REVIEW ARTICLE

Bone Morphogenetic Protein (BMP) signaling in development and human diseases

Richard N. Wang a, b, Jordan Green a, b, Zhongliang Wang b, c, Youlin Deng b, c, Min Qiao b, c, Michael Peabody b, Qian Zhang b, c, Jixing Ye b, d, Zhengjian Yan b, c, Sahitya Denduluria b, Olumuyiwa Idowu a, b, Melissa Li b, Christine Shen b, Alan Hu b, Rex C. Haydon b, Richard Kang b, James Mok b, Michael J. Lee b, Hue L. Luu b, Lewis L. Shi b, *

* The University of Chicago Pritzker School of Medicine, Chicago, IL 60637, USA
b Molecular Oncology Laboratory, Department of Orthopaedic Surgery and Rehabilitation Medicine, The University of Chicago Medical Center, Chicago, IL 60637, USA
b, c Departments of Orthopaedic Surgery, Medicine, and Gynecology, the Affiliated Hospitals of Chongqing Medical University, Chongqing 400016, China
d School of Bioengineering, Chongqing University, Chongqing, China

Received 11 July 2014; accepted 15 July 2014
Available online 27 July 2014

KEYWORDS
BMP signaling; Development; Genetics; Mouse knockout; Pathogenesis; Signal transduction

Abstract Bone Morphogenetic Proteins (BMPs) are a group of signaling molecules that belongs to the Transforming Growth Factor-β (TGF-β) superfamily of proteins. Initially discovered for their ability to induce bone formation, BMPs are now known to play crucial roles in all organ systems. BMPs are important in embryogenesis and development, and also in maintenance of adult tissue homeostasis. Mouse knockout models of various components of the BMP signaling pathway result in embryonic lethality or marked defects, highlighting the essential functions of BMPs. In this review, we first outline the basic aspects of BMP signaling and...
then focus on genetically manipulated mouse knockout models that have helped elucidate the role of BMPs in development. A significant portion of this review is devoted to the prominent human pathologies associated with dysregulated BMP signaling.

Introduction

The activity of Bone Morphogenetic Proteins (BMPs) was first observed in the mid-1960s when it was discovered they could induce ectopic bone formation.1 It was not until the late 1980s, however, when the first BMPs were characterized and cloned, that individual BMPs could be studied biochemically.2 Many studies have since demonstrated the ability of BMPs to induce mesenchymal stem cells to differentiate into bone, confirming their role in bone and cartilage formation. BMPs are part of the Transforming Growth Factor-β (TGF-β) superfamily of proteins (Fig. 1A), which includes TGF-βs, activins, inhibins, Growth Differentiation Factors (GDFs), Glial Derived Neurotrophic Factors (GDNFs), Nodal, Lefty, and anti-Müllerian hormone. Since their initial discovery, they have been shown to affect a wide variety of cell types and processes beyond bone and osteogenesis. They are important morphogens in embryogenesis and development, and also regulate the maintenance of adult tissue homeostasis.

Many processes in early development are dependent on BMP signaling for cell growth, apoptosis, and differentiation.3-6 BMPs also play important roles in maintaining adult tissue homeostasis, such as the maintenance of joint integrity, the initiation of fracture repair, and vascular remodeling.7-9 Because of these diverse functions in all organ systems, it has been suggested that BMPs deserve to be called body morphogenetic proteins.10 Due to their ubiquitous expression and importance as regulators throughout the body, deficiency in BMP production or ubiquitous expression and importance as regulators throughout the body, deficiency in BMP production or recruitment can activate different pathways15. The existence of preformed oligomeric complexes adds an additional layer of intricacy; indeed, binding to preformed receptor complexes versus BMP-induced receptor recruitment can activate different pathways.15

Upon formation of a heterotetrameric complex, the constitutively active type II receptor transphosphorylates the type I receptor at a glycine—serine rich motif known as the GS domain. This activates the type I receptor and allows phosphorylation of the immediately downstream substrate proteins known as the receptor-regulated Smads (R-Smads) at a C-terminal SSXS motif.13 The R-Smads involved in BMP signaling are Smad1, Smad5, and Smad8 (Smad1/5/8). R-Smads then associate with the co-mediator Smad (co-Smad) Smad4, and this complex translocates to the nucleus where it functions as a transcription factor with coactivators and corepressors to regulate gene expression. Inhibitory Smads (I-Smads), Smad6 and Smad7 (Smad6/7), are involved in feedback inhibition of the signaling pathway.16

Several non-canonical, Smad-independent signaling pathways for BMPs have been identified. BMP4, for example, was found to activate TAK-1, a serine—threonine kinase of the MAPKKK family.17,18 In addition to the MAPK pathway, BMP signaling has been found to affect PI3K/Akt, Pkc, Rho-GTPases, and others.19 Interestingly, BMPs can have temporal regulation of signaling via the canonical Smad pathway or non-canonical pathways.20 The specific
pathway that is activated upon ligand-receptor interaction is thus likely dependent upon the extracellular environment, other cellular activity, and crosstalk with other pathways, such as Wnt signaling.

BMP signaling is extensively regulated by extracellular, intracellular, and membrane modulators. Extracellular modulators act as agonists or antagonists of BMP signaling. For example, BMP antagonists include the CAN (Cerberus and DAN) family of proteins, Twisted gastrulation, Chordin and Crossveinless 2, and Noggin. Intracellular regulators of BMP signaling include microRNAs, I-SMADS, phosphatases such as PP1 and PP2A that dephosphorylate the receptor and R-Smad, and FK506-binding protein 1A (FKBP1A or FKBP12) that binds the G5 domain of type I receptors to inhibit receptor internalization. Crosstalk with other signaling pathways, such as Wnt signaling, likely adds another layer of control. Co-receptors in the plasma membrane that interact with type I and type II receptors further add a level of regulation. For instance, Endoglin is a co-receptor that has been shown to be important in vascular growth and disease. Biological consequences of BMP signaling

More than 15 known BMPs are structurally related and can be further categorized into subgroups based on amino acid or nucleotide similarity. In particular, BMP2/4, BMP5/6/7/8, BMP9/BMP10, and BMP12/13/14 (GDF5/6/7) are subgroups based on phylogenetic analysis (Fig. 1A). Analysis with amino acid (Fig. 1A, panel a) or nucleotide (Fig. 1A, panel b) sequences of BMPs yields similar clustering patterns. BMP1, while able to induce bone and cartilage development, is a metalloprotease that functions in collagen maturation as a procollagen C-proteinase and is not part of the TGF-β superfamily. Although the name implies that all members are inducers of bone, some BMPs can act as inhibitors of bone formation. For example, BMP3 is a negative regulator of bone density, and BMP13 is a strong inhibitor of bone formation. BMP2, 4, 6, 7, and 9 are commonly referred to as the osteogenic BMPs, based on their potent bone-inducing activity. For instance, BMP2 is indispensable for endochondral bone formation.
Many organ systems have one or more BMPs that are critical for development. BMP4 serves to regulate limb development, and BMP13 has a modulatory role in the development of the eye. BMP7, important for eye development, is also crucial in the kidney. BMP8 has a demonstrated role in spermatogenesis, BMP12 is needed for seminal vesicle development, and BMP15 is classically associated with ovarian function.

BMP signaling in embryogenesis

BMPs are essential during embryogenesis, most prominently for mesoderm formation and cardiac development. Knocking out BMP2 or BMP4 results in embryonic lethality, and BMP1, BMP7, and BMP11 knockouts die shortly after birth (Table 1). BMP2 deficient mice have malformation of amnion and chorion and cardiac defects. BMP4 deficient mice do not have differentiation of mesoderm, implying that BMP4 is essential for developmental processes as early as gastrulation. These mice also have no primordial germ cells (PGCs). BMP4 heterozygotes are viable but have a wide variety of abnormalities. Mice lacking BMP1 fail to close the ventral body wall and have gut herniation as a result.

Deleting BMPR-1A, ActR-1A, and BMPR-2 results in embryonic lethality, and ActR-2B knockouts die shortly after birth (Table 1). The defects seen in these mice are consistent with the above phenotypes due to lack of BMP signaling. For instance, BMPR-1A is necessary for gastrulation and mesoderm formation. ActR-1A is also necessary for gastrulation in the mouse embryo, with mice deficient in this receptor showing disruption of mesoderm formation.
Table 1  Knockout phenotypes and biological consequences for the major players in BMP signaling.

| Signaling molecule | Phenotype                                                                 |
|--------------------|---------------------------------------------------------------------------|
| BMP1               | Die after birth, failure of ventral body wall closure\(^{36}\)             |
| BMP2               | Embryonically lethal, defects in amnion/chorion and cardiac development\(^{37}\); limb: spontaneous fractures and impaired fracture repair\(^{36}\); chondrocyte: severe chondrodysplasia\(^{39}\); cardiac progenitor: abnormal heart valve development\(^{38}\); myocardium: defects in myocardial patterning\(^{39}\) |
| BMP3               | Increased bone density\(^{28}\)                                          |
| BMP4               | Embryonically lethal, lack of mesoderm formation, \(^{40}\) no PGCs, \(^{41,42}\) no lens induction\(^{43}\); heterozygotes: various organ abnormalities\(^{44}\); hypomorph: AVCD, \(^{45}\) HSC microenvironment defect\(^{46}\); limb bud mesoderm: defective digit patterning\(^{47}\); adipocyte: enlarged adipocytes and impaired insulin sensitivity\(^{48}\); other targeted: loss-of-trachea phenotype, \(^{49}\) abnormal branchial arch arteries and outflow tract septation, \(^{50}\) defects in mandibular development, \(^{50}\) defects in vestibular apparatus\(^{11}\) |
| BMP5               | Short ear phenotype\(^{52}\); smaller and weaker bones\(^{53}\)             |
| BMP6               | Delay in sternum ossification\(^{54}\); smaller long bones\(^{55}\); decreased fertility\(^{56}\) |
| BMP7               | Die after birth, defects in kidney and eye development\(^{57}\); defects in skeletal patterning\(^{58}\); impaired corticogenesis\(^{59}\); decreased brown fat, \(^{60}\) diminished Langerhans cell number\(^{61}\); inducible deletion: precocious differentiation of kidney progenitor cells\(^{62}\); limb: no effect\(^{63}\); podocyte: defective kidney development\(^{64}\) |
| BMP8               | Germ cell degeneration\(^{65}\); defective PGC formation\(^{65}\); germ cell deficiency and infertility\(^{66}\) |
| BMP9/GDF2          | Abnormal lymphatic development\(^{67,68}\)                                |
| BMP10              | Reduced cardiomyocyte proliferation\(^{69}\)                            |
| BMP11/GDF11        | Die after birth, defects in A-P patterning\(^{70}\); smaller pancreas\(^{71}\); reduced β-cell numbers\(^{72}\); kidney agenesis\(^{73}\); slender spinal cord neuron differentiation\(^{74}\); increased olfactory neurogenesis\(^{75}\); retinal abnormalities\(^{76}\) |
| BMP12/GDF7         | Increased endochondral bone growth\(^{77}\); smaller bone cross-sectional parameters\(^{78}\); no effect on tail tendon phenotype\(^{79}\); subtle effects on Achilles tendon\(^{80}\); defective dorsal interneuron formation\(^{81}\); sterile with seminal vesicle defects\(^{82}\) |
| BMP13/GDF6         | Bone fusions in wrists and ankles\(^{83}\); accelerated coronal suture fusion\(^{83}\); eye and neural defects\(^{84,85}\); Klippel–Feil syndrome\(^{86}\); males: lower tail tendon collagen\(^{87,88}\) |
| BMP14/GDF5         | Brachypodism\(^{89}\); malformations in bones of limb, sternum, and digits\(^{90}\); delayed fracture healing\(^{91,92}\); impaired joint formation and osteoarthritis\(^{93}\); weaker Achilles tendon\(^{94}\); increased scarring after myocardial infarction\(^{95}\); altered skin properties\(^{96}\) |
| BMP15              | Males: normal and fertile; females: subfertile with decreased fertilization and ovulation rates\(^{97}\) |

Smad factors

| Smad1              | Die mid-gestation, defects in allantois formation, \(^{98}\) no PGCs, \(^{99,100}\); chondrocyte: delayed calvarial bone development\(^{101}\); osteoblast: osteopenia\(^{101}\); lung epithelium: severe neonatal respiratory failure\(^{102}\) |
| Smad5              | Die mid-gestation, multiple embryonic and extraembryonic defects\(^{103}\); defective PGC formation\(^{104}\); left-right asymmetry\(^{105}\) |
| Smad8              | Dispensable for development\(^{106}\); defective pulmonary vascular remodeling\(^{9}\) |
| Smad4              | Embryonically lethal, gastrulation defects\(^{107}\); heterozygotes: gastric and duodenal polyps\(^{108,109}\); adult: anemia\(^{110}\); osteoblast: lower bone mass\(^{111}\); chondrocyte: hearing loss and inner ear malformation\(^{112,113}\); disorganized growth plate\(^{114}\); muscle: muscle atrophy\(^{115}\); cardiomyocyte: cardiac hypertrophy\(^{116}\); endothelial cell: embryonically lethal\(^{117,118}\); vascular smooth muscle: embryonically lethal\(^{119}\); CNS: cerebellar defects\(^{120}\); eye: defects in lacrimal gland\(^{121}\); Sertoli and Leydig cells: testicular dysgenesis\(^{122}\); preovulatory follicles: follicle atresia\(^{123}\); ovary: defects in folliculogenesis\(^{124}\); skin: aberrant wound healing\(^{125}\); hair loss and squamous cell carcinoma\(^{126}\); keratinocyte: accelerated epithelialization\(^{127}\); head and neck: head and neck squamous cell carcinoma\(^{128}\); odontoblast: keratocystic odontogenic tumors\(^{129}\) |
| Smad6              | Defects in axial and appendicular skeletal development\(^{130}\); multiple cardiovascular abnormalities\(^{31}\) |
| Smad7              | Embryonically lethal, cardiovascular defects\(^{132}\); defects in axial and appendicular skeletal development\(^{133}\); growth retardation and reduced viability\(^{134}\); renal fibrosis and inflammation\(^{135}\); defective eye development\(^{136}\); sclerodermat\(^{137}\); altered B cell response\(^{138}\); T cell: reduced disease and CNS inflammation\(^{139}\) |

BMP receptors (continued on next page)
and no PGCs. These phenotypes are again recapitulated in mouse knockouts of intracellular regulators. Smad1, Smad5, Smad4, and Smad7-knockouts are all embryonically lethal, but interestingly Smad8 is dispensable for development (Table 1). Smad1 plays an essential role in fusion of amnion and chorion, and mutants have defects in extraembryonic structures as well, including left-right asymmetry and significantly reduced PGC number.

**BMP signaling in skeletal system**

BMPs play a crucial role in bone and cartilage formation, providing the namesake for this family of proteins, as well as in adult homeostasis of bone function. Though BMPs were initially discovered to induce bone formation, BMP3 has been shown to be a negative regulator of bone density. Some BMPs may have redundant roles in bone formation, as conditional deletion of BMP7 initially discovered to induce bone formation, BMP3 has been shown to be a negative regulator of bone density. Some BMPs may have redundant roles in bone formation, as conditional deletion of BMP7.

**Table 1** (continued)

| Signaling molecule | Phenotype |
|--------------------|-----------|
| BMPR-1A (ALK3)     | Embryonically lethal, no mesoderm formation; mesoderm: embryonically lethal, omphaloleole-like defect; osteoblast: increased bone mass; chondrocytes: no long bone growth; liver: iron overload; cardiomyocyte: heart valve defects and EC defects; endocardium: EC defects; vascular smooth muscle cells: impaired vascular remodeling; lung epithelium: defects in lung development; neonatal respiratory distress; urteric bud: dysplastic renal phenotype; hypothalamus (neurons in feeding center): hypophagia and death; eye: lack of lens and retina growth; Leydig cell: abnormal Leydig cells; granulosa cell: subfertile; facial primordia: lip and palate defects; hair follicle: impaired differentiation of inner root sheath; dental epithelia: switched differentiation of crown epithelium to root lineage |
| BMPR-1B (ALK6)     | Defects in appendicular skeleton; retinal defects; females: irregular estrous cycles |
| ActR-1A (ALK2, ACVR1) | Embryonically lethal, defects in mesoderm formation and gastrulation, no PGCs; surface ectoderm: smaller lens; neural crest: cranifacial defects, cardiac outflow tract defects; liver: iron overload; endothelium: defects in AV septa, valves, and EC formation; cushion mesenchyme: bicuspid aortic valve; uterus: delayed implantation and sterility |
| BMPR-2             | Embryonically lethal, defects in mesoderm and gastrulation, cardiac defects; heterozygotes: PAH; lung epithelium: predisposed to PAH |
| ActR-2A (ACVR2A)   | Defective reproductive performance and sexual behavior |
| ActR-2B (ACVR2B)   | Die after birth, complicated cardiac defects and left-right asymmetry |

The short ear and brachypodism mutations, associated with BMP5 and BMP14, respectively, are specific named loci and phenotypes in the mouse. These mice are both viable and fertile, but have various skeletal defects. BMP5 mutants have shorter and weaker bones, while the brachypodism mutation results in mice having altered length and number of bones in the limb. Examination of BMP14 mutants revealed that BMP14 also coordinates bone and joint formation during digit development. BMP5/14 double mutants have additional defects compared to single mutants, suggesting a likely synergistic function. Along with BMP14, BMP4 regulates digit patterning and the apical ectodermal ridge. A BMP14 mutant with severe joint malformations exhibited early-onset osteoarthritis (OA), offering a potential model for the study of the disease. Interestingly, BMP14 is implicated in OA in humans, although the mechanism is not clear (see OA section). Mice lacking BMP6 are also viable and fertile, but show reduced size of long bones and delayed sternal ossification, which is slightly exacerbated in BMP5/BMP6 double mutants. The GDF5/6/7 family of BMPs (BMP12/13/14) are important in normal formation of bones and joints, and there is increasing evidence of their role in tendon and ligament biology. BMP12 is thought to play a role in the structural integrity of bone. Mutation in BMP13 causes defects at multiple sites, including the wrist and ankle. These sites are distinct from BMP14 mutants, and BMP13/14 double mutants show additional defects. BMP13 knockout mice have accelerated coronal suture fusion, indicating an inhibitory role of BMP13 in osteogenic differentiation. Furthermore, mutations in BMP13 are distinguished by fusion of carpal and tarsal bones. An in vitro study confirmed the strong inhibitory role of this BMP. Examination of tendon phenotype in mice with mutations in this subgroup has revealed the role of BMP in tendon biology. BMP14 deficiency results in structurally weaker tendon and altered mechanics, BMP13 deficiency results in significantly lower tendon collagen in males, and BMP12 deficiency has only a subtle effect on tendon phenotype although BMP14 levels were higher in these mice. This suggests these proteins might have overlapping roles,
and BMP14 may be able to compensate for BMP12 deficiency.

BMPs also regulate cartilage development, which is usually coordinated with bone formation. BMP2 is considered a main player during endochondral bone development for chondrocyte proliferation and maturation.30 Chondrocyte-specific knockout of BMP2 results in a severe chondrodysplasia phenotype. BMP12, on the other hand, may serve as a negative regulator of chondrogenesis, since BMP12 deficient mice have accelerated hypertrophic chondrocyte kinetics.77 The Smad pathway is essential to mediate signaling in chondrocytes. Chondrocyte-specific deletion of Smad1 leads to delayed calvarial bone development.101 I-Smads are significant during cartilage development as well. Smad6 is needed for inhibition of endochondral bone formation, with knockouts showing abnormal growth plates; loss of Smad7 also results in abnormalities at the growth plate.130,133

BMPs play a prominent role in adult bone homeostasis and fracture healing. BMP2, while dispensable for bone formation, is required for the initiation of fracture healing.8 In limb-specific BMP2 knockouts, the bones lacking BMP2 have spontaneous fractures and an inability to initiate the early stages of fracture healing. BMP14 deficiency is associated with a delay in the fracture healing process, presumably due to a delay in the recruitment of cells and chondrocyte differentiation, but long-term healing is not compromised.91,92 These results emphasize the importance of BMPs as inducers of proliferation and differentiation in post-natal life.

The ability of BMPs to induce bone and cartilage formation is the basis for understanding the mechanism of certain diseases, as well as the potential use of recombinant human BMPs in treatments. Cleft palate (CP) is a recognizable birth defect that has various etiologies. BMPs are known to have a role in palate morphogenesis in development, and haploinsufficiency of BMP2 has been associated with syndromic CP.182 A family with a BMP2 deletion was examined, with the conclusion that BMP2 haploinsufficiency has high penetrance but variable expressivity. BMP4 also plays an important role in maxillofacial development, as three variants in BMP4 were identified as potential risk factors for nonsyndromic cleft lip/palate.183 We further discuss three other bone diseases linked to BMP signaling.

Fibrodysplasia ossificans progressiva (FOP)
FOP is a congenital disease that causes heterotopic ossification of soft tissues, such as skeletal muscle, through the endochondral pathway.184 The classic phenotype is caused by a constitutively activating mutation in ACVR1, the gene for ActR-1A (ALK2), due to an R206H mutation in the GS domain, and accounts for at least 98% of classic presentations.185 Other gain-of-function mutations have also been identified.186,187

The constitutive activity appears to be dependent on non-enzymatic cooperation with type II receptors, but not type II receptor kinase activity or ligand participation.188,189 Determination of the crystal structure of the cytoplasmic domain of ActR-1A complexed with FKBP12, an intracellular negative regulator of BMP signaling, revealed that FOP mutations disrupt critical interactions and decrease FKBP12 binding, which is consistent with other findings.190 Impaired binding of FKBP12 likely contributes to leaky activity of the type I BMP receptor and increased BMP pathway activity.191 The subcellular distribution of ActR-1A and FKBP12 may also play an important role.192 In addition to the canonical pathway, the BMP-p38 MAPK signaling pathway is disrupted in FOP.193 Because there is no known effective treatment of FOP, the focus is on prevention of heterotopic ossification. A selective small-molecule inhibitor of the type I BMP receptor, LDN-193189, inhibits activation of Smad1/5/8 and results in reduction of ectopic ossification.194 Recently, a novel class of small-molecule inhibitor of BMP signaling was discovered.195 Anti-sense oligonucleotides targeting the overactive ActR-1A receptor are another avenue of investigation.196

Osteogenesis imperfecta (OI)
OI, or "brittle bone disease," is a heritable disorder characterized by bone fragility, deformity, and growth deficiency, and is etiologically related to type I collagen, one of the main components of the extracellular matrix.97 Type I collagen is synthesized as a procollagen I precursor with N- and C-terminal propeptides that must be cleaved for maturation and proper fibril assembly. Classic OI has autosomal dominant inheritance and is due to mutations in type I collagen genes, but rare forms have been discovered with autosomal recessive inheritance and are indirectly related to type I collagen. Two children of a consanguineous Egyptian family were diagnosed with severe autosomal recessive OI, and found to have a F249L homozygous missense mutation in the protease domain of BMP1.198 BMP1 is a metalloproteinase known to have procollagen C-proteinase activity that cleaves the C-propeptides from procollagens I-III.199 Another study of two individuals of a consanguineous Turkish family with autosomal recessive OI found a homozygous G12R mutation in the signal peptide of BMP1.200

Osteoarthritis (OA)
OA is a disease involving degeneration of the articular cartilage in synovial joints, such as the knee, hip, and hand. The molecular mechanisms of pathogenesis are not fully understood, but there appears to be a genetic component.201 An association between two polymorphisms in intron I of the BMP5 gene and OA has been demonstrated, and suggests that variability in gene expression of BMP5 is a susceptibility factor for the disease.202 The 5' UTR of BMP14 is also implicated as a susceptibility factor.203 Over-expression of BMP2 was found in OA tissues and may be involved in the response to cartilage degeneration.204 However, crosstalk between the BMP pathway and Wnt/ß-catenin pathway revealed that BMP2 contributes to both chondrocyte hypertrophy and cartilage degradation.205 This dual role of BMPs in OA has been discussed and explains why both increased cartilage anabolism and catabolism are observed.204 Elevated serum BMP2 and BMP4 is evidence of disease and has been proposed as indicators for disease severity and joint arthroplasty.206 Only a couple of studies have investigated OA progression, and these found no association with BMP2 or BMP5.206,207

BMP7 has been known to protect cartilage and inhibit degradation in models of OA.208,209 Investigation into the
protein.219,220 microRNA modulation of BMP4 is also implicated in the development of BE.221

Juvenile polyposis (JP) and colorectal cancer
It has been relatively well-established that deletion of Smad4 in mice leads to gastric and duodenal polyps. While homozygotes are embryonically lethal, heterozygotes develop polyps with loss of heterozygosity.108,109 This is highlighted in the corresponding human disease called JP. JP is an autosomal dominant syndrome in which affected individuals develop cystic polyps in the stomach and intestines with an increased risk for colorectal cancer. Mutations in Smad4 and BMPR-1A have been linked to the development of JP, and together they are responsible for around 40% of JP cases.222–225 The genetic mutations represent a downregulation of the BMP signaling pathway, indicating a significant event in the pathogenesis of JP. Both nonsense and missense mutations of Smad4 have been identified in patients and result in reduced BMP signaling, with nonsense mutations causing a more significant reduction.

Missense mutations in BMPR-1A do not lead to decreased expression of the receptor, but rather cause localization to the cytoplasm instead of the plasma membrane.226 Deletions in chromosome 10q23, encompassing the PTEN and BMPR-1A genes, cause aggressive polyposis and numerous congenital anomalies, such as facial dysmorphism.227 It was recently shown that the BMP-Smad1 pathway functions as a tumor suppressor and stabilizes the well-known p53 tumor suppressor.228 Disruption of this interaction is thus suspected to play a role in tumorigenesis and the development of JP and cancer. The discovery of the relationship between BMPR-1A mutations and JP led to speculation of the role of BMP in colorectal cancer. Indeed, the BMP pathway is inactivated in the majority of sporadic colorectal cancer cases.229 BMP signaling has now been suggested to be involved in the initiation and progression of gastrointestinal cancer.

Studies have identified numerous potential markers for colorectal cancer, but further investigation is needed to clarify the associated mechanism. BMP11 may be a diagnostic and prognostic marker in colorectal cancer patients, as tumors with high BMP11 expression have a higher frequency of lymph node metastases, more cancer-related deaths, and decreased overall survival.230 Another study investigated potential markers for colorectal neoplasia screening, identifying BMP3, as well as three other genes, as highly methylated and silenced compared with normal epithelia.231 Interestingly, BMP3 was also found to be a powerful methylation marker in a stool assay for the detection of pancreatic cancer.232 In both these studies, however, many other markers were also identified, so it remains to be seen which have real significance. The relevance of silencing BMP3 has been investigated in the pathogenesis of JP and cancer. The discovery of the relationship between BMPR-1A mutations and JP led to speculation of the role of BMP in colorectal cancer. Indeed, the BMP pathway is inactivated in the majority of sporadic colorectal cancer cases.229 BMP signaling has now been suggested to be involved in the initiation and progression of gastrointestinal cancer.

Studies have identified numerous potential markers for colorectal cancer, but further investigation is needed to clarify the associated mechanism. BMP11 may be a diagnostic and prognostic marker in colorectal cancer patients, as tumors with high BMP11 expression have a higher frequency of lymph node metastases, more cancer-related deaths, and decreased overall survival.230 Another study investigated potential markers for colorectal neoplasia screening, identifying BMP3, as well as three other genes, as highly methylated and silenced compared with normal epithelia.231 Interestingly, BMP3 was also found to be a powerful methylation marker in a stool assay for the detection of pancreatic cancer.232 In both these studies, however, many other markers were also identified, so it remains to be seen which have real significance. The relevance of silencing BMP3 has been investigated in the onset of colorectal cancer development, but the lack of known BMP3-interacting proteins is a hindrance to understanding. BMP3 inactivation does appear to be important in early polyp formation and colorectal tumor development.233 One study using data from population-based case-control studies found significant variation in certain BMP genes in colon and rectal cancer.234 Genetic variations in
BMP signaling and human diseases

BMP-1A, BMPR-1B, BMPR-2, BMP2, BMP4 were all associated with risk of developing colon cancer, with the most high-risk phenotypes conferring a 20–30% increased risk. BMPR-2, BMPR-1B, and BMP2 were associated with rectal cancer.

BMP signaling in cardiovascular and pulmonary systems

BMP signaling has an established role in development of the heart, the first functional organ in the embryo, and is a continuing area of investigation. BMP2 homozygous mutants are embryonically lethal, with malformation of the amnion and chorion and also developmental abnormalities of the heart. BMP2 expression is detected in extraembryonic mesoderm as well as myocardium, and the signaling from myocardium has been demonstrated by multiple studies to be critical for endocardial cushion (EC) formation. Signaling from myocardium to the underlying endothelium to form ECs depends on an epithelial-mesenchymal transformation (EMT) mediated by BMP2. The ECs eventually give rise to the mature heart valves and septa, ultimately allowing for formation of a four-chambered heart. Conditional deletion of BMP2 in cardiac progenitors prevents this process, and the heart valve region becomes differentiated chamber myocardium. Deletion of BMP2 in atrioventricular (AV) myocardium further revealed the role of BMP2 in EC EMT, as well as in formation of cardiac jelly and patterning of AV myocardium.

Together with BMP2, BMP4 plays an essential role in the AV septation of the heart. BMP4 signaling from the myocardium to endocardium is involved in the process, and conditional inactivation leads to AV canal defect (AVCD). BMP4 is also required for outflow tract septation as demonstrated by conditional knockout. Several other BMPs play a role in the developing heart. BMP6 and BMP7, for example, are expressed in the ECs, although neither is essential during cardiogenesis. However, double mutants have defective EC development. BMP10 plays an essential role in maintaining cardiac growth during cardiogenesis, as BMP10 knockout mice have dramatically reduced cardiomyocyte proliferation. Smad6 and Smad7 mutants both show developmental defects in the outflow tract.

Murine knockout models of BMP receptors and BMP modulators in cardiac development have also been explored. Since multiple BMP knockouts show developmental abnormalities of the heart, it is not surprising that lack of BMP receptors presents with similar phenotype. Mouse knockouts have shown that BMPR-2 regulates outflow tract and AV cushion development. Mutation of BMPR-1A is embryonically lethal, even if only in cardiomyocytes and vascular smooth muscle. BMPR-1A signaling in the AV myocardium is required for EC formation and development of AV valves from the ECs. ActR-1A is also crucial for AV cushion development, specifically the EMT required in EC formation. Ablation of ActR-1A signaling in neural crest cells results in impaired migration of these cells to form the outflow tract. Deletion of ActR-1A in cushion mesenchyme results in bicuspid aortic valve.

BMP4 is a prominent signaling molecule in lung development. Knocking out Smad1 in lung epithelium disrupts branching morphogenesis and ultimately results in severe neonatal respiratory failure. It is thought that BMP4 and Smad1 signaling crosstalks with Wnt signaling to regulate lung development. Knocking out BMPR-1A, expressed mainly in airway epithelial cells, also disrupts branching morphogenesis and airway formation and causes neonatal respiratory distress. Smad8 mutants have defective pulmonary vascular remodeling, with a resulting pulmonary arterial hypertension (PAH) phenotype. This highlights the role of BMP signaling in adult tissue homeostasis, and the PAH phenotype due to Smad8 mutation is observed in humans as well. BMPR-2 deletion has also been established to give rise to PAH, and the mechanism is likely through a Smad-dependent manner.

Pulmonary arterial hypertension (PAH)

PAH is characterized by high pulmonary artery pressure and resulting heart failure. There are two types: idiopathic pulmonary arterial hypertension (IPAH) and hereditary pulmonary arterial hypertension (HPAH), with heterozygous germline mutations in BMPR-2 found in more than 70% of patients with HPAH and 20% of patients with IPAH. The pathogenesis of disease is not well understood, and a variety of factors other than BMPR-2 are likely involved. In HPAH, the autosomal dominant disease, only about 20% of carriers get the disease, and the low penetrance might be explained by changes in BMPR-2 alternative splicing. BMPR-2 mutations influence the disease expression more obviously in males than in females.

Mutations that lead to decreased BMP signaling have been found in the ligand-binding domain, kinase domain, and long cytoplasmic tail. Although mutant receptors may be expressed at lower level or not at all, it is also possible they have altered cellular localization. A few mutants were shown to associate abnormally with caveolae and clathrin-coated pits, and disruption of these domains restored BMP signaling to wild-type levels. BMPR-2 also interacts with the cytoskeleton, and mutant receptors may cause cytoskeletal defects related to the development of PAH. Several differences compared to wild-type are noted when BMPR-2 expression is disrupted in pulmonary arterial smooth muscle cells (PASMCs), including reduced BMP2 and BMP4 signaling but enhanced BMP6 and BMP7 signaling. This results in loss of the anti-proliferative effects of BMP4 and also activation of the p38-MAPK pathway, leading to aberrant PASMC proliferation and lack of apoptosis. In addition, there is increased TGF-β1 signaling and reduced BMP signaling, so the pathogenesis of disease is expected to be in part due to abnormal crosstalk between the two pathways. Mutations in Smad8 have been linked to PAH, possibly due to a non-redundant role of Smad8 in microRNA processing. Interestingly, PAH is sometimes associated with hereditary hemorrhagic telangiectasia (HHT). Defects in the ALK1 or Endoglin gene can lead to HHT, with associated PAH. Sildenafil is an established treatment for PAH, and was shown to enhance BMP4-induced phosphorylation of Smad1/5 and the resulting downstream BMP pathway. By restoring some of the BMP pathway function in BMPR-2 deficiency, the anti-proliferative effects of BMP4 were
partly re-established. Especially important are the downstream Id proteins that regulate PASMC proliferation by inhibiting the cell cycle. A recent study showed that ataluren might be an effective treatment for HPAH caused by nonsense mutations by causing ribosomal read-through of the premature stop codon. This is promising in an age of personalized medicine in which treatments are tailored based on one’s genetics.

**Hereditary hemorrhagic telangiectasia (HHT)**

HHT is an autosomal dominant disease associated with abnormal and fragile blood vessel formation in skin and mucosa. HHT type 1 is due to mutations in Endoglin, a BMP abnormal and fragile blood vessel formation in skin and Hereditary hemorrhagic telangiectasia (HHT) tailored based on one’s genetics. 

HHT type 2 is due to loss-of-function in a combined JP-HHT syndrome that predisposes to thoracic aortic disease. The discovery of BMP9 as the ligand for ALK1 showed that mutations in the receptor lead to defective BMP9 signaling. ALK1 is mainly expressed in arterial endothelial cells, and the defective BMP9 signaling likely leads to decreased levels of Id1 and Id3. Subsequent increased expression of VEGFR2 is suggested as the mechanism of abnormal endothelial cell sprouting. Other non-Smad pathways, such as the MAPK cascade, likely also play an important role in the process. This process reveals the role of BMP9 as a regulator of angiogenesis in the adult. Because ALK1 normally has anti-angiogenic effects and is mainly expressed in endothelial cells, it makes for a potentially interesting target in diseases related to angiogenesis, such as cancer. BMP9 might be considered in such treatments, since it is one of the main ligands of the receptor.

**BMP signaling in urinary system**

Initiation of mature kidney development involves the ureteric bud, which originates from the caudal end of the mesonephric duct, invaginating into metanephric mesenchyme. The metanephric mesenchyme ultimately gives rise to structures from the glomerulus to distal convoluted tubule, while the ureteric bud is the precursor to all structures distal to the collecting duct. BMP signaling is one mediator of the interaction between ureteric bud and metanephric mesenchyme. Abnormal interaction due to improper BMP signaling causes malformations of the kidney, in particular a group of disorders termed congenital anomalies of the kidney and urinary tract (CAKUT), which includes renal agenesis, dysplasia, ureteropelvic junction obstruction (UPJO), and others. BMP11 knockout mice, for example, have a spectrum of renal abnormalities, with the majority having bilateral renal agenesis. BMP11 likely plays a role in directing the ureteric bud from the mesonephric duct towards the metanephric mesenchyme, as mutant BMP11 embryos show a failure of ureteric bud formation. Control of ureteric bud branching is mediated by BMPR-1A signaling, as conditional knockout of the receptor in ureteric bud results in abnormal bud branching. These mice have renal dysplasia phenotype and decreased number of collecting ducts.

Renal hypodysplasia (RHD) is characterized by reduced kidney size or maldevelopment of the renal tissue. BMP4 mutations were identified in RHD patients, consistent with BMP4 being mainly expressed in the mesenchyme surrounding the branching ureter. Homozygous mutations in BMP4 and DACH1 were found in a patient with Bilateral Cystic Renal Dysplasia. Proper signaling of BMP4 and BMP7 is necessary for complete development of the urethra during embryonic development. BMP4 is a major factor in the signaling cascade involved in controlling embryonic urethral development, and BMP7 helps regulate the growth of the urethral plate epithelium as well as the proper closure of the distal urethra. This is consistent with missense mutations in BMP4 and BMP7 being strongly associated with hypospadias. Mutations in BMP4 can also lead to UPJO. The ureteropelvic junction is the last to canalize and the most common site of obstruction in the fetus. It is believed that these mutations cause UPJO due to loss of canonical Smad4 signaling mediated in part by decreased levels of BMP4, although the mechanism is at least partially Smad4-independent.

BMP7 has emerged as one of the most critical BMPs for kidney development. Mice lacking BMP7 have severe kidney defects in addition to eye defects. Early nephrogenic tissue interactions establishing the organ do not appear to be affected, but renal dysplasia ultimately results from lack of continued growth and development. This indicates a role for BMP7 in maintaining proliferation. After establishing a small number of nephrons, further development is arrested, and there is massive apoptosis of kidney progenitor cells. Using an inducible BMP7 knockout system, it was shown that BMP7 preserves undifferentiated kidney progenitor cells by preventing their differentiation into nephron. In effect, this serves as a regulator of kidney nephron number. Podocyte-specific knockout of BMP7 revealed a BMP7-mediated regulatory axis between glomeruli and proximal tubules during kidney development.

Recent research has shown that the presence of BMP signaling may be protective against renal disease, evidence of BMP function in adult homeostasis. Progression of chronic kidney disease (CKD) is mainly determined by renal fibrosis, which is characterized by an accumulation of extracellular components that leads to loss of renal parenchyma and function. BMP7, highly expressed in podocytes, distal tubules, and collecting ducts, has been shown to be protective against CKD. Exogenous administration of BMP7 or transgenic overexpression of BMP7 reduces overall renal fibrosis and nephrocyte apoptosis. Additionally, BMP7 signaling has been shown to be protective against hypertensive nephrosclerosis, another major cause of CKD. BMP5 may have a similar role.

**BMP signaling in neurological and ophthalmic systems**

BMP signaling plays an important role in many aspects of the development of the eye, including the formation of the retina, lens, iris, and ciliary body. Eye development begins with paired optic vesicles, diverticula of the forebrain that contact the surface ectoderm and develop into optic cups. The surface ectoderm thickens to form the lens placode, which in part gives rise to the lens. There is signaling
between the optic vesicle and surface ectoderm mediating these transitions. BMP4 and BMP7 are implicated in the progression from optic vesicle to optic cup. Furthermore, BMP4 is an important ligand from the optic cup in lens induction, and BMP7 is expressed in the lens placode to regulate lens induction as well. Germline mutations in BMP7 prevent lens formation. As in the case of the kidney, early tissue interactions establishing the organ are unaffected in BMP7 mutants, but the mice exhibit anophthalmia. BMPR-1A is essential for lens and retinal growth. Although lens formation occurred with conditional knockout of ActR-1A in surface ectoderm, the lenses were smaller, due to an increase in apoptosis of lens epithelial cells. BMP13 is implicated in maintaining cell survival in the eye and central nervous system, as loss of BMP13 results in retinal apoptosis and smaller eye size. Retinal apoptosis is also associated with loss of BMPR-1B, which is exclusively expressed in the ventral retina during development.

In humans, frameshift, missense, and Kozak sequence mutations have been identified in BMP7 in individuals with developmental eye abnormalities and extra-ocular features. BMP7 is suggested to have an important role in optic fissure closure, and thus may play a role in coloboma, a condition in which there is a hole or gap in some part of the eye. The incomplete penetrance and variability in phenotype expression in families examined demonstrates the complex nature of BMP-related diseases. There are not extensive reports of BMP4 loss-of-function mutations and the resulting effects, but it is known to be associated with ocular defects, as well as digit and brain anomalies. BMP4 is also known to be associated with anophthalmia—microphthalmia (A–M), but with large degrees of variable expressivity, sometimes with polysyndactyly. One report indicated a role for BMP4 in short stature, hyperextensibility, hernia, ocular depression, Rieger anomaly, and teething delay (SHORT) syndrome.

Another role of BMP in the neurological system is neurogenesis, and neural defects are associated with loss of BMP function in mouse models. BMP12 signaling in the spinal cord leads to differentiation of specific neuron classes, suggesting a role for BMPs in determining neuron identity in the CNS. BMP11 is also involved in spinal cord neurogenesis through its ability to induce cell cycle exit and instead promote differentiation of progenitors. On the other hand BMP11, secreted from neurons themselves, serves as an inhibitory signal in the generation of new neurons from progenitors in the olfactory epithelium. It plays a role in retina development as well. Further highlighting the importance of BMP regulation in neural development is the role of BMP7 in corticogenesis. BMP7 deletion results in reduced cortical thickening and impaired neurogenesis. Interestingly, BMPR-1A is important in establishment of neurons involved in regulating feeding behavior.

### BMP signaling in reproductive system

Several BMPs play an important role in aspects of reproductive system development and biology, from PGC formation to seminal vesicle development. As mentioned previously, knocking out some BMPs, such as BMP4, results in lack of formation of PGCs. Fertility is affected by several BMPs. BMP15 is the prominent BMP associated with ovarian function, specifically granulosa cell proliferation. Male BMP15 knockout mice are normal and fertile, while females are subfertile and have decreased ovulation and fertilization rates. BMP6 knockout females are demonstrated to have decreased fertility as well, with a decrease in ovulated eggs. BMP8 is most often associated with male reproductive system development, including spermatogenesis and development of epididymis. Maintenance of germ cells and spermatogenesis, and formation of PGCs, are dependent on BMP8.

A couple of BMP receptors have been shown to be important in pregnancy. BMPR-2 is required for maintenance of pregnancy and uterine function after implantation. Signaling in the uterus during implantation requires ActR-1A.

### BMP signaling in adipogenesis

Adipocyte development first involves the generation of pre-adipocytes from mesenchymal stem cells, followed by differentiation of pre-adipocytes into adipocytes. Mesenchymal stem cells have the ability to differentiate along several lineages, including osteocytes, chondrocytes, myocytes, fibroblasts, and adipocytes. The specific lineage is in part regulated by BMPs. The osteogenic BMPs, in particular BMP9, are strong inducers of osteocyte differentiation. However, BMP2 and BMP4 have been shown to be capable of inducing pluripotent stem cells into adipocytes, mediated primarily by the Smad pathway rather than a non-canonical pathway.

Obesity is characterized by an increase in white adipose tissue accumulation, which results from an increase in adipocyte size and/or an increase in adipocyte number. Mice studies have generally led to the belief that BMP4 induces stem cells to differentiate along the white adipocyte lineage, whereas BMP7 induces brown adipocyte differentiation. However, recent studies have indicated that BMP4 induces white-to-brown transition. Expressing BMP4 in white adipocytes of mice leads to white adipocyte cells with brown adipocyte characteristics, suggesting a role for BMP4 in altering insulin sensitivity. BMPR-1A has been associated with obesity because its activity has been shown to favor differentiation into adipocytes as opposed to osteoblasts. BMPR-2 has also been implicated in obesity. BMP7 treatment of diet-induced obese mice leads to increased energy expenditure and decreased food intake. This is likely linked to the ability of BMP7 to induce brown adipogenesis, and presents as a potentially interesting avenue for disease treatment.

### Conclusions and future directions

BMP signaling is critical in embryogenesis and is involved in development of many organ systems, as well as many aspects of adult tissue homeostasis. BMPs mediate processes important in development, such as cell proliferation, differentiation, and apoptosis. Deletion of various components of the BMP pathway is embryonically lethal or presents with marked abnormalities. Thus, conditional
knockout mouse models have aided tremendously in studying BMP function, and have provided much insight into the roles of BMP signaling in development. Adult tissues also rely on BMP signaling for homeostasis, and examples include fracture repair initiation and pulmonary vascular remodeling. Several players in BMP signaling have been determined to be the causative agent of human disease, while others have been shown to have a strong association. While these associations have been determined, the mechanisms of pathogenesis need to be fully understood. Thus, many critical biological questions pertaining to BMP signaling remain unanswered: What are the upstream signals governing BMP signaling? How are the distinct biological outcomes of a given BMP in different cell and tissue types regulated? Conversely, it remains to be understood how the actions of different BMPs exerted on the same cell or tissue types are coordinated. Furthermore, the extensive crosstalk with other major signaling pathways, such as the Wnt pathway, needs to be fully elucidated. Ultimately, a better understanding of BMP signaling should facilitate the clinical management of the BMP signaling-associated diseases, and may lead to the development of innovative and efficacious therapies, especially in the field of regenerative medicine.

Conflicts of interest

All authors have none to declare.

Acknowledgments

The reported work was in part supported by research grants from the National Institutes of Health (AR50142 and AR054381 to RCH and HHL). RW, JG, and OI were recipients from the National Institutes of Health (AR50142 and AR054381 to RCH and HHL). AH was a recipient of the Pritzker Summer Research Fellowship funded through a NIH T-35 training grant (NIDDK). AH was a recipient of the Urban Leadership Fellowship from Miami University.

References

1. Urist MR. Bone: formation by autoinduction. Science. 1965; 150(3698):893–899.
2. Wozney JM, Rosen V, Celeste AJ, et al. Novel regulators of bone formation: molecular clones and activities. Science. 1988;242(4885):1528–1534.
3. Hemmati-Brivanlou A, Thomsen GH. Ventral mesodermal patterning in Xenopus embryos: expression patterns and activities of BMP-2 and BMP-4. Dev Genet. 1995;17(1):78–89.
4. Zou H, Niswander L. Requirement for BMP signaling in interdigital apoptosis and scale formation. Science. 1996;272(5262):738–741.
5. Stewart A, Guan H, Yang K. BMP-3 promotes mesenchymal stem cell proliferation through the TGF-beta/activin signaling pathway. J Cell Physiol. 2010;223(3):658–666.
6. Kobayashi T, Lyons KM, McMahon AP, Kronenberg HM. BMP signaling stimulates cellular differentiation at multiple steps during cartilage development. Proc Natl Acad Sci U S A. 2005;102(50):18023–18027.
7. Bobacz K, Gruber R, Soleiman L, Erlacher L, Smolen JS, Graninger WB. Expression of bone morphogenetic protein 6 in healthy and osteoarthritic human articular chondrocytes and stimulation of matrix synthesis in vitro. Arthritis Rheum. 2003;48(9):2501–2508.
8. Tsuji K, Bandyopadhyay A, Harfe BD, et al. BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. Nat Genet. 2006;38(12):1424–1429.
9. Huang Z, Wang D, Ihida-Stansbury K, Jones PL, Martin JF. Defective pulmonary vascular remodeling in Smad8 mutant mice. Hum Mol Genet. 2009;18(15):2791–2801.
10. Wagner DO, Sieber C, Bhushan R, Bürgermann JH, Graf D, Knaus P. BMPs: from bone to body morphogenetic proteins. Sci Signal. 2010;3(107). mr1.
11. Harrison CA, Al-Musawi SL, Walton KL. Prodomates regulate the synthesis, extracellular localization and activity of TGF-β superfamily ligands. Growth Factors Chur Switz. 2011;29(5):174–186.
12. Heldin CH, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature. 1997;390(6659):465–471.
13. Horbelt D, Denakis A, Knaus P. A portrait of transforming growth factor β superfamily signalling: background matters. Int J Biochem Cell Biol. 2012;44(3):469–474.
14. De Caestecker M. The transforming growth factor-beta superfamily of receptors. Cytokine Growth Factor Rev. 2004;15(1):1–11.
15. Nohe A, Hassel S, Ehrlich M, et al. The mode of bone morphogenetic protein (BMP) receptor oligomerization determines different BMP-2 signaling pathways. J Biol Chem. 2002;277(7):5330–5338.
16. Heldin C-H, Moustakas A. Role of Smads in TGFβ signaling. Cell Tissue Res. 2012;347(1):21–36.
17. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-β family signaling. Nature. 2003;425(6958):577–584.
18. Yamaguchi K, Shirakabe K, Shibuya H, et al. Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. Science. 1995;270(5244):2008–2011.
19. Zhang YE. Non-Smad pathways in TGF-β signaling. Cell Res. 2009;19(1):128–139.
20. Broege A, Pham L, Jensen ED, et al. Bone morphogenetic proteins signal via SMAD and mitogen-activated protein (MAP) kinase pathways at distinct times during osteoclastogenesis. J Biol Chem. 2013;288(52):37230–37240.
21. Corradini E, Babitt JL, Lin HY. The RGM/DRAGON family of BMP co-receptors. Cytokine Growth Factor Rev. 2009;20(5–6):389–398.
22. Walsh DW, Godson C, Brazil DP, Martin F. Extracellular BMP antagonist regulation in development and disease: tied up in knots. Trends Cell Biol. 2010;20(5):244–256.
23. Yao D, Doré Jr JJ, Leof EB. FKBP12 is a negative regulator of transforming growth factor-beta receptor internalization. J Biol Chem. 2000;275(17):13149–13154.
24. Toporsian M, Jerkic M, Zhou Y-Q, et al. Spontaneous adult-onset pulmonary arterial hypertension attributable to hereditary hemorrhagic telangiectasia. Arterioscler Thromb Vasc Biol. 2010;30(3):509–517.
25. Mueller TD, Nickel J. Promiscuity and specificity in BMP receptor activation. FEBS Lett. 2012;586(14):1846–1859.
26. Kessler E, Takahara K, Biniaminov L, Brusel M, Greenspan DS. Bone morphogenetic protein-1: the type I procollagen C-proteinase. Science. 1996;271(5247):360–362.
27. Shen B, Bhargav D, Wei A, et al. BMP-13 emerges as a potential inhibitor of bone formation. Int J Biol Sci. 2009;5(2):192–200.
28. Daluiski A, Engstrand T, Bahamonde ME, et al. Bone morphogenetic protein-3 is a negative regulator of bone density. Nat Genet. 2001;27(1):84–88.
BMP signaling and human diseases

29. Luu HH, Song W-X, Luo X, et al. Distinct roles of bone morphogenetic proteins in osteogenic differentiation of mesenchymal stem cells. J Orthop Res Off Publ Orthop Res Soc. 2007;25(5):665–677.

30. Shu B, Zhang M, Xie R, et al. BMP2, but not BMP4, is crucial for chondrocyte proliferation and maturation during endochondral bone development. J Cell Sci. 2011;124(Pt 20): 3428–3440.

31. Selever J, Liu W, Lu M-F, Behringer RR, Martin JF. Bmp4 in limb bud mesoderm regulates digit pattern by controlling AER development. Dev Biol. 2004;276(2):268–279.

32. Asai-Coakwell M, French CR, Berry KM, et al. GDF6, a novel locus for a spectrum of ocular developmental anomalies. Am J Hum Genet. 2007;80(2):306–315.

33. Zhao GQ, Liaw L, Hogan BL. Bone morphogenetic protein -15. Identification of target cells and biological functions. J Biol Chem. 2000;275(50):39523–39528.

34. Suzuki N, Labosky PA, Furuta Y, et al. Failure of ventral body wall closure in mouse embryos lacking a procollagen C-proteinase encoded by Bmp1, a mammalian gene related to Drosophila tolloid. Dev Camb Engl. 1996;122(11):3587–3595.

35. Zhang H, Bradley A. Mice deficient for Bmp2 are viable and have defects in amnion/chorion and cardiac development. Dev Camb Engl. 1996;122(10):2977–2986.

36. Rivera-Feliciano J, Tabin CJ. Bmp2 instructs cardiac progenitors to form the heart-valve-inducing field. Dev Biol. 2006;295(2):580–588.

37. Ma L, Lu M-F, Schwartz RJ, Martin JF. Bmp2 is essential for cardiac cushion epithelial-mesenchymal transition and myocardial patterning. Dev Camb Engl. 2005;132(24):5601–5611.

38. Winnier G, Blessing M, Labosky PA, Hogan BL. Bone morphogenetic protein 4 is required for mesoderm formation and patterning in the mouse. Genes Dev. 1995;9(17):2105–2116.

39. Lawson KA, Dunn NR, Roelen BA, et al. Bmp4 is required for the generation of primordial germ cells in the mouse embryo. Genes Dev. 1999;13(4):424–436.

40. De Sousa Lopes SM, Roelen BAJ, Monteiro RM, et al. BMP signaling mediated by ALK2 in the visceral endoderm is necessary for the generation of primordial germ cells in the mouse embryo. Genes Dev. 2004;18(15):1838–1849.

41. Furuta Y, Hogan BL. BMP4 is essential for lens induction in the mouse embryo. Genes Dev. 1998;12(23):3764–3775.

42. Dunn NR, Winnier GE, Hargett LT, Schrick JJ, Fogo AB, Hogan BL. Haploinsufficient phenotypes in Bmp4 heterozygous null mice and modification by mutations in Gli3 and Alx4. Dev Biol. 1997;188(2):235–247.

43. Jiao K, Kulessa H, Tompkins K, et al. An essential role of Bmp4 in the atrioventricular septation of the mouse heart. Genes Dev. 2003;17(19):2362–2367.

44. Goldman DC, Bailey AS, Pfaffle DL, Al Masri A, Christian JL, Fleming WH. BMP4 regulates the hematopoietic stem cell niche. Blood. 2009;114(20):4393–4401.

45. Qian S-W, Tang Y, Li X, et al. BMP4-mediated brown fat-like changes in white adipose tissue alter glucose and energy homeostasis. Proc Natl Acad Sci U S A. 2013;110(9):E798–E807.

46. Li Y, Gordon J, Manley NR, Lintingtung Y, Chiang C. Bmp4 is required for tracheal formation: a novel mouse model for tracheal agenesis. Dev Biol. 2008;322(1):145–155.

47. Qian S-W, Tang Y, Xie R, et al. BMP2, but not BMP4, is crucial for chondrocyte proliferation and maturation during endochondral bone development. J Cell Sci. 2011;124(Pt 20): 3428–3440.

48. Chang W, Lin Z, Kulessa H, Hebert J, Hogan BLM, Wu DK. Bmp4 is essential for the formation of the vestibular apparatus that detects angular head movements. PloS Genet. 2008;4(4):e1000050.

49. Liu W, Selever J, Wang D, et al. Bmp4 signaling is required for outflow-tract septation and branchial-arch artery remodeling. Proc Natl Acad Sci U S A. 2004;101(13):4489–4494.

50. Liu W, Selever J, Murali D, et al. Threshold-specific requirements for Bmp4 in mandibular development. Dev Biol. 2005;283(2):282–293.

51. Kriegsmann K, Bland AE, Gruber JM, et al. The mouse short ear mouse embryo. J Biol Chem. 1996;271(3):2986–3000.

52. Kingsley DM, Bland AE, Gruber JM, et al. The mouse short ear mouse embryo. J Biol Chem. 1996;271(3):2986–3000.

53. Kriegsmann K, Bland AE, Gruber JM, et al. The mouse short ear mouse embryo. J Biol Chem. 1996;271(3):2986–3000.

54. Kriegsmann K, Bland AE, Gruber JM, et al. The mouse short ear mouse embryo. J Biol Chem. 1996;271(3):2986–3000.

55. Kriegsmann K, Bland AE, Gruber JM, et al. The mouse short ear mouse embryo. J Biol Chem. 1996;271(3):2986–3000.

56. Kriegsmann K, Bland AE, Gruber JM, et al. The mouse short ear mouse embryo. J Biol Chem. 1996;271(3):2986–3000.

57. Kriegsmann K, Bland AE, Gruber JM, et al. The mouse short ear mouse embryo. J Biol Chem. 1996;271(3):2986–3000.
69. McPherron AC, Lawler AM, Lee SJ. Regulation of anterior/posterior patterning of the axial skeleton by growth/differentiation factor 11. Nat Genet. 1999;22(3):260–264.

70. Harmon EB, Apelqvist AA, Smart NG, Osborne DH, Kim SK. GDF11 modulates NGN3–islet progenitor cell number and promotes beta-cell differentiation in pancreas development. Dev Cereb. 2004;13(14):256–267.

71. Esquela AF, Lee SJ. Regulation of metanephric kidney development by growth/differentiation factor 11. Dev Biol. 2003;257(2):356–370.

72. Shi Y, Lu J-P. Gdf11 facilitates temporal progression of neurogenesis in the developing spinal cord. J Neurosci Off J Soc Neurosci. 2011;31(3):883–893.

73. Wu H-H, Ivkovic S, Murray RC, et al. Autoregulation of neurogenesis by GDF11. Neuron. 2003;37(2):197–207.

74. Kim J, Wu H-H, Lander AD, Lyons KM, Matzuk MM, Caiol AL. GDF11 controls the timing of progenitor cell competence in developing retina. Science. 2005;308(5730):1927–1930.

75. Mikic B, Ferreira MP, Battaglia TC, Hunziker EB. Accelerated hypertrophic chondrocyte kinetics in GDF-7 deficient murine tibial growth plates. J Orthop Res Off Publ Orthop Res Soc. 2008;26(7):986–990.

76. Maloul A, Rossemeier K, Mikic B, Pogue V, Battaglia T. Geometric and material contributions to whole bone structural behavior in GDF-7-deficient mice. Connect Tissue Res. 2006;47(3):157–162.

77. Mikic B, Entwistle R, Rossemeier K, Bierwert L. Effect of GDF-7 deficiency on tail tendon phenotype in mice. J Orthop Res Off Publ Orthop Res Soc. 2008;26(6):834–839.

78. Mikic B, Bierwert L, Tsou D. Achilles tendon characterization in GDF-7 deficient mice. J Orthop Res Off Publ Orthop Res Soc. 2006;24(4):831–841.

79. Lee KJ, Mendelsohn M, Jessell TM. Neuronal patterning by BMPs: a requirement for GDF7 in the generation of a discrete class of commissural interneurons in the mouse spinal cord. Genes Dev. 1998;12(21):3394–3407.

80. Settle SH, Rountree RB, Sinha A, Thacker A, Higgins K, Kingsley DM. Multiple joint and skeletal patterning defects caused by single and double mutations in the mouse Gdf6 and Gdf5 genes. Dev Biol. 2003;254(1):116–130.

81. Clendenning DE, Mortlock DP. The BMP ligand Gdf6 prevents differentiation of coronal suture mesenchyme in early cranial development. PloS One. 2012;7(5):e36789.

82. Asai-Coakwell M, March L, Dai XH, et al. Contribution of growth differentiation factor 6-dependent cell survival to early-onset retinal dystrophies. Hum Mol Genet. 2013;22(7):1432–1442.

83. Naito M, Hensey C. Eye and neural defects associated with loss of GDF6. BMC Dev Biol. 2006;6:43.

84. Tassabehji M, Fang ZM, Hilton EH, et al. Mutations in Gdf6 are associated with vertebral segmentation defects in Klippel-Feil syndrome. Hum Mutat. 2008;29(8):1017–1027.

85. Mikic B, Rossemeier K, Bierwert L. Identification of a tendon phenotype in GDF6 deficient mice. Anat Rec Hoboken. 2009;292(3):396–400.

86. Mikic B, Rossemeier K, Bierwert L. Sexual dimorphism in the effect of GDF-6 deficiency on murine tendon. J Orthop Res Off Publ Orthop Res Soc. 2009;27(12):1603–1611.

87. Storm EE, Huyhn TV, Copeland NG, Jenkins NA, Kingsley DM, Lee SJ. Lmip alteration in brachypodism mice due to mutations in a new member of the TGF-beta-superfamily. Nature. 1994;368(6472):639–643.

88. Storm EE, Kingsley DM. GDF5 coordinates bone and joint formation during digit development. Dev Biol. 1999;209(1):11–27.

89. Chen H, Shi S, Acosta L, et al. BMP10 is essential for maintaining cardiac growth during murine cardiogenesis. Dev Cereb. 2004;13(14):2219–2231.

90. Cibadora A, Jizjerdi D, Zhang J, Kline A, Balian G, Hurwitz S. BMP-14 deficiency inhibits long bone fracture healing: a biochemical, histologic, and radiographic assessment. J Orthop Trauma. 2005;19(9):629–634.

91. Coleman CM, Scheremetia BH, Boyce AT, Mauck RL, Tuan RS. Delayed fracture healing in growth differentiation factor 5-deficient mice: a pilot study. Clin Orthop. 2011;469(10):2915–2924.

92. Masuya H, Nishida K, Furuiichi T, et al. A novel dominant-negative mutation in Gdf5 generated by ENU mutagenesis impairs joint formation and causes osteoarthritis in mice. Hum Mol Genet. 2007;16(19):2366–2375.

93. Mikic B, Schalet BJ, Clark RT, Gaschen V, Hunziker EB. GDF-5 deficiency in mice alters the ultrastructure, mechanical properties and composition of the Achilles tendon. J Orthop Res Off Publ Orthop Res Soc. 2001;19(3):365–371.

94. Mikic B, Ferreira MP, Battaglia TC, Hunziker EB. Accelerated hypertrophic chondrocyte kinetics in GDF-7 deficient murine tibial growth plates. J Orthop Res Off Publ Orthop Res Soc. 2008;26(7):986–990.

95. Maloul A, Rossemeier K, Mikic B, Pogue V, Battaglia T. Geometric and material contributions to whole bone structural behavior in GDF-7-deficient mice. Connect Tissue Res. 2006;47(3):157–162.

96. Mikic B, Entwistle R, Rossemeier K, Bierwert L. Effect of GDF-7 deficiency on tail tendon phenotype in mice. J Orthop Res Off Publ Orthop Res Soc. 2008;26(6):834–839.

97. Mikic B, Bierwert L, Tsou D. Achilles tendon characterization in GDF-7 deficient mice. J Orthop Res Off Publ Orthop Res Soc. 2006;24(4):831–841.

98. Lee KJ, Mendelsohn M, Jessell TM. Neuronal patterning by BMPs: a requirement for GDF7 in the generation of a discrete class of commissural interneurons in the mouse spinal cord. Genes Dev. 1998;12(21):3394–3407.

99. Settle SH, Rountree RB, Sinha A, Thacker A, Higgins K, Kingsley DM. Multiple joint and skeletal patterning defects caused by single and double mutations in the mouse Gdf6 and Gdf5 genes. Dev Biol. 2003;254(1):116–130.

100. Clendenning DE, Mortlock DP. The BMP ligand Gdf6 prevents differentiation of coronal suture mesenchyme in early cranial development. PloS One. 2012;7(5):e36789.
109. Taketo MM, Takaku K. Gastro-intestinal tumorigenesis in Smad4 mutant mice. *Cytokine Growth Factor Rev*. 2000;11(1–2):147–157.

110. Pan D, Schomber T, Kalberer CP, et al. Normal erythropoiesis but severe polyposis and bleeding anemia in Smad4-deficient mice. *Blood*. 2007;110(8):3049–3055.

111. Tan X, Weng T, Zhang J, et al. Smad4 is required for maintaining normal murine postnatal bone homeostasis. *J Cell Sci*. 2007;120(Pt 13):2162–2170.

112. Archambeault DR, Yao HH-C. Loss of smad4 in Sertoli and Leydig cells leads to testicular dysgenesis and hemorrhagic tumor formation in mice. *Dev Dyn Off Publ Am Assoc Anat*. 2009;238(8):1897–1908.

113. Pangas SA, Li X, Robertson EJ, Matzuk MM. Premature cardinal maturation of chondrocytes in the growth plate. *Dev Biol*. 2013;382(2):375–384.

114. Sun J, Liu Y-H, Chen H, et al. Deficient Alk3-mediated BMP signaling controls muscle mass. *Nat Genet*. 2013;45(11):1309–1318.

115. Wang J, Xu N, Feng X, et al. Targeted disruption of Smad4 in cardiac myocytes results in cardiac hypertrophy and heart failure. *Circ Res*. 2005;97(8):821–828.

116. Lan Y, Liu B, Yao H, et al. Essential role of endothelial Smad4 in normal organization of the cartilage growth plate. *Dev Biol*. 2005;284(2):311–322.

117. Wang J, Xu N, Feng X, et al. Targeted disruption of Smad4 in Leydig cells leads to testicular dysgenesis and hemorrhagic tumor formation in mice. *Dev Dyn Off Publ Am Assoc Anat*. 2009;238(8):1897–1908.

118. Qi X, Yang G, Yang L, et al. Essential role of Smad4 in maintaining cardiac myocyte proliferation during murine embryonic heart development. *Dev Biol*. 2007;311(1):136–146.

119. Mao X, Denebedittis P, Sun Y, et al. Vascular smooth muscle cell Smad4 gene is important for mouse vascular development. *Arterioscler Thromb Vasc Biol*. 2012;32(9):2171–2177.

120. Zhou Y-X, Zhao M, Li D, et al. Cerebellar deficits and hyperactivity in mice lacking Smad4. *J Biol Chem*. 2003;278(43):42313–42320.

121. Liu Y, Lin D. Necessity of Smad4 for the normal development of the mouse lacrimal gland. *Jpn J Ophthalmol*. 2014;58(3):298–306.

122. Archambeault DR, Yao HH-C. Loss of smad4 in Sertoli and Leydig cells leads to testicular dysgenesis and hemorrhagic tumor formation in mice. *Biopol Reprod*. 2014;90(3):62.

123. Yu C, Zhang Y-L, Fan H-Y. Selective Smad4 knockout in ovarian-specific conditional deletion of the bone morphogenetic protein receptor 1A signaling is dispensable for hematopoietic development but essential for vessel and hemangioblastic development of the mouse lacrimal gland. *Dev Biol*. 2005;298(2):381–392.

124. Pangas SA, Li X, Robertson EJ, Matzuk MM. Premature cardinal maturation of chondrocytes in the growth plate. *Dev Biol*. 2013;382(2):375–384.

125. Owens P, Engelking E, Haeger SM, Wang X-J. Epidermal Smad4 deletion results in aberrant wound healing. *Am J Pathol*. 2010;176(1):122–133.

126. Qiao W, Li AG, Owens P, Xu X, Wang X-J, Deng C-X. Hair follicle defects and squamous cell carcinoma formation in Smad4 conditional knockout mouse skin. *Oncogene*. 2006;25(2):207–217.

127. Yang L, Li W, Wang S, et al. Smad4 disruption accelerates keratinocyte reepithelialization in murine cutaneous wound repair. *Histochim Cell Biol*. 2012;138(4):573–582.

128. Bornstein S, White R, Malkoski S, et al. Smad4 loss in mice causes spontaneous head and neck cancer with increased genomic instability and inflammation. *J Clin Invest*. 2009;119(11):3408–3419.

129. Gao Y, Yang G, Wang K, et al. Disruption of Smad4 in odontoblasts causes multiple keratocystic odontogenic tumors and tooth malformation in mice. *Mol Cell Biol*. 2009;29(21):5941–5951.

130. Estrada KD, Retting KN, Chin AM, Lyons KM. Smad6 is essential to limit BMP signaling during cartilage development. *J Bone Miner Res Off J Am Soc Bone Mineral Res*. 2011;26(10):2498–2510.

131. Galvin KM, Donovan MJ, Lynch CA, et al. A role for smad6 in development and homeostasis of the cardiovascular system. *Nat Genet*. 2000;24(2):171–174.

132. Chen Q, Chen H, Zheng D, et al. Smad7 is required for the development and function of the heart. *J Biol Chem*. 2009;284(1):292–300.

133. Estrada KD, Wang W, Retting KN, et al. Smad7 regulates terminal maturation of chondrocytes in the growth plate. *Dev Biol*. 2013;382(2):375–384.

134. Tojo M, Takebe A, Takahashi S, et al. Smad7-deficient mice show growth retardation with reduced viability. *J Biochem (Tokyo)*. 2012;151(6):621–631.

135. Chung ACK, Huang XR, Zhou L, Heuchel R, Lai KN, Lan HY. Disruption of the Smad7 gene promotes renal fibrosis and inflammation in unilateral ureteral obstruction (UUO) in mice. *Nephrol Dial Transpl Off Pbl Eur Dial Transpl Assoc - Eur Ren Assoc*. 2009;24(5):1443–1454.

136. Zhang R, Huang H, Cao P, Wang Z, Chen Y, Pan Y. Smad-related protein 7 (Smad7) is required for embryonic eye development in the mouse. *J Biol Chem*. 2013;288(15):10275–10285.

137. Dong C, Zhu S, Wang T, et al. Deficient Smad7 expression: a putative molecular defect in scleroderma. *Proc Natl Acad Sci U S A*. 2002;99(6):3908–3913.

138. Li R, Rosendahl A, Brodin G, et al. Deletion of exon I of Smad7 in mice results in altered B cell responses. *J Immunol Balt Md*. 2006;176(11):6777–6784.

139. Kleiter I, Song J, Lukas D, et al. Smad7 in T cells drives T helper 1 responses in multiple sclerosis and experimental autoimmune encephalomyelitis. *Brain J Neurol*. 2010;133(Pt 4):1067–1081.

140. Mishina Y, Suzuki A, Ueno N, Behringer RR. Bmpr encodes a type I bone morphogenetic protein receptor that is essential for gastrulation during mouse embryogenesis. *Genes Dev*. 1995;9(24):3027–3037.

141. Park C, Lavine K, Mishina Y, Deng C-X, Ornitz DM, Choi K. Bone morphogenetic protein receptor 1A signaling is dispensable for hematopoietic development but essential for vessel and atrioventricular endocardial cushion formation. *Dev Camb Engol*. 2006;133(17):3473–3484.

142. Sun J, Liu Y-H, Chen H, et al. Deficient Alk3-mediated BMP signaling causes prenatal omphalocoe-like defect. *Biochem Biophys Res Commun*. 2007;360(1):238–243.

143. Kamiya N, Ye L, Kobayashi T, et al. BMP signaling controls muscle mass. *Nat Genet*. 2010;42(7):7692.

144. Park C, Lavine K, Mishina Y, Deng C-X, Ornitz DM, Choi K. Bone morphogenetic protein receptor 1A signaling is dispensable for hematopoietic development but essential for vessel and atrioventricular endocardial cushion formation. *Dev Camb Engol*. 2006;133(17):3473–3484.

145. Sun J, Liu Y-H, Chen H, et al. Deficient Alk3-mediated BMP signaling causes prenatal omphalocoe-like defect. *Biochem Biophys Res Commun*. 2007;360(1):238–243.

146.using multiple keratocystic odontogenic tumors and tooth malformation in mice. *Blood*. 2011;118(15):4224–4230.

147. Gaussin V, Van de Putte T, Mishina Y, et al. Endocardial cushion and myocardial defects after cardiac myocyte-specific conditional deletion of the bone morphogenetic protein receptor ALK3. *Proc Natl Acad Sci U S A*. 2002;99(5):2878–2883.

148. Gaussin V, Morley GE, Cox L, et al. ALK3/Bmpr1a receptor is required for development of the atrioventricular canal into valves and annulus fibrosus. *Circ Res*. 2005;97(3):219–226.

149. Song L, Fassler R, Mishina Y, Jiao K, Baldwin HS. Essential functions of Alk3 during AV cushion morphogenesis in mouse embryonic hearts. *Dev Biol*. 2007;301(1):276–286.
in neural crest cells. Dev Camb Engl. 2004;131(14):3481–3490.

168. Wang J, Sridurongrit S, Dudas M, et al. Atrioventricular cushion transformation is mediated by ALK2 in the developing mouse heart. Dev Biol. 2005;286(1):299–310.

169. Thomas PS, Sridurongrit S, Ruiz-Lozano P, Kaartinen V. Deficient signaling via ALK2 (Acvrl1) leads to bicuspid aortic valve development. Plos One. 2012;7(4):e35539.

170. Clementi C, Triipurani SK, Large MJ, et al. Activin-like kinase 2 functions in peri-implantation uterine signaling in mice and humans. PLoS Genet. 2013;9(11):e1003863.

171. Nagashima T, Li Q, Clementi C, Lydon JP, DeMayo FJ, Matuzk MM. BMPR2 is required for postimplantation uterine function and pregnancy maintenance. J Clin Invest. 2013;126(5):2539–2550.

172. Beppu H, Kawabata M, Hamamoto T, et al. BMP type II receptor is required for gastrulation and early development of mouse embryos. Dev Biol. 2000;221(1):249–258.

173. Beppu H, Malhotra R, Beppu Y, Lepore JJ, Parmacek MS, Bloch KD. BMP type II receptor regulates positioning of outflow tract and remodeling of atrioventricular cushion during cardiacogenesis. Dev Biol. 2009;331(2):167–175.

174. Beppu H, Ichinose F, Kawai N, et al. BMPR-II heterozygous mice have mild pulmonary hypertension and an impaired pulmonary vascular remodeling response to prolonged hypoxia. Am J Physiol Lung Cell Mol Physiol. 2004;287(6):L1241–L1247.

175. West J, Harral J, Lane K, et al. Mice expressing BMPR2R899X transgene in smooth muscle develop pulmonary vascular lesions. Am J Physiol Lung Cell Mol Physiol. 2008;295(5):L744–L755.

176. Hong K-H, Lee YJ, Lee E, et al. Genetic ablation of the BMPR2 gene in pulmonary endothelium is sufficient to predispose to pulmonary arterial hypertension. Circulation. 2008;118(7):722–730.

177. Matuzk MM, Kumar TR, Bradley A. Different phenotypes for mice deficient in either activins or activin receptor type II. Nature. 1995;374(6520):356–360.

178. Ma X, Reyna A, Mani SK, Matuzk MM, Kumar TR. Impaired male sexual behavior in activin receptor type II knockout mice. Biol Reprod. 2005;73(6):1182–1190.

179. Wreford NG, Rajendra Kumar T, Matzuk MM, de Kretser DM. Analysis of the testicular phenotype of the follicle-stimulating hormone beta-subunit knockout and the activin type II receptor knockout mice by stereological analysis. Endocri- nology. 2001;142(7):2916–2920.

180. Oh SP, Li E. The signaling pathway mediated by the type IIB activin receptor controls axial patterning and lateral asymmetry in the mouse. Genes Dev. 1997;11(14):1812–1826.

181. Storm EE, Kingsley DM. Joint patterning defects caused by single and double mutations in members of the bone morphogenetic protein (BMP) family. Dev Camb Engl. 1996;122(12):3969–3979.

182. Williams ES, Uhas KA, Bunke BP, Garber KB, Martin CL. Cleft palate in a multigenerational family with a microdeletion of 20p12.3 involving BMP2. Am J Med Genet A. 2012;158A(10):2616–2620.

183. Suazo J, Tapia JC, Santos JL, Castro VG, Colombo A, Blanco R. Risk variants in BMP4 promoters for nonsyndromic cleft lip/palate in a Chilean population. BMC Med Genet. 2011;12:163.

184. Kaplan FS, Le Merrer M, Glaser DL, et al. Fibrodysplasia ossificans progressiva. Best Pract Res Clin Rheumatol. 2008;22(1):191–205.

185. Kaplan FS, Xu M, Seemann P, et al. Classic and atypical fibrodysplasia ossificans progressiva (FOP) phenotypes are caused by mutations in the bone morphogenetic protein (BMP) type I receptor ACVR1. Hum Mutat. 2009;30(3):379–390.
221. Van Baal JWPM, Verbeek RE, Bus P, et al. microRNA-145 in Barrett’s oesophagus: regulating BMP4 signalling via GATA6. Gut. 2013;62(5):664–675.

222. Jee MJ, Yoon SM, Kim EJ, et al. A novel germline mutation in exon 10 of the SMAD4 gene in a familial juvenile polyposis. Gut Liver. 2013;7(6):747–751.

223. Howe JR, Roth S, Ringold JC, et al. Mutations in the SMAD4/DPC4 gene in juvenile polyposis. Science. 1998; 280(5366):1086–1088.

224. Howe JR, Bair JL, Sayed MG, et al. Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis. Nat Genet. 2001;28(2):184–187.

225. Carr JC, Dahdaleh FS, Wang D, Howe JR. Germline mutations in SMAD4 disrupt bone morphogenetic protein signaling. J Surg Res. 2012;174(2):211–214.

226. Howe JR, Dahdaleh FS, Carr JC, Wang D, Sherman SK, Howe JR. BMPR1A mutations in juvenile polyposis affect cellular localization. J Surg Res. 2013;184(2):739–745.

227. Sepfer S, Zhang L, Lawson CE, Cojcin J, Attard T, Ardinger HH. Aggressive juvenile polyposis in children with chromosome 10q23 deletion. World J Gastroenterol. 2013;19(14):2286–2292.

228. Chau JF, Toda D, Wang Z, et al. A crucial role for bone morphogenetic protein-Smad1 signalling in the DNA damage response. Nat Commun. 2012;3(836).

229. Kodach LL, Wiercinska E, de Miranda NFCC, et al. The bone morphogenetic protein signaling in vascular diseases. J Med Genet. 2003;40(12):865–871.

230. Abdalla SA, Gallione CJ, Barst RJ, et al. Primary pulmonary hypertension in families with hereditary haemorrhagic telangiectasia. Eur Respir J. 2004;23(3):373–377.

231. Yang J, Li X, Al-Lamki RS, et al. Sildenafil potentiates bone morphogenetic protein signaling in pulmonary arterial smooth muscle cells and in experimental pulmonary hypertension. Arterioscler Thromb Vasc Biol. 2013;33(1):34–42.

232. Yang J, Li X, Li Y, et al. Id proteins are critical downstream effectors of BMP signaling in human pulmonary arterial smooth muscle cells. Am J Physiol Lung Cell Mol Physiol. 2013;305(4):L312–L321.

233. Choe E-J, Kim YH, Choe S, et al. Enhanced responses to angiogenic cues underlie the pathogenesis of hereditary hemorrhagic telangiectasia 2. PloS One. 2013;8(5):e63138.

234. Mitchell D, Pobre EG, Mulivor AW, et al. ALK1-Fc inhibits multiple mediators of angiogenesis and suppresses tumor growth. Mol Cancer Ther. 2010;9(2):379–388.

235. Weber S, Taylor JC, Winyard P, et al. SIX2 and BMP4 mutations in children with renal agenesis associate with anomalous kidney development. J Am Soc Nephrol. 2009;20(5):891–903.

236. Cogar J, Austin E, Hedges L, et al. Role of BMPR2 alternative splicing in heritable pulmonary arterial hypertension. Circulation. 2012;126(15):1907–1916.

237. Liu D, Wu W-H, Mao Y-M, et al. BMPR2 mutations influence phenotype more obviously in male patients with pulmonary arterial hypertension. Circ Cardiovasc Genet. 2012;5(5):511–518.

238. Jiang Y, Nohe A, Brandon B, et al. Trapping of BMP receptors in distinct membrane domains inhibits their function in pulmonary arterial hypertension. Am J Physiol Lung Cell Mol Physiol. 2011;301(2):L218–L227.

239. Johnson JA, Hennessy AR, Perrien DS, et al. Cytoskeletal defects in Bmp2-associated pulmonary arterial hypertension. Am J Physiol Lung Cell Mol Physiol. 2012;302(5):L474–L484.

240. Yu PB, Beppu H, Kawai N, Li E, Bloch KD. Bone morphogenetic protein (BMP) type II receptor deletion reveals BMP ligand-specific gain of signaling in pulmonary artery smooth muscle cells. J Biol Chem. 2005;280(26):24443–24450.

241. Dewachter L, Adnot S, Guignabert C, et al. Bone morphogenetic protein signaling in heritable versus idiopathic pulmonary hypertension. Eur Respir J. 2009;34(5):1100–1110.

242. Upton PD, Davies RJ, Tajacic T, Morrell NW. Transforming growth factor-β1 represses bone morphogenetic protein-mediated Smad signaling in pulmonary artery smooth muscle cells via Smad3. Am J Respir Cell Mol Biol. 2013;49(6):1135–1145.

243. Drake KM, Zygmunt D, Mavrakis L, et al. Altered MicroRNA processing in heritable pulmonary arterial hypertension: an important role for Smad-8. Am J Respir Crit Care Med. 2011;184(12):1400–1408.

244. Johnson JA, Hemnes AR, Perrien DS, et al. Functional analysis identifies ALK1-1 as the predominant cause of pulmonary hypertension related to hereditary haemorrhagic telangiectasia. J Med Genet. 2003;40(12):865–871.

245. Rodenburg RE, Flanagan JA, Sankelo M, et al. Molecular and functional analysis identifies ALK1 as the predominant cause of pulmonary hypertension related to hereditary haemorrhagic telangiectasia. J Med Genet. 2003;40(12):865–871.

246. Abadlla SA, Gallione CJ, Barst RJ, et al. Primary pulmonary hypertension in families with hereditary haemorrhagic telangiectasia. Eur Respir J. 2004;23(3):373–377.

247. Mitchell D, Pobre EG, Mulivor AW, et al. ALK1-Fc inhibits multiple mediators of angiogenesis and suppresses tumor growth. Mol Cancer Ther. 2010;9(2):379–388.

248. Weber S, Taylor JC, Winnyard P, et al. SIX2 and BMP4 mutations associate with anomalous kidney development. J Am Soc Nephrol. 2008;19(5):891–903.

249. Mitchell D, Pobre EG, Mulivor AW, et al. ALK1-Fc inhibits multiple mediators of angiogenesis and suppresses tumor growth. Mol Cancer Ther. 2010;9(2):379–388.

250. Tabatabaeifar M, Schlingmann K-P, Litwin M, et al. Functional analysis of BMP4 mutations identified in pediatric CASKUT patients: a diagnostic tool for novel ACVRL1 mutations. Blood. 2010;116(9):1604–1612.

251. Choe E-J, Kim YH, Choe S, et al. Enhanced responses to angiogenic cues underlie the pathogenesis of hereditary hemorrhagic telangiectasia 2. PloS One. 2013;8(5):e63138.

252. Mitchell D, Pobre EG, Mulivor AW, et al. ALK1-Fc inhibits multiple mediators of angiogenesis and suppresses tumor growth. Mol Cancer Ther. 2010;9(2):379–388.
ureteropelvic junction obstruction. *J Am Soc Nephrol JASN*. 2012;23(4):618–628.

261. Wetzel P, Haag J, Câmpean V, et al. Bone morphogenetic protein-7 expression and activity in the human adult normal kidney is predominantly localized to the distal nephron. *Kidney Int.* 2006;70(4):717–723.

262. Mitu G, Hirschberg R. Bone morphogenetic protein-7 (BMP7) in chronic kidney disease. *Front Biosci J Virtual Libr.* 2008;13:4726–4739.

263. Bramlage CP, Tampe B, Koziolek M, et al. Bone morphogenetic protein (BMP)-7 expression is decreased in human hypertensive nephrosclerosis. *BMC Nephrol.* 2010;11:31.

264. Bramlage CP, Müller GA, Tampe B, et al. The role of bone morphogenetic protein-5 (BMP-5) in human nephrosclerosis. *J Nephrol.* 2011;24(5):647–655.

265. Graw J. Eye development. *Curr Top Dev Biol.* 2010;90:343–386.

266. Wyatt AW, Osborne RJ, Stewart H, Ragge NK. Bone morphogenetic protein 7 (BMP7) mutations are associated with variable ocular, brain, ear, palate, and skeletal anomalies. *Hum Mutat.* 2010;31(7):781–787.

267. Takenouchi T, Nishina S, Kosaki R, et al. Concurrent deletion of BMP4 and OTX2 genes, two master genes in ophthalmoogenesis. *Eur J Med Genet.* 2013;56(1):50–53.

268. Bakrania P, Efthymiou M, Klein JC, et al. Mutations in BMP4 cause eye, brain, and digit developmental anomalies: overlap between the BMP4 and hedgehog signaling pathways. *Am J Hum Genet.* 2008;82(2):304–319.

269. Hayashi S, Okamoto N, Makita Y, Hata A, Imoto I, Inazawa J. Heterozygous deletion at 14q22.1-q22.3 including the BMP4 gene in a patient with psychomotor retardation, congenital corneal opacity and feet polydactyly. *Am J Med Genet A.* 2008;146A(22):2905–2910.

270. Reis LM, Tyler RC, Schilter KF, et al. BMP4 loss-of-function mutations in developmental eye disorders including SHORT syndrome. *Hum Genet.* 2011;130(4):495–504.

271. Huang H, Song T-J, Li X, et al. BMP signaling pathway is required for commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. *Proc Natl Acad Sci U S A.* 2009;106(31):12670–12675.

272. Elsen M, Raschke S, Tennagels N, et al. BMP4 and BMP7 induce the white-to-brown transition of primary human adipose stem cells. *Am J Physiol Cell Physiol.* 2014;306(5):C431–C440.

273. Böttcher Y, Unbehauen H, Klötting N, et al. Adipose tissue expression and genetic variants of the bone morphogenetic protein receptor 1A gene (BMPR1A) are associated with human obesity. *Diabetes.* 2009;58(9):2119–2128.

274. Schleinitz D, Klötting N, Böttcher Y, et al. Genetic and evolutionary analyses of the human bone morphogenetic protein receptor 2 (BMPR2) in the pathophysiology of obesity. *PloS One.* 2011;6(2):e16155.

275. Townsend KL, Suzuki R, Huang TL, et al. Bone morphogenetic protein 7 (BMP7) reverses obesity and regulates appetite through a central mTOR pathway. *FASEB J Off Publ Fed Am Soc Exp Biol.* 2012;26(5):2187–2196.