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REVIEW ARTICLE

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

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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

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Peer review under responsibility of Chongqing Medical University.

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.

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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- β s, 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 functionality usually leads to marked defects or severe pathologies (Fig. 2). Here, we review the mouse knockout models that have helped elucidate the role of BMPs in development and also emphasize some of the prominent human pathologies associated with deficiencies related to BMP signaling.

Bmp signaling: canonical and non-canonical pathways

BMPs are synthesized as precursor proteins with an N-terminal signal peptide, a prodomain for folding and secretion, and a C-terminal mature peptide.¹¹ Precursors are formed in the cytoplasm as dimeric pro-protein complexes, which are cleaved by pro-protein convertases to generate N- and C-terminal fragments. The C-terminal mature fragment is capable of binding to its receptor, with the non-covalently associated prodomain playing an important regulatory role.

BMPs can signal through both canonical and non-canonical pathways. In the canonical signaling pathway, they initiate the signal transduction cascade by binding to cell surface receptors and forming a heterotetrameric complex comprised of two dimers of type I and type II serine/threonine kinase receptors (Fig. 1B).¹² Both receptor types have a short extracellular domain, a single transmembrane domain, and an intracellular domain with serine/threonine kinase activity. There are a total of seven type I receptors (ALK1-7) for the TGF- β family of ligands, three of which bind BMPs: type 1A BMP receptor (BMPR-1A or ALK3), type 1B BMP receptor (BMPR-1B or ALK6), and type 1A activin receptor (ActR-1A or ALK2).¹³ There are a total of four type II receptors for the TGF- β family, three of which are known to interact with BMPs: type 2 BMP receptor (BMPR-2), type 2 activin receptor (ActR-2A), and type 2B activin receptor (ActR-2B). While BMPR-1A, BMPR-1B, and BMPR-2 are specific to BMPs, ActR-1A, ActR-2A, and ActR-2B can function as receptors for activins, which are also members of the TGF- β superfamily. The mechanism of the heterotetrameric signaling complex formation can vary. For example, BMP6 and BMP7 interact with type II receptors and recruit type I receptors, whereas BMP2 and BMP4 preferentially bind type I receptors and recruit type II receptors.¹⁴ 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, P/kc, 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

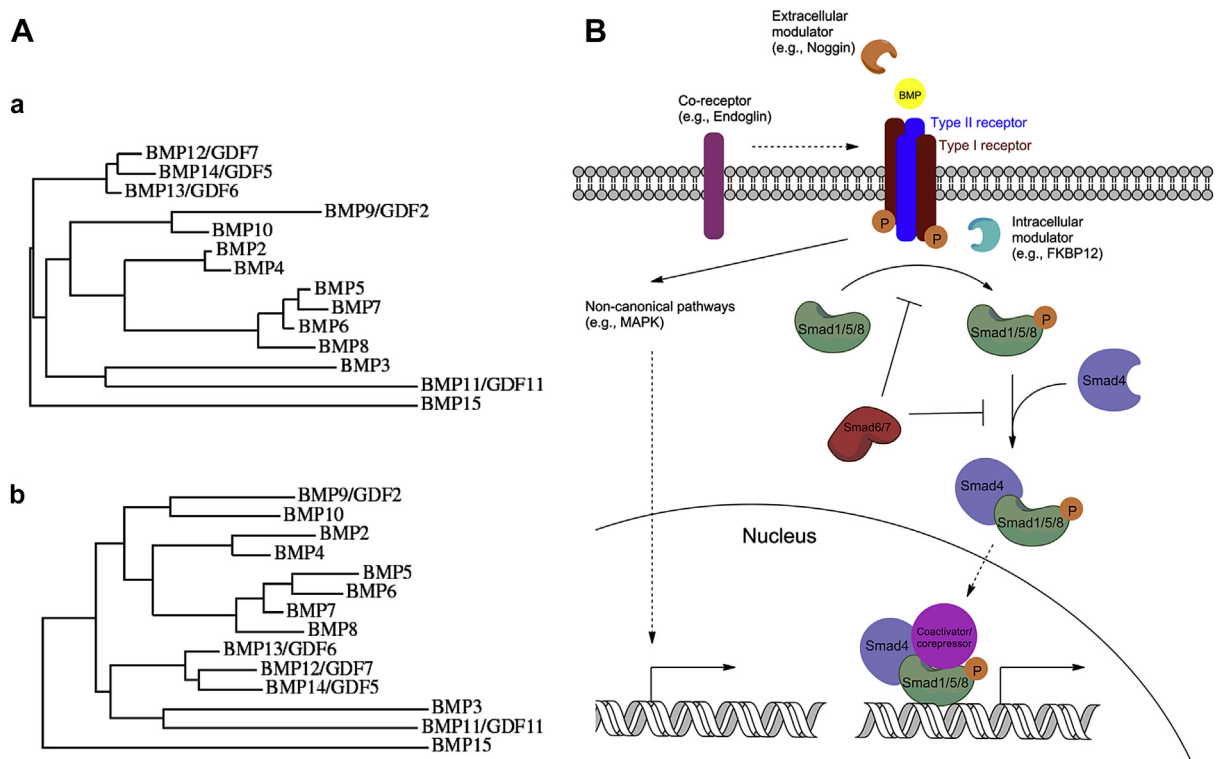


Figure 1 BMP Family and Signaling Pathways. (A) Phylogenetic analysis of the human BMP family members. The human BMP full-length precursor protein sequences and coding region sequences were analyzed using *Phylogeny.fr*. The branch length is proportional to the number of substitutions per site. (a) Phylogenetic analysis was performed using the amino acid sequence of human BMPs. (b) Phylogenetic analysis was carried out using the coding region of human BMPs. (B) BMPs signal via the canonical, Smad-dependent pathway or various non-canonical pathways. In the canonical pathway, BMPs initiate the signal transduction cascade by binding to type I or type II serine/threonine kinase receptors and forming a heterotetrameric complex. The constitutively active type II receptor then transphosphorylates the type I receptor, and the type I receptor phosphorylates the R-Smads (Smad1/5/8). Phosphorylated Smad1/5/8 associates with the co-Smad (Smad4), and the complex translocates to the nucleus where it further associates with coactivators or corepressors to regulate gene expression. Various non-canonical pathways, including the MAPK cascade, can also lead to regulation of gene expression. BMP signaling is modulated extracellularly (e.g., Noggin), intracellularly (e.g., FKBP12, microRNAs, phosphatases, and I-Smads), and by co-receptors in the plasma membrane (e.g., Endoglin).

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.²¹ 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.²² 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 GS domain of type I receptors to inhibit receptor internalization.²³ 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.²⁴

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).²⁵ 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.²⁶ 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.^{27,28} BMP2, 4, 6, 7, and 9 are commonly referred to as the osteogenic BMPs, based on their potent bone-inducing activity.²⁹ For instance, BMP2 is indispensable for endochondral bone formation.³⁰

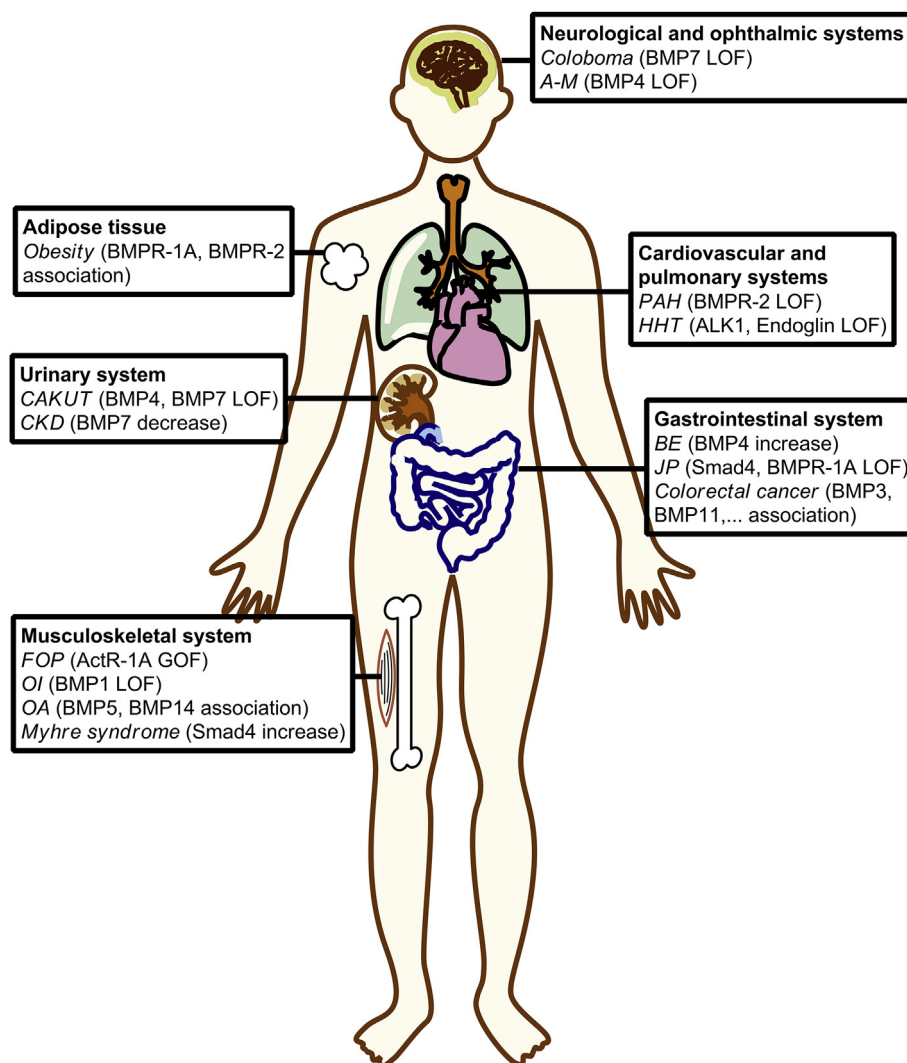


Figure 2 Representative members of the BMP signaling pathway that have been demonstrated to cause or be associated with human diseases. The mutations associated with human pathologies may be gain-of-function or loss-of-function. In some instances, higher or lower gene expression is correlated with disease. Because BMP signaling is involved in multiple organ systems, there are associated pathologies with most organ systems. Abbreviations: LOF, loss-of-function; GOF: gain-of-function; CAKUT, congenital anomalies of the kidney and urinary tract; CKD, chronic kidney disease; FOP, fibrodysplasia ossificans progressiva; OI, osteogenesis imperfecta; OA, osteoarthritis; A–M, anophthalmia–microphthalmia; PAH, pulmonary arterial hypertension; HHT, hereditary hemorrhagic telangiectasia; BE, Barrett esophagus; JP, juvenile polyposis.

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.^{31,32} 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.^{33–35}

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).⁴¹ BMP4 heterozygotes are viable but have a wide variety of abnormalities.³³ Mice lacking BMP1 fail to close the ventral body wall and have gut herniation as a result.³⁶

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.¹⁴⁰ 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 ; <i>limb</i> : spontaneous fractures and impaired fracture repair ⁸ ; <i>chondrocyte</i> : severe chondrodysplasia ³⁰ ; <i>cardiac progenitor</i> : abnormal heart valve development ³⁸ ; <i>myocardium</i> : 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 ; <i>heterozygotes</i> : various organ abnormalities ⁴⁴ ; <i>hypomorph</i> : AVCD, ⁴⁵ HSC microenvironment defect ⁴⁶ ; <i>limb bud mesoderm</i> : defective digit patterning ³¹ ; <i>adipocyte</i> : enlarged adipocytes and impaired insulin sensitivity ⁴⁷ ; <i>other targeted</i> : loss-of-trachea phenotype, ⁴⁸ abnormal branchial arch arteries and outflow tract septation, ⁴⁹ defects in mandibular development, ⁵⁰ defects in vestibular apparatus ⁵¹
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 ; <i>inducible deletion</i> : precocious differentiation of kidney progenitor cells ⁶² ; <i>limb</i> : no effect ⁶³ ; <i>podocyte</i> : defective kidney development ⁶⁴
BMP8	Germ cell degeneration ³³ ; 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 ; slower 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 ³⁴
BMP13/GDF6	Bone fusions in wrists and ankles ⁸² ; accelerated coronal suture fusion ⁸³ ; eye and neural defects ^{84,85} ; Klippel–Feil syndrome ⁸⁶ ; <i>males</i> : 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	<i>Males</i> : normal and fertile; <i>females</i> : subfertile with decreased fertilization and ovulation rates ⁹⁷
Smad factors	
Smad1	Die mid-gestation, defects in allantois formation, ⁹⁸ no PGCs ^{99,100} ; <i>chondrocyte</i> : delayed calvarial bone development ¹⁰¹ ; <i>osteoblast</i> : osteopenia ¹⁰¹ ; <i>lung epithelium</i> : 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 ; <i>heterozygotes</i> : gastric and duodenal polyps ^{108,109} ; <i>adult</i> : anemia ¹¹⁰ ; <i>osteoblast</i> : lower bone mass ¹¹¹ ; <i>chondrocyte</i> : hearing loss and inner ear malformation, ^{112,113} disorganized growth plate ¹¹⁴ ; <i>muscle</i> : muscle atrophy ¹¹⁵ ; <i>cardiomyocyte</i> : cardiac hypertrophy ¹¹⁶ ; <i>endothelial cell</i> : embryonically lethal ^{117,118} ; <i>vascular smooth muscle</i> : embryonically lethal ¹¹⁹ ; <i>CNS</i> : cerebellar defects ¹²⁰ ; <i>eye</i> : defects in lacrimal gland ¹²¹ ; <i>Sertoli and Leydig cells</i> : testicular dysgenesis ¹²² ; <i>preovulatory follicles</i> : follicle atresia ¹²³ ; <i>ovary</i> : defects in folliculogenesis ¹²⁴ ; <i>skin</i> : aberrant wound healing, ¹²⁵ hair loss and squamous cell carcinoma ¹²⁶ ; <i>keratinocyte</i> : accelerated reepithelialization ¹²⁷ ; <i>head and neck</i> : head and neck squamous cell carcinoma ¹²⁸ ; <i>odontoblast</i> : keratocystic odontogenic tumors ¹²⁹
Smad6	Defects in axial and appendicular skeletal development ¹³⁰ ; multiple cardiovascular abnormalities ¹³¹
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 ; scleroderma ¹³⁷ ; altered B cell response ¹³⁸ ; <i>T cell</i> : reduced disease and CNS inflammation ¹³⁹
BMP receptors	

(continued on next page)

Table 1 (continued)

Signaling molecule	Phenotype
BMPR-1A (ALK3)	Embryonically lethal, no mesoderm formation ¹⁴⁰ ; <i>mesoderm</i> : embryonically lethal, ¹⁴¹ omphalocele-like defect ¹⁴² ; <i>osteoblast</i> : increased bone mass ¹⁴³ ; <i>chondrocytes</i> : no long bone growth ¹⁴⁴ ; <i>liver</i> : iron overload ¹⁴⁵ ; <i>cardiomyocyte</i> : heart valve defects and EC defects ^{146,147} ; <i>endocardium</i> : EC defects ¹⁴⁸ ; <i>vascular smooth muscle cells</i> : embryonically lethal ¹⁴⁹ ; <i>adult vascular smooth muscle cells</i> : impaired vascular remodeling ¹⁵⁰ ; <i>lung epithelium</i> : defects in lung development, ¹⁵¹ neonatal respiratory distress ¹⁵² ; <i>ureteric bud</i> : dysplastic renal phenotype ¹⁵³ ; <i>hypothalamus (neurons in feeding center)</i> : hypophagia and death ¹⁵⁴ ; <i>eye</i> : lack of lens and retina growth ¹⁵⁵ ; <i>Leydig cell</i> : abnormal Leydig cells ¹⁵⁶ ; <i>granulosa cell</i> : subfertile ¹⁵⁷ ; <i>facial primordia</i> : lip and palate defects ¹⁵⁸ ; <i>hair follicle</i> : impaired differentiation of inner root sheath ¹⁵⁹ ; <i>dental epithelia</i> : switched differentiation of crown epithelia to root lineage ¹⁶⁰
BMPR-1B (ALK6)	Defects in appendicular skeleton ¹⁶¹ ; retinal defects ¹⁶² ; <i>females</i> : irregular estrous cycles ¹⁶³
ActR-1A (ALK2, ACVR1)	Embryonically lethal, defects in mesoderm formation and gastrulation, ¹⁶⁴ no PGCs ⁴² ; <i>surface ectoderm</i> : smaller lens ¹⁶⁵ ; <i>neural crest</i> : craniofacial defects, ¹⁶⁶ cardiac outflow tract defects ¹⁶⁷ ; <i>liver</i> : iron overload ¹⁴⁵ ; <i>endothelium</i> : defects in AV septa, valves, and EC formation ¹⁶⁸ ; <i>cushion mesenchyme</i> : bicuspid aortic valve ¹⁶⁹ ; <i>uterus</i> : delayed implantation and sterility, ¹⁷⁰ mid-gestation abnormalities ¹⁷¹
BMPR-2	Embryonically lethal, defects in mesoderm and gastrulation, ¹⁷² cardiac defects ¹⁷³ ; <i>heterozygotes</i> : PAH ^{174,175} ; <i>lung epithelium</i> : predisposed to PAH ¹⁷⁶
ActR-2A (ACVR2A)	Defective reproductive performance and sexual behavior ^{177–179}
ActR-2B (ACVR2B)	Die after birth, complicated cardiac defects and left-right asymmetry ¹⁸⁰

and no PGCs.^{42,164} 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).¹⁰⁶ Smad1 plays an essential role in fusion of amnion and chorion, and mutants have defects in extraembryonic structures and germ cell formation.^{99,140} Smad5 knockouts exhibit multiple embryonic and extraembryonic defects as well, including left-right asymmetry and significantly reduced PGC number.^{103–105}

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.²⁸ Deletion of BMPR-1A in osteoblasts also surprisingly leads to increased bone mass.¹⁴³ Some BMPs may have redundant roles in bone formation, as conditional deletion of BMP7 from limb has no noticeable effect.⁶³ However, BMP7 deficient mice have skeletal patterning defects confined to the ribs, skull, and hindlimbs.⁵⁸ Additionally, BMP11 plays important roles in skeletal patterning during development, as BMP11 mutants have altered Hox gene expression and abnormal anterior-posterior (A-P) patterning of the axial skeleton.⁷⁰

The *short ear* and *brachypodism* mutations, associated with BMP5 and BMP14, respectively, are specific named loci and phenotypes in the mouse.^{52,89} 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.⁵³ Examination of BMP14 mutants revealed that BMP14 also coordinates bone and joint formation during digit development.⁹⁰ BMP5/14 double mutants have additional defects compared to single mutants, suggesting a likely synergistic function.¹⁸¹ Along with BMP14, BMP4 regulates digit patterning and the apical ectodermal ridge.³¹ A BMP14 mutant with severe joint malformations exhibited early-onset osteoarthritis (OA), offering a potential model for the study of the disease.⁹³ 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.^{54,55}

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.⁷⁸ Mutation in BMP13 causes defects at multiple sites, including the wrist and ankle.⁸² 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.⁸³ Furthermore, mutations in BMP13 are distinguished by fusion of carpal and tarsal bones.⁸⁶ An *in vitro* study confirmed the strong inhibitory role of this BMP.²⁷ 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.^{79,87,94} 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.¹⁹⁷ 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 Overexpression 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/ β -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

potential applicability of this for treatment in humans is in progress. A Phase I trial to evaluate safety in using BMP7 for the treatment of OA found no major adverse events and also a dose-dependent trend in symptom improvement.²¹⁰ These results have suggested potential therapeutic applications of BMP7 for OA. Investigations of BMP2 as an injectable therapeutic agent to stimulate cartilage repair have also shown promise in an animal model.²¹¹

BMP signaling in muscle

Not many pathways are known to regulate muscle growth in adulthood other than myostatin, which is a member of the TGF- β family and a known negative regulator.¹¹⁵ It binds to activin type II receptors (ActR-2A and ActR-2B) and activin type I receptors (ALK4 and ALK5), leading to Smad2/3 phosphorylation and subsequent complexing with Smad4 to affect gene expression. Recently, it has been shown that the BMP pathway is an important hypertrophic, as well as anti-atrophic, signal in adult muscle.¹¹⁵ Constitutively active type I BMP receptor can lead to substantial muscle hypertrophy. Decreased levels of phosphorylated Smad2/3 perhaps leads to release of Smad4, which is then recruited into BMP signaling. Another study also concluded BMP signaling was a positive regulator of skeletal muscle.²¹² These results demonstrate the emerging evidence of BMP signaling in adult muscle homeostasis.

Mutations in Smad4 in humans result in Myhre syndrome, which is associated with muscle hypertrophy.²¹³ The Smad4 mutations lead to increased stability of the protein and result in downregulation of the Myostatin pathway and variable response of the BMP pathway. Further investigations need to be done, but it seems the muscle hypertrophy is mainly due to inhibition of Myostatin signaling. Muscle-specific knockout of Smad4 in mice leads to muscle atrophy, consistent with the above finding.¹¹⁵

BMP signaling in gastrointestinal system

BMP signaling is required for normal growth and morphogenesis of the developing gastrointestinal tract.²¹⁴ The underlying smooth muscle of the embryonic gut is also dependent upon BMP signaling, especially BMP2, for proper development.²¹⁵ Gastric patterning requires BMP signaling, as deletion of BMPR-1A in foregut endoderm leads to anteriorization of the stomach.²¹⁶ BMP11 is important in pancreatic development, and BMP11 deficient mice have a pancreas half the size of wild-type mice.⁷¹

Barrett esophagus (BE)

BE is a metaplasia associated with esophagitis from gastroesophageal reflux disease, in which the squamous epithelium of the esophagus is replaced by columnar epithelium. BMP4 and the downstream phosphorylated Smad1/5/8 were found to be upregulated in BE patient samples.^{217,218} The BMP pathway is thus implicated in the trans-differentiation of squamous epithelium to columnar epithelium in inflamed esophageal mucosa, but the pathogenesis is not well understood. Initial upregulation of the Hedgehog pathway may lead to upregulation of target genes, including BMP4, subsequently reprogramming the

squamous epithelium to the more favorable columnar type for protection.^{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 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

BMPR-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.³⁷ 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 BMP2.³⁸ 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.³⁹

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).⁴⁵ BMP4 is also required for outflow tract septation as demonstrated by conditional knockout.⁴⁹ 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.²³⁵ BMP10 plays an essential role in maintaining cardiac growth during cardiogenesis, as BMP10 knockout mice have dramatically reduced cardiomyocyte proliferation.⁶⁹ Smad6 and Smad7 mutants both show developmental defects in the outflow tract.^{131,132}

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.¹⁷³ Mutation of BMPR-1A is embryonically lethal, even if only in cardiomyocytes and vascular smooth muscle.¹⁴⁹ BMPR-1A signaling in the AV myocardium is required for EC formation and development of AV valves from the ECs.^{146,147} ActR-1A is also crucial for AV cushion development, specifically the EMT required in EC formation.¹⁶⁸ Ablation of ActR-1A signaling in neural crest cells results in impaired migration of these cells to form the outflow tract.¹⁶⁷ Deletion of ActR-1A in cushion mesenchyme results in bicuspid aortic valve.¹⁶⁹

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.¹⁰² 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.^{151,152} Smad8 mutants have defective pulmonary vascular remodeling, with a resulting pulmonary arterial hypertension (PAH) phenotype.⁹ 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.^{174–176}

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.²³⁶ 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.²³⁷ BMPR-2 mutations influence the disease expression more obviously in males than in females.²³⁸

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.²³⁹ BMPR-2 also interacts with the cytoskeleton, and mutant receptors may cause cytoskeletal defects related to the development of PAH.²⁴⁰ 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.²⁴¹ 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.²⁴² In addition, there is increased TGF- β 1 signaling and reduced BMP signaling, so the pathogenesis of disease is expected to be in part due to abnormal crosstalk between the two pathways.²⁴³ Mutations in Smad8 have been linked to PAH, possibly due to a non-redundant role of Smad8 in microRNA processing.²⁴⁴ Interestingly, PAH is sometimes associated with hereditary hemorrhagic telangiectasia (HHT). Defects in the ALK1 or Endoglin gene can lead to HHT, with associated PAH.^{24,245,246}

Sildenafil is an established treatment for PAH, and was shown to enhance BMP4-induced phosphorylation of Smad1/5 and the resulting downstream BMP pathway.²⁴⁷ 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 PASM proliferation by inhibiting the cell cycle.²⁴⁸ 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.²⁴⁹ 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 co-receptor, while HHT type 2 is due to loss-of-function in ALK1.²³⁶ Smad4 mutations are known to be associated with a combined JP-HHT syndrome that predisposes to thoracic aortic disease.²⁵⁰ The discovery of BMP9 as the ligand for ALK1 showed that mutations in the receptor lead to defective BMP9 signaling.²⁵¹ 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 VEGR2 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.²⁵² 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.²⁵³ 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.²⁵⁴ BMP11 knockout mice, for example, have a spectrum of renal abnormalities, with the majority having bilateral renal agenesis.⁷³ 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.¹⁵³ 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.²⁵⁵ Homozygous mutations in BMP4 and DACH1 were found in a patient with Bilateral Cystic Renal Dysplasia.²⁵⁶ 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.²⁵⁷ This is consistent with missense mutations in BMP4 and BMP7 being strongly associated with hypospadias.²⁵⁸ Mutations in BMP4 can also lead to UPJO.²⁵⁹ 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.²⁶⁰

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.⁵⁷ 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.⁶² 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.⁶⁴

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.²⁶¹ Exogenous administration of BMP7 or transgenic overexpression of BMP7 reduces overall renal fibrosis and nephrocyte apoptosis.²⁶² Additionally, BMP7 signaling has been shown to be protective against hypertensive nephrosclerosis, another major cause of CKD.²⁶³ BMP5 may have a similar role.²⁶⁴

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.²⁶⁵ 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.⁴³ Germline mutations in BMP7 prevent lens formation.⁵⁸ As in the case of the kidney, early tissue interactions establishing the organ are unaffected in BMP7 mutants, but the mice exhibit anophthalmia.⁵⁷ BMPR-1A is essential for lens and retinal growth.¹⁵⁵ 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.¹⁶⁵ 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.^{84,85} Retinal apoptosis is also associated with loss of BMPR-1B, which is exclusively expressed in the ventral retina during development.¹⁶²

In humans, frameshift, missense, and Kozak sequence mutations have been identified in BMP7 in individuals with developmental eye abnormalities and extra-ocular features.²⁶⁶ 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.^{267–269} One report indicated a role for BMP4 in short stature, hyperextensibility, hernia, ocular depression, Rieger anomaly, and teething delay (SHORT) syndrome.²⁷⁰

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.⁸¹ BMP11 is also involved in spinal cord neurogenesis through its ability to induce cell cycle exit and instead promote differentiation of progenitors.⁷⁴ 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.⁷⁵ It plays a role in retina development as well.⁷⁶ 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.⁵⁹ Interestingly, BMPR-1A is important in establishment of neurons involved in regulating feeding behavior.¹⁵⁴

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.³⁴ 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.⁹⁷ BMP6 knockout females are demonstrated to have decreased fertility as well, with a decrease in ovulated eggs.⁵⁶ 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.^{33,65,66}

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.¹⁷¹ Signaling in the uterus during implantation requires ActR-1A.¹⁷⁰

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.²⁷¹

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.⁶⁰ However, recent studies have indicated that BMP4 induces white-to-brown transition.²⁷² 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.⁴⁷ BMPR-1A has been associated with obesity because its activity has been shown to favor differentiation into adipocytes as opposed to osteoblasts.²⁷³ BMPR-2 has also been implicated in obesity.²⁷⁴ BMP7 treatment of diet-induced obese mice leads to increased energy expenditure and decreased food intake.²⁷⁵ 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 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.

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