expressed postnatally in early osteoblasts, we deleted the BMP4 and BMP2 genes. In the single BMP4 bone selective conditional knock-out, an osteopenic phenotype developed rapidly after birth and continued up to 1½ years. Osterix and BMP2 expression were reduced in the BMP-4 cKO animals. By 9 months, some of the osteopenia could be accounted for by increased osteoclast activity. The mineral quality in the bones of BMP-4 cKO were compromised with less mineral to matrix and an altered mineral organization in the bone. Similar experiments were carried out with the 3.6 collagen 1a1-Cre model and BMP-2 floxed mice. Targeted deletion of BMP2 resulted in a similar osteopenic phenotype as seen in the BMP-4 cKO animals. However, there was an increase in BMP4 expression and increased phosphor-Smad1/5/8 levels, suggesting that there is a compensation mechanism in place or the cell population distribution in the bone is dramatically altered. We could clearly show reduced late osteoblast gene expression of osteocalcin and collagen type 1a1, suggesting the population of osteoblast had not progressed to these later stages in the BMP-2 bone selective deletions. In the analysis of the bone marrow stem cell population from control heterozygotes and BMP-2 cKO animals, we also showed a greatly reduced number of mesenchymal stem cells as classified as CFU-Fs. Thus BMP4 and BMP2 have unique roles in postnatal bone biology, and BMP-2 has at least two roles in bone formation, one an early role in osteoblast commitment and another role in late differentiation to mineral-matrix producing osteoblast.

Keywords: bone morphogenetic protein 2 and 4, osteoblast growth and differentiation, mesenchymal stem cell, osteopenia, bone modeling and remodeling, conditional knock-out.
The role of bone morphogenetic protein 4 (BMP-4) in tooth development

BMP-4 is expressed throughout the main stages of embryonic tooth formation: initiation, bud, cap, bell (cytodifferentiation), and secretory stages. BMP-4 has both antagonistic and stimulatory regulatory roles during early stages of tooth development. BMP-4 expression is very high in preodontoblasts but not in preameloblasts. Final secretory stage of tooth development shows BMP-4 expression in odontoblasts and ameloblasts. Conditional deletion of BMP-4 using a BMP-4 floxed mouse and 3.6 Col1a1-Cre model, resulted in decreased dentin formation, decrease BMP signaling in odontoblasts, and decreased expression of osterix, DMP1, and DSPP. Deletion of BMP-4 in early odontoblasts also disrupted and delayed enamel formation. These overall results demonstrate a necessary role for the BMP-4 gene in tooth formation after birth. Low levels of BMP-4 are normally expressed in the periodontal ligament, and removal of BMP-4 from PDL cells also disrupted the formation of the collagen matrix in the PDL and the formation of the cementum around the roots of the teeth. Potential for use of BMPs in development of new teeth and stem differentiation are now open for future studies.

Keywords: bone morphogenetic protein, tooth morphogenesis, tooth cytodifferentiation, odontoblasts, dentin, dentinogenesis, ameloblasts, amelogenesis, dentin tubules, BMP4 signaling,
as soluble signals of tissue morphogenesis sculpting the multicellular mineralized structures of the periodontal tissues with functionally oriented periodontal ligament fibers inserted into newly formed cementum. Amino acid sequence variations in the carboxy terminal domain confer specialized and pleiotropic activities to each isoform, the molecular basis of the structure-activity profile of each morphogenetic protein. The presence of multiple forms of BMPs/OPs has a therapeutic significance, and the choice of a suitable isoform is a formidable challenge for the practicing skeletal reconstructor and periodontologist alike. Naturally-derived BMPs/OPs induce cementum, alveolar bone and de novo induction of a true periodontal ligament in the primate Papio ursinus. Tissue morphogenesis induced by hOP-1 and hBMP-2 is qualitatively different when the morphogens are applied singly, indicating the critical role of the structure-activity profile amongst BMPs/OPs in controlling pleiotropic tissue induction and morphogenesis. hOP-1 in contact with dentin extracellular matrix is cementogenic; in contrast, hBMP-2 does not have a significant effect on cementogenesis and formation of a periodontal ligament both in canine and primate models. Furcation defects of Papio ursinus with root surfaces long-term exposed to periodontal disease and implanted with 0.5 and 2.5 mg of gamma-irradiated hOP-1 per gram of bovine collagenous matrix as carrier show regeneration of alveolar bone and the induction of cementogenesis with Sharpey's fibers embedded in the regenerated bone and newly formed cementum. The induction of the complex tissue morphologies of the periodontal tissues develops as a mosaic structure in which the osteogenic proteins of the TGF-β supergene family singly, synergistically and synchronously initiate and maintain tissue induction and morphogenesis.

Key words: bone morphogenetic proteins, tissue induction and morphogenesis, cementogenesis, periodontal ligament regeneration

Summary
David J.J. de Gorter, Carola Krause, Clemens W.G.M. Löwik, Rutger L. van Bezooijen and Peter ten Dijke
Control of bone mass by sclerostin: inhibiting BMP- and WNT-induced bone formation

In an aging world population osteoporotic fractures have become an important public health problem and consume considerable health resources. Targeting the osteocyte-derived, negative regulator of bone formation sclerostin in osteoporotic patients could potentially serve as an anabolic treatment to restore lost bone. Mutations in the coding region or in regulatory elements of SOST, the gene encoding sclerostin, underlie the high bone mass disorders sclerosteosis and Van Buchem disease, respectively. Sclerostin was found to inhibit osteoblast differentiation induced by BMPs and WNTs, growth factors playing a crucial role in bone formation. In this chapter, we discuss our current understanding of the mechanism of action by which sclerostin controls bone formation, and how this information can be applied in the treatment of osteoporotic patients.

Keywords: sclerostin, SOST, antagonist, bone formation, osteoblast, osteocyte, sclerosteosis, Van Buchem disease, osteoporosis, BMP, WNT

Summary
Susan Chubinskaya, Mark Hurtig and David C. Rueger
Bone morphogenetic proteins in cartilage biology
Four years ago we reviewed the role of bone morphogenetic proteins (BMPs) in articular cartilage repair in the last edition of this series on BMPs. Since that time, our understanding of the function of BMPs and especially BMP-7, also called osteogenic protein-1 (OP-1), in cartilage homeostasis and repair has substantially increased. Here we summarize the information accumulated on BMPs with the emphasis on BMP-7 from *in vitro* and *ex vivo* studies with cartilage cells and tissues as well as from *in vivo* studies of cartilage repair in various animal models. The primary focus is on articular chondrocytes and cartilage, but studies will also be reviewed covering nonarticular cartilage, particularly from the intervertebral disc. The data show that among BMPs, BMP-7 occupies a unique place because unlike other members of the family, it exhibits in addition to strong pro-anabolic activity very prominent anti-catabolic properties. In the current review, we unravel some of the underlying mechanisms of the anabolic and anti-catabolic activities of BMP-7 that provide a better understanding of the interactions between BMP-7 and signaling pathways and highlight the overall role BMP-7 and other BMPs in human cartilage homeostasis. In regard to *in vivo* activities, we describe animal studies demonstrating that BMP-7, BMP-2 and GDF-5 (also called CDMP-2 or MP-52) have various abilities to repair cartilage in models of degenerated articular cartilage, including focal osteochondral and chondral defects and osteoarthritis, as well as models of degenerated intervertebral disc cartilage. Together, our findings indicate a significant promise for BMPs and particularly BMP-7 as therapeutics for cartilage repair and regeneration.

**Summary**

*Slobodan Vukicevic, Petra Simic, Lovorka Grgurevic, Fran Borovecki and Kuber Sampath*

**Systemic administration of bone morphogenetic proteins**

BMPs act predominantly in the local microenvironment. However, after systemic application they circulate and accumulate in different organs. Due to their multiple regenerative activity, BMPs have been systemically tested in preclinical models for the following indications: bone formation in a model of osteoporosis, kidney regeneration in models of acute and chronic renal failure, liver regeneration, ischemic coronary infarction and stroke, and in a nude mouse model of different human cancers. These studies suggested that following their systemic use, BMPs support organ regeneration recapitulating the embryonic development and therefore might serve as novel molecules in regenerative medicine.

**Keywords:** systemic use of BMPs, osteoporosis, stroke, myocardial infarction, breast cancer, prostate cancer, tumor biology, diabetes, acute kidney failure, chronic kidney failure, biological fluids, urine, plasma, blood
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