

Embryonic Skeletogenesis and Craniofacial Development

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Abstract Bone morphogenetic proteins (BMPs) are originally identified with their ability to induce heterotopic ossification. Several decades of studies have demonstrated that BMPs have pleiotropic functions in numbers of tissues for many different aspects. This review focuses on the effects of BMP signaling on skeletogenesis and craniofacial development. We will summarize recent progresses on in vitro studies, animal models, and human genetics to uncover highly context-dependent functions of BMP signaling, including unexpected outcomes, and the mechanisms of how BMP signaling regulates bone mass. We will also summarize reported findings about BMP signaling-related genes identified as causes of human diseases in skeletal system such as chondrodysplasia, facial cleft, and craniosynostosis.

Keywords Osteoblast • Chondrocyte • Osteocyte • Osteoclast • Mesenchyme • Neural crest • BMP signaling • Wnt signaling • Hedgehog • FGF • Facial process • Cleft palate • Cleft lip • Craniosynostosis • Chondrodysplasia • Temporomandibular joint

1 Introduction

Bone morphogenetic proteins (BMPs) were discovered and named in 1965 by Marshall Urist, who initially identified the ability of a then unknown factor in the bone to induce ectopic bones in muscle [204]. In the past 50 years, the osteogenic function of BMPs has been extensively examined [188]. The US Food and Drug

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Administration (FDA) has approved BMP2 and BMP7 for clinical use in long bone open fractures, nonunion fractures, and spinal fusion. Therefore, the exogenous role of BMPs in the bone is well known in orthopedics. However, it is crucial to understand endogenous or physiological roles of BMPs during skeletogenesis and bone remodeling.

BMP signaling plays important roles in a variety of cell types in the skeleton including osteoblasts, chondrocytes, and osteoclasts. The osteogenic function of BMPs and BMP signaling has been further investigated over the last decade using gene-targeting technology in animals. This chapter focuses on the physiological roles of BMP signaling on bone formation, bone resorption, and bone mass control, specifically via its action on osteoblasts or chondrocytes by reviewing mouse genetic studies of skeletal development and bone remodeling. This chapter also focuses on roles of BMP during craniofacial development including formation of calvaria and mandible.

2 Embryonic Skeletogenesis

2.1 Developmental Stages of Ossification

One of the key components derived from the paraxial mesoderm is the bone. The skeleton which includes the bone is generated from three distinct lineages: (1) the somites which generate the axial skeleton, (2) the lateral plate mesoderm which generates the limb skeleton, and (3) the cranial neural crest which generates the branchial and craniofacial bones and cartilage. The skeleton in mammals is formed through two distinct processes during embryogenesis: intramembranous ossification and endochondral ossification [51, 110]. Both processes involve the transformation of a preexisting mesenchymal tissue into the bone tissue as they are called “bone formation” or “osteogenesis.” The intramembranous ossification is a direct conversion of mesenchymal tissue into the bone, which primarily occurs in flat bones including the skull, the mandible, and the clavicle. On the other hand, endochondral ossification, which occurs in long bones, is an indirect conversion of mesenchymal tissue into the bone; i.e., the mesenchymal tissue differentiates into cartilage and this cartilage is later replaced by the bone.

2.2 BMP and Osteogenesis

At cellular levels, the *in vivo* physiological process of “osteogenesis” or “bone formation” can be described as two distinct processes: (1) intramembranous ossification through osteoblastogenesis that is direct differentiation of mesenchymal cells into bone cells (i.e., osteoblasts) and (2) the endochondral ossification, which includes an initial chondrogenesis that is differentiation from mesenchymal cells into cartilage

cells (i.e., chondrocytes) followed by the apoptosis of chondrocyte secondary differentiation from osteoblast precursor to osteoblasts via osteogenesis. Therefore, “osteogenesis” encompasses osteoblastogenesis and chondrogenesis. The key molecules of BMP pathway involved in osteogenesis are listed (Table 1). Note that BMPRIA is a potent receptor of BMP2 and BMP4 [66], as is ACVRI for BMP7 [136].

In mice, BMP2 is expressed in a variety of sites including the developing limb buds [134], mesenchymal derivatives of which undergo endochondral ossification. The osteogenic (i.e., anabolic) roles of BMPs have been extensively examined over 50 years, and human recombinant BMP2, BMP4, BMP6, and BMP7 proteins have been vigorously used for mammalian cells to induce their differentiation in culture. To induce chondrogenesis or osteogenesis, primary cells or pluripotent mesenchymal cell lines such as C3H10T1/C3H10T2 [43], C2C12 [99], ATDC5 [186], N1511 [90], MC3T3 [13], and ST2 [219] have been treated with BMPs. In these cells, BMPs directly activate *Sox9* and *Cbfa1*, transcriptional master genes required for chondrogenesis and osteoblastogenesis, respectively [114, 160, 232], to secondarily induce expression of chondrogenic (i.e., aggrecan, ColII, ColIX, ColX, etc.) or osteogenic (i.e., ALP, osteocalcin, BSP, Col1, etc.) markers. Based on the accumulated evidence of anabolic actions of BMPs, BMP2 and BMP7 have been approved by the US FDA for clinical application [56, 62]. It is noted that average circulating serum levels of BMPs are around 300~600 pg/ml [102, 206] while a typical dosage range of BMPs in culture experiments is 0~300 ng/ml. Also expression levels of BMPs by primary osteoblasts and pluripotent mesenchymal cell lines are quite low demonstrating a significant discrepancy between levels of BMPs found in tissues and those used for pharmacological experiments.

In addition to BMP signaling, the impacts of Wnt signaling on skeletogenesis and bone formation have been investigated for a decade [16, 57, 63, 64, 109]. The relationship of BMP signal with Wnt signal in the skeletal system is of interest. In vitro experiments using pluripotent mesenchymal cell lines or primary osteoblasts to test the interaction between BMP and Wnt signaling in osteoblasts have yielded both synergistic and antagonistic results: C2C12 cells and primary osteoblasts induce *Wnt3a* expression and stabilize Wnt/ β -catenin signaling upon BMP2 treatment [7, 33, 141]. Alternatively, C3H10T1/2 cells treated with Wnt3a induce BMP4 expression [215]. These facts suggest the presence of a positive autocrine loop

Table 1 The key molecules in BMPs’ signaling cascade regarding osteogenesis

Function	Key molecules
Antagonists	Noggin, Chordin, Gremlin
Ligands	BMP2, BMP4, BMP6, BMP7
Type I receptors	BMPRIA/ALK3, ACVRI/ALK2, BMPRII/ALK6
Type II receptors	BMPRII, ActRIIA, ActRIIB
R-Smad	Smad1, Smad5, Smad8
Co-Smad	Smad4
I-Smad	Smad6, Smad7
Non-Smad pathways	p38 MAPK, TAK1

between BMP and Wnt signaling pathways [33, 171]. In contrast, primary osteoblasts show increased Wnt canonical signaling when BMP signaling is inhibited upon treatment with Dorsomorphin, an inhibitor for BMP type I receptors [92]. Wnt3a treatment represses BMP2-dependent *Id1* expression in C2C12 cells [152]. Similarly, treatment of cultured skull bone with a BMP antagonist *Noggin* increases Wnt canonical signaling [95]. Moreover, one study investigated intracellular cross talk between BMP and Wnt pathways using uncommitted bone marrow stromal cells [127]. Dishevelled homolog 1 (Dvl1) is a cytoplasmic protein known to act as a signaling molecule for Wnt pathway. This study found that BMP2 antagonizes Wnt3a-induced proliferation and Wnt/ β -catenin activation through an interaction between Smad1 and Dvl1. Another intracellular interaction via Pten/Akt pathway has been reported in hair follicle stem/progenitor cells [234]; however, this pathway is less likely functional in osteoblasts [68]. Taken together, these facts suggest that both positive and negative feedback loops are present between the two signaling pathways, BMP and Wnt, in a context-dependent manner.

2.3 Functional Studies in Animal Models

As detailed in Chap. 4, the BMP family members are involved with early patterning of the mouse embryo. Conventional knockout mice for the key genes (i.e., BMP2, BMP4, and BMP7 and their receptors BMPRIA and ACVRI) are lethal, and, thus, it is not possible to investigate bone development and remodeling using these mouse models [49, 61, 132, 143, 146, 216, 233]. To avoid the embryonic lethality, a strategy of conditional knockout mice using a Cre-loxP system has been employed.

Both osteoblasts and chondrocytes are derived from mesenchymal cells and are responsible for the bone and cartilage, respectively. Recent animal studies have been designed to investigate the physiologic function of BMP signaling in these different cell types (mesenchymal cells, chondrocytes, and osteoblasts) independently (Table 2). Interestingly, BMP signaling both in chondrocytes and mesenchymal cells positively controls bone size and mass while negatively controls the same in osteoblasts. Accumulated evidence has revealed similarities between mesenchymal cells and chondrocytes and differences between these cells and osteoblasts regarding how BMP signaling affects their behavior (i.e., bone size).

2.3.1 BMP and Osteoblasts

An osteoblast-specific conditional deletion of *Bmpr1a* using the *Og2-Cre* mouse line, in which Cre recombination is restricted in differentiated osteoblasts under the osteocalcin promoter, was first reported in 2004 [145]. The co-Smad, Smad4, was also conditionally deleted in osteoblasts using another *Og2-Cre* mouse line [197]. Interestingly, these two studies demonstrated that the response of osteoblasts after loss of BMP signaling is age dependent; trabecular bone volume is lower in young

Table 2 Mouse studies of BMP signaling in different cell type

	Promoter to drive transgene or Cre	BMP signal	Stage	Bone mass	Ref.
<i>Chondrocyte</i>					
<i>Bmpr1a</i> cKO	Gdf5-Cre	Down	E12.5–E16.5, 7w, 9M	Reduced	[176]
Double knockout of <i>Bmpr1a</i> and <i>Bmpr1b</i>	Col2-Cre	Down	E12.5–E16.5	Reduced	[227]
<i>Bmp4</i> overexpression	Col11a2	Up	E18.5	Increased	[202]
Noggin overexpression	Col11a2	Down	E18.5	Reduced	[202]
Double cKO of Smad1 and Smad5	Col2-Cre	Down	E12.5–newborn	Reduced	[172]
<i>Bmpr1a</i> cKO	Aggrecan-CreER	Down	2, 4, 8, 20w	Reduced	[86]
<i>Acvr1</i> cKO	Col11a2-Cre	Slightly down	E17.5, P0	Not reported	[174]
<i>Mesenchymal cell</i>					
Double cKO of <i>Bmp2</i> and <i>Bmp4</i>	Prx1-Cre	Down	E10.5–newborn, 3w	Reduced	[11]
<i>Bmp2</i> cKO	Prx1-Cre	Down	5M	Reduced	[201]
<i>Bmpr2</i> cKO	Prx1-Cre	Normal ^a	2 M	Increased	[130]
<i>Osteoblast</i>					
<i>Bmpr1a</i> cKO	Ogl2-Cre	Down	3M 10M	Reduced Increased	[145]
<i>Smad4</i> cKO	Ogl2-Cre	Down	3~12w 11M	Reduced Increased	[197]
<i>Bmp4</i> overexpression	2.3 kb Col1	Up	E18.5	Reduced	[158]
Noggin overexpression	2.3 kb Col1	Down	E17.5, 3w	Increased	[158]
<i>Bmpr1a</i> cKO	3.2 kb Col1-CreER	Down	E18.5, 3w, 5M	Increased	[92, 94, 95]
<i>Acvr1</i> cKO	3.2 kb Col1-CreER	Down	E18.5, 3w, 5M	Increased	[91]
<i>Osteoclast</i>					
<i>Bmpr1a</i> cKO	Ctsk-Cre	Down	8w	Increased	[157]
<i>Osteocyte</i>					
<i>Bmpr1a</i> cKO	Dmp1-Cre	Down	1M, 2M, 4M	Increased	[93, 124]

^aActivin signal is increased while BMP signal is unchanged

mutant mice but higher in aged mutant mice. In addition, the activity of osteoclasts is reduced in aged osteoblast-specific *Bmpr1a*-deficient mice, which may have led to the complex skeletal phenotype [145, 197]. These facts suggest that BMP signaling in differentiated osteoblasts controls the balance between bone formation by osteoblasts and resorption by osteoclasts, thereby affecting the final outcome of the amount of bone mass in an age-dependent manner. Increased bone mass in *Bmpr1a*-deficient mice appeared to be challenging to the general concept of BMPs as osteogenic inducers.

Comprehensive functions of BMP signaling in skeletogenesis have been further investigated and led to a new paradigm that alternation of Wnt signal by BMP is the key modulator of skeletal development. The loss of function of BMP signaling via BMPRIA in osteoblasts upregulates Wnt canonical signaling during embryonic and postnatal bone development, suggesting a negative regulation of Wnt signaling by BMP [92, 95]. These studies show that the upregulation of Wnt signaling is at least in part mediated by suppression of Wnt inhibitors including *Sost*/sclerostin and *Dkk1* because both *Sost*/sclerostin and *Dkk1* are direct targets of BMP signaling (Fig. 1). In addition, *Sost* expression was severely downregulated in *Bmpr1a*-deficient bones as assessed by microarray analysis [92, 95]. Interestingly, both Smad-dependent and Smad-independent pathways appear to contribute to *Dkk1* expression, whereas *Sost*/sclerostin requires only Smad-dependent signaling, suggesting differential regulation of these genes by BMP signaling via BMPRIA [92]. BMP and Wnt signaling regulate the development and remodeling of many tissues and interact synergistically or antagonistically in a context- and age-dependent manner in vivo [17, 77]. Lastly, the role of BMPRIA in osteocytes was recently investigated by conditional disruption of *Bmpr1a* using *Dmp1-Cre* mouse line from two independent groups [93, 124]. The resulting mutant mice demonstrated an increased bone mass concomitant with accelerated cell proliferation and SOST reduction [93, 124]. It is interesting that the increased bone phenotype was much stronger in the osteocyte-specific condition (i.e. *Dmp1Cre*:*Bmpr1a* mice) compared

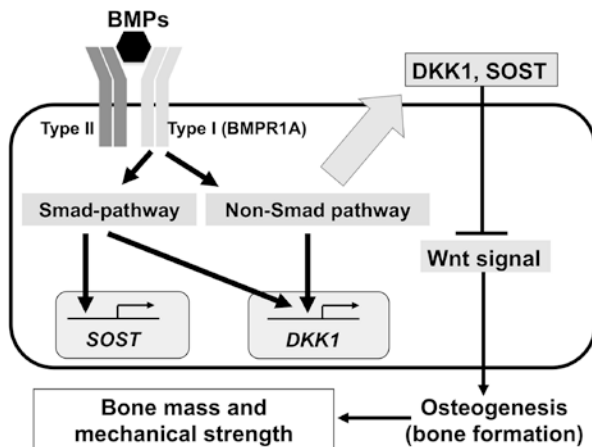


Fig. 1 A proposed model of the relationship between the BMP signaling via BMPRIA and the canonical Wnt signaling in osteoblasts. Both *Dkk1* and *Sost*/sclerostin are downstream targets of the BMP signaling. The BMP signaling upregulates *Sost* expression primarily through the Smad-dependent signaling while it upregulates *Dkk1* expression through both the Smad and non-Smad signaling pathways (p38 MAPK). As *DKK1* and *SOST*/sclerostin act as Wnt signaling inhibitors, BMP signaling in osteoblasts, in turn, inhibits osteogenesis and decreases bone mass. *DKK1* and *Sost*/sclerostin play an important role in regulating bone mass and mechanical strength as downstream effectors of BMPRIA signaling in bone by taking balances between BMP signaling and Wnt signaling

with osteoblast-specific condition (i.e. Col1Cre:Bmpr1a mice). In addition, similar to the Col1Cre:Bmpr1a mice, Wnt signal is activated while RANKL is suppressed in the Dmp1Cre:Bmpr1a mice [93]. This fact is very intriguing because recent reports show osteocytes as a primary source of RANKL production [153, 219] and therefore BMPRIA can be a key molecule in osteocytes by regulating RANKL production.

Similarly, the loss of function of BMP signaling in osteoblasts via ACVR1, another type I receptor, results in increased bone mass [91]. In this mouse model, upregulation of Wnt canonical signaling is observed concomitant with reduction in *Dkk1* and *Sost* expression during embryonic and postnatal bone development [91]. Because the resulting *Acvr1* mutant mice show similar bone phenotypes to those found in *Bmpr1a* mutant mice, despite structural and functional similarities between two receptors, the other does not compensate loss of one receptor.

Sost/sclerostin was originally reported as a member of the BMP antagonist DAN family [111, 214]. Although DAN family members modulate both BMP and Wnt signaling in *Xenopus* [19, 79, 167], recent studies suggest a primary role of *Sost/sclerostin* in Wnt signaling in mouse and humans: *Sost/sclerostin* is not a BMP antagonist [207] but rather a Wnt inhibitor [208] that binds the Wnt co-receptors low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6) [123, 183]. It is known that both DKK1 and *Sost/sclerostin* inhibit Wnt/ β -catenin signaling by binding to co-receptors. As both *Dkk1* and *Sost/sclerostin* are secreted proteins expressed by osteoblasts, their role in regulating bone mass has been investigated using human and mouse genetic approaches. Although conventional knockouts of *Dkk1* die in utero from defective head induction and limb formation [151], mice heterozygous for *Dkk1* (*Dkk1*^{+/-} mice) exhibit a high bone mass (HBM) phenotype [150], while overexpression of *Dkk1* in osteoblasts causes osteopenia [118]. In addition, increased *DKK1* expression in bone marrow has also been associated with lytic bone lesions in patients with multiple myeloma [199].

Similar to *Dkk1*^{+/-} mice, conventional knockouts of *Sost* are viable and exhibit increased bone mass [122]. In humans, the loss of function and hypomorphic mutations in *SOST* cause sclerosteosis [9, 30] and van Buchem disease [10, 191], respectively, with a high bone mass (HBM) phenotype. These mutants share the HBM phenotypes with other gain of function of LRP5 mutation effects, due to defect in *Dkk1*-mediated regulation of LRP5 in humans [26, 125, 209] and overexpression of *Lrp5* in mice [6]. In contrast, the loss of function of LRP5 leads to OPPG with low bone mass [59], which is similar to the bone phenotype of mice overexpressing *Sost* [214]. In addition, recent genome-wide SNP-based analyses identified a significant association between bone mineral density and the *SOST* gene locus [76, 194, 226]. Consistent with these observations, conditional knockouts of *Bmpr1a*, which show reductions in expressions of *Dkk1* and *Sost*, show an HBM phenotype [92–95]. Furthermore, increased expression of *Dkk1* and *Sost* in osteoblasts by constitutive activation of BMPRIA signaling is associated with a partial rescue of the bone phenotype of *Bmpr1a*-deficient mice [92]. These facts support the interpretation that *Dkk1* and *Sost/sclerostin* act physiologically as inhibitors of Wnt canonical signaling and therefore as negative regulators of bone mass.

2.3.2 BMP and Chondrocytes

When BMP signaling was enhanced by overexpression of *Bmp4* in chondrocytes using a chondrocyte-specific Cre mouse line, the mutant mice demonstrated an increase in bone mass [202]. By contrast, when the BMP signaling was attenuated by overexpression of *Noggin*, an antagonist for BMPs (BMP2, BMP4, BMP5, BMP6, and BMP7) [236], in chondrocytes, the mutant mice showed a decrease in bone mass [202]. Similarly, the loss of function of BMP signaling via BMPRIA in chondrocytes, which is a potent receptor for BMP2 and BMP4, demonstrated impairment of articular cartilage and growth plate cartilage, resulting in decreased bone size [86, 176, 227]. Mice deficient for *Bmpr1a* or *Bmpr1b* in chondrocytes can form intact cartilage during skeletal development, while double mutant embryos deficient for both *Bmpr1a* and *Bmpr1b* exhibit a severe defect in cartilage (i.e., chondrodysplasia) around embryonic day 12.5 (E12.5) to E16.5 [227]. These facts suggest a possible functional compensation mechanism between BMPRIA and BMPRIB in chondrocytes during early cartilage development in growth plates [227]. Mice deficient in *Acvr1* in chondrocytes using a *Col2-Cre*-driven conditional deletion are viable but exhibit defects in the development of cranial and axial structures [174]. The mutant mice exhibit shortened cranial base, and cervical vertebrae are hypoplastic. Unlike compound mutant mice for *Bmpr1a* and *Bmpr1b*, compound mutant mice for *Acvr1* and *Bmpr1b* can develop cartilage primordia and subsequent bones through endochondral ossification [174], suggesting that BMP signaling through ACVR1 plays a relatively minor role compared with other type 1 receptors during chondrogenesis.

Recent study using *aggreCAN CreERT2-Cre* mice to conditionally disrupt *Bmpr1a* in chondrocytes demonstrated a severe reduction in bone length and bone mass in the mutant femur at the age of 1 month [86], indicating a more distinct role of BMPRIA in chondrocytes postnatally which is not redundant with other receptors. Note that cell proliferation assessed by BrdU incorporation was strikingly reduced in the mutant mice at 2 weeks of age, which may reduce the size of cartilaginous foundation during the process of endochondral bone formation, leading finally to reduced bone length and mass. Taken together, these facts strongly demonstrate that BMP signal in chondrocytes plays a positive and potent role in regulating bone mass.

2.3.3 BMP and Mesenchymal Cells

Similar to chondrocytes, BMP signaling in mesenchymal cells contributes to an increase in bone mass (Table 2). A mesenchymal cell-specific Cre mouse line, *Prx1-Cre*, is used for these studies since Cre is active in mesenchymal cells as early as E9.5 in this line [128]. The simultaneous disruption of *Bmp2* and *Bmp4* in mesenchymal cells resulted in impairment of osteogenesis with reduced bone size [11]. Disruption of *Bmp2* in mesenchymal cells impaired the initiation of fracture healing, presumably due to a defect in endochondral bone formation after a bone

fracture, in which chondrocytes derived from mesenchymal cells play an important role [201]. These facts demonstrate the necessity of BMP signaling in mesenchymal cells for proper bone mass during development and remodeling. Recently, the role of type 2 receptor, BMPRII, in the skeleton was investigated using the *Prx1-Cre* mouse line. The resulting mutant mice are expectedly normal probably due to the compensation mechanism by other type 2 receptors, ACVR2A and ACVR2B, suggesting BMPRII is not required for endochondral ossification in the limb [54]. The same group further investigated the mutant mice and found increased bone mass at 2 months after birth [130]. While BMP signal is unchanged, activin signal is impaired in mutant mice, leading to increased osteoblast activity. This study raises the possibility that type 2 receptor segregation and/or competition could be a generalized mechanism by which BMP and activin signaling interact.

2.3.4 BMP and Osteoclasts

A putative coupling theory in bone metabolism states that in general, bone anabolism is locally induced by bone catabolism [71]. Osteoblasts control bone resorption by expressing RANK ligand (RANKL) and its decoy receptor, osteoprotegerin (OPG) [112, 187]. BMPs induce osteoclastogenesis via the RANKL-OPG pathway in an osteoblast-dependent manner. Exogenous treatment of BMP2 in vitro induces osteoclastogenesis by upregulating RANKL while treatment with BMP antagonist Noggin blocks osteoclastogenesis [1, 80, 159, 163]. In vivo studies using genetically engineered mutant mice demonstrated similar results (Table 2). Gain of function of BMP signaling by *Bmp4* overexpression in osteoblasts results in an increase of osteoclastogenesis and reduced bone mass [158]. In contrast, the loss of function of BMP signaling by disruption of *Bmpr1a* or *Noggin* overexpression results in reduction of osteoclastogenesis, leading to an increase of bone mass [145, 158] due to a decrease in the RANKL-OPG ratio [94, 95]. Taken together, these facts indicate that BMP signal has an indirect positive role in osteoclast function through osteoblast as a secondary effect. In a nonhuman primate bone defect model, treatment with BMP2 increases the size of the defect in association with increased osteoclast number and bone resorption, which is followed by bone formation [182].

In addition, it is also possible that BMPs directly control osteoclasts since *Bmp2* and its receptor *Bmpr1a* both are expressed in osteoclasts [55, 98]. When BMP signaling through BMPRII is conditionally ablated in osteoclasts using a *cathepsin K* promoter (*CtsK*) to drive Cre, bone mass increased in association with reduced osteoclast number in the bone as expected [157] (Table 2). Interestingly, both bone formation rate and osteoblast number assessed by bone histomorphometric analysis are greater in the mutant mice compared to their control littermates. This evidence suggests a possibility that BMPRII signaling in osteoclasts negatively regulates osteoblast function through its downstream target genes within osteoclasts.

Several recent reports have emerged revealing factors secreted by osteoclasts such as sphingosine-1-phosphate regulate osteogenesis [164]. It is an interesting future direction how BMP signaling involves osteoclast-mediated osteoblast differentiation.

2.3.5 BMP and Other Cell Types in Skeletal System

Angiogenesis is another necessary step in new bone formation in skeletal development as well as in bone remodeling after fracture [31, 97]. Both BMP2 and BMP7 are known to induce angiogenesis by associating with other growth factors such as VEGF (vascular endothelial growth factor), bFGF (basic fibroblast growth factor), and TGF- β 1 [40]. Overexpression of BMP9 in muscle induces heterotopic bone formation similar to BMP2 [34, 166]. As BMP9 is abundantly expressed in endothelial cells that are a primary cell type for angiogenesis [38], it is possible that BMP signaling in endothelial cells synergizes anabolic bone formation. The mechanism and origin of precursor cells for heterotopic bone formation, which is pathologically observed in fibrodysplasia ossificans progressiva (FOP) patients, is under investigation [96, 129, 229]. Taken together, the fact that BMPs implanted subcutaneously induce ectopic bone and increase bone mass [204] is likely due to the primary effects of BMP signaling on cells that are positive regulators for bone mass, including mesenchymal cells, chondrocytes, and endothelial cells (Table 3).

The current application of BMP therapy via systemic and local treatment can affect multiple cell types simultaneously in bone tissue including mesenchymal cells, chondrocytes, osteocytes, osteoblasts, osteoclasts, and endothelial cells, because typically a BMP2-soaked collagen sponge is applied around bone defects in orthopedic surgeries and the BMP2 diffuses to other tissues around the bone. Thus, it is important to evaluate the effects of BMPs on more than just osteoblasts. In addition to these cell types, we recently investigated the effects of high-dose BMP2 on periosteum and found that high concentration of BMP2 can reduce cell proliferation and increase apoptosis via DKK1 and SOST by inhibiting Wnt activity in human primary periosteal cells [102]. Interestingly, a lower concentration of BMP2 (i.e., 50–200 ng/ml) shows a trend of decreased caspase activity which is

Table 3 A variety of cell types possibly affected by BMP therapy in the bone

Cell types that can increase bone mass	Cell types that can reduce bone mass
Mesenchymal cells	Osteoclasts
Chondrocytes	Osteoblasts · osteocytes ^a
Osteoblasts · osteocytes	Periosteal cells
Endothelial cells	

^aNote that both osteoblast and osteocyte may have an indirect effect on bone mass through osteoclast activation via RANKL

opposite to the effect of higher concentrations of BMP2 (500–2000 ng/ml) that shows an increased caspase activity, suggesting a “biphasic nature” of BMP2 depending on its concentration. Note that BMP2 belongs to the TGF-beta superfamily and TGF-beta also has biphasic effects in a concentration-dependent manner with distinct molecular mechanisms [218]. This study is clinically significant because BMP2 is generally applied around the periosteum in orthopedic surgeries for fracture repair and spinal fusion and, therefore, it is important to delineate the effects of the BMP2 concentration on human periosteum-derived cells. In addition, the BMP2 concentration of clinical applications is extremely high (i.e., 1.5 mg/ml [InFUSE Bone Graft/LT-CAGE Lumbar Tapered Fusion Device. Summary of safety and effective data premarket approval application P000058, 2002, US Food and Drug Administration, Silver Spring, MD, <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cftopic/pma/pma.cfm?num=P000058>]), compared with the BMP2 concentration of cell basis studies (i.e., 0~300 ng/ml) as described before. It is possible that the negative role of BMP2 on cell proliferation leads to a reduction in bone mass because the cell proliferation is an initial phase prior to the cell differentiation phase that is required for new bone formation (Table 3).

The potential effects of BMP signal on mesenchymal cells, chondrocytes, and osteoblasts have been discussed. It is possible that chondrocytes or mesenchymal cells increase bone mass by responding to BMPs while osteoblasts or osteocytes reduce net bone mass (Fig. 2). This possibility supports a physiological role of BMPs in endogenous bone formation and remodeling, while the current view that BMPs enhances bone formation reflects a pharmacological role. Apparently, BMP signal has a different function depending on each context (i.e., endogenous vs. exogenous, low dose vs. high dose, chondrocyte vs. osteoblast).

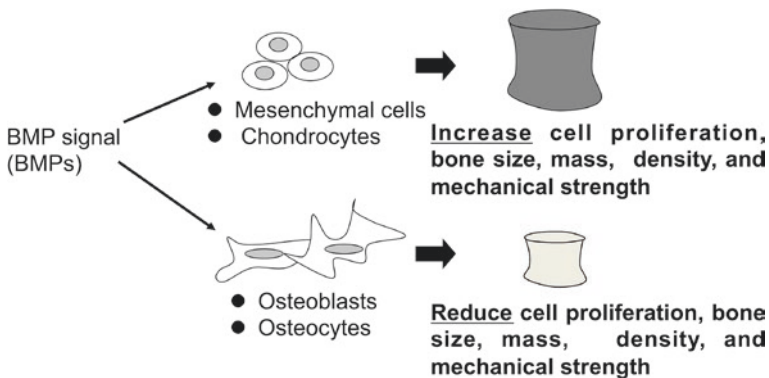


Fig. 2 Possible effects of BMP signal induced by BMPs on mesenchymal cells, chondrocytes, osteoblasts, and osteocytes. Based on the recent progresses shown in Tables 2 and 3, it is possible that BMP signaling in chondrocytes or mesenchymal cells can function to increase cell proliferation, bone size, mass, density, and mechanical strength while BMP signaling in osteoblasts or osteocytes may have opposite outcomes through regulating balance between bone formation and resorption

Table 4 Skeletal abnormalities associated with the molecules in BMP signaling

Gene	Disease	Ref.
BMP2 regulatory element	Brachydactyly type A2	[37]
BMP4	Poly/syndactyly	[8]
CDMP1/GDF5	Acromesomelic chondrodysplasia	[198]
	Brachydactyly type A1	[41]
	Brachydactyly type C	[168]
GDF6	Hemi-vertebrae, polydactyly, Klippel–Feil, rib malformation, spondylothoracic dysostosis	[5]
GDF3	Scoliosis, Klippel–Feil, vertebral fusion	[225]
SOST	Scleroosteosis	[9, 30]
	Van Buchem disease	[10, 191]
BMPR1B	Brachydactyly type A2	[116]
	Acromesomelic chondrodysplasia	[42]
ALK2	Fibrodysplasia ossificans progressiva	[185]
NOGGIN	Brachydactyly type B	[115]

2.4 BMP and Bone-Related Diseases

Studies of human mutations also suggest the importance of BMP signaling for skeletogenesis and bone-related diseases such as chondrodysplasia and fibrodysplasia ossificans progressiva [185, 198]. Mutations in genes involving BMP signaling associated with skeletal abnormalities in humans are summarized in Table 4 [5, 8–10, 30, 37, 41, 42, 115, 116, 168, 191, 225]. While the association of each molecule with its skeletal abnormality is known (Table 4), precise molecular mechanisms including tissue source and cell type responsible for the pathogenesis are still under investigation.

3 Craniofacial Development

3.1 Head Induction

Soon after implantation and before gastrulation, one group of cells formed at the distal tip of the visceral endoderm moves along one direction to form the anterior visceral endoderm (AVE). The AVE acts as a signaling center to instruct underneath epiblast (embryonic ectoderm) to form the future head [103, 193]. Nodal signaling plays a critical role for migration of the AVE [44, 222]. BMP signaling mediated by BMPR1A is critical to orient migration of the AVE [147, 221]. Similarly, BMPR1A signaling in epiblast regulates functions in the AVE for head induction [39]. The loss of *Bmp4* and *Bmp2* affect normal head formation; however, usage of different

receptors in this context is not fully understood [32, 216, 233]. These facts suggest that BMP signaling is critical for induction of the head structure around the gastrulation stage and causes of some of craniofacial abnormalities may be traced back to such early stages.

3.2 Facial Development and Abnormalities

Fetuses (by the end of 5 weeks for humans and at 10.5 days of mice) develop the frontonasal prominence (FNP) [154] (Fig. 3). Neural crest cells (NCCs) formed at the dorsal ectodermal midline in vertebrate embryos migrate laterally and ventrally on all axial levels [24]. Cranial neural crest cells (CNCCs) migrate into the FNP and branchial arches and differentiate to most of the facial tissues. The FNP further splits into four processes, a pair of the medial nasal process and a pair of the lateral nasal processes [195, 200] (Fig. 3). The maxillary and mandibular processes are derived from first branchial arch. The face is formed by fusion of these primordial structures, namely, four processes developed from the FNP and the paired maxillary and mandibular processes. Fusion of the two medial nasal processes at the midline provides the continuity of the nose, the middle upper lip, and the primary palate. Fusion of the medial nasal and maxillary prominences provides the continuity of the upper lip and jaw.

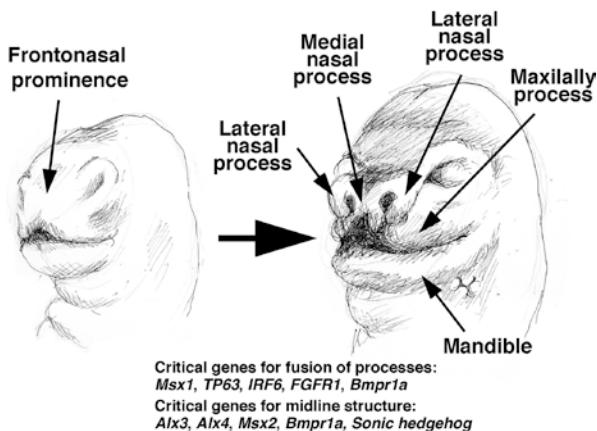


Fig. 3 Facial development and fusion of facial processes. During early facial development, pairs of the medial nasal processes and the lateral nasal processes are developed from the frontonasal prominences. Pairs of the maxillary process and the mandibular processes are developed from the first and second pharyngeal arches, respectively. Failure of the fusion of these processes causes facial clefts as detailed in the text. BMPs and related molecules play a critical role in the fusion process

3.2.1 BMP and Cleft Lip

Failure of fusions of any processes will develop facial cleft. For examples, failure of fusion between the medial nasal and the maxillary processes results in uni- or bilateral cleft lip and that between the lateral nasal and the maxillary processes results in oblique facial cleft. The fusion of these processes is critical for formation of the lip and the alveolar ridge in the primary palate. Following closure of the primary palate, closure of the secondary palate takes place by elevation of the palatal shelves. In some cases, these facial clefts occur alone (cleft lip without cleft palate), while other cases, these clefts accompany cleft palate (cleft lip with cleft palate) [45]. Studies in human genetics and animal models reveal several genes involved in development of cleft lips such as mutations in *MSX1*, tumor protein 63 (*TP63*), interferon regulatory factor 6 (*IRF6*), and fibroblast growth factor receptor 1 (*FGFR1*) [27, 28, 45]. Since *MSX1* is one of the established downstream targets of BMP signaling, involvement of BMP signaling during fusion process for lip formation has been speculated. Disruption of *Bmpr1a* in a dental epithelial-specific manner using *Nestin-Cre* results in bilateral cleft lip in association with increased apoptosis in the medial nasal processes [126] (Fig. 3).

3.2.2 BMP, Facial Cleft, and Midline Structure

Craniofacial syndromes that include median facial cleft are believed to be caused by dysplasia of the frontonasal prominence [181]. When the fusion between left and right medial nasal processes fails, that likely results in midface clefting [23]. In humans, it is reported that mutations in aristaless-related homeobox transcription factor 3 and 4 (*Alx3* and *Alx4*) are identified in frontonasal dysplasia patients (FND OMIM ID, 136760; FND2 OMIM ID, 613451) [22, 203, 205]. FND is characterized by hypertelorism, severely depressed nasal bridge and ridge, and bifid nasal tip. In the mouse, similar phenotypes are seen in *Alx3/Alx4* or *Alx1/Alx4* compound mutant mice [23, 169]. A significant increase of apoptosis is detected in the outgrowing frontonasal prominence at E10, which is proposed to be the underlying cause of the subsequent nasal cleft [23]. Potential involvement of BMP signaling in FND is poorly understood. However, it is reported that a gain-of-function mutation in *Msx2* causes midface clefting [217]. Neural crest-specific expression of *caBmpr1a* results in short nasal septum due to increased cell death [67] (Fig. 3). The amount of Hedgehog signaling is known to be strongly associated with alterations in midline facial structures [29, 231]. Since BMP signaling and Hedgehog signaling regulate each other in highly context-dependent manner, it is possible to speculate that BMP signaling may also play a critical role in midline development and failure of precise control of signaling activity may result in medial facial cleft and FND.

There are several evidences indicating that increased BMP signaling leads to a reduction or loss of the midline structure. Noggin mutant mice develop a microform of holoprosencephaly (HPE) [113]. The fact that compound mutations of Noggin and Chordin results in variable forms of HPE [104] suggests that levels of BMP

signaling are associated with severity of HPE. It is reported that BMP ligands interact with NODAL, another TGF-beta superfamily ligand, and it is possible that increased availability of BMP ligands because of the loss of their binding antagonists (Noggin and Chordin) secondarily influences NODAL signaling activity that plays a critical role in head formation soon after gastrulation [223, 224]. Alternatively, but not exclusively, it is also possible to speculate that increased BMP signaling activity may suppress Hedgehog activity. In tooth development, BMP signaling has been shown to negatively regulate Hedgehog signaling activity [117]. It is reported that disruption of *Shh* in mice results in holoprosencephaly and cyclopia [35] and mutations in *SHH* in human are associated with holoprosencephaly [20, 175, 190]. Thus, there exists a possibility of cross talk with increased BMP signaling activity suppressing Hedgehog signaling leading to midline hypoplasia.

3.2.3 BMP and Cleft Palate

During palatogenesis, first a pair of palatal shelves is formed downward around 7 weeks of gestation in humans and E11.5 in mice with interposition of the tongue. Fetal growth allows downward movement of the tongue to reorient palatal shelves to medial direction around 8 weeks in human and E13–14 in mice. These shelves grow, come closer, and then fuse to separate the oral and nasal cavity by 9 weeks in human and E15.5 in mice [45, 107] (Fig. 4). Thus, failure of fetal growth, movement of tongue reorientation of the palatal shelves, and/or growth of the palatal shelves may result in cleft palate. The final step of palatogenesis is dissolution of the medial edge epithelium (MEE) likely due to the cell death of this population. Persistence of the MEE results in submucosal cleft, i.e., the soft tissue has fused, while underlying palatal bone and muscle layer remain unfused.

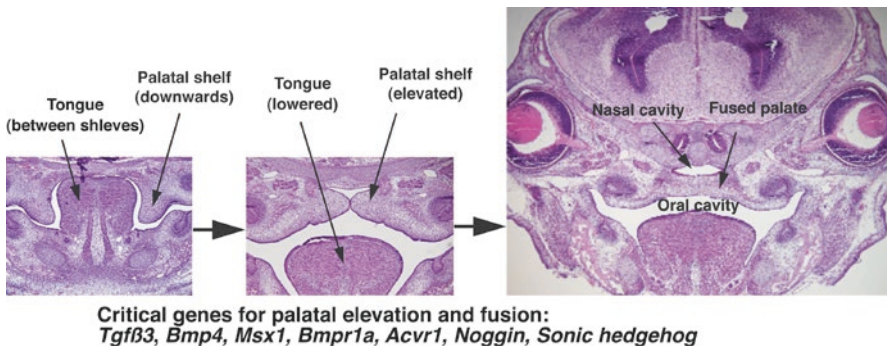


Fig. 4 Development of the palatal shelf and formation of the secondary palate. During mid-gestation, a pair of palatal shelves are formed from the mandibular processes and grow downwards. Along with the growth of the mandibular, the position of the tongue lowers allowing the shelves elevate. The elevated shelves further grow to reach each other and then fuse together to form the secondary palate. The primary palate is formed anterior to the secondary palate as a derivative of the medial nasal process and the frontonasal prominence

Mice homozygous for *Tgfb3* null mutation develop cleft palate demonstrating for the first time that TGF β superfamily signaling plays a critical role in palatogenesis [89]. Tissue-specific inactivation of *Tgfb1*, a type 1 receptor for TGF β , using *Wnt1-Cre* or *K14-Cre* also results in cleft palate [46, 47]. Involvement of BMP signaling in palatogenesis was initially suggested in a retinoic acid-induced cleft palate model [74, 131], where pathogenesis coincided with downregulation of BMP ligands such as *Bmp2*, *Bmp3*, *Bmp4*, *Bmp5*, and *Bmp7*. *Msx1*-null mice also develop cleft palate [180]. *Msx1* is expressed in mesenchymal tissues in anterior palatal shelves, and the loss of *Msx1* results in downregulation of *Bmp4* [4, 235]. Detailed analyses suggest that in the palatal shelves, BMP4 induces *Shh* expression that in turn induces *Bmp2* expression that positively regulates cell proliferation [4].

Neural crest-specific disruption of *Acvr1*, one of the type 1 receptors for BMPs, results in cleft palate along with multiple craniofacial defects including a hypomorphic mandible [48]. Neural crest-specific disruption of *Bmpr1a* results in mid-gestation lethality due to cardiac malfunctions [156, 192]. When the said cardiac malfunction is compensated by administration of isoproterenol, a beta-adrenergic agonist, the mutant embryos can survive until term and develop reduced projection of facial structures [148] and cleft palate [119]. In addition to the cleft lip mentioned above, deletion of *Bmpr1a* using Nestin-Cre resulted in cleft palate [126]. However, deletion of *Bmpr1a* in a neural crest-specific manner using *Wnt1-Cre* resulted in anterior clefting only [119], suggesting that BMP signaling mediated by ACVR1 and BMPR1A positively regulates proliferation of the cells in the anterior palatal shelf mesenchyme.

A BMP antagonist Noggin is highly expressed in the palatal shelf epithelium [138]. Disruption of Noggin results in cleft palate [70] suggesting that increased BMP signaling activity also affect normal palatogenesis. In the anterior regions of the secondary palate, the loss of *Noggin* results in upregulation of *Bmp2* expression leading to an increase of cell proliferation. In the posterior regions of the secondary palate, in contrast, the loss of *Noggin* induces ectopic expression of *Tgfb3* that coincides to ectopic fusion of palatal shelves to epithelia of the oral cavity and tongue [70]. Expression of a constitutively active form of *Bmpr1a* in the oral epithelium also leads to the similar phenotype [70]. Taken together, these facts suggest that suppression of BMP signaling is critical to prevent premature or ectopic fusions of palatal shelves to maintain structural integrity within the oral cavity. In contrast, expression of a constitutively active form of *Acvr1* in the oral epithelium using K14-Cre results in submucosal cleft in association with a reduced cell death in the MEE [155]. These results might suggest that BMP signaling mediated by different receptors plays distinct roles during palatogenesis. Further investigation is required to address this exciting hypothesis.

3.3 Calvarial Vault and Cranial Base

Mammalian craniofacial skeleton consists of a little more than 20 bones. Bones comprising the cranial vault are generated through intramembranous ossification. In contrast, bones in cranial base are generated through endochondral ossification. The

majority of cranial bones and cartilage residing in the anterior part of the head are derived from cranial neural crest cells (CNCCs), whereas the posterior part of elements is from paraxial mesoderm [137, 144, 177, 189, 212]. BMP signaling components are highly expressed in the migrating cranial neural crest cells and later in the cranial cartilage and bone [135]. These reports suggest that BMP signaling regulates skeletal development by organizing neural crest cell proliferation and cell death [36]. Both CNCC-derived and paraxial mesoderm-derived osteoprogenitor cells undergo intramembranous ossification to generate corresponding skull elements. Interestingly, osteoblasts from neural crest-derived bones show a higher level of activation of FGF signaling pathways compared with osteoblasts from paraxial mesoderm-derived bones [121, 170]. Osteoblasts from neural crest-derived bones also show lower apoptotic response when stimulated by TGF β signaling [120]. Regenerative ability of skull defects in the frontal bone is higher than that in parietal bones [18]. Taken together, these results suggest that neural crest-derived bones are more proliferative and less apoptotic than paraxial-derived bones due to enhanced signaling of FGF, BMP, and Wnt signaling pathways with a reduction in the TGF-beta pathway [184].

Sutures are a fibrous connective tissue found between bones in the cranial vault and cranial base. Sutures are critical growth sites in the skull. Mesenchymal cells proliferate and differentiate into osteoblasts that deposit collagen fibers and minerals to the bony plates to increase their size. Genetic studies in mice demonstrate that nasal and metopic sutures, which connect nasal bones and frontal bones, are of neural crest origin [83]. Coronal sutures are of mesodermal origin and are formed between the neural crest-derived frontal bones and the mesoderm-derived parietal bones. The sagittal suture is formed between the two mesoderm-derived parietal bones and is of neural crest origin. Since sutures are critical for growth of the skull, premature fusion of sutures results in cessation of skull growth at the site of fusion causing a pathological condition called craniosynostosis resulting in increased intracranial pressure and skull deformity [144, 149, 173].

3.3.1 BMP and Skull Formation

BMP signaling alters the homeobox *Msx* genes which are important for normal skull development [15, 21, 140]. A conventional gain-of-function mutation in *Msx2* results in skeletal defects such as mandibular hypoplasia and aplasia of interparietal bone [217]. BMP signaling plays crucial roles in regulation of cranial suture morphogenesis [101]. BMP signaling components such as *Bmp2*, *Bmp4*, *Msx1*, and *Msx2* are expressed in sagittal suture during its development [101]. Local application of BMP4 protein into mouse calvarial explants induces expression of *Msx* genes and obliteration of the mid-sutural space [101], which is probably through a BMP-responsive element located proximal to the *Mxs2* promoter [12]. Both *Msx1* and *Msx2* mutant mice develop persistent calvarial foramina [78, 179, 180]. Compound heterozygous mutant mice for *Msx1* and *Msx2* lack formation of the frontal and parietal bones [78]. These results suggest that BMP signaling plays a critical role through expression of *Msx1* and *Msx2* on osteoblast differentiation for normal skull vault formation.

3.3.2 BMP and Suture Formation

Fibroblast growth factor (FGF) family is known to play critical role during facial development and cranial vault formation [65, 142, 144]. Gain-of-function mutations in FGF signaling are known to cause some types of craniosynostosis [162, 213]. For example, two missense mutations (S252W and P253R) have been found in the IgII-LgIII linker region of FGFR2 and are associated with Apert syndrome [149, 162]. Gain-of-function mutations in *MSX2* also result in Boston-type craniosynostosis in human (OMIM ID: 604757) by inducing premature fusion in cranial sutures [82]. Noggin is present in postnatal sutures, and its expression is under negative regulation of FGF signaling. *Fgf* gain-of-function mutations in syndromic forms of craniosynostosis might inappropriately reduce Noggin expression such that the suture loses its patency [211]. Direct involvement of BMP signaling in skull deformity and craniosynostosis was recently demonstrated. Enhanced BMP signaling through constitutively active form of *Bmpr1a* (*caBmpr1a*) in neural crest cells results in craniosynostosis through premature fusion of the anterior frontal suture in mice [95, 105]. Increased BMP signaling in neural crest cells also leads to craniofacial skeletal defects. Constitutive activation of *Bmpr1a* in neural crest cell lineage using *P0-Cre* or *Wnt1-Cre* leads to increased level of cell death in skeletal primordia. These mutant mice exhibited bone and cartilage defects of nasomaxillary complex such as nasal bone and nasal septum [60, 67, 105].

In contrasting to neural crest-specific augmentation of BMP signaling activity, osteoblast-specific augmentation of BMPRI1A signaling does not cause overt skull deformity [105]. Increased apoptosis is found in the skull vault in this animal model, and the skull deformity is rescued by prevention of cell death by inhibition of p53 together suggesting that augmented BMP signaling increases p53-dependent cell death resulting in depletion of osteogenic progenitor cells leading to premature suture fusion [67, 105]. This is an interesting finding since it is believed that premature fusion of cranial sutures is a result of increased bone formation within the cranial suture [52].

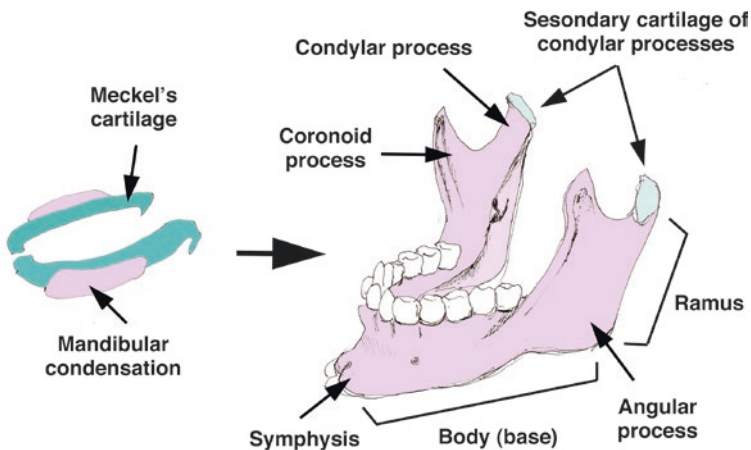
In the craniosynostosis mouse model caused by neural crest-specific augmentation of BMPRI1A signaling, it is shown that only a small increase in BMP signaling (50 %) is enough to result in skull deformity and craniosynostosis [105]. It is reasonable to speculate that several folds of changes in BMP signaling may result in early embryonic lethality [53, 75]. Recent genome-wide association studies find single-nucleotide polymorphisms (SNPs) located in proximity to BMP-related genes that are associated with skull morphology such as sagittal suture craniosynostosis [88]. Direct connection between mutations in BMP-related genes and human skull deformity has not been demonstrated; however, gain-of-function mutation in *MSX2*, a known downstream target gene of BMP signaling, results in Boston-type craniosynostosis as mentioned earlier [82]. Suture mesenchymal cells isolated from craniosynostosis patients show mutations in glypican-1 and glypican-3 (*GPC1* and *GPC3*) that negatively regulate BMP signaling [50]. It is possible to speculate that SNPs found in proximity of *BMP2* may alter enhancer activity to increase BMP signaling in the sagittal suture [88]. Taken together, these circumstantial evidences imply that some human cases may be caused by augmented BMP signaling in suture mesenchymal cells.

3.3.3 BMP and Cranial Base

Unlike calvarial vault, bones in the calvarial base (ethmoid, presphenoid, and basisphenoid) are formed through endochondral ossification. Very little is known about involvement of BMP signaling in the calvarial base. Cartilage structures called synchondrosis connect between the bones in the skull base. The ethmoid bone and the basisphenoid bone are articulated to the frontal and the basioccipital bones, respectively, through synchondrosis. *Bmp2*, 3, 4, 5, and 6 are expressed in cranial base with a temporally dynamic manner [100]. Development of growth plates in synchondrosis are tightly regulated by SHH and FGF signaling like the ones in long bones [139, 228]. Expression of inhibitor of differentiation 2 (*Id2*) is regulated in part by BMP-Smad signaling. The mutant mice for *Id2* are born without overt abnormalities; however, they show a narrower hypertrophic zone in the synchondrosis postnatally [178].

3.4 Mandibular Development and Temporomandibular Joint Formation

The mandible that forms the lower jaw is unique among bones in the body because it is formed through both intramembranous ossification and endochondral ossification [161] (Fig. 5). The body (or base) of the mandible and the ramus undergo



Critical genes for mandibular growth:
Bmp2, Bmp4, Bmp7, Tak1, Bmpr1a, Acvr1

Fig. 5 Mandibular development. A pair of mandibular condensation occurs along with Meckel's cartilage that forms the body of mandibular. Another pair of condensation forms posteriorly to give rise ramus of mandibular that eventually fused with the body of the mandibular. Secondary cartilage is developed at the tip of the condylar process and participates formation of the temporomandibular joint

intramembranous ossification; however, processes from these bones such as condyle, coronoid, and symphysis undergo endochondral ossification. The body of the mandible forms along with the Meckel's cartilage; however, cells in the Meckel's cartilage do not contribute the body. The body and the ramus form separately then fused together [45]. Cartilage is formed between 10 and 14 weeks in the human fetus at the head of condyle, coronoid, and symphysis. These cartilages are called as secondary cartilage since the cartilage primordia for endochondral ossification are formed at 5 weeks. The endochondral bone growth driven by the condylar cartilage is the most significant contributor to mandibular growth. The condylar process articulates to the temporal bone to form the temporomandibular joint (TMJ) [72, 73].

3.4.1 BMP and Mandible

Bmp2 and *Bmp7* are expressed at early stages of the developing Meckel's cartilage, while *Noggin* expression persists and is continuous [210]. *Noggin*-deficient mice that result in increased pSmad1/pSmad5/pSmad9 develop a significantly thicker Meckel's cartilage that is later ossified instead of degenerating [210]. In contrast, the growth of Meckel's cartilage is reduced in *Bmp7*-deficient mice [108]. This animal model develops small mandible (micrognathia) leading to cleft palate since the palatal shelves can fuse when whole upper jaws are cultured in vitro [108, 237]. Similar skeletal defects are observed when *Tak1*, a downstream component critical for non-Smad signaling pathway, is disrupted in a neural crest-specific manner and the cleft palate phenotype is rescued when the whole upper jaws are cultured [230]. These suggest that compromised BMP signaling during mandibular development may be one of the causes of the Pierre Robin syndrome [196].

Neural crest-specific disruption of both *Bmp2* and *Bmp4* using *Wnt1*-Cre results in mandibular and cranial bone defects in mice [25]. Subsequent analyses demonstrate that BMP signaling is required for self-renewal of cranial neural crest cells, and thus the loss of BMP signaling results in micrognathia and enlarged frontal fontanelle phenotype [25]. Similar skeletal phenotypes are reported in neural crest-specific mutant mice for *Acvr1* [48]. In contrast, overexpression of *Bmp4* in neural crest cells leads to syngnathia, a rare human bony birth defect manifested by a bony connection between maxilla and mandible [69].

3.4.2 BMP and the Temporomandibular Joint

The temporomandibular joint (TMJ) forms between the condyle process and the temporal bone in the calvarial vault and plays a critical role in jaw movement during chewing and articulating sound while speaking. The secondary cartilage found in the TMJ is different from primary cartilages by the fact that cells in the prechondroblastic layer produce type 1 collagens rather than type 2 collagens [73]. Cells in the prechondroblastic layer are dual potent, i.e., they can differentiate into either

cartilage or bone depending on their mechanical environment [58, 133]. Direct transformation of chondrocytes in condylar cartilage into osteoblasts is recently demonstrated *in vivo* using a lineage tracing technique [87]. Genes affecting growth and differentiation of primary cartilages such as *Sox9*, *Shh*, and *Pthrp* play important roles in normal TMJ development [72, 84]. Neural crest-specific disruption of *Bmpr1a* results in malformation of TMJ including failure of articular disc separation from a hypoplastic condyle [60]. Similarly, cartilage-specific removal of *Bmpr1a* also develops chondrodysplastic phenotypes in TMJ, and mandibular condyle growth is significantly compromised [85]. In the global *Bmp7* mutant mice, the secondary cartilage does not form at the anterior end of the mandible (symphysis) [106]. In this animal model, condylar cartilage however seems to be developed suggesting that requirement of BMP signaling activity in the secondary cartilage may be different depending on anatomical sites.

4 Perspective and Conclusions

The current review elucidates how BMP signal has multifaceted functions in different cell types, ages, and anatomical sites of bones. Knowledge gained from studies on genetically altered animal models and human genetics demonstrates that functions of BMP signaling are highly context dependent and that alterations of BMP signaling in one tissue type secondarily affect behavior of other tissues. It is noteworthy that levels of BMP2 or BMP7 clinically used for fracture healing are very high compared with endogenous levels of BMPs. The functions of BMPs that we have learned from clinical applications may be better applied to understand pathogenesis of genetically induced and trauma-induced heterotopic ossifications [2, 3, 165, 185]. It is now an established concept that both bone mass and bone quality such as collagen cross-linking and mineral crystallinity are important factors contributing to biomechanical properties of bones [14, 81]. How BMP signaling influences bone quality in addition to bone mass in a physiological condition is an interesting future direction.

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