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Common mechanisms in development and disease: BMP signaling in craniofacial development

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Abstract

BMP signaling is one of the key pathways regulating craniofacial development. It is involved in the early pattering of the head, the development of cranial neural crest cells, and facial patterning. It regulates development of its mineralized structures, such as cranial bones, maxilla, mandible, palate, and teeth. Targeted mutations in the mouse have been instrumental to delineate the functional involvement of this signaling network in different aspects of craniofacial development. Gene polymorphisms and mutations in BMP pathway genes have been associated with various non-syndromic and syndromic human craniofacial malformations. The identification of intricate cellular interactions and underlying molecular pathways illustrate the importance of local fine-regulation of Bmp signaling to control proliferation, apoptosis, epithelial-mesenchymal interactions, and stem/progenitor differentiation during craniofacial development. Thus, BMP signaling contributes both to shape and functionality of our facial features. BMP signaling also regulates postnatal craniofacial growth and is associated with dental structures life-long. A more detailed understanding of BMP function in growth, homeostasis, and repair of postnatal craniofacial tissues will contribute to our ability to rationally manipulate this signaling network in the context of tissue engineering.

Keywords

Craniofacial development; craniofacial malformations; tooth morphogenesis; cleft palate; BMP signaling; gene targeting; congenital malformations

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1. Introduction

Our head is a complex structure, home to several specialized tissues. The majority of congenital malformations involve craniofacial tissues, and BMP signaling is one of the key signaling pathways regulating their development. This review considers BMP signaling in the context of early facial patterning and the subsequent development of several of its mineralized structures. The development of skull and face also occurs in relationship with the brain [1].

Holoprosencephaly (HPE), a common developmental disorder characterized by forebrain midline defects due to failure of its separation into two hemispheres, typically also causes facial midline defects. Gain of Mutations in the BMP signaling pathway can cause HPE [2, 3], reviewed in [4], but will not be considered further.

The first section of this review considers the development of the facial primordium and cranial neural crest (CNC) cells. CNC is a multipotent cell lineage that contributes to all facial tissues, including bone, cartilage, teeth, nerves, and connective tissue, amongst others. This and the section on craniofacial skeletogenesis illustrate how CNC cells are affected by mutations in the BMP pathway. The section on tooth development shows how epithelial-mesenchymal interactions during tooth morphogenesis are regulated by BMP signaling. The involvement of BMP signaling in tooth mineralization and tooth root development is less well understood, though important functions in the development of these structures are emerging.

The fourth section considers BMP signaling in the context of palate morphogenesis. Palate development relies on the coordinated development of several orofacial structures; encompassing palatal shelves themselves, but also tongue and mandible. The development of all these structures is regulated by BMP signaling. Several mutations in the BMP pathway cause cleft palate, a pathology resulting from disturbed palate morphogenesis.

The last sections place BMP signaling into context with common cellular and molecular mechanisms and provide an outlook for future research directions including tissue engineering.

2. BMP signaling in development of the cranial neural crest and facial primordium

Neural crest cells are derived from dorsal midline ectoderm of developing vertebrate embryos [5]. Neural crest cells migrate downwards beside the neural tube and laterally under the surface ectoderm at all axial levels. The cells formed at craniofacial levels migrate into the frontonasal process and pharyngeal arches, and give rise to nearly all tissues of the face and neck. After cranial neural crest cells have completed their migration, facial growth is dominated by regional growth centers and the final differentiation of tissues occurs. Several growth signaling pathways such as BMP, WNT, SHH, and FGF are known to play crucial roles for these events [6-8].

Improper neural crest cell development leads to numerous congenital birth defects, such as Treacher-Collins syndrome (TCS) and DiGeorge syndrome (DGS). TCS is autosomal dominant disorder characterized by abnormal craniofacial development such as micrognathia, hypoplastic zygomatic arches, microtia, and coloboma of the eyelid (OMIM ID:154500) [9-11]. DGS is usually sporadic and results from de novo deletion within chromosome 22. DGS is characterized by hypoplasia of the parathyroid glands, hypoplasia or aplasia of thymus gland, and cardiac malformations (OMIM ID:188400) [12, 13].

2.1 Embryonic craniofacial development

The face is formed by fusion or merger of the five identifiable primordial structures, the frontonasal prominence (FNP) and the paired maxillary and mandibular processes [14]. Fetuses develop the FNP by the end of 5 weeks in humans and at 10.5 days in mice. The frontal portion of the FNP forms the forehead. The FNP splits into the frontonasal process, a pair of the medial nasal process and a pair of the lateral nasal processes due to the invagination of an ectodermal placode (Fig. 1A). The expanding nasomedial limbs merge at the midline to form the primordium, which differentiates into the middle part of nose, philtrum, premaxilla, primary palate, and a portion of the nasal septum (Fig. 1B) [15]. The maxillary and mandibular processes are derived from first branchial arch (Fig. 1A) [16]. The paired maxillary processes expand medially toward each other and the nasomedial processes. This merger between maxillary processes and nasomedial process contributes to formation of the lip (Fig. 1B) [15].

2.2 BMP and facial patterning

BMP signaling components are important factors in the growth of facial processes. *Bmp2* and *Bmp4* are expressed in maxillary and mandibular processes [17]. *Bmp5* and *Bmp7* are also expressed in branchial arches at the early gastrulation stage [18]. *Bmpr1a* is nearly ubiquitously expressed in the mouse embryo including craniofacial region [19, 20]. *Acvr1* is expressed in the first two pharyngeal arches [21].

Alteration of these BMP signaling components leads to abnormal craniofacial development. Some of the *Bmp2* heterozygous mutants exhibit open neural tube defects at E9.5, while all *Bmp2* homozygous null mutants die at early embryonic stage [22, 23]. This neural tube phenotype is caused by p53-mediated apoptosis and administration of a p53 inhibitor partially rescues the phenotype in heterozygous mutants. *Bmp4* homozygous mutants die between embryonic day (E) 6.5 and 9.5 with abnormal head morphology [24]. Although homozygous *Bmp5* null mutants are viable and fertile [25], *Bmp5* and *Bmp7* double knockout mice have a reduced size of their branchial arches and die at E10.5 [18]. The reduced size of their forebrain and branchial arches is associated with an abnormal pattern of apoptotic cells in the cranial area (Fig. 1C).

Msx1 and *Msx2*, direct downstream targets of BMP signaling, are also expressed in developing facial processes including FNP, maxillary processes, and mandibular processes in chick embryos [26, 27]. Down-regulation of *Msx1* and *Msx2* by applying retinoic acid results in inhibition of upper beak outgrowth in chick [28].

2.3 BMP and fusion of facial processes

Cleft lip occurs because of a failure of fusion between the medial and lateral nasal processes and the maxillary processes. The fusion of these processes creates not only the lip but also the alveolar ridge during primary palate formation. Closure of the secondary palate by elevation of the palatal shelves follows that of the primary palate. That means an interference with lip closure can affect formation of the palate. About 60% of patient with cleft lip also have cleft palate (OMIM ID:119530). Cleft palate is explained in a subsequent section and this part of the review focuses on the cleft lip without cleft palate.

In humans, several genetic lesions are known to cause cleft lip such as mutations in *MSX1*, tumor protein 63 (*TP63*), interferon regulatory factor 6 (*IRF6*), and fibroblast growth factor receptor 1 (*FGFR1*) [29]. Animal studies have dissected the function of BMP signaling in cleft lip etiology. Dental epithelial-specific *Bmpr1a* knockout mice using *Nestin-Cre* exhibit elevated apoptosis in the fusing region of the mutant mesial nasal processes and bilateral cleft lips [30] (Fig. 1D).

The failure of the fusion between left and right medial nasal processes most probably leads to mid-facial clefting [31]. In human, mutation in aristaless-like (*ALX*) gene family is known to cause frontonasal dysplasia (FND) (OMIM ID:613451), characterized by hypertelorism, severely depressed nasal bridge and ridge, bifid nasal tip. Similar phenotypes are also seen in *Alx3/Alx4 or Alx1/Alx4* compound mutant mice [31, 32]. These mutants exhibit increased apoptotic cells in the outgrowing front nasal processes [31] (Fig. 1E). These reports suggest that *Alx* genes play an important role in the morphogenesis of craniofacial midline structures. Interestingly, cytogenetic location of *ALX4* is in 11p11.2 and the sequence contains the region homologous to the *MSX2* gene [33].

BMP signaling members also contribute to abnormal facial morphogenesis in the midline region. A gain-of function mutation in *Msx2* results in midfacial clefting [34]. Further, a gain-of-function mutation in *Bmpr1a* results in a short nasal septum and increased cell death in the nasal septal primordia [35]. These reports suggest that cell death might be affected by BMP signaling in developing facial processes. Further investigations are necessary to reveal the relationships between BMP signaling and apoptosis in craniofacial development.

3. Craniofacial skeletogenesis

The mammalian craniofacial skeleton consists of more than 20 bones and cartilages. The size and shape of those components are strictly determined. The craniofacial skeleton can be divided into 3 major elements such as calvaria, mandible, and nasomaxillary complex [36]. The majority of the cranial bones and cartilage are derived from cranial neural crest cells [37, 38]. Mandible, nasomaxillary complex, and the anterior cranial bones are derived from neural crest, whereas the posterior part is derived from paraxial mesoderm [39]. BMP signaling components along with other signaling molecules play crucial roles in morphogenesis of these elements [40, 41]. Importantly, BMP signaling components are highly expressed in the migrating cranial neural crest cells and later in cranial cartilage and bone [41]. These reports suggest that BMP signaling regulates skeletal development by organizing neural crest cell proliferation and cell death [40].

3.1 BMP and skull formation

BMP signaling alters the expression of *Msx* homeobox genes, TGFß family ligands, and growth and differentiation factors (*Gdfs*), which are all important for normal skull development [17, 42, 43]. A conventional gain-of-function mutation in *Msx2* results in skeletal defects such as mandibular hypoplasia and aplasia of the interparietal bone [34]. However, the pathogenetic mechanisms of those phenotypes are unknown. *Gdf5*, *6* and *7* were firstly identified in a screen for BMP related genes corresponding to brachypodism [44]. The *Gdf5* null mutant mouse exhibits a brachypodism phenotype, characterized by reduced long bone length in the limbs [44]. A mutation in *Gdf6* leads to absence of the coronal suture [45].

Increased BMP signaling in neural crest cells also leads to craniofacial skeletal defects. Constitutive activation of *Bmpr1a* in the neural crest cell linage using *P0-Cre* or *Wnt1-Cre* leads to increased cell death in skeletal primordia. These mutant mice exhibit bone and cartilage defects of the nasomaxillary complex, such as nasal bone and nasal septum [35, 46, 47].

During skull development, the cranial sutures serve as growth centers. Premature fusion of the sutures results in reduced skull growth leading to increased intracranial pressure that causes skull deformity termed craniosynostosis. BMP signaling plays crucial roles in the regulation of cranial suture morphogenesis [48]. BMP signaling components such as *Bmp2*, *Bmp4*, *Msx1*, and *Msx2* are expressed in the sagittal suture during its development [48]. Local application of BMP4 protein into mouse calvaria explants induced expression of *Msx* genes and led to obliteration of the mid sutural space [48]. Enhanced BMP signaling through a constitutively active form of BMPR1A results in induction of craniosynostosis [46]. Gain-of-function mutations in *MSX2* also result in Boston-type craniosynostosis in humans (OMIM ID:604757) [49]. Noggin is present in post-natal 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 [50].

3.2. BMP and mandibular development

In the early embryonic stage, the mandible develops adjacent to Meckel's cartilage, a first pharyngeal arch derivative. Meckel's cartilage is a transient structure providing early structural stability to the mandible prior to the development of the mandibular bone. *Bmp2* and *Bmp7* are expressed at early stages of the developing Meckel's cartilage; *Noggin* expression is continuous [51]. Noggin-deficient mice show a significantly thicker cartilage along with increased pSmad1/5/8 staining leading to ossification rather than degeneration of Meckel's cartilage [51]. *Bmp7*-deficient mice show reduced growth of Meckel's cartilage, which fails to form a symphysis at its tip [52]. The subsequent development of the mandible begins as a condensation of mesenchyme just lateral to Meckel's cartilage and proceeds entirely as an intramembranous bone formation. BMP signaling also play roles in mandibular morphogenesis. *Bmp7*-deficient mice have a shorter maxilla and mandible (micrognathia) [52]. *Bmp2*, and *Bmp4* conditional compound mutants using *Wnt1-Cre* exhibit mandibular and cranial bone defects in a dose dependent manner [53]. In this study,

Bmp2 deletion in the *Bmp4* conditional knockout background resulted in worsening of micrognathia and enlarged frontal fontanelle phenotype. These studies conclude that BMP signaling is required for self-renewal of cranial neural crest cells [53]. Similar skeletal phenotypes are seen in *Acvr1:Wnt1-Cre* conditional mutant embryos [21]. Directed *Bmp4* expression in neural crest cells leads to a bony fusion of the mandible with the maxilla, reminiscent of the rare human bony syngnathia birth defect [54].

4. BMP and tooth development

4.1 Tooth Morphogenesis

Mammalian tooth development is regulated by sequential and reciprocal epithelial-mesenchymal interactions. As shown in Fig. 2A, it is initiated by thickening of the dental lamina, a band of oral epithelial tissue, to form the dental placode. Tooth morphogenesis starts with its invagination into the underlying neural crest derived mesenchyme, leading to the bud stage. Condensation of the mesenchyme surrounding the bud leads to the cap stage, when the primary enamel knot appears, a signaling center thought to shape tooth morphology, and lateral epithelial protrusions develop. During the bell stage cell differentiation occurs and the first tooth hard tissue is being deposited. Secondary and tertiary enamel knots form to define the crown shape. Tooth mineralization begins late in embryogenesis and continues during root formation towards the end of the first postnatal week. In the mouse, teeth erupt by the second week concomitant with the development of the periodontal tissue that anchors the tooth to the alveolar bone. For a detailed recent review on tooth development refer to [55]. *Bmps* are expressed in all stages of tooth morphogenesis (dental placode, bud, cap and bell stages), and expression continuous during the secretory stages and root development [56-58], and remains prominent in molar teeth of adult mice (Fig. 2).

4.2 BMP signaling in tooth initiation and morphogenesis

BMP signaling regulates tooth formation from the earliest stages. Competition between BMP and Noggin regulates the size of the mouse incisor placode, which determines the number of incisors [59], and mice overexpressing Noggin in the oral epithelium lack mandibular molars [60, 61]. BMP4 is an important signal driving tooth morphogenesis from bud to cap transition. *In vitro* experiments established that Bmp4 regulates expression of *Msx1* [62, 63] and *Pax9* [64]. Mice lacking *Msx1* or *Pax9* arrest tooth developmental at the bud stage [64, 65]. Deletion of *Bmp4* from dental mesenchyme led altered expression of *Msx1* and *Pax9*, and to arrest of the mandibular molars at the bud stage [66]. Tooth agenesis and hypodontia are the most common tooth malformations in humans. Mutations in MSX1 or PAX9 are known to cause selective tooth agenesis of the second premolar and third molar in humans [67, 68]. Arrest of tooth development at the bud stage has also been observed in mice carrying an epithelial deletion of *Bmpr1a* [69], indicating the importance of mesenchyme-derived BMP signals to drive development of tooth epithelium. *Bmp2, Bmp4 and Bmp7* are expressed in the primary enamel knot, but their roles has not been addressed directly. Loss of the BMP antagonist Ectodin/Usag-1, which is regulated in cultured tooth explants by BMP2 and BM7 [70] results in supernumerary tooth formation [71]. BMP

signaling also regulates *Sox2*-positive dental epithelial stem cells in incisors and molars through Smad-dependent inhibition of the Shh-Gli1 axis [72, 73].

4.3 BMP and tooth mineralization

Differentiation of epithelium-derived ameloblasts (enamel forming cells) and mesenchyme-derived odontoblast (dentin forming cells) starts around the bell stage. *Bmp2, Bmp4, and Bmp7* have all been shown to strongly induce Ameloblastin expression, a protein associated with enamel mineralization [74]. Loss of Follistatin, a Bmp and Activin antagonist expressed in enamel-free lingual dental epithelium, resulted in ectopic enamel deposition, whereas it's overexpression inhibited ameloblast differentiation [74]. Deletion of *Bmp2* in odontoblasts (Osx-Cre) led to deregulated expression of *Amel* and *Enam*, two enamel matrix proteins, and *Klk4* and *Mmp20*, two enamel processing proteases [75]. In humans, mutations in *MMP20, KLK4* and *ENAM* are associated with Amelogenesis Imperfecta (AI) [76-81]. In AI enamel fails to form (hypoplastic form) or mature properly (hypocalcified, hypermatured forms) [82]. Bmp2 also regulates the differentiation of odontoblasts and thus dentin formation [83]. Though the molecular mechanism by which mesenchymal BMP2 regulates the maturation of epithelial ameloblasts has not been elucidated, these findings indicate that BMP-dependent epithelial-mesenchymal interactions also regulate the mineralization stage.

4.4 BMP and root formation

Differentiation of the root starts after birth in mice, is regulated by epithelial-mesenchymal interactions, and involves HERS (Hertwig's Epithelial Root Sheath) and the mesenchymal dental follicle and dental papilla cells. *Bmp2, Bmp3, Bmp4,* and *Bmp7* are expressed in the tooth root [57]. Inactivation of *Bmp2* in mesenchymal cells affects terminal differentiation of root and periodontal ligament [84]. Inactivation of *Smad4* in dental epithelial cells results in arrested molar root development [85]. Deletion of *Bmpr1a* from dental epithelium at the differentiation stage promoted differentiation of crown epithelium into cementum or root epithelial cells. [86]. Overexpression of *Noggin* in oral epithelium results in various phenotypic alterations including disrupted root development [60]. Together, these studies indicate that BMP signaling regulates epithelial-mesenchymal interactions during root development. In humans, mutations in the BMP antagonist *GREM2* are associated with microdontia, taurodontism and a short tooth root [87]. *Grem2* null mice have defects in maxillary and mandibular incisors, but no information on molar root development is available [88].

4.5 BMP in adult teeth

There is comparatively little information available on BMP involvement in development and of cementum and periodontal ligament, though several BMP ligands are expressed in these structures [57]. Inactivation of *Bmp2* in mesenchymal cells affects development of the periodontal ligament [84]. BMP signaling has also been associated with orthodontic tooth movement [89, 90]. As shown in Fig. 2B, *Bmp7, Gremlin1,* and *Twisted Gastrulation* are expressed in the root and pulp of 4-6 month old mouse teeth. In addition, distinct expression is also observed in several structures of the periodontium (gingiva, periodontal ligament, alveolar bone). Life-long persistence of the Bmp signaling network indicates its potential

involvement in alveolar bone remodeling in response to mastication forces and homeostasis of the gingival and periodontal tissues.

5. BMP signaling during palate morphogenesis

5.1 Palatogenesis

Palatogenesis is a highly regulated multi-step process that starts during the 6th embryonic week in humans and at E11.5 in the mouse, when the initial growth of a pair of palatal shelves at either side of maxillary processes can be observed. Palatal shelves mainly consist of neural crest mesenchyme derived from the first branchial arch encapsulated by a multi-layer of ectodermal epithelial cells. At the initial steps of palatal shelves development, the oral cavity is relatively small and the tongue is interposed between left and right palatal shelves forcing the early palatal shelves to grow downwards. With the expansion of the lower part of oral cavity and downward/forward movement of the tongue the palatal shelves re-orientate, grow in medial direction, and fuse separating oral and nasal cavity [91]. Reorientation, medial growth, and shelve apposition occur rapidly, between E14.0 to E15.0 in the mouse, and shelves fuse by E15.5, as part of which the medial edge epithelium is dissolved. Disturbances to growth, elevation, or fusion of the palatal shelves may result in cleft palate (Fig. 3). Failure of dissolution of the medial edge epithelium can result in sub-mucosal cleft, a situation where the soft tissue has fused, but underlying palatal bone and muscle layer remain separated. The mouse is a well-accepted model to study palate development, as many gene loci associated with cleft palate in humans when deleted result in cleft palate in the mouse [29, 92].

5.2 BMP and palatal shelve growth, elevation, and fusion

Early evidence for involvement of BMP signaling in palatal shelves growth came from work showing deregulated BMP expression in the retinoic acid-induced cleft palate model [93, 94] and *Msx1*-null mice [65]. *Msx1* expressed in the anterior mesenchyme during initial palatal shelves growth regulates expression of *Bmp4* [95, 96]. Loss of *Msx1* results in short palatal shelves associated with impaired cell proliferation, a phenotype that is rescued by transgenic expression of *Bmp4* [96]. *Bmp4* induces *Shh* expression, which in turn regulates *Bmp2*. *Bmp2* appears to be the primarily responsible factor regulating cell proliferation [96]. Differences in *Bmp2* expression correlate with differences in proliferation also in turtles and birds [97]. Deletion of *Bmpr1a* in the craniofacial primordium using Nestin-Cre results in cleft palate [30], whereas a neural crest-specific deletion using Wnt1-Cre causes anterior clefting only [98]. This supports the role of BMP signaling in regulating proliferation in the anterior palatal shelve mesenchyme (Fig 3A). *Noggin* is strongly expressed in palatal shelve epithelium [99] and loss of Noggin also results in cleft palate [100]. Within the anterior palate Noggin negatively affects *Bmp2* expression and hence cell proliferation. In the posterior region, loss of Noggin leads to spontaneous fusion of the palate epithelium with epithelia of oral cavity and tongue [100]. Fusion of palatal shelves at their midline is dependent on TGFß3. At the sites of palate-mandible fusion ectopic expression of *Tgfß3* is observed [100]. Expression of a constitutively active Bmpr1a in the oral epithelium also causes ectopic epithelial fusions [100]. Together, this implies that repression of Bmp

signaling in the oral epithelium is critical to maintain its integrity and prevent premature or ectopic fusion of palatal shelves (Fig. 3A).

5.3 BMP and cleft palate due to other factors

Pierre Robin Sequence stands for a series of developmental events encompassing micrognathia, glossoptosis, and cleft palate. It is thought that micrognathia interferes with the downward movement of the tongue, which in turn disturbs downward movement of the tongue, which is required for palatal shelves reorientation (OMIM ID:261800, [101], Fig 3B). Several loss-of-function mutations in the BMP signaling pathway molecules cause micrognathia [21, 52, 53]. As loss of *Bmp7* does neither affect expression of *Msx1* or *Bmp4*, nor does it alter proliferation or apoptosis in the palatal shelves [52], the cleft palate might be caused by a Pierre Robin Sequence. *Tak1*, a member of Smad-independent BMP pathway also plays crucial roles in craniofacial morphogenesis. *Tak1: Wnt1-Cre* conditional mutants have a cleft palate, likely the result of micrognathia and malformed tongue rather than changes in the palatal shelves [102, 103]. *Bmp11* mutants exhibit abnormal palate, but no detailed information is available [104]. BMP signaling is also involved in regulating maturation and ossification of the fused palate. A constitutively active mutation of *Acvr1* in oral epithelium causes a submucosal cleft palate in mice [105]. Enhanced BMP signaling resulted in altered proliferation and apoptosis in the medial edge epithelium leading to its persistence, thereby preventing fusion of palatal mesenchyme and muscle tissue (Fig. 3C).

5.4 BMP and cleft palate in humans

Cleft lip/cleft palate in humans has been associated with altered Bmp signaling. Mutations in the genes for transcription factor MSX1 [106] and gene polymorphisms in *BMP4* and *NOGGIN* identified by linkage analysis and GWAS have been associated with non-syndromic cleft lip/palate ([107] and references therein, [108-110]. *GREMLIN1* has also been associated with cleft palate, which was supported by detailed burden analysis [111]. Microdeletions of 20p12.3 or 14q22-23, which encompass the *BMP2* [112] or *BMP4* [113] loci respectively have been associated with syndromic forms of cleft palate. Mutations in *BMP7* cause a variety of craniofacial malformations including cleft and high-arched palate [114]. The burden is now to demonstrate how these genetic associations, polymorphisms, and mutations functionally alter Bmp signaling in the affected tissues and how this contributes to the observed morbidities.

6. Common mechanism and outlook

6.1 Fine tuning of Bmp signaling

BMP signaling fulfills many diverse functions during craniofacial development. It regulates tissue patterning and organ development, facial growth and morphology. Though many of these structures are mineralized structures, BMP signaling also regulates proliferation and apoptosis in stem and progenitor cells, cell polarization and migration, epithelial-mesenchymal interactions during organ development and differentiation. Craniofacial development is highly coordinated, and many structures develop concomitant and in dependence of each other, as evidenced during palate development. Both an increase and decrease in BMP signaling can affect the development of craniofacial structures, underlining

the importance of balanced and locally regulated Bmp signaling. Fine control of production of BMP ligands and BMP antagonists in a precise spatio-temporal manner appears to be critical for this. Several BMPs (e.g. *Bmp2*, *Bmp4*, *Bmp5*) are located in gene deserts that contain a large number of cis-regulatory elements for such tissue- and cell specific gene expression (reviewed in [115]). SNPs in *BMP4* associated with non-syndromic cleft palate might relate to changes in tissue-specific enhancers, in contrast to the gene deletion associated with syndromic cleft palate.

It is thus likely that the outcome of BMP signaling is highly context dependent and dosage dependent. Differences in gene dosage of *Bmp4* and *Noggin* affect the shape of the mouse mandible [116]. Gene dosage in *Noggin* affects susceptibility to arthritis [117], or, beyond mineralized structures, gene dosage in *Bmp4* affects vascular remodeling [118, 119]. Juvenile polyposis (OMIM ID: 174900) can be caused by mutations in *BMPR1A*, which are also associated with dysmorphic facial features. The fact that the craniosynostosis phenotypes caused by expression of the constitutively activated *Bmpr1a* can be rescued by heterozygous null mutation of *Bmpr1a* suggests that 50% increase of signaling activity is enough to cause morphological abnormalities [46]. It is thus possible that small differences in BMP signaling contribute to the variability of human craniofacial features as well as variations in tissue physiology or disease susceptibility.

6.2 BMP in the regulation of cell apoptosis

The complexity of BMP signaling is also reflected by the multiple ways it can regulate apoptosis. Apoptosis due to augmented BMP signaling is seen in several craniofacial structures [35, 120, 121]. Smad-dependent BMP signaling prevents degradation of p53, and increased levels of p53 augment downstream targets, such as Bax and caspase-3 to promote apoptosis. Bmp-mediated apoptosis occurs also via interference with the cyclin-dependent kinase inhibitor p21 (WAF1/CIP1). BMP2 and BMP4 enhance *p21* expression, which leads to cell cycle arrest and subsequent apoptosis, as shown in the tooth enamel knot [122]. P21 also regulates differentiation of osteoblasts [123], providing a possible link between tissue patterning (proliferation and apoptosis) and tissue differentiation (ossification). In contrast, dissolution of Meckel's cartilage, which in part is mediated via p53-dependent apoptosis [124], requires termination rather than active Bmp signaling [51].

6.3 BMP crosstalk with other signaling pathways

The BMP4-Shh-BMP2 signaling cascade associated with mesenchymal proliferation in the developing anterior palate is well documented [96, 98]. Interaction of BMP with Shh signaling is also seen in the regulation of the epithelial stem cell fate in the developing tooth [73] or the fusion of the facial primordia [125]. Crosstalk between BMP and Wnt/ß-catenin signaling has been demonstrated for early tooth development [61]. Despite the importance of Wnt signaling for craniofacial tissue development and homeostasis [126], this crosstalk has not been widely investigated. For comparison, BMP /Wnt crosstalk regulates stem/progenitor cell proliferation and differentiation in the intestinal crypt (reviewed in [127]) or the developing brain, where BMP signaling promotes Wnt-dependent cell proliferation via stabilization of Lef1 [128]. Bmp signaling regulates cytodifferentiation and epithelial-

mesenchymal interactions often in crosstalk with FGF and Notch signaling [129, 130]. The same pathways also regulate osteoblast differentiation and ossification (reviewed in [131]).

6.4 BMP signaling beyond tissue morphology

Most studies investigating BMP signaling in craniofacial development report on morphological features only (e.g. size and shape of mandible, nasal bone, skull bones), but do not investigate if these changes also affect the quality of the mineralized or non-mineralized structures. Bmp signaling controls tooth mineralization [75, 83], bone formation and homeostasis (reviewed in [132-134]). Thus, it is possible to speculate that BMP signaling may regulate quality of minerals such as crystallinity and mineral matrix ratio (MMR). On that basis it might be expected that morphological changes due to altered BMP signaling go along with changes in tissue characteristics such as alterations in collagen crosslinking. In addition to the growth factor signaling, mechanotransduction is another important mechanism by which tissue formation is regulated [135]. How BMP signaling and mechanotransduction pathways interact to regulate bone remodeling is an emerging field [136-138].

7. Summary and Outlook

BMP signaling not only regulates the embryonic development of craniofacial tissues, it also emerges as an important regulator of postnatal craniofacial tissues. BMPs are used in the clinic for bone healing applications, including regeneration of alveolar bone defects. Despite significant progress in the field, our understanding on how BMP signaling regulates tissue homeostasis or repair remains fragmented. Functional genomics studies in the mouse that have been so instrumental for revealing the importance of BMP signaling during embryonic development should therefore embrace adult tissues and their pathologies.

Systematic massive sequencing of human genomes is expected to reveal novel sequence variations in the BMP signaling pathway genes. If and how such differences will affect tissue morphology and function is difficult to predict, not least due to the complexity of the BMP signaling network itself, but also because of its extensive crosstalk to other signaling pathways, such as FGF, HH, WNT, and NOTCH signaling.

Possibly the most important lesson from all these studies is the importance of balanced BMP signaling and its local fine-regulation to organize and maintain tissues. This has important and direct implications for the application of recombinant BMPs or BMP antagonists for tissue engineering *in* situ, and their current rather general way of application is likely not to achieve the desired results. There are significant efforts under way to develop smart scaffolds and recombinant BMP anchored to specific structures. Their availability should clearly improve the prospects of successful clinical applications. As a last point, BMPs are emerging important immune regulators [139-141] and thus could also play a role in inflammation. Future studies will need to establish whether and how this affects tissue repair, potentially adding further complexity to this pathway.

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Highlights

- BMP signaling is one of the key pathways regulating craniofacial development.
- BMP signaling is involved in the early pattering of the head, the development of cranial neural crest cells, and facial patterning.
- BMP signaling regulates development of mineralized structures, such as cranial bones, maxilla, mandible, palate, and teeth.
- Targeted mutations in the mouse have been instrumental to delineate the functional involvement of BMP signaling in different aspects of craniofacial development.
- Gene polymorphisms and mutations in BMP pathway genes have been associated with various non-syndromic and syndromic human craniofacial malformations.
- Balanced BMP signaling and its local fine-regulation is critical to organize and maintain craniofacial tissues. This has direct implications for the development of clinical applications using recombinant BMPs or BMP antagonists for tissue engineering *in situ*.

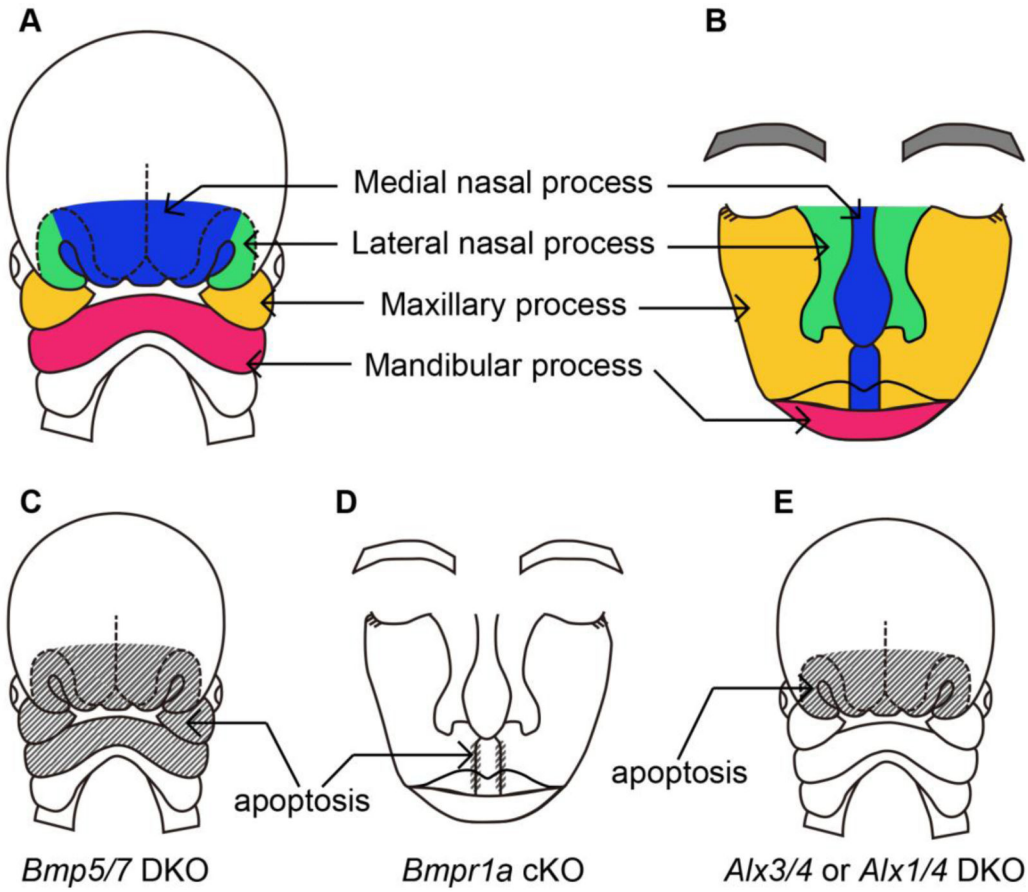

Figure 1.

Schematic representations of fusion of the facial processes. (A) A diagram of human craniofacial structures at 31 day after conception. (B) A diagram of the contribution of the embryonic facial processes to the structures of adult face. The medial nasal process contributes the central part of the nose and the philtrum. The lateral nasal processes form outer parts of the nose. Maxillary processes form lateral part of the upper lip and the cheeks. Lower lip is derived from mandibular processes. (C-E) Schematic representations of affected areas (stripe) and resulted phenotypes caused by mutations in each gene.

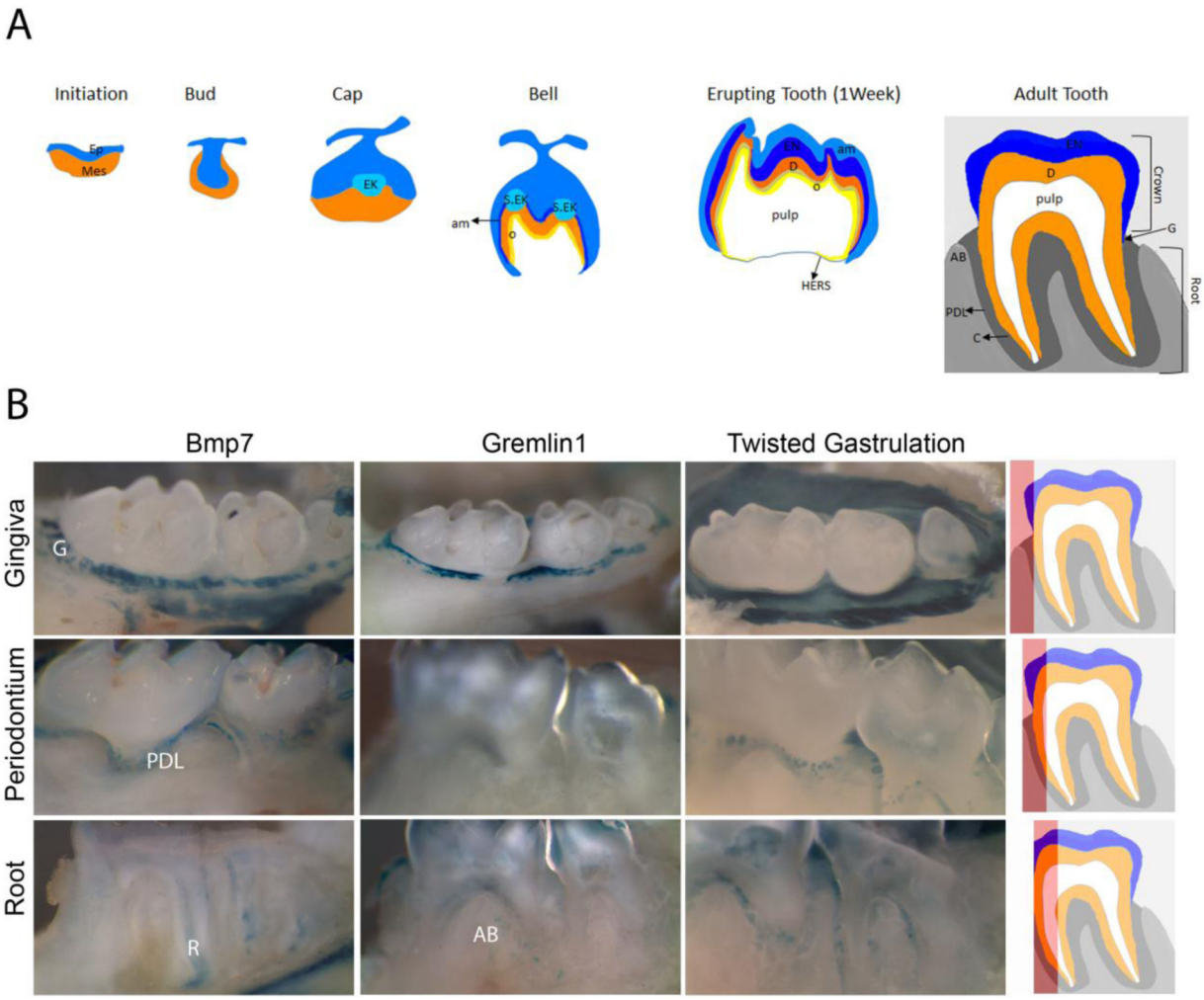

Figure 2.

BMP signaling in teeth. (A) Schematic representation of tooth morphogenesis and of an adult tooth. (B) Whole mount lacZ stained molar teeth from 4-6 month old mice. Scheme on the right hand side indicates visible area (red) when viewed from the left. Gingiva = unmanipulated tooth, Periodontium = gingival tissue resected, Root = Periodontium and tooth partly mechanically grounded off. Ep-Epithelium, Mes-Mesenchyme, EK-Enamel Knot, S.EK-Secondary Enamel Knot, o-odontoblasts, EN-Enamel, D-Dentin, am-Ameloblast, AB-Alveolar Bone, PDL-Periodontal Ligament, C-Cementum, G- Gingiva. Fig 2B adapted from [142]

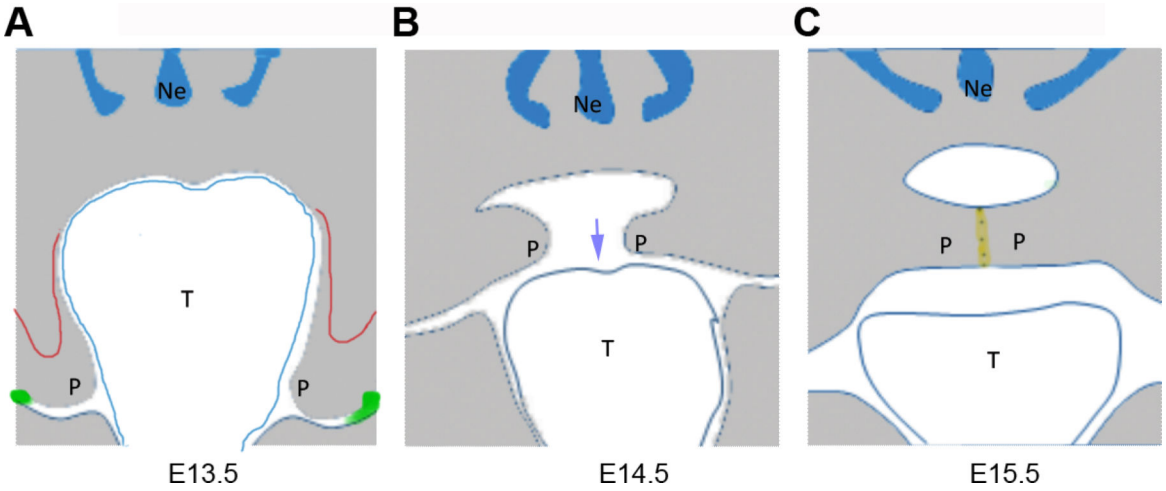

Figure 3.

Schematic representation of palate development and major developmental disturbances. (A) E13.5 palatal shelves. Red = small anterior shelve due to failure to grow, green = ectopic fusion of palate with oral epithelium. (B) E14.5 palatal shelves. Blue arrow indicates tongue contraction. (C) E15.5 palatal shelves. yellow = failure to remove midline epithelial seam cells

Table 1

BMP signaling mouse mutants associated with defects in tooth development: red = mutation in mesenchyme, blue = mutation in epithelia

	Mice	Defects	References
Bmp2	*SP7/Osx* -Cre Conditional KO *SP7/Osx*-Cre Conditional KO *Collal*-Cre Conditional KO	Root and periodontium development Enamel formation Odontoblast differentiation, delay in amelogenesis	[84] [75] [83]
Bmp4	*Wnt1*-Cre Conditional KO	Tooth arrest at bud stage	[66]
Bmp7	Null	Various craniofacial deformities with missing maxillary molars and defective mandible.	[58]
Follistatin	*K14* transgenic expression	Enamel patterning and ameloblast differentiation	[74]
Noggin	*K14* transgenic expression *K14* transgenic *expression*	Root/crown patterning defects Arrested tooth development at early bud stage.	[60] [61]
Grem2	Null	Incisors most severely affected with dentin and enamel defects.	[88]
Ectodin	Null	Supernumerary teeth	[71]
Bmpr1a	K14-Cre Conditional KO *(Krt5)-rtTA* inducible cond. KO at E14.5	Tooth arrest at bud stage Cementoblast like cells in crown of epithelia. Defects in differentiation of crown epithelia	[69] [86]