An inductive signalling network regulates mammalian tooth morphogenesis with implications for tooth regeneration

Z. Li*, M. Yu* and W. Tian*,†

*State Key Laboratory of Oral Disease, West China School of Stomatology, Sichuan University, Chengdu, 610041, China, and †Department of Oral and Maxillofacial Surgery, West China College of Stomatology, Sichuan University, Chengdu, 610041, China

Received 26 March 2013; revision accepted 18 May 2013

Abstract

Sequential and reciprocal epithelial–mesenchymal interactions, essential throughout such aspects of tooth morphogenesis as patterning, size and number of teeth, involves a well-ordered series of inductive and permissive signals that exert global control over cell proliferation, differentiation and organogenesis. In particular, growth factors, transcription factors and their corresponding receptors, as well as other soluble morphogens, make up a regulatory network at the molecular level that synergistically or antagonistically controls intra-/inter-cellular signal transduction during odontogenesis. This review summarizes recent advances in the study of crucial signalling pathways, for example of BMPs, Wnt, Notch, Shh and FGF, with emphasis on the potential integrated signalling network responsible for tooth formation. Our work probes into the complexity of these inductive signalling pathways to promote the understanding of tooth regeneration. Additionally, our study provides further insights into therapeutic strategies for various dental abnormalities in patterning and number, such as tooth agenesis and supernumerary teeth.

Introduction

Teeth are embryologically ectodermal organs that form by sequential and reciprocal interactions between the odontogenic epithelium and neural crest-derived mesenchyme (1). Mammalian tooth organogenesis mainly

involves three fundamental stages: initiation, bud stage and morphogenesis. During initiation, growth factors induce epithelial cell proliferation restricted to a certain limit, to form a dental lamina, thus determining site of tooth formation. Subsequently, cells of the thickened epithelial layer unceasingly proliferate and the epithelium invaginates into adjacent areas of mesenchymal cells to form a tooth bud (2). Specific folding of the epithelial tissue results in subsequent cap- and bell-morphological alterations, which are accompanied by formation of a primary and secondary enamel knot. Tooth buds sequentially differentiate and bring into being the following essential tissues, dentin and endodontium, under modulation of a specific range of molecular signals, and finally erupt into the epidermal tissue.

To our knowledge, the tooth seems to be one of the simplest organs in human body, but its developmental is regulated by various molecules, in distinct stages (Fig. 1), and a complex set of inductive signalling networks from initiation to terminal differentiation. Numerous studies have been performed to investigate canonical signalling pathways of bone morphogenetic proteins (BMPs), Wnt signalling, Notch signalling, fibroblast growth factors (FGFs) and Sonic hedgehog (Shh), which can function synergistically or antagonistically during tooth development (3–6). Here we provide an account of current states of knowledge on signalling research and aim to provide a comprehensive picture of tooth regeneration and tooth abnormalities.

Core molecules and signalling in tooth development

BMP pathway

Gene knockout and defect experiments, as well as spatiotemporal control of BMP gradient models, have achieved considerable advances in understanding the role of BMP signalling during embryonic development.

Correspondence: M. Yu, State Key Laboratory of Oral Disease, West China School of Stomatology, Sichuan University, Chengdu 610041, China. Tel/Fax : +86 28 85503499; E-mail: yumei925@hotmail.com, and W. Tian, Department of Oral and Maxillofacial Surgery, West China College of Stomatology, Sichuan University, Chengdu 610041, China. Tel/Fax : +86 28 85503499; E-mail: drtwd@sina.com

Stem cells from exfoliated deciduous teeth (SHED) have not been found to express markers (such as *Dspp*, *Dmp1* and *Mepe*), of odontoblastic differentiation, when seeded in deproteinized tooth slice/scaffolds and cultured *in vitro* (9). This indicated that the process of

© 2013 John Wiley & Sons Ltd

Figure 2. Inductive signalling network regulating tooth morphogenesis. Together, (a) bone morphogenetic protein (BMP) signalling pathway, (b) Wnt signalling pathway, (c) Shh pathway, (d) fibroblast growth factor (FGF) signalling pathway and (e) Notch pathway constitute an inductive signalling network and are joined by core molecular links.

In the BMP pathway, SMAD4 acts as a central regulator and elimination of SMAD4 results in downregulation of downstream targets *Msx2* and *Shh*. However, this downregulation is not sufficient to block BMP signalling, which suggests a SMAD4-independent pathway within the BMP cascade (14).

Wnt signalling

Wnt family proteins are evolutionarily conserved secretory proteins that mediate multiple developmental processes during embryogenesis. The canonical Wnt signalling pathway has been unravelled with efforts from multidisciplinary studies (15,16). Once Wnt proteins bind to Frizzled (Fz)/low density lipoprotein (LDL) receptor-related protein (LRP) coreceptor complex at the cell surface, signals are transmitted to cytoplasmic proteins, including Dishevelled (Dvl), glycogen synthase kinase-3 β (GSK-3 β), casein kinase I- γ (CK1 γ), Axin, Adenomatous Polyposis Coli (APC), and β -catenin,

which act as integral transcription regulators in the Wnt signalling pathway. With phosphorylation of DVL, activation of GSK-3 β is inhibited, allowing β -catenin to accumulate and subsequently translocate into the nucleus to interact with transcriptional lymphoid enhancer-binding factor1/T cell-specific transcription factor (LEF/TCF), and to activate Wnt-specific target genes (4). In contrast, when Wnt ligand is absent, β -catenin phosphorylated by GSK-3 β and CK1 γ is degraded via the ubiquitination pathway. In the nucleus, binding of transcriptional repressors, such as Groucho, Grg, CtBP, Kaiso and other factors, to LEF/TCF, blocks transcription of Wnt target genes.

Recently, Wnt/ β -catenin signalling pathway function in tooth development has been extensively investigated, and better understanding of regulation of Wnt signalling is now available. Several studies have revealed that Dkks (Dickkopf), particularly *Dkk1*, are putative antagonists of Wnt signalling that bind its receptor complex (17); these proteins are expressed dynamically and

spatiotemporally during tooth developmental stages, indicating that they have significant functions in dental patterning and tooth formation (18). Furthermore, *Dkk1* has also been shown to have expression restricted to the incisor region, in a mouse model (18). A further important regulator of the Wnt/ β -catenin pathway is WISE (also called *Sostdc1*, *USAG-1* and *ectodin*). Loss-/gain-of-function studies indicate that overexpression of *Wise* reduces Wnt signalling and tooth number (19). Moreover, binding of WISE to LRP4, which acts as an antagonist to Lrp5/6-mediated Wnt signalling, inhibits the Wnt pathway (20). Wnt/ β -catenin signalling has previously been reported to contribute to ectodermal organ regeneration (16,21). Study on conditional β -catenin^{*ex3K14/+*} mutant mice, as well as computer simulations, have indicated that induction of enamel knots can lead to continuous generation of supernumerary teeth by stabilizing β -catenin expression (22). Previous studies have also shown that ablation of epithelial APC function, or constitutive activation of β -catenin, also lead to supernumerary tooth formation (23), uncovering substantial potential for *de novo* tooth regeneration. Most Wnt family gene expression is restricted to the dental epithelium; however, as deficient mice die too young for phenotype analysis to have been performed, the specialized function for each member remains unclear. Maybe a conditional up/downregulated approach could constitute an intelligent option for such studies.

Notch signalling pathway

The Notch signalling pathway functions critically during differentiation of the dental epithelium and mesenchyme, generation of cusp patterns and formation of teeth. In mammals, there exist four already-known Notch receptors (*Notch1*, *Notch2*, *Notch3* and *Notch4*). They are all type I transmembrane proteins with extra- and intracellular domains, synthesized in the endoplasmic reticulum and sequentially transported by secretory pathways involved in the trans-Golgi network. After being trans-activated by Notch ligands (DLL-type ligands Delta-like1, Delta-like3 and Delta-like4 and JAG-type ligands *Jagged1* and *Jagged2*), Notch proteins are cleaved at site 1 (S1) by a Furin-like convertase, then localized to the plasma membrane. Receptors release the Notch intracellular domain through cleavage of ADAM proteinase and γ -secretase complex at S2 and S3/S4, respectively. In canonical Notch signalling, after entering nuclei, Notch intracellular domain is activated by binding to transcription regulator CSL, resulting in transcriptional regulation of target genes, including those of Ski-interacting protein (*Skip*), Mastermind-like proteins *Maml1*, *Maml2* and *Maml3*, and co-activator *p300* (24,25).

Among all Notch ligands, *Jagged2* performs notably in tooth development. *Jagged2* mutant mice die immediately after birth with abnormal odontogenesis; they are characterized by fusion of palatal and mandibular regions (26). Additionally, in *Jagged2*^{*-/-*} mice, lack of apoptosis and presence of dental abnormalities, such as misshapen crown morphology and additional cusps occur. These findings demonstrate the pivotal role of *Jagged2* in tooth development (27). In normal human and neoplastic odontogenic tissues, mRNA expression of *Notch1*, *Notch2*, *Notch3*, *Delta1* and *Jagged1* has been detected in odontogenic epithelium, implying a role for Notch signalling in dental diseases (28). Research in ameloblastoma tumours has shown that *Notch1*, *Notch3*, *Notch4*, *Jagged1* and *Delta1*, but not *Notch2* and *Jagged2*, were expressed in the epithelium. In endothelial cells and fibroblasts, *Notch4* was highly expressed, but not other Notch members and their ligands (29). NOTCH1 protein and *Hes1* mRNAs are expressed in stratum intermedium cells in rat incisor organ cultures, while inner enamel epithelial cells and ameloblasts expressed *Jagged1*. Furthermore, more stratum intermedium cells grew under the conditions of recombinant JAGGED1 protein in HAT-7 cultures, but fewer grew in the presence of anti-Jagged1 (30). Parathyroid-hormone-related protein (PTHrP) induced *Jagged1* expression in a cAMP/PKA-dependent manner in periodontal ligament cells, then JAGGED1 promoted receptor activator of nuclear factor- κ B ligand (RANKL)-induced osteoclastogenesis (31). Interestingly, Notch signalling can be activated by overexpressed *Jagged1* in post-natal human dental pulp stem cells; however, further studies showed that overexpression of *Notch1* intracellular domain and *Jagged1* inhibited odontoblastic differentiation of dental pulp stem cells *in vitro*, while *Jagged1* also caused absence of formation of mineralized tissues *in vivo* (32). The Notch pathway composes a complex containing various receptors, ligands and transcripts that are active in tooth formation. More research is needed to determine direct targets of this pathway and how they participate in dental differentiation.

Shh signalling pathway

Sonic hedgehog is the critically studied Hh ligand implicated in initiation and patterning of teeth by activation of downstream gene expression (Fig. 2). Vertebral Shh signalling is generally considered to be transmitted and mediated by the GLI family of transcription factors including Gli1, Gli2 and Gli3. Once secreted Shh binds to Hh receptor Patched (Ptc) protein, its normal inhibition of Smoothed (Smo) (a further seven-membrane-spanning receptor), is blocked, and downstream effector

Gli1/2/3 is activated through positive regulator Fused (Fu). Meanwhile, Shh signal can also abrogate negative effects of kinesin-related protein Costal2 (Cos2), cAMP-dependent protein kinase (PKA) and Suppressor of Fused (Sufu) (33). Otherwise, Ptc inhibits Smo protein and normally GLI, FU, SUFU and COS2 are found in a complex in the absence of Shh signalling (34).

At early stages of vertebrate tooth formation, Shh is detected in the dental lamina, enamel knots, ameloblasts and tooth cusps (19,35,36), implicating Shh as having global control on tooth formation. In the Shh signalling pathway, primary cilia exert a specific negative regulatory effect on Shh activity that functions to repress tooth formation, which further indicates that Shh is responsible for controlling number of teeth (20). In a recent study, Shh signalling was found to be associated with enamel formation, acting as a molecular marker of pre-ameloblasts. It was shown to be essential for generation of ameloblasts by inhibiting the Hh pathway (19), however, the precise mechanism of Shh function and regulation is still under its veil. Consistently, vestigial tooth rudiments mesial to the first molar do not undergo apoptosis, but instead survive and develop into a functional tooth in *Gas1* (an antagonist of Shh protein) mutant mice, characterized by increase in Shh signalling *in vivo* (20).

All the above signalling molecules have their respective contributions in each biological process, and it is reported that proteins including BMPs and Wnts can activate enamel knot differentiation, while Shh and FGFs inhibit this process and promote cell proliferation and growth (37).

FGF pathways

The FGF pathway is essential for regulation of tissue patterning and organogenesis during mammalian embryogenesis and self-renewal. After FGF ligands bind and activate four FGF receptors (FGFRs) in a heparan sulphate glycosaminoglycan, this process sequentially results in recruitment, phosphorylation and activation of *Frs2*, *Grb2*, *Sos*, *Sh2* and *Shp2*, and eventually leads to proliferation and development in organogenesis through Ras-dependent ERK, p38 MAPKs or JNK pathways (38,39) (Fig. 2d). During odontogenesis, a number of molecules participates in regulation of epithelial *Spry2* and mesenchymal *Spry4*, preventing diastema tooth formation by inhibiting FGF ligands involved in FGF-mediated bidirectional signalling between the epithelium and mesenchyme (5). Furthermore, SPROUTYs can prevent formation of epithelial-mesenchymal FGF signalling loops and inhibit differentiation from self-renewing stem cells to ectopic ameloblasts (40). Some studies have

shown that transcription factor RUNX2 can modulate epithelial FGF signals and *Fgf3* expression in the dental mesenchyme during mouse odontogenesis (41); in the dental epithelium of FGFR mutant mice, expression of *Tbx1*, amelogenin expression inducer, has been shown to be downregulated (42). Interestingly, removal of FGF20 in an *Eda* (which is crucial for ectodermal organogenesis) gain-of-function mouse model results in an *Eda* loss-of-function phenotype, and further research studies have verified that FGF20 acts as an intermediate in functions of the *Eda* pathway during molar development (43).

Inductive signalling network

The signalling pathways described above control most events in tooth organogenesis, such as pattern, number and shape of teeth. Furthermore, these pathways regulate each other during the developmental process, and crosstalk between them determines the fate of cells and organs appropriately. Among the epithelial-mesenchymal interactions in tooth development, the FGF signalling pathway occupies the upstream domain. Experiments *in vitro* have indicated that FGF2, FGF4 and FGF8 upregulate expression of *Jagged2*, a ligand for mammalian Notch proteins. Downstream gene expression is then regulated through the Notch pathway (27). However, one study has also reported that both epithelial and mesenchymal FGF proteins can induce Shh expression in the epithelium (44). Furthermore, functions of epithelial FGF signals can be mediated by RUNX2, a nuclear regulator, in BMP signalling, as *Fgf3* in the dental mesenchyme may be a direct target gene of RUNX2 (41). The BMP signalling pathway plays a critical role in odontogenesis, and its common mediator, Smad4, antagonistically targets Wnt inhibitors *Dkk1* and *Sfrp1*, which might increase Wnt signalling. Binding of SMAD7-AXIN in the cytoplasm and SMAD4- β -catenin-LEF1 binding in the nucleus further support the important link between these two pathways during tooth formation (45). Interestingly, *Jagged2* expression was found to be downregulated in recombinant cultures of dental epithelium and dental mesenchyme isolated from embryonic tooth germ, when beads of *Bmp2* or *Bmp4* were placed superficially (27). *Msx1*-independent mesenchymal BMP4 activity, involving the transcriptional complex of β -catenin, *Tcf1* and *Lef1*, maintained continuous Shh expression in the dental epithelium, which regulated *Lef1* expression in the mesenchyme (46). Moreover, Shh participates in a Wnt-Shh-Wise negative feedback loop where secreted Shh in the enamel knot moves laterally to induce Wise, an inhibitor of Wnt receptors LRP5 and LRP6; thus Wise can downregulate Wnt signalling,

which can also move laterally to lead to *Lef1* and *Sp5* activation (47). In addition, Wnt and its nuclear mediator, LEF1, are essential for epithelial FGF4 signalling, whose targets are the *Fgf* genes expressed in the dental mesenchyme (44). Although there is lack of consensus between various experimental approaches, the study of these inductive signalling networks involving key signalling pathways and links, may reflect their adaptability and provide a comprehensive view of how cells orchestrate these pieces of information and respond in an accurate, efficient and balanced manner in tooth development.

Future perspectives

The inductive signalling network described here integrates molecules and signalling pathways involved in mouse odontogenesis, casting light upon mechanisms of tooth regeneration. Accumulated data have proved that overexpression of Shh and Wnt/ β -catenin lead to supernumerary teeth, thus these may be potential targets for driving stem cells to *de novo* tooth formation according to the complex signalling network in odontogenesis. Currently, tooth regeneration is regarded as having great potential for clinical applications and is attracting more and more attention due to its ability to provide physiological and functional superiority in mastication and better responsiveness to noxious stimulation, in contrast to artificial teeth. Based on distinct mechanisms of tooth development, there are two possible approaches to tooth regeneration by various kinds of stem cells. The first involves creation of a bioengineered tooth unit for mature organ replacement; more recently, for example, a tooth unit comprising a mature tooth, periodontal ligament and alveolar bone has successfully been transplanted into an extensive bone defect of the murine lower jaw; this unit displayed physiological tooth function after grafting (48). The second approach is to directly induce stem-cell proliferation and differentiation into a mature functional tooth in the defective area. Recent advances in adult cell reprogramming show that forced expression of transcription factors, such as *Oct4*, *Sox2*, *cmyc*, *Klf4*, *Nanog* and *Lin28*, can result in creation of induced pluripotent stem-cell lines from adult differentiated cells, and provide the basis for stem cells for tooth regeneration (49). More recently, *Sox2* has been identified as a marker of dental epithelial stem cells that expressed restricted to the oral epithelium throughout early incisor development, contributing to future stem-cell research studies (50). Subsequently, Juuri *et al.* have studied the *Sox2* expression pattern in mammalian molars and found that there existed dormant capacity for successional tooth renewal (51). But whether *Sox2* expression inhibits

or maintains proliferation of progenitor cells still needs to be verified by further functional experiments (51). Furthermore, stem cells in the cervical loop epithelium, whose population should depend on survival factor FGF10 in the dental mesenchyme, can give rise to enamel-forming ameloblasts, controlled by FGF signals derived from the dental mesenchyme (52).

A further potentially significant application for this inductive signalling network involves human congenital dental malformation due to improper molecular regulation of odontogenesis, such as tooth agenesis or enamel dysplasia. Currently, genetic defects of *Msx1*, *Pax9* and *Axin2* in non-syndromic tooth agenesis and *Pitx2* in Rieger syndrome have been identified (53–56). Additionally, amelogenesis imperfecta involving hereditary developmental malformations of tooth enamel can be caused heterogeneously, resulting in distinct phenotypic forms. Mutations of *AMELX*, *MMP20*, *KLK4* and *WDR72* can lead to hypomatured amelogenesis imperfecta. Although either smooth or rough hypoplastic type can occur once *AMELX* and *ENAM* are mutated, only the hypocalcified type is caused by mutations in *FAM83H*, with some variation in expressivity (57–59).

In summary, mutation/dysfunction of key molecular regulators, which also serve as components of the inductive signalling network, and balance intra-signalling pathways in the early stages of tooth development, particularly at the transition point between stages, results in dental malformation.

In conclusion, although rapid expansion of our knowledge of molecular mechanisms during odontogenesis has emerged in the past several years, this field is still in its infancy, and more work needs to be performed before we can easily and efficiently solve problems in dentistry, including tooth regeneration and human congenital dental malformation. Several questions remain to be answered. What are the differences in molecular regulation of tooth development among different species, such as mice and humans? How much influence could the pathways involved in tooth morphogenesis/development have on tooth regeneration and therapies for dental malformation? How can researchers control or take advantage of spatiotemporal expression of potential functional genes? In the near future, these challenges entail research in diverse fields, including systems biology, biomaterials, stem-cell biology and endodontics. However, our current work represents a comprehensive analysis of an inductive signalling network at the molecular level; this work describes regulation of cell maintenance, proliferation and differentiation during odontogenesis and provides fascinating insights into potential approaches for transforming the way we practise dentistry.

Acknowledgements

This work was supported by grant from National Basic Research Program of China (973 Program, 2010CB944800). We thank Qi-jia Yu (Sichuan University) for his artwork and critical reviews of this manuscript.

References

- 1 Lumsden AG (1988) Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development* **103**(Suppl), 155–169.
- 2 Zhang YD, Chen Z, Song YQ, Liu C, Chen YP (2005) Making a tooth: growth factors, transcription factors, and stem cells. *Cell Res.* **15**, 301–316.
- 3 Ahn Y, Sanderson BW, Klein OD, Krumlauf R (2010) Inhibition of Wnt signaling by Wise (Sostdc1) and negative feedback from Shh controls tooth number and patterning. *Development* **137**, 3221–3231.
- 4 Chen J, Lan Y, Baek JA, Gao Y, Jiang R (2009) Wnt/beta-catenin signaling plays an essential role in activation of odontogenic mesenchyme during early tooth development. *Dev. Biol.* **334**, 174–185.
- 5 Klein OD, Minowada G, Peterkova R, Kangas A, Yu BD, Lesot H *et al.* (2006) Sprouty genes control diastema tooth development via bidirectional antagonism of epithelial-mesenchymal FGF signaling. *Dev. Cell* **11**, 181–190.
- 6 O'Connell DJ, Ho JW, Mammoto T, Turbe-Doan A, O'Connell JT, Haseley PS *et al.* (2012) A Wnt-bmp feedback circuit controls inter-tissue signaling dynamics in tooth organogenesis. *Sci. Signal.* **5**, ra4.
- 7 Schwappacher R, Weiske J, Heining E, Ezerski V, Marom B, Henis YI *et al.* (2009) Novel crosstalk to BMP signalling: cGMP-dependent kinase I modulates BMP receptor and Smad activity. *EMBO J.* **28**, 1537–1550.
- 8 Chan MC, Hilyard AC, Wu C, Davis BN, Hill NS, Lal A *et al.* (2010) Molecular basis for antagonism between PDGF and the TGF beta family of signalling pathways by control of miR-24 expression. *EMBO J.* **29**, 559–573.
- 9 Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG *et al.* (2003) SHED: stem cells from human exfoliated deciduous teeth. *Proc. Natl. Acad. Sci. USA* **100**, 5807–5812.
- 10 Casagrande L, Demarco FF, Zhang Z, Araujo FB, Shi S, Nor JE (2010) Dentin-derived BMP-2 and odontoblast differentiation. *J. Dent. Res.* **89**, 603–608.
- 11 Lee HK, Park JT, Cho YS, Bae HS, Cho MI, Park JC (2012) Odontogenic ameloblast-associated protein (ODAM), via phosphorylation by bone morphogenetic protein receptor type IB (BMPRI-IB), is implicated in ameloblast differentiation. *J. Cell. Biochem.* **113**, 1754–1765.
- 12 Torres CB, Alves JB, Silva GA, Goes VS, Nakao LY, Goes AM (2008) Role of BMP-4 during tooth development in a model with complete dentition. *Arch. Oral Biol.* **53**, 2–8.
- 13 Wang XP, Suomalainen M, Jorgez CJ, Matzuk MM, Werner S, Thesleff I (2004) Follistatin regulates enamel patterning in mouse incisors by asymmetrically inhibiting BMP signaling and ameloblast differentiation. *Dev. Cell* **7**, 719–730.
- 14 Xu X, Han J, Ito Y, Bringas P Jr, Deng C, Chai Y (2008) Ectodermal Smad4 and p38 MAPK are functionally redundant in mediating TGF-beta/BMP signaling during tooth and palate development. *Dev. Cell* **15**, 322–329.
- 15 Andl T, Reddy ST, Gaddapara T, Millar SE (2002) WNT signals are required for the initiation of hair follicle development. *Dev. Cell* **2**, 643–653.
- 16 Gat U, DasGupta R, Degenstein L, Fuchs E (1998) De novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. *Cell* **95**, 605–614.
- 17 Ren S, Johnson BG, Kida Y, Ip C, Davidson KC, Lin SL *et al.* (2013) LRP-6 is a coreceptor for multiple fibrogenic signaling pathways in pericytes and myofibroblasts that are inhibited by DKK-1. *Proc. Natl. Acad. Sci. USA* **110**, 1440–1445.
- 18 Fjeld K, Kettunen P, Furmanek T, Kvinnsland IH, Luukko K (2005) Dynamic expression of Wnt signaling-related Dickkopf1, -2, and -3 mRNAs in the developing mouse tooth. *Dev. Dyn.* **233**, 161–166.
- 19 Seidel K, Ahn CP, Lyons D, Nee A, Ting K, Brownell I *et al.* (2010) Hedgehog signaling regulates the generation of ameloblast progenitors in the continuously growing mouse incisor. *Development* **137**, 3753–3761.
- 20 Ohazama A, Johnson EB, Ota MS, Choi HJ, Pomtaveetus T, Oommen S *et al.* (2008). Lrp4 modulates extracellular integration of cell signaling pathways in development. *PLoS One* **3**, e4092.
- 21 Ito M, Yang Z, Andl T, Cui C, Kim N, Millar SE *et al.* (2007) Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding. *Nature* **447**, 316–320.
- 22 Jarvinen E, Salazar-Ciudad I, Birchmeier W, Taketo MM, Jernvall J, Thesleff I (2006) Continuous tooth generation in mouse is induced by activated epithelial Wnt/beta-catenin signaling. *Proc. Natl. Acad. Sci. USA* **103**, 18627–18632.
- 23 Wang XP, O'Connell DJ, Lund JJ, Saadi I, Kuraguchi M, Turbe-Doan A *et al.* (2009) Apc inhibition of Wnt signaling regulates supernumerary tooth formation during embryogenesis and throughout adulthood. *Development* **136**, 1939–1949.
- 24 Goodfellow H, Krejci A, Moshkin Y, Verrijzer CP, Karch F, Bray SJ (2007) Gene-specific targeting of the histone chaperone asf1 to mediate silencing. *Dev. Cell* **13**, 593–600.
- 25 Komatsu H, Chao MY, Larkins-Ford J, Corkins ME, Somers GA, Tukey T *et al.* (2008) OSM-11 facilitates LIN-12 Notch signaling during *Caenorhabditis elegans* vulval development. *PLoS Biol.* **6**, e196.
- 26 Mitsiadis TA, Regaudiat L, Gridley T (2005) Role of the Notch signalling pathway in tooth morphogenesis. *Arch. Oral Biol.* **50**, 137–140.
- 27 Mitsiadis TA, Graf D, Luder H, Gridley T, Bluteau G (2010) BMPs and FGFs target Notch signalling via jagged 2 to regulate tooth morphogenesis and cytodifferentiation. *Development* **137**, 3025–3035.
- 28 Kumamoto H, Ohki K (2008) Detection of Notch signaling molecules in ameloblastomas. *J. Oral Pathol. Med.* **37**, 228–234.
- 29 Siar CH, Nakano K, Han PP, Nagatsuka H, Ng KH, Kawakami T (2010) Differential expression of Notch receptors and their ligands in desmoplastic ameloblastoma. *J. Oral Pathol. Med.* **39**, 552–558.
- 30 Harada H, Ichimori Y, Yokohama-Tamaki T, Ohshima H, Kawano S, Katsube K *et al.* (2006) Stratum intermedium lineage diverges from ameloblast lineage via Notch signaling. *Biochem. Biophys. Res. Commun.* **340**, 611–616.
- 31 Nakao A, Kajiya H, Fukushima H, Fukushima A, Anan H, Ozeki S *et al.* (2009) PTHrP induces Notch signaling in periodontal ligament cells. *J. Dent. Res.* **88**, 551–556.
- 32 Zhang C, Chang J, Sonoyama W, Shi S, Wang CY (2008) Inhibition of human dental pulp stem cell differentiation by Notch signaling. *J. Dent. Res.* **87**, 250–255.
- 33 Walterhouse DO, Yoon JW, Iannaccone PM (1999) Developmental pathways: Sonic hedgehog patched-GLI. *Environ. Health Perspect.* **107**, 167–171.
- 34 Dai P, Akimaru H, Tanaka Y, Maekawa T, Nakafuku M, Ishii S (1999) Sonic hedgehog-induced activation of the Gli1 promoter is mediated by GLI3. *J. Biol. Chem.* **274**, 8143–8152.

- 35 Fraser GJ, Graham A, Smith MM (2006) Developmental and evolutionary origins of the vertebrate dentition: molecular controls for spatio-temporal organisation of tooth sites in osteichthyans. *J. Exp. Zool. B Mol. Dev. Evol.* **306**, 183–203.
- 36 Keranen SV, Kettunen P, Aberg T, Thesleff I, Jernvall J (1999) Gene expression patterns associated with suppression of odontogenesis in mouse and vole diastema regions. *Dev. Genes. Evol.* **209**, 495–506.
- 37 Jernvall J, Thesleff I (2012) Tooth shape formation and tooth renewal: evolving with the same signals. *Development* **139**, 3487–3497.
- 38 Matsumoto T, Turesson I, Book M, Gerwins P, Claesson-Welsh L (2002) p38 MAP kinase negatively regulates endothelial cell survival, proliferation, and differentiation in FGF-2-stimulated angiogenesis. *J. Cell Biol.* **156**, 149–160.
- 39 Venkataraman G, Raman R, Sasisekharan V, Sasisekharan R (1999) Molecular characteristics of fibroblast growth factor-fibroblast growth factor receptor-heparin-like glycosaminoglycan complex. *Proc. Natl. Acad. Sci. USA* **96**, 3658–3663.
- 40 Klein OD, Lyons DB, Balooch G, Marshall GW, Basson MA, Peterka M *et al.* (2008) An FGF signaling loop sustains the generation of differentiated progeny from stem cells in mouse incisors. *Development* **135**, 377–385.
- 41 Aberg T, Wang XP, Kim JH, Yamashiro T, Bei M, Rice R *et al.* (2004) Runx2 mediates FGF signaling from epithelium to mesenchyme during tooth morphogenesis. *Dev. Biol.* **270**, 76–93.
- 42 Mitsiadis TA, Tucker AS, De Bari C, Cobourne MT, Rice DPC (2008) A regulatory relationship between Tbx1 and FGF signaling during tooth morphogenesis and ameloblast lineage determination. *Dev. Biol.* **320**, 39–48.
- 43 Haara O, Harjunmaa E, Lindfors PH, Huh SH, Fliniaux I, Aberg T *et al.* (2012) Ectodysplasin regulates activator-inhibitor balance in murine tooth development through Fgf20 signaling. *Development* **139**, 3189–3199.
- 44 Kratochwil K, Galceran J, Tontsch S, Roth W, Grosschedl R (2002) FGF4, a direct target of Lef1 and Wnt signaling, can rescue the arrest of tooth organogenesis in Lef1(–/–) mice. *Genes Dev.* **16**, 3173–3185.
- 45 Li JY, Huang XF, Xu X, Mayo J, Bringas P, Jiang RL *et al.* (2011) SMAD4-mediated WNT signaling controls the fate of cranial neural crest cells during tooth morphogenesis. *Development* **138**, 1977–1989.
- 46 Fujimori S, Novak H, Weissenböck M, Jussila M, Gonçalves A, Zeller R *et al.* (2010) Wnt/beta-catenin signaling in the dental mesenchyme regulates incisor development by regulating Bmp4. *Dev. Biol.* **348**, 97–106.
- 47 Cho SW, Kwak S, Woolley TE, Lee MJ, Kim EJ, Baker RE *et al.* (2011) Interactions between Shh, Sostdc1 and Wnt signaling and a new feedback loop for spatial patterning of the teeth. *Development* **138**, 1807–1816.
- 48 Oshima M, Mizuno M, Imamura A, Ogawa M, Yasukawa M, Yamazaki H *et al.* (2011) Functional tooth regeneration using a bioengineered tooth unit as a mature organ replacement regenerative therapy. *PLoS One* **6**, e21531.
- 49 Bei M (2009) Molecular genetics of tooth development. *Curr. Opin. Genet. Dev.* **19**, 504–510.
- 50 Juuri E, Saito K, Ahtiainen L, Seidel K, Tummers M, Hochedlinger K *et al.* (2012) Sox2⁺ stem cells contribute to all epithelial lineages of the tooth via Sfrp5⁺ progenitors. *Dev. Cell* **23**, 317–328.
- 51 Juuri E, Jussila M, Seidel K, Holmes S, Wu P, Richman J *et al.* (2013) Sox2 marks epithelial competence to generate teeth in mammals and reptiles. *Development* **140**, 1424–1432.
- 52 Harada H, Toyono T, Toyoshima K, Yamasaki M, Itoh N, Kato S *et al.* (2002) FGF10 maintains stem cell compartment in developing mouse incisors. *Development* **129**, 1533–1541.
- 53 Lammi L, Arte S, Somer M, Jarvinen H, Lahermo P, Thesleff I *et al.* (2004) Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. *Am. J. Hum. Genet.* **74**, 1043–1050.
- 54 Pawlowska E, Janik-Papis K, Poplawski T, Blasiak J, Szczepanska J (2010) Mutations in the PAX9 gene in sporadic oligodontia. *Orthod. Craniofac. Res.* **13**, 142–152.
- 55 Wang Y, Zhao H, Zhang X, Feng H (2003) Novel identification of a four-base-pair deletion mutation in PITX2 in a Rieger syndrome family. *J. Dent. Res.* **82**, 1008–1012.
- 56 Xuan K, Jin F, Liu YL, Yuan LT, Wen LY, Yang FS *et al.* (2008) Identification of a novel missense mutation of MSX1 gene in Chinese family with autosomal-dominant oligodontia. *Arch. Oral Biol.* **53**, 773–779.
- 57 Chan HC, Mai L, Oikonomopoulou A, Chan HL, Richardson AS, Wang SK *et al.* (2010) Altered enamelin phosphorylation site causes amelogenesis imperfecta. *J. Dent. Res.* **89**, 695–699.
- 58 Kuchler EC, Menezes R, Callahan N, Costa MC, Modesto A, Meira R *et al.* (2011) MMP1 and MMP20 contribute to tooth agenesis in humans. *Arch. Oral Biol.* **56**, 506–511.
- 59 Stephanopoulos G, Garefalaki ME, Lyroutdia K (2005) Genes and related proteins involved in amelogenesis imperfecta. *J. Dent. Res.* **84**, 1117–1126.