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TGF-β Signalling and Tooth Development

Xiao Feng HUANG[1], Yang CHAI[2]

Members of the transforming growth factor-β (TGF-β) superfamily are critical regulators that control cell proliferation, differentiation and apoptosis. TGF-β signalling also regulates the morphogenesis of many developing organs. The development of mouse tooth germ, which is a good model for organogenesis, provides a powerful tool for elucidating the molecular mechanisms that control organogenesis. As ectodermal appendages, the tooth organ arises from complex and progressive interactions between an ectoderm, the oral epithelium and an underlying mesenchyme. Their morphogenesis is regulated by conserved signalling pathways, including TGF-β. In this review, the essential function of the TGF-β superfamily will be discussed in detail, including TGF-β, bone morphogenetic proteins (BMP), activin, etc, during tooth crown patterning and following tooth root development. The review also highlights recent advances in the understanding of Smad-dependent and Smad–independent pathways in regulating tissue–tissue interactions during patterning of tooth crown and root.
Key words: *TGF-β, tooth development, BMP, Smad, root development*

Organ regeneration, in which fully functional bioengineered organs can be developed to replace lost or damaged ones, is a promising and challenging goal. The approaches that have been adopted in regenerative medicine are based on current understanding of embryonic development, stem-cell biology and tissue-engineering technology[1-5]. The tooth is one of the best models for developmental biology and organ regeneration. In recent years, multiple studies have addressed the process of tooth development and its reciprocal induction events[1,6-10]. The study of tooth regeneration has progressed as well. Tooth germ has been bioengineered with cell compartmentalisation *in vitro*, and it has also survived and developed into a mature tooth by transplantation *in vivo*[2]. Thus, tooth organ regeneration is becoming a reality.

The development of a functional tooth organ still requires information and insight from basic research, including the study of tooth development and tooth eruption. Tooth development in mice begins at embryonic day (E) 9.5. The reciprocal interactions between the dental epithelium and cranial neural crest (CNC)-derived mesenchyme result in the induction of previously quiescent genes that determine the fate of undifferentiated cells[8-10]. Signals from members of the hedgehog (Hh)[11-13], Wnt[14-16], fibroblast growth factor (FGF)[17-21] and the transforming growth factor-β (TGF-β) superfamily[21-28] are involved in the epithelial-mesenchymal interactions required for regulating tooth development.

TGF-β was initially identified by the pioneering work of de Larco and Todaro[29]. TGF-βs are secreted proteins recognised as multifunctional growth factors that mediate a variety of biological functions essential for gastrulation, organogenesis, embryonic and postnatal development, and oncogenesis[30,31]. TGF-βs are

1 Department of Stomatology, Beijing Friendship Hospital, Capital Medical University, Beijing, P.R. China.
2 Center for Craniofacial Molecular Biology, School of Dentistry, University of Southern California, Los Angeles, CA, USA and Adjunct Professor, Peking University School of Stomatology, Beijing, P.R. China.

Corresponding author: Dr Yang CHAI, Center for Craniofacial Molecular Biology (CCMB), School of Dentistry, University of Southern California, 2250 Alcazar St. CSA 103, Los Angeles, CA 90033 USA. E-mail: ychai@usc.edu

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important regulators during craniofacial development, including tooth formation[32]. How these conserved signals regulate the development of the mammalian tooth remains an interesting and challenging issue.

In the present review, the roles of TGF-β superfamily members in tooth development are discussed in detail, with a focus on recent advances.

TGF-β signalling pathway

The TGF-β superfamily of cytokines is a large group of extracellular growth factors including TGF-β, bone morphogenetic proteins (BMP), activin, growth and differentiation factor (GDF), inhibins, anti-Müllerian hormone (AMH), nodal and myostatin[30,31]. In the canonical TGF-β signalling pathway, the TGF-β ligand binds to the TGF-β type II receptor (Tgfbr2) and triggers heterodimerisation with a TGF-β type I receptor (Tgfbr1)[33]. Following heterodimerisation, the Tgfbr2 serine/threonine kinase transphosphorylates Tgfbr1, resulting in the propagation of a phosphorylation signal to downstream substrate Smad proteins (R-Smads), which form a complex with the common Smad (Smad4). The Smad complex then translocates into the nucleus where it binds with transcriptional coactivators and/or co-repressors to regulate the expression of an array of target genes[30,31].

Most mammalian cells express members of the TGF-β receptor family, some of which may be utilised by multiple TGF-β ligands. The activated type I receptor phosphorylates Smad proteins in the cytoplasm. The type I receptors for TGF-β, activin, nodal and myostatin (ALK 4, 5 and 7) phosphorylate Smads 2 and 3, whereas the BMP and AMH type I receptors (ALK 1, 2, 3 and 6) phosphorylate Smads 1, 5 and 8[30,34-37]. Smad4 is a central mediator of the canonical TGF-β signalling pathway[34,38,39]. However, Smad4 is not essential for TGF-β responses. Some TGF-β responses occur in the absence of Smad4. TGF-β activates mediators other than Smad4, such as PP2A phosphatases, Rho family members and the mitogen-activated protein kinases (MAPKs), including ERK, Jun N-terminal kinase (JNK), p38 and PI3K kinases[40-43]. Moreover, some genetic studies suggest that certain Smad-dependent effects may not require Smad4[44,45]. Smad6 and Smad7 are structurally divergent from other members of the Smad family and function as inhibitors of TGF-β, activin and BMP signalling[46-48].

Tooth development

As an epidermis-derived organ, the tooth is one of the best-studied models of craniofacial organogenesis to identify and analyse specific signalling cascades that play essential roles in regulating patterning and morphogenesis during development. Embryonic development of the tooth relies on a series of sequential and reciprocal homotypic and heterotypic molecular interactions between the oral epithelium and the underlying mesenchyme that derive from the CNC[1,6,9,10,49-51]. The factors that trigger the initiation of tooth development remain to be determined. Prior to the initiation of tooth morphology, BMP4 and FGF8, two important early signals in the oral epithelia, are expressed in the incisor and molar regions, respectively[22,52]. By E9.5, several secreted ligands and transcription factors are expressed in the oral epithelia, such as Pitx2 and Shh, and the underlying mesenchyme, such as Barx1 and Msx1/2, suggesting that the identity of the tooth organ has been determined at this point[52-57].

The first morphological sign of mammalian tooth development is a thickening of the oral epithelium, named the dental placode, at E11.5. Cells of the epithelial thickening proliferate and invaginate further into the CNC-derived mesenchyme to form a tooth bud (E12 to 13). Next, the epithelium expands deeper to form a tooth germ at the cap (E13 to 14) and the bell (E16) stage. At the cap stage, a cluster of epithelial cells in the center of the tooth bud, named the primary enamel knot, acts a signalling center that controls the process of tooth morphogenesis. The enamel knot produces signalling molecules essential for the formation of the tooth organ and then undergoes programmed cell death at the late cap stage. In multicuspid teeth, secondary enamel knots occur at the sites of future cusps after the disappearance of the primary enamel knot. The function of the secondary enamel knots, which differ from the primary enamel knot, is to trigger the folding of the inner enamel epithelium and the formation of the cusps. The ameloblasts and odontoblasts, cells that produce the hard tissue of the tooth, begin to differentiate after the cusp pattern is formed. Mesenchymal cells adjacent to the basement membrane differentiate into dentine-producing odontoblasts and start to form predentine. After deposition of predentine, the adjacent layer of epithelial cells differentiate into ameloblasts and secrete enamel matrix for the organisation of enamel and the formation of the hard tissue of the crown[1,6,8-10](Fig 1).

At the newborn stage in mice, the crown formation is almost completed. The tooth organ is comprised of dental epithelium, dental papilla and dental follicle. The latter two structures are derived from the CNC[51]. The dental epithelium contains the outer enamel epithelium (OEE), inner enamel epithelium (IEE), stellate reticulum and stratum intermedium. Following crown

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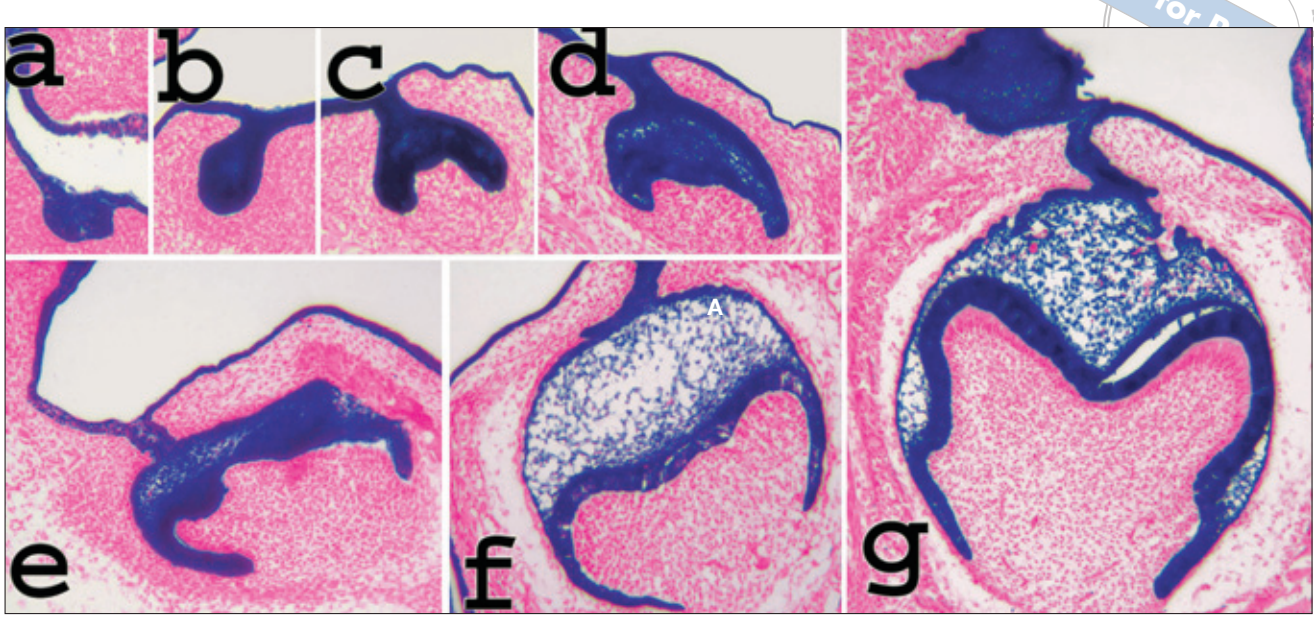

Fig 1 The development of tooth crown in mice: X-gal staining of sections showing molars in *K14-Cre;R26* mice at lamina stage, E12.5 (a); bud stage, E13.5 (b); cap stage, E14.5 (c); E15.5 (d); bell stage, E16.5 (e); later bell stage, E17.5 (f) and newborn stage (g).

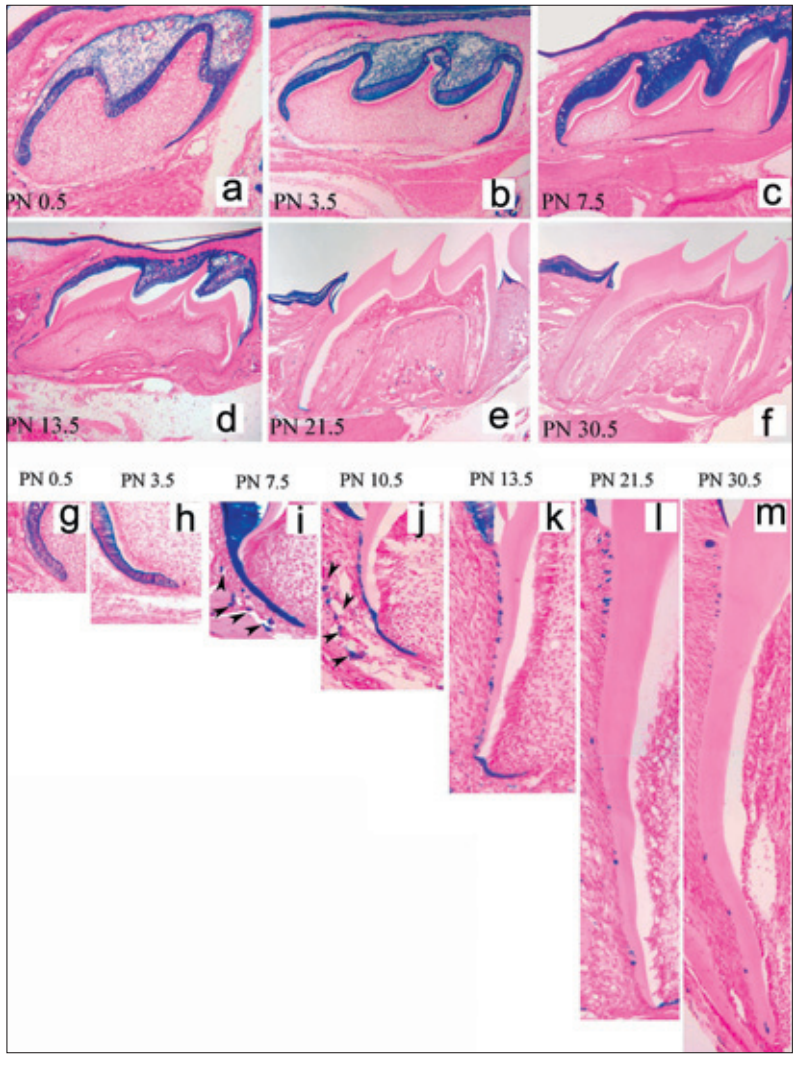

Fig 2 The development of a tooth root in mice. X-gal staining of sections showing upper molars of *K14-Cre;R26R* mice (a to f) and lateral surface of the root (g to m). After birth, cells from the ameloblast layer and the outer enamel epithelium are elongated and begin to form a bilayer (a, b, g and h). At PN 7.5, the Hertwig's epithelial root sheath (HERS) forms a bilayer of flat cells and extends in an apical direction at the interface between dental follicle and dental pulp (c and i). Dissociation of HERS is observed at PN 10.5 (j). The developed root and the HERS are dissociated further after PN 10.5, and HERS cells remain detectable on the surface of the root until the mature and functional tooth at PN 30.5 (d to f and k to m).

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development, the IEE and OEE form a bilayer epithelial structure, named the Hertwig's epithelial root sheath (HERS). The HERS migrates apically, and then participates in root formation and the completion of tooth organ development. Morphologically, HERS is a structural boundary between two dental mesenchymal tissues, the dental papilla and follicle. This strategic position suggests an important role for HERS during critical interactions between the dental epithelium and mesenchyme. During root development, HERS induces differentiation of the dental papilla cells into odontoblasts. Interestingly, HERS breaks up into epithelial rests and cords, but these are still connected to each other by an epithelial network, allowing dental follicle cells to come in contact with the outer surface of the root. Both dental follicle cells and HERS cells can differentiate into cementoblasts and lay cementum on the root, which is required for root maturation[58]. Cells of the outer layer of the dental follicle differentiate into fibroblasts and osteoblasts that, together with cementum, contribute to the periodontium[59,60]. The interplay of dental follicle cells, osteoblasts and osteoclasts is necessary for mineralised teeth to erupt and later to reach a position for functional occlusion[61] (Fig 2).

Expression patterns of members of the TGF-β family during tooth development

Hundreds of growth and transcription factors, including TGF-β, are involved in tooth development[6,9,10]. Members of the TGF-β superfamily mediate a variety of biological functions for embryonic development, including tooth development. TGF-β family members, such as activin, BMP2 to 7, TGF-β1 to 3, and their intracellular transducers, such as Smad1 to 7, are expressed in a time- and tissue-specific manner and serve as regulators for tooth development (Table 1).

Activin-βA

During tooth development, activin-βA is expressed in the dental mesenchyme, but not in the epithelium, from the initiation to the late bell stage. It is also expressed in the dental follicle at the bell stage[62].

BMP

BMP2 expression is detectable in dental epithelia during the initiation, bud and cap stages. It is also expressed in both epithelia and mesenchyme at the late bell stage and in odontoblasts and ameloblasts at the secretion and differentiation stages[63,64]. During root development, BMP2 is expressed in the dental papilla and odontoblasts[65]. BMP2 is also expressed in the epithelia of the diastema bud[66]. BMP3 is expressed in the dental papilla and dental follicle at the cap stage. It is also detectable in the dental papilla after the bell stage and in odontoblasts and cementoblasts during root development[64,65]. BMP4 is localised in both the epithelia and mesenchyme at the initiation stage, then in the dental mesenchyme and the enamel knot at the cap and bell stages. At the secretion and differentiation stages, it is detected in ameloblasts and odontoblasts. BMP4 is also expressed in dental papilla and odontoblasts during root development[64,65,67]. BMP5 is only expressed in pre-ameloblasts and ameloblasts after the late bell stage, and BMP6 is only expressed in the dental mesenchyme at the bud and cap stages. BMP7 expression is detectable in the dental epithelia throughout tooth development and also in odontoblasts after the bell stage. It is also expressed in dental papilla and odontoblasts during root development[64,65].

TGF-β

TGF-β1 is expressed in dental epithelia and mesenchyme at the bud and cap stages, and also in pre-ameloblasts, ameloblasts, pre-odontoblasts and odontoblasts[68]. TGF-β2 expression is detectable in dental papilla and pre-odontoblasts, but not in dental epithelium. TGF-β3 is mainly expressed in the stellate reticulum at the cap and bell stages, and in the dental mesenchyme after the bell stage[69].

Smad

Smad1 is present in the dental lamina and mesenchyme throughout tooth development. At the early bell stage, Smad1 is expressed in the enamel knot. Smad2 is expressed in the dental epithelium and mesenchyme at the initiation, bud stage and cap stage. At the bell stage, Smad2 is expressed in the inner enamel organ epithelium and dental mesenchyme. Smad3 is expressed in the dental epithelium and mesenchyme at the lamina, bud and cap stages. At the bell stage, Smad3 is localised to the inner enamel organ epithelium and adjacent dental mesenchyme. Smad4 is detectable in the dental epithelium and mesenchyme at all stages, in the enamel knot at the cup and bell stages, and also in the HERS and dental pulp during root development[25]. Smad5 is present in the dental lamina and mesenchyme at the lamina stage. At the bud and cap stages, Smad5 is primarily detectable in the dental epithelium. At the early bell stage, Smad5 is mainly localised to the inner enamel organ epithelium, including the enamel knot and mesenchyme. At the late bell stage, Smad5 expression is restricted to the inner enamel organ epithelium, dental mesenchyme and follicle. Smad6 and Smad7 expression is detectable in the

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Table 1 Expression of relevant members of the TGF-β and Smad families during tooth development .

	Initiate S		Bud S		Cap S		Bell S		Dif & Sec S		Root S	
	DE	DM	DE	DM	DE	DM	DE	DM	DE	DM	DE	DM
Activin-βA	n	y	n	y	n	y	n	y	n	y	-	-
BMP2	y	-	y	-	y	-	y	y	-	-	-	y
BMP3	-	-	-	-	-	y	-	y	-	y	-	y
BMP4	y	y	n	y	y	y	n	y	n	y	n	y
BMP5	n	n	n	n	n	n	n	n	y	n	Y	n
BMP6	-	-	n	y	n	y	-	-	-	-	-	-
BMP7	y	n	y	n	y	n	y	n	y	y	y	y
TGF-β1	n	n	y	y	y	y	y	y	y	y	y	y
TGF-β2	-	-	-	-	-	-	n	n	n	y	n	y
TGF-β3	-	-	-	-	y	n	y	n	n	y	n	y
Smad1	y	y	y	y	y	y	y	y	y	y	-	-
Smad2	y	y	y	y	y	y	y	y	y	y	-	-
Smad3	y	y	y	y	y	y	y	y	y	y	-	-
Smad4	y	y	y	y	y	y	y	y	y	y	y	y
Smad5	y	y	y	-	y		y	y	y	y	-	-
Smad6	y	y	y	y	y	y	y	y	y	y	-	-
Smad7	y	y	y	y	y	y	y	y	y	y	-	-

S: stage, y: yes, n: no, -: unknown, DE: dental epithelium, DM: dental mesenchyme

dental lamina and mesenchyme at the laminar, bud and cap stages. Smad6 expression shifts to become predominately detectable in the inner enamel organ epithelium and adjacent dental mesenchyme at the early and late bell stages, respectively[70].

Function of TGF-β members in the regulation of tooth development

TGF-β and tooth development

Multiple lines of evidence suggest that odontoblast maturation is controlled by TGF-β signalling. TGF-β ligands and TGF-β1 to 3, are detectable in the inner dental epithelium and in polarising and functional odontoblasts. Analysis of the expression patterns of TGF-β suggests that they contribute to odontoblast terminal differentiation[68,69]. Studies using mice with a TGF-β1 null mutation or overexpression of TGF-β1 support an essential role for TGF-β1 signalling in dentine mineralisation[71,72]. Moreover, exogenous TGF-β2 induced dentine sialophosphoprotein (DSPP) expression in wild type dental mesenchymal cells[73]. Loss of TGF-β signalling in the CNC-derived mesenchyme in *Wnt1-Cre;Tgfbr2*$^{fl/fl}$ mutant mice affects the terminal differentiation of odontoblasts. Expression of Type I collagen (Col1) and DSPP, which are differentiation markers for odontoblasts, is diminished in the CNC-derived mesenchyme in the absence of Tgfbr274. Tooth root development is also affected in *Wnt1-Cre;Tgfbr2*$^{fl/fl}$ mutant mice. However, the molecular and cellular mechanism of TGF-β signalling in regulating root formation is still unknown[73].

Activin

Activin-βA expression is localised in the presumptive tooth mesenchyme of all teeth, where it is an essential early mesenchymal signal in tooth development that is required for patterning of the murine dentition[74]. Beads with activin protein can induce the expression of the ectodysplasin A receptor (EDAR), which is a member of the tumour necrosis factor (TNF) family and important in the epithelium for tooth development[75]. In newborn activin-βA mutant mice, all teeth are absent, except maxillary molars, which exhibit normal development. Activin target gene expression is down-regulated in the dental epithelium of all tooth germs of activin-βA mutant mice, including the maxillary molars. This indicates that other factors may compensate for the loss of activin-βA activity in the maxillary molars[74].

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BMP signalling and tooth development

BMP signalling has multiple roles in the initiation of tooth development, including regulating the distance between adjacent secondary enamel knots to control the positioning of cusps, and in root development[22,64,75-78]. BMP is expressed in a gradient in the oral ectoderm of the first branchial arch[22]. Early in the tooth initiation stage, BMP2, 4 and 7 expression is detectable in the dental epithelia, and they play an important role in epithelial-mesenchymal interactions that induce tooth formation[64]. BMP mediates epithelial-mesenchymal interactions via regulation of the expression of Shh[57] and transcription factors, such as members of the Pitx or Msx family[9,79]. Repression of BMP signalling by Noggin, which is a wide-range inhibitor of BMP, results in the transformation of tooth shape from incisor to molar[22] and the decreased expression of Shh[57]. Moreover, tooth development is arrested at the bud stage in *Bmpr1a*-deficient mice, indicating that BMP signalling is essential for tooth development[23,24].

During the bud-to-cap stage transition, a BMP/Msx signal loop mediates reciprocal interactions between the epithelium and mesenchyme that triggers tooth initiation and crown morphogenesis. BMP4 induces the expression of Msx1 and Msx2 in the mesenchyme; then, in a feedback mechanism, Msx1 further induces BMP4 expression in the mesenchyme. Ectopic BMP4 expression driven by the mouse Msx1 promoter partially rescues tooth development in Msx1-deficient mice[55,57,80].

At the cap and bell stages, BMP4 signalling to form the enamel knot is crucial for the progression of tooth development[54,55]. BMP4 signalling in the mesenchyme also has been proposed to induce expression of p21 and cell cycle arrest in cells of the prospective enamel knot. BMP4 functions to regulate tooth organ shape by controlling cell cycle progression and apoptosis[78]. BMP2, 4 and 7 up-regulate the expression of ectodin, another inhibitor of BMP and Wnt signalling, in the enamel knot. Thus, the extent of BMP signalling is negatively controlled by the domain of ectodin expression that surrounds the enamel knot[81]. Mice deficient in ectodin have an expanded domain of BMP signalling, increased expression of Shh and enlarged primary enamel knots during early morphogenesis. The final dentition is composed of large molars with a longitudinal crest connecting cusps and supernumerary teeth[82]. In subsequent development, BMP4 is expressed in pre-odontoblasts and its expression is sharply down-regulated after their differentiation, whereas BMP2 expression is up-regulated during the terminal differentiation of odontoblasts[83,84]. During root development, BMP2, 3 and 7 are expressed in early odontoblasts in the apical region. Strong BMP3 expression is detectable in cementoblasts, as well as the dental follicle and osteoblasts. BMP4 expression appeared early in the pre-odontoblast cells adjacent to the epithelial root sheath. However, when pre-odontoblasts differentiated and started to secrete dentine matrix, BMP4 expression was down-regulated almost completely. BMP4 and BMP7 are also detectable in ameloblasts, alveolar bone and the periodontal ligament-mesenchyme[65]. In *in vitro* experiments, ectopic BMP4 protein in the mesenchyme regulates HERS development by preventing elongation and maintaining cell proliferation[85]. Moreover, mild inhibition of BMP signalling in K14-Noggin mice results in severe crown and root defects, suggesting that BMP signalling is essential for tooth hard tissue formation and root development[86].

Smad4 dependent and independent TGF-β signalling during tooth development

Smad4, the center of the canonic TGF-β signalling pathway, plays important roles in regulating cell differentiation and proliferation during tooth development. In mice lacking Smad4 expression in the CNC-derived dental mesenchyme (*Wnt1-Cre;Smad4*$^{fl/fl}$), tooth germ-like structures are not detectable beyond the dental lamina stage. Conditional inactivation of Smad4 in the oral epithelium results in the delayed differentiation of the inner enamel epithelium at the newborn stage. In addition, dental cusp formation is severely affected and there is a disorganised cell mass located in the dental epithelium. Therefore, Smad4 appears to be crucial for dental epithelium patterning. Disrupting the TGF-β/BMP signalling pathway by eliminating Smad4 in the dental epithelium results in down-regulation of Msx2 and Shh, which are downstream targets of BMP signalling and are essential for the patterning of dental cusps[54,56]. However, conditional inactivation of Smad4 in the oral epithelium results in much milder phenotypes than those seen in the corresponding receptor mutant mice (*K14-Cre;Bmpr1a*$^{fl/fl}$)[23], leading to the hypothesis that a Smad4-independent pathway compensates in *K14-Cre;Smad4*$^{fl/fl}$ conditional knockout mice. In fact, p38 MAPK functions redundantly with Smad4 to mediate TGF-β/BMP signalling during tooth development, and tooth development can only be arrested at the bud stage by blocking both Smad4 and p38 MAPK *in vitro*[26].

During tooth root development, conditional inactivation of Smad4 in the dental epithelium results not only in abnormal enamel and dentine formation but also defective tooth root development. In the absence of

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Smad4 in the epithelium, HERS can enlarge, but fails to elongate. Root development halts at the initiation stage. Shh and Nfic are essential for root development[87,88]. Without Smad4 in the dental epithelium, expression of Shh in the dental epithelia and Nfic in the dental mesenchyme is dramatically decreased. Interestingly, ectopic Shh can induce Nfic expression in the dental mesenchyme and rescue root development in *K14-Cre;Smad4*$^{fl/fl}$, but not Nfic$^{-/-}$ mice. Thus, TGF-β/BMP signalling relies on a Smad4-dependent mechanism in regulating Nfic expression via Shh signalling to control tooth root development[25].

Summary

TGF-β signalling is essential for tooth development and in regulating early tooth morphogenesis, subsequent odontoblast differentiation and root development. Members of the TGF-β superfamily, such as TGF-β, BMP, activin and their downstream targets, such as Smad, play critical roles in odontogenesis. TGF-β mediates tooth and root development not only though the canonical Smad-dependent pathway but also through a Smad-independent pathway. Ultimately, the functional outcome of TGF-β/BMP signalling in regulating tooth development is determined by the cell type upon which TGF-β/BMP signals. This is because the unique transcription activators/repressors as well as co-factors will associate with the Smad complex to initiate cell-type specific events to control ameloblast, odontoblast and other specific cellular differentiation during tooth morphogenesis.

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References

1. Chai Y, Slavkin HC. Prospects for tooth regeneration in the 21st century: a perspective. Microsc Res Tech 2003;60:469-479.
2. Kazuhisa N, Ritsuko M, Yasumitsu S, Kentaro I, Yusuke T, Miho O et al. The development of a bioengineered organ germ method. Nat Methods 2007;4:227-230.
3. Langer RS, Vacanti JP. Tissue engineering: the challenges ahead. Sci Am 1999;280:86-89.
4. Brockes JP, Kumar A. Appendage regeneration in adult vertebrates and implications for regenerative medicine. Science 2005;310:1919-1923.
5. Atala A. Tissue engineering, stem cells and cloning: current concepts and changing trends. Expert Opin Biol Ther 2005;5:879-892.
6. Chai Y, Maxson RE Jr. Recent advances in craniofacial morphogenesis. Dev Dyn 2006;235:2353-2375.
7. Lumsden AG. Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. Development 1988;103:155-169.
8. Miletich I, Sharpe PT. Normal and abnormal dental development. Hum Mol Genet 2003;12:69-73.
9. Jernvall J, Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev 2000;92:19-29.
10. Thesleff I, Sharpe PT. Signalling networks regulating dental development. Mech Dev 1997;67:111-23.
11. Cobourne MT, Miletich I, Sharpe PT. Restriction of sonic hedgehog signaling during early tooth development. Development 2004;131:2875-2885.
12. Cobourne MT, Hardcastle Z, Sharpe PT. Sonic hedgehog regulates epithelial proliferation and cell survival in the developing tooth germ. J Dent Res 2001;80:1974-1979.
13. Gritli-Linde A, Bei M, Maas R, Zhang XM, Linde A, McMahon AP. Shh signaling within the dental epithelium is necessary for cell proliferation, growth and polarization. Development 2002;129:5323-5237.
14. Sarkar L, Sharpe PT. Expression of Wnt signalling pathway genes during tooth development. Mech Dev 1999;85:197-200.
15. Kratochwil K, Galceran J, Tontsch S, Roth W, Grosschedl R. FGF4, a direct target of LEF1 and Wnt signaling, can rescue the arrest of tooth organogenesis in Lef1 (-/-) mice. Genes Dev 2002;16:3173-3185.
16. Sasaki T, Ito Y, Xu X, Han J, Bringas P Jr, Maeda T et al. LEF1 is a critical epithelial survival factor during tooth morphogenesis. Dev Biol 2005;278:130-143.
17. Kettunen P, Laurikkala J, Itaranta P, Vainio S, Itoh N, Thesleff I. Associations of FGF-3 and FGF-10 with signaling networks regulating tooth morphogenesis. Dev Dyn 2000;219:322-332.
18. Kettunen P, Thesleff I. Expression and function of FGFs-4, -8, and -9 suggest functional redundancy and repetitive use as epithelial signals during tooth morphogenesis. Dev Dyn 1998;211:256-268.
19. Takamori K, Hosokawa R, Xu X, Deng X, Bringas P Jr, Chai Y. Epithelial fibroblast growth factor receptor 1 regulates enamel formation. J Dent Res 2008;87:238-43.
20. De Moerlooze L, Spencer-Dene B, Revest J, Hajihosseini M, Rosewell I, Dickson C. An important role for the IIIb isoform of fibroblast growth factor receptor 2 (FGFR2) in mesenchymal–epithelial signalling during mouse organogenesis. Development 2000;127:483-492.
21. Neubuser A, Peters H, Balling R, Martin GR. Antagonistic interactions between FGF and BMP signaling pathways: a mechanism for positioning the sites of tooth formation. Cell 1997;90:247-255.
22. Tucker AS, Matthews KL, Sharpe PT. Transformation of tooth type induced by inhibition of BMP signaling. Science 1998;282:1136-1138.
23. Andl T, Ahn K, Kairo A, Chu EY, Wine-Lee L, Reddy ST et al. Epithelial bmpr1a regulates differentiation and proliferation in postnatal hair follicles and is essential for tooth development. Development 2004;131:2257-2268.
24. Liu W, Sun X, Braut A, Mishna Y, Behringer RR, Mina M, Martin JF. Distinct functions for bmp signaling in lip and palate fusion in mice. Development 2005;132:1453-1461.
25. Huang X, Xu X, Bringas P Jr, Hung YP, Chai Y. Smad4-Shh-Nfic signaling cascade-mediated epithelial-mesenchymal interaction is crucial in regulating tooth root development. J Bone Miner Res 2010; 25:1167-1178.
26. Xu X, Han J, Ito Y, Bringas Jr. P, Deng C, Chai, Y. Ectodermal Smad4 and p38 MAPK are functionally redundant in mediating TGF-beta/BMP signaling during tooth and palate development. Dev Cell 2008;15:322-329.

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27. Ko SO, Chung IH, Xu X, Oka S, Zhao H, Cho ES et al. Smad4 is required to regulate the fate of cranial neural crest cells. Dev Biol 2007;312:435-447.
28. Oka S, Oka K, Xu X, Sasaki T, Bringas P Jr, Chai Y. Cell autonomous requirement for TGF-beta signaling during odontoblast differentiation and dentin matrix formation. Mech Dev 2007;124:409-15.
29. De Larco JE, Todaro GJ. Growth factors from murine sarcoma virus-transformed cells. Proc Natl Acad Sci U S A 1978;75:4001-4005.
30. Massagué J. How cells read TGF-beta signals. Nat Rev Mol Cell Biol 2000;1:169-178.
31. Siegel PM, Massagué J. Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat Rev Cancer 2003;3:807-821.
32. Chai Y, Ito Y, Han J. Tgf-β signaling and its functional significance in regulating the fate of cranial neural crest cells. Crit Rev Oral Biol Med 2003;14:78-88
33. Massagué J, Weis-Garcia F. Serine/threonine kinase receptors: mediators of transforming growth factor beta family signals. Cancer Surv 1996;27:41-64.
34. Zhang Y, Feng XH, Wu RY, Derynck R. Receptor-associated Mad homologues as effectors of the TGF-beta response. Nature 1996;383:168-172.
35. Massagué J. Receptors of the TGF beta family. Cell 1992;69: 1067-1070.
36. Massagué J. TGF-β signaling: receptors, transducers, and Mad proteins. Cell 1996;85:947-950.
37. Massagué J. TGF-β signal transduction. Annu Rev Biochem 1998;67:753-791.
38. Lagna G, Hata A, Hemmati-Brivanlou A, Massagué J. Partnership between DPC4 and Smad proteins in TGF-β signaling pathways. Nature 1996;383:832-836.
39. Wu R, Zhang Y, Feng X, Derynck R. Heteromeric and homomeric interactions correlate with signaling activity and functional cooperativity of Smad3 and Smad4 (DPC4). Mol Cell Biol 1997;17:2521-2528.
40. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-β family signalling. Nature 2003;425:577-584.
41. Adachi-Yamada T, Nakamura M, Irie K, Tomoyasu Y, Sano Y, Mori E et al. p38 mitogen-activated protein kinase can be involved in transforming growth factor β superfamily signal transduction in Drosophila wing morphogenesis. Mol Cell Biol 1999;19:2322-2329.
42. Hartsough MT, Mulder KM. Transforming growth factor β activation of p44mapk in proliferating cultures of epithelial cells. J Biol Chem 1995;270:7117-7124.
43. Yu L, Hebert MC, Zhang YE. TGF-β receptor-activated p38 MAP kinase mediates Smad-independent TGF-β responses. EMBO J 2002; 21:3749-3759.
44. Subramanian G, Schwarz RE, Higgins L, McEnroe G, Chakravarty S, Dugar S, Reiss M. Targeting endogenous transforming growth factor beta receptor signaling in SMAD4-deficient human pancreatic carcinoma cells inhibits their invasive phenotype1. Cancer Res 2004;64:5200-5211.
45. Wisotzkey RG, Mehra A, Sutherland DJ, Dobens LL, Liu X, Dohrmann C et al. Medea is a Drosophila Smad4 homolog that is differentially required to potentiate DPP responses. Development 1998;125:1433-1445.
46. Massague J. How cells read TGF-b signals. Nat Rev Mol Cell Biol 2000;1:169-178.
47. Itoh S, Itoh F, Goumans M J, ten Dijke, P. Signaling of transforming growth factor-β family members through Smad proteins. Eur J Biochem. 2000;267:6954-6967.
48. Moustakas A, Souchelnytskyi S. Heldin CH. Smad regulation in TGF-β signal transduction. J Cell Sci 2001;114:4359-4369.
49. Slavkin HC, Bavetta LA. Morphogenetic expressions during odontogenesis: a tool in developmental biology. Clin Orthop Relat Res 1968;59:97-109.
50. Slavkin HC, Bavetta LA. Odontogenic epithelial-mesenchymal interactions *in vitro*. J Dent Res 1968;47:779-785.
51. Chai Y, Jiang X, Ito Y, Bringas P Jr, Han J, Rowitch DH et al. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development 2000;127:1671-1679.
52. Liu W, Selever J, Lu MF, Martin JF. Genetic dissection of Pitx2 in craniofacial development uncovers new functions in branchial arch morphogenesis, late aspects of tooth morphogenesis and cell migration. Development 2003;130:6375-6385.
53. Tissier-Seta JP, Mucchielli ML, Mark M, Mattei MG, Goridis C, Brunet JF. Barx1, a new mouse homeodomain transcription factor expressed in cranio-facial ectomesenchyme and the stomach. Mech Dev 1995;51:3-15.
54. Bei M, Kratochwil K. Maas RL. BMP4 rescues a non-cell-autonomous function of Msx1 in tooth development. Development 2000;127:4711-4718.
55. Chen Y, Bei M, Woo I, Satokata I, Maas R. Msx1 controls inductive signaling in mammalian tooth morphogenesis. Development 1996;122:3035-3044.
56. Dassule HR, Lewis P, Bei M, Maas R, McMahon AP. Sonic hedgehog regulates growth and morphogenesis of the tooth. Development 2000;127:4775-4785.
57. Zhang Y, Zhang Z, Zhao X, Yu X, Hu Y, Geronimo B et al. A new function of BMP4: dual role for BMP4 in regulation of Sonic hedgehog expression in the mouse tooth germ. Development 2000;127:1431-1443.
58. Huang X, Bringas P, Slavkin HC, Chai Y. Fate of HERS during tooth root development. Dev Biol 2009;334:22-30
59. Thomas HF. Root formation. Int J Dev Biol 1998;39:231-237.
60. Diekwisch TG. The developmental biology of cementum. Int J Dev Biol 2001;45:695-706.
61. Huang XF, Zhao YB, Zhang FM, Han PY. Comparative study of gene expression during tooth eruption and orthodontic tooth movement in mice. Oral Dis 2009;15:573-579.
62. Ferguson CA, Tucker AS, Christensen L, Lau AL, Matzuk MM, Sharpe PT. Activin is an essential early mesenchymal signal in tooth development that is required for patterning of the murine dentition. Genes Dev 1998;12:2636-2649.
63. Wang XP, Suomalainen M, Jorgez CJ, Matzuk MM, Werner S, Thesleff I. Follistatin regulates enamel patterning in mouse incisors by asymmetrically inhibiting BMP signaling and ameloblast differentiation. Dev Cell 2004;7:719-730.
64. Aberg T, Wozney J, Thesleff I. Expression patterns of bone morphogenetic proteins (Bmps) in the developing mouse tooth suggest roles in morphogenesis and cell differentiation. Dev Dyn 1997;210:383-396.
65. Yamashiro T, Tummers M, Thesleff I. Expression of bone morphogenetic proteins and Msx genes during root formation. J Dent Res 2003;82:172-176.
66. Keränen SV, Kettunen P, Aberg T, Thesleff I, Jernvall J. Gene expression patterns associated with suppression of odontogenesis in mouse and vole diastema regions. Dev Genes Evol 1999;209:495-506.
67. Aberg T, Wang XP, Kim JH, Yamashiro T, Bei M, Rice R et al. Runx2 mediates FGF signaling from epithelium to mesenchyme during tooth morphogenesis. Dev Biol 2004;270:76-93.
68. Vaahtokari A, Vainio S, Thesleff I. Associations between transforming growth factor beta 1 RNA expression and epithelial-mesenchymal interactions during tooth morphogenesis. Development 1991;113:985-994.
69. Pelton RW, Dickinson ME, Moses HL, Hogan BL. *In situ* hybridization analysis of TGF beta 3 RNA expression during mouse development: comparative studies with TGFβ1 and β2. Development 1990;110:609-620 .

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70. Xu X, Jeong L, Han J, Ito Y, Bringas P Jr, Chai Y. Developmental expression of Smad1-7 suggests critical function of TGF-β/BMP signaling in regulating epithelial-mesenchymal interaction during tooth morphogenesis. Int J Dev Biol 2003;47:31-39.
71. D'Souza RN, Cavender AC, Sood R, Tarnuzzer R, Dickinson D, Roberts A, Letterio J. Dental abnormalities in mice lacking a functional transforming growth factor-β1 (TGF-β1) gene indicate a role for TGF-β1 in biomineralization. Int J Oral Biol 1998;23:119-131.
72. Thyagarajan T, Sreenath T, Cho A, Wright JT, Kulkarni AB. Reduced expression of dentin sialophosphoprotein is associated with dysplastic dentin in mice overexpressing transforming growth factor-β1 in teeth. J Biol Chem 2001;276:11016-11020.
73. Oka S, Oka K, Xu X, Sasak T, Bringas P Jr, Chai Y. Cell autonomous requirement for TGF-β signaling during odontoblast differentiation and dentin matrix formation. Mech Dev 2007;124:409-415.
74. Ferguson CA, Tucker AS, Christensen L, Lau AL, Matzuk MM, Sharpe PT. Activin is an essential early mesenchymal signal in tooth development that is required for patterning of the murine dentition. Genes Dev 1998;12:2636-2649.
75. Laurikkala J, Mikkola M, Mustonen T, Aberg T, Koppinen P, Pispa J et al. TNF signaling via the ligand-receptor pair ectodysplasin and edar controls the function of epithelial signaling centers and is regulated by Wnt and activin during tooth organogenesis. Dev Biol 2001;229:443-455.
76. Neubüser A, Peters H, Balling R, Martin GR. Antagonistic interactions between FGF and BMP signaling pathways: a mechanism for positioning the sites of tooth formation. Cell 1997;90:247-255.
77. Tucker AS, Al Khamis A, Sharpe PT. Interactions between Bmp-4 and Msx-1 act to restrict gene expression to odontogenic mesenchyme. Dev Dyn. 1998;212:533-539.
78. Jernvall J, Aberg T, Kettunen P, Keränen S, Thesleff I. The life history of an embryonic signaling center: BMP-4 induces p21 and is associated with apoptosis in the mouse tooth enamel knot. Development 1998;125:161-169.
79. Tucker AS and Sharpe PT. Molecular genetics of tooth morphogenesis and patterning: the right shape in the right place. J Dent Res 1999;78:826-834.
80. Zhao X, Zhang Z, Song Y, Zhang X, Zhang Y, Hu Y et al. Transgenically ectopic expression of bmp4 to the msx1 mutant dental mesenchyme restores downstream gene expression but represses shh and bmp2 in the enamel knot of wild type tooth germ. Mech Dev 2000;99:29-38.
81. Laurikkala J, Kassai Y, Pakkasjarvi L, Thesleff I, Itoh N. Identification of a secreted BMP antagonist, ectodin, integrating BMP, FGF, and SHH signals from the tooth enamel knot. Dev Biol 2003;264:91-105.
82. Kassai Y, Munne P, Hotta Y, Penttila E, Kavanagh K, Ohbayashi N et al. Regulation of mammalian tooth cusp patterning by ectodin. Science 2005;309:2067-2070.
83. Nakashima M. Induction of dentin formation on canine amputated pulp by recombinant human bone morphogenetic proteins (BMP)-2 and -4. J Dent Res 1994;73:1515-22.
84. Nakashima M, Nagasawa H, Yamada Y, Reddi AH. Regulatory role of transforming growth factor-beta, bone morphogenetic protein-2, and protein-4 on gene expression of extracellular matrix proteins and differentiation of dental pulp cells. Dev Biol 1994;162:18-28.
85. Hosoya A, Kim JY, Cho SW, Jung HS. BMP4 signaling regulates formation of Hertwig's epithelial root sheath during tooth root development. Cell Tissue Res 2008;333:503-509.
86. Plikus MV, Zeichner-David M, Mayer JA, Reyna J, Bringas P, Thewissen JG et al. Morphoregulation of teeth: modulating the number, size, shape and differentiation by tuning Bmp activity. Evol Dev 2005;7:440-457.
87. Nakatomi M, Morita I, Eto K, Ota MS. Sonic hedgehog signaling is important in tooth root development. J Dent Res 2006;85:427-31.
88. Steele-Perkins G, Butz KG, Lyons GE, Zeichner-David M, Kim HJ, Cho MI, Gronostajski RM. Essential role for NFI-C/CTF transcription-replication factor in tooth root development. Mol Cell Biol 2003;23:1075-1084.