REVIEW ARTICLE

Fibroblast growth factor signaling in mammalian tooth development

Chun-Ying Li · Jan Prochazka · Alice F. Goodwin ·
Ophir D. Klein

Abstract In this review, we discuss the central role of fibroblast growth factor (FGF) signaling in mammalian tooth development. The FGF family consists of 22 members, most of which bind to four different receptor tyrosine kinases, which in turn signal through a cascade of intracellular proteins. This signaling regulates a number of cellular processes, including proliferation, differentiation, cell adhesion and cell mobility. FGF signaling first becomes important in the presumptive dental epithelium at the initiation stage of tooth development, and subsequently, it controls the invagination of the dental epithelium into the underlying mesenchyme. Later, FGFs are critical in tooth shape formation and differentiation of ameloblasts and odontoblasts, as well as in the development and homeostasis of the stem cell niche that fuels the continuously growing mouse incisor. In addition, FGF signaling is critical in human teeth, as mutations in genes encoding FGF ligands or receptors result in several congenital syndromes characterized by alterations in tooth number, morphology or enamel structure. The parallel roles of FGF signaling in mouse and human tooth development demonstrate the conserved importance of FGF signaling in mammalian odontogenesis.

C.-Y. Li
Zhongshan Hospital of Dalian University, Dalian 116001, China

C.-Y. Li · J. Prochazka · A. F. Goodwin · O. D. Klein (✉)
Department of Orofacial Sciences and Program in Craniofacial
and Mesenchymal Biology, University of California San
Francisco, 513 Parnassus ave, HSE1508, San Francisco,
CA 94143, USA
e-mail: ophir.klein@ucsf.edu

O. D. Klein
Department of Pediatrics and Institute for Human Genetics,
University of California San Francisco, 513 Parnassus ave,
HSE1508, San Francisco, CA 94143, USA

Introduction

Every species has a unique array of teeth. Humans develop two sets of dentition, one deciduous (milk teeth) and one permanent, with four types of teeth in a continuous row. Unlike humans, mice only have one set of teeth with two different tooth types: three molars proximally and one incisor distally in each quadrant, separated by a toothless region called the diastema. In contrast to the molars, mouse incisors grow continuously throughout the life of the animal, and this continuous growth is fueled by somatic stem cells that reside in the proximal portion of the incisor and give rise to the differentiated cell types of the tooth [1]. Therefore, the mouse provides an excellent model to study both the genetics and molecular mechanisms of tooth patterning and the function of stem cells in tooth development.

The stages of early tooth development are similar in all mammals. In the mouse, tooth formation is initiated by a signal from the oral epithelium at around embryonic day (E) 9.5 [2, 3], and the thickening of the prospective dental epithelium is the first visible sign of tooth development. The epithelium signals to the underlying mesenchyme to initiate odontogenesis, and the mesenchyme induces the localized thickening of the oral epithelium to form the dental lamina at the position of the future teeth. The dental lamina then grows into the underlying mesenchyme at the sites of tooth formation, and the odontogenic mesenchymal cells condense around the invaginating epithelium to form the tooth bud. The development of the tooth crown and acquisition of tooth shape occurs during the subsequent cap and bell stages.

During tooth development, two sets of transient signaling centers called enamel knots (EKs) form in the epithelium. The primary EK (pEK) appears at the bud stage at the tip of the dental epithelium, and it expresses several signaling molecules that regulate the bud to cap transition by controlling cell proliferation and apoptosis and later determine cusp morphogenesis [4, 5]. Molar teeth develop a secondary EK (sEK), which determines the multicuspid pattern of molar crowns. During the transition from bud to cap stage, the mesenchymal cells nearest to the tip of the epithelial bud give rise to the dental papilla. At the later bell stage, the odontoblasts differentiate from the dental papilla and produce the dentin matrix, while the ameloblasts arise from the epithelium and secrete the enamel matrix [6].

Fibroblast growth factor (FGF) signaling induces the growth and differentiation of many different cell types in the embryo [7–11]. The role of FGFs as inductive embryonic signals was first reported during mesoderm formation in Xenopus embryos [12], and further studies in Drosophila [13] and mammals [14] showed that FGFs are widely required for development in animals. Here, we focus on the roles of FGF signaling in mammalian tooth development and review how FGFs regulate dental positioning, initiation, invagination and differentiation during tooth formation. We also discuss how FGFs control the function of stem cells in the continuously growing incisor in mouse and how dysregulation of FGF signaling in humans affects dental development.

Fibroblast growth factors and their receptors

The FGF family is one of the largest growth factor families, consisting of 22 members that share 13–71 % sequence homology in mammals [15]. Most FGFs mediate their biological responses as extracellular proteins by binding to and activating cell surface tyrosine kinase FGF receptors (FGFRs) [15, 16]. FGFs can be subdivided into several subfamilies based on sequence similarities and functional properties such as receptor specificity and binding affinity [15–17] (Table 1). Among these FGFs, FGF11–14 function as intracellular proteins, called iFGFs, which act in an FGFR-independent manner [18]. A principal role of FGF signaling during embryonic tooth development, as discussed below, is the regulation of morphogenesis between the epithelial layer and underlying mesenchyme. Similar processes have also been observed in the development of lungs [19], salivary glands [20], mammary glands [21], limb buds [22], brain [23], and other organs.

FGFRs are four related transmembrane proteins consisting of an extracellular ligand binding domain, a single transmembrane domain, and an intracellular tyrosine

Table 1 FGF families and their members

FGF family	FGF members
FGF1	FGF1, FGF2
FGF4	FGF4, FGF5, FGF6
FGF7	FGF3, FGF7, FGF10, FGF22
FGF8	FGF8, FGF17, FGF18
FGF9	FGF9, FGF16, FGF20
FGF11	FGF11, FGF12, FGF13, FGF14
FGF19/15	FGF19/15, FGF21, FGF23

kinase domain. *Fgfr1-3* mRNA undergoes alternative splicing events that can result in three alternative versions of the Ig-like domain III of the extracellular component of FGFR; this, in turn, can alter ligand binding properties of the extracellular domain. Individual splice forms are called IIIa, IIIb, IIIc in FGFRs 1–3 [24–26]; *Fgfr4* mRNA is not alternatively spliced in this region [27]. The *FgfrIIIa* splice form encodes a protein with a terminal Ig-like domain resulting in a soluble FGF binding protein without known signaling function [28]. The splice variants *IIIb* and *IIIc* influence specificity of ligand binding and appear to be regulated in a tissue-dependent manner. *IIIb* splice forms are predominantly expressed in epithelial lineages and transduce signals initiated by FGF ligands expressed in the mesenchyme. The *IIIc* splice variant expression is restricted to mesenchymal lineages and is responsible for transduction of signaling from FGF ligands expressed in the epithelium [29–33].

The dimerization of receptors results in trans-phosphorylation and activation of FGFRs [34], which initiates signaling through multiple downstream intracellular pathways. The cytosolic domain of the activated receptor binds a range of adaptor proteins, including growth factor receptor-bound protein 2 (GRB2) and SHP2 [35–38]. These recruit the guanosine nucleotide exchange factors (GEFs) son-of-sevenless (SOS) 1 and 2, which convert the small GTPase Ras from inactive Ras-GDP to activated Ras-GTP. Once activated, Ras signals through multiple effector pathways, including RAF/MEK/ERK, phosphatidylinositol-3-kinase (PI3K)/AKT, T-cell lymphoma invasion and metastasis 1 (TIAM1)/Rac, and Ral guanine nucleotide dissociation stimulator (RALGDS)/Ral [39–42]. In the simplest terms, Ras activates RAF (RAF-1, ARAF, BRAF, and CRAF), which phosphorylates and activates MEK1/2, which in turn activates ERK1/2. Activated ERK1/2 phosphorylates several targets, including transcription factors of the ETS family, such as JUN and ELK1, which promote cell cycle progression and proliferation [43]. Activated Ras can also bind PI3K [44, 45], which activates phosphoinositide-dependent kinase 1 (PDK1) and AKT to promote cell cycle progression and

cell survival [46]. The TIAM-1/Rac pathway is involved in cytoskeletal remodeling [47, 48], while RALGDS/Ral is involved in endocytosis, exocytosis, and actin skeleton organization [49]. The contribution of each downstream effector pathway to various biological processes is an active area of research, and complex interactions between the pathways have yet to be fully characterized.

Since the intensity and duration of FGF signaling are critical for controlling various cellular functions, the pathway has multiple regulators. One such group of regulators is encoded by the Sprouty genes, whose gene products negatively regulate FGF signaling. Although the biochemical functions of Sprouty proteins are still unclear, it is known that Ras signaling induces expression of Sprouty genes, and it is thought that Sprouty proteins bind GRB2, preventing SOS localization and activation of Ras [50], and RAF, interfering with its interaction with downstream MEK [51, 52].

Expression of FGFs, FGFRs, and Sprouty genes during tooth development

To date, the expression of twelve FGF ligands has been reported at different stages of tooth development (Fig. 1). Four FGFs, *Fgf8, Fgf9, Fgf10* and *Fgf17*, and two FGF receptors, *Fgfr2IIIb* and *Fgfr1IIIc*, are expressed early in the prospective tooth region (Fig. 1a) [53–55]. The expression of *Fgfr2IIIb* and *Fgfr1IIIc* is maintained throughout tooth development. When the epithelium in the prospective tooth region becomes thickened to form the dental lamina (Fig. 1b), *Fgf10* expression in the epithelium is diminished [55], while six other FGFs are expressed in the prospective tooth area: *Fgf8, Fgf9, Fgf15* and *Fgf20* in the epithelium, *Fgf10* and *Fgf18* in the mesenchyme. As the epithelium within the dental lamina continues to form the epithelial bud (Fig. 1c), the expression of *Fgf9* and *Fgf20* is maintained in the epithelium, and expression of *Fgf4* is initiated. *Fgf10* and *Fgf18* continue to be expressed in the mesenchyme, and *Fgf3* is expressed in both epithelium and mesenchyme. In addition to *Fgfr1IIIc*, *Fgfr2IIIc* expression is established in the mesenchyme at this stage. After formation of the pEK (Fig. 1d), *Fgf3, Fgf4, Fgf9, Fgf15* and *Fgf20* are restricted to this structure. Other FGFs are expressed as follows: *Fgf3, Fgf10* and *Fgf18* in the mesenchyme, and *Fgf16* and *Fgf17* in both epithelium and mesenchyme of the cervical loop. *Fgfr2IIIb*, *Fgfr1IIIb* and *Fgfr1IIIc* are expressed in the enamel epithelium, and *Fgfr1IIIc* and *Fgfr2IIIc* in the buccal mesenchyme.

At the late bell stage (Fig. 1e), *Fgf4* and *Fgf20* are expressed at the tip of the forming cusps in the secondary enamel knot (sEK), and *Fgf9* and *Fgf16* in the differentiating ameloblasts (Fig. 1f). In the mesenchyme, *Fgf3* is restricted to the dental papilla, *Fgf10* to the differentiating odontoblasts, and *Fgf15* to the mesenchyme underlying the sEK. *Fgfr1IIIb* and *Fgfr1IIIc* are expressed in the differentiating ameloblasts, and *Fgfr1IIIb* and *Fgfr1IIIc* are expressed in the odontoblasts [55]. In the incisor at the bell stage, *Fgf1*, *Fgf9*, *Fgf16* and *Fgf17* are expressed in the epithelium of the cervical loop, and in the mesenchyme, six FGFs are detected at this point: *Fgf3, Fgf7, Fgf10, Fgf16, Fgf18* and *Fgf21* [54, 56].

Sprouty (Spry) genes are expressed in different tissue compartments during tooth development [57]. At the cap stage, *Spry1* is expressed at low levels in the diastema buds and at higher levels in the first molar (M1) tooth germs. *Spry2* is abundantly expressed in the epithelium adjacent to dental mesenchyme, including the diastema and M1 tooth germ. *Spry4* is almost exclusively expressed in the dental mesenchyme in both diastema and M1 tooth germs. *Spry3*, which is expressed in adult brain and testis, is not expressed in the tooth germ.

FGFs are critical in the determination of the presumptive tooth epithelium and formation of the dental lamina

Vertebrate organogenesis is often initiated at sites that are histologically indistinguishable from the surrounding tissues. In murine tooth formation, the first signals are derived from the presumptive tooth epithelium at E9.5 [58]. The oral ectoderm thickens in the prospective tooth-forming regions, and the expression of *Fgf8*, *Fgf9*, and *Fgf17* in the epithelium at this stage suggests that these FGFs may signal to initiate tooth formation [54, 56]. Early work showed that FGF8 protein is sufficient to induce *Pax9* expression at E9.5, which marks sites of prospective odontogenesis in mice and is required for tooth development beyond the bud stage [59]. Conditional deletion of *Fgf8* with Nestin-Cre in the ectoderm of the first branchial arch (BA1) resulted in decreased expression of *Pax9* in the presumptive molar region, and molar tooth formation was arrested at the initiation stage. The expression of *Pax9* was not affected in the presumptive incisor region, and the incisor developed normally in this mutant. FGF8 is essential for mesenchymal cell survival, since without FGF8, the mesenchymal cells undergo apoptosis in the proximal region of BA1 [60]. These data suggested that FGF8 is a key inductive signal in the mesenchyme during initiation of molar odontogenesis. However, deletion of *Fgf8* resulted in agenesis of the entire posterior portion of BA1, and therefore, conditional deletion of *Fgf8* after proper formation of the mandible and before the initiation of molar odontogenesis will be needed to provide a clearer picture of FGF8 function during tooth development.
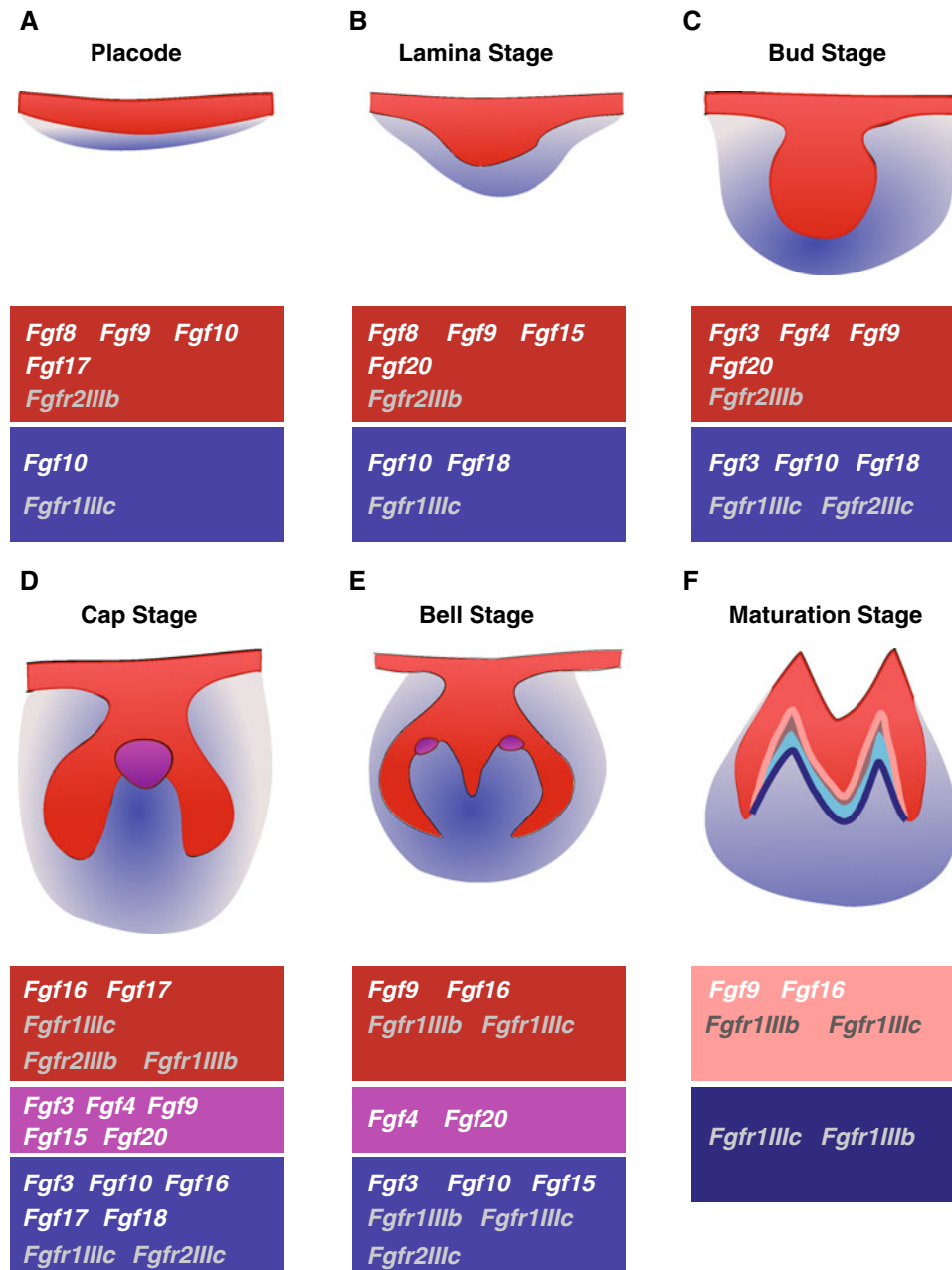
A **Placode**

B **Lamina Stage**

C **Bud Stage**

Fgf8 *Fgf9* *Fgf10* *Fgf17*
Fgfr2IIIb

Fgf10
Fgfr1IIIc

Fgf8 *Fgf9* *Fgf15* *Fgf20*
Fgfr2IIIb

Fgf10 *Fgf18*
Fgfr1IIIc

Fgf3 *Fgf4* *Fgf9* *Fgf20*
Fgfr2IIIb

Fgf3 *Fgf10* *Fgf18*
Fgfr1IIIc *Fgfr2IIIc*

D **Cap Stage**

E **Bell Stage**

F **Maturation Stage**

Fgf16 *Fgf17*
Fgfr1IIIc
Fgfr2IIIb *Fgfr1IIIb*

Fgf3 *Fgf4* *Fgf9* *Fgf15* *Fgf20*

Fgf3 *Fgf10* *Fgf16* *Fgf17* *Fgf18*
Fgfr1IIIc *Fgfr2IIIc*

Fgf9 *Fgf16*
Fgfr1IIIb *Fgfr1IIIc*

Fgf4 *Fgf20*

Fgf3 *Fgf10* *Fgf15*
Fgfr1IIIb *Fgfr1IIIc*
Fgfr2IIIc

Fgf9 *Fgf16*
Fgfr1IIIb *Fgfr1IIIc*

Fgfr1IIIc *Fgfr1IIIb*

Fig. 1 Expression of FGFs and their receptors in molar development. **a** At the placode (initiation) stage, *Fgf8*, *Fgf9*, *Fgf10* and *Fgf17* are expressed in epithelium (*red*) together with *Fgfr2IIIb*. *Fgf10* is also expressed in mesenchyme (*blue*) with *Fgfr1IIIc*. **b** At the dental lamina stage, expression of *Fgf8*, *Fgf9*, *Fgf15*, *Fgf20 and Fgfr2IIIb* is localized in epithelium and *Fgf10*, *Fgf18* and *Fgfr1IIIc* in mesenchyme. **c** At bud stage, *Fgf3*, *Fgf4*, *Fgf9*, *Fgf20* and *Fgfr2IIIb* are expressed in epithelium and *Fgf3*, *Fgf10*, *Fgf18*, *Fgfr1IIIc* and *Fgfr2IIIc* are expressed in mesenchyme. **d** At cap stage, *Fgf16*, *Fgf17* and *Fgfr1IIIb*, *Fgfr1IIIc* and *Fgfr2IIIb* are expressed in dental epithelium (*red*). Expression of *Fgf3*, *Fgf4*, *Fgf9*, *Fgf15* and *Fgf20* is restricted to the enamel knot (*violet*). Mesenchyme forms the dental papilla (*blue*) at this stage which expresses *Fgf3*, *Fgf10*, *Fgf16*, *Fgf17*, *Fgf18*, *Fgfr1IIIc* and *Fgfr2IIIc*. **e** At bell stage, *Fgf9*, *Fgf16*, *Fgfr1IIIb* and *Fgfr1IIIc* are expressed in dental epithelium. *Fgf4* and *Fgf20* are expressed only in the secondary enamel knots (*violet*). *Fgf3*, *Fgf10*, *Fgf15*, *Fgfr1IIIb*, *Fgfr1IIIc* and *Fgfr2IIIc* are expressed in the mesenchyme of the dental papilla. **f** During maturation stage, the expression of *Fgf9* and *Fgf16* is localized to ameloblasts (*pink*) derived from the epithelium (*red*) together with *Fgfr1IIIb* and *Fgfr1IIIc*. Only odontoblasts (*dark blue*) from mesenchymal cells (*light blue*) retain expression of *Fgfr1IIIb* and *Fgfr1IIIc*. At this stage, ameloblasts secrete enamel matrix (*grey*) and odontoblast secrete dentin (*cyan*)

Another FGF family member, *Fgf10,* is expressed in both epithelium and mesenchyme at this early stage [55]. *Fgf10* null mice develop teeth, although the formation of

the stem cell compartment in the apical incisor bud is disrupted [61], and deletion of another gene expressed at early stages, *Fgf9*, does not affect tooth formation in mice

[62, 63]. These data indicate that neither FGF9 nor FGF10 is involved in positioning of the tooth sites, or that there is redundancy between these FGF ligands during tooth initiation. *Fgf17*, a more recently studied gene, is expressed at the initiation stage and belongs to the FGF8 subfamily. *Fgf17* is expressed in the prospective molar but not incisor epithelial region, suggesting that like FGF8, FGF17 plays a role in the positioning of the presumptive molar sites [54]. FGF8 is thought to play a critical role in tooth type determination [59, 64], and FGF17 may participate in this process as well. *Bmp2* and *Bmp4* antagonize the inductive effects of *Fgf8* on *Pax9* expression at E10, prior to thickening of the dental ectoderm, and it has been suggested that odontogenesis is initiated only in regions in which the inducer FGF is present, its antagonists (BMPs) are absent, and the mesenchyme is competent to respond to the inducer.

At the prospective tooth-forming region, the epithelium thickens to form a multilayered epithelium that later forms the dental lamina. At this stage, the expression of *Fgf10* is downregulated [55]. Expression of *Fgf8* and *Fgf9* in the epithelium persists, expression of *Fgf15* is initiated on the lingual side of the dental lamina, and *Fgf20* is expressed at the tip of the dental lamina, suggesting that these genes play a role during the thickening of the epithelium [54].

Mouse molecular genetic approaches targeting individual FGF genes during tooth development have provided some understanding of the role of FGF signaling in dental lamina formation. Deletion of *Fgf9*, *Fgf10* or *Fgf20* does not appear to affect the thickening of oral epithelium or dental lamina formation [63, 65]. These results may be due to compensatory effects among the FGFs, and conditional deletion of FGFs in combination at this stage is needed to determine their effects on lamina formation. FGF18 is a newly identified FGF that belongs to the FGF8 subfamily. Unlike other FGF8 family members, which are expressed in the epithelium, *Fgf18* mRNA is found at the buccal side of the mesenchyme at the lamina stage. Its function in tooth development is still unknown, and further study is required to determine whether FGF18 plays a role during odontogenesis. During these early stages of tooth development, *Fgf2rIIIb* is expressed in the odontogenic epithelium, and *Fgf1rIIIc* is expressed in the underlying mesenchyme [56].

FGF signaling regulates invagination of the dental epithelium

The dental lamina invaginates into the mesenchyme and induces mesenchymal condensation around the epithelium, which forms a tooth bud and later cap. The expression pattern of FGFs (Fig. 1) indicates that, at the invagination and bud stages, FGF signaling is activated in the

epithelium by FGF3 and FGF10 binding to Fgfr2IIIb. In the mesenchyme, FGF4, FGF8 and FGF20 likely bind FGFR1IIIc, and FGF4, FGF8, FGF9, FGF16, FGF18 and FGF20 bind FGFR2IIIc [53, 54]. In *Fgfr2* mutant mice, tooth development is arrested after epithelial thickening, and mesenchymal condensation is not observed in the *Fgfr2*$^{-/-}$ tooth germ. Although mesenchymal *Fgf3* and *Fgf10* expression is detectable, the epithelial expression of *Fgf3* in *Fgfr2* mutants is diminished [66].

FGF3 and FGF10 signal through FGFR2IIIb, suggesting that these FGFs are critical in the transition to the bud stage [67, 68]. However, mice with single mutations in *Fgf3* or *Fgf10* do not show any defect in early tooth development, and tooth germs proceed to cap stage normally. Further study in the *Fgf3*$^{-/-}$*;Fgf10*$^{-/-}$ double null mutants revealed that, in the embryo, molar development is arrested prior to the bud stage, indicating that *Fgf3* and *Fgf10* can compensate for each other during dental epithelium invagination [69, 70].

Fgf9 is strongly expressed in the tip of the bud epithelium (Fig. 1). *Fgf9* null mice do not show any defect in tooth bud invagination, although the differentiation of progenitor cells in the incisor is affected [62, 63]. Interestingly, exogenous FGF9 protein rescues the epithelium invagination defect in *Runx2*$^{-/-}$ tooth germs [62, 71], which suggests that FGF9 is a required downstream target of RUNX2 in tooth invagination. These findings highlight the compensatory effects that occur between FGF9 and other FGFs expressed in the epithelium. FGF9 also positively regulates the homeobox-containing transcription factor *Msx1*, which is an essential molecule for bud invagination [56, 72].

PITX2 is an important transcription factor that is regulated by FGF signaling during tooth bud invagination. Two molecules, BMP4 and FGF8, initially control the expression of *Pitx2* in the oral epithelium; FGF8 positively regulates *Pitx2* expression and BMP4 represses it [73]. In the absence of *Pitx2*, the expression of *Fgf8* in the oral epithelium is diminished [74, 75]. FGF8 and BMP4 may act as positive–negative feedback regulators to control *Pitx2* expression and regulate invagination of the tooth bud.

At E13.5, FGF4 expression initiates at the tip of the epithelium. *Fgf4* expression is diminished in *Lef1* null tooth germs at E13, resulting in a defect in mesenchyme condensation [76], and exogenous FGF4 protein rapidly induces the expression of *Fgf3* in dental mesenchyme and fully rescues the developmental arrest of *Lef1*$^{-/-}$ tooth germs [77]. These data indicate that *Fgf4* is a transcriptional target of WNT signaling. FGF18 is present in the mesenchyme, except underneath the bud epithelium, at this stage, and further investigation is required to understand the function of this protein in odontogenesis [54]. FGF20 expression is restricted in the epithelium to the tip of the

tooth bud. Like *Fgf9*, deletion of *Fgf20* in teeth does not disrupt early tooth development, and mutant mice form teeth normally [63]. These redundant roles will make it necessary to analyze double or triple deletion of FGFs to clearly understand gene function at this stage.

FGF signaling regulates tooth shape and cusp formation

Tooth shape characteristics are determined during embryonic development. The pEK, a signaling center that regulates tooth size and shape, is composed of non-proliferating cells [4] that express signaling molecules and their antagonists, including FGFs, Sprouty genes, Shh, several WNTs, BMPs and Follistatin [78]. The pEK cells themselves do not express FGF receptors and thus are unable to respond to the mitogenic stimuli of FGFs [56]. The lack of proliferation in the pEK combined with extensive proliferation around it may regulate the epithelial folding and the bud to cap transition [4, 79]. In multicuspid teeth, the pEK induces the formation of the sEKs. A network of activators and inhibitors has been suggested to determine the spatial arrangement of the sEKs [80, 81]. The secreted molecules from the sEKs regulate the proliferation and differentiation of the epithelium, which specifies the position and shape of the cusps and thus determines the shape of the tooth crown.

The size of the pEK in the molar is responsible for shaping the invaginated dental epithelium. If the pEK is too small, the folding of the dental epithelium and formation of cervical loop and sEKs are affected, which results in reduction of tooth size and cusp number; this occurs, for example, in mice that are null for ectodysplasin (*Eda*) or *Traf6*, members of the TNF-α family that regulate tooth development [82, 83]. Compromising the signaling from the pEK by altering its size or shape leads to changes in the arrangement of the sEKs in the molar, resulting in cusp defects. Experimentally, manipulating the level of gene expression in the EKs also results in variation of molar morphologies. For example, modulation of SHH, BMP and WNT signaling results in altered molar shapes and cusp patterns [84–88].

In terms of the FGF family, *Fgf4* and *Fgf9* are strongly expressed in the pEK and sEKs, and these proteins maintain *Fgf3* expression in the dental mesenchyme. FGF4 in the EK may stimulate proliferation and thereby regulate the growth of tooth cusps [4]. In addition to stimulating cell division, FGF4 prevents apoptosis in the dental mesenchyme and epithelium [5, 89]. However, inactivating either *Fgf4* or *Fgf9* individually has no effect on tooth number or shape [62, 63].

Another FGF family member, *Fgf20,* is expressed in the anterior bud of the dental lamina as well as in the EK, where *Fgf3*, *Fgf4*, *Fgf9* and *Fgf15* are also expressed [54, 56, 90]. FGF20 lies downstream of EDA during tooth development, as the expression of *Fgf20* is significantly

decreased in $Eda^{-/-}$ molars, whereas the $K14\text{-}Eda$ mice show increased expression of *Fgf20* [63]. Deletion of *Fgf20* in mice results in smaller molars with a mildly altered anterior cusp morphology, but the overall cusp pattern of the *Fgf20* mutants appears normal, and thus, FGF20 is involved in the regulation of tooth size and fine-tuning of anterior cusp patterning. Deletion of both *Fgf9* and *Fgf20* has a significant additive effect, shortening the EK length significantly compared to the length of either single mutant, demonstrating the redundant functions of the two FGFs [63].

Mesenchymal FGFs also affect tooth shape during development. In $Fgf3^{-/-};Fgf10^{+/-}$ mice, the molars are small, similar to *Fgf20* null molars [63, 70], and FGF10 protein partially rescues the $Eda^{-/-}$ molar phenotype in vitro [82]. Thus, reduction of either epithelial or mesenchymal FGF signaling can cause similar effects on tooth formation.

Besides the regulation of individual tooth size and shape, FGF signaling also tightly regulates tooth number and arrangement within the dentition. Adult mice have a reduced dentition of three molars and one incisor in each quadrant, and rudimentary tooth buds have been described in mouse embryos in the incisor and cheek regions [91]. These have their own signaling centers, which resemble EKs of functional teeth [92]. The rudiments arrest at the bud stage or possibly fuse with the first molar primordium to give rise to the anterior extension of the crown of the lower M1, called the anteroconid [93]. Supernumerary teeth have been reported in several mutant mice, and these are mostly located at the putative site of the premolar. These teeth are thought to represent revitalization of evolutionarily suppressed tooth rudiments. The first transgenic mouse line discovered with ectopic teeth was the *Eda* overexpressor *(K14-Eda)* [94]. Later experiments showed that deletion of *Fgf20* in this genetic background increased the frequency of extra tooth formation, but deletion of *Fgf20* alone was not sufficient to induce extra molar formation [63]. Supernumerary teeth anterior to the first molar as well as extra incisors are also present in knockouts of Sprouty genes, presumably from rescued vestigial buds [57, 95]. These results demonstrate a role for FGFs as stimulators of tooth formation and for Sprouty genes as important endogenous inhibitors of FGF activity in tooth formation.

FGF signaling regulates ameloblast and odontoblast differentiation

At the later bell stage, dental papilla cells differentiate into odontoblasts that produce a dentin matrix. This matrix induces the epithelium to differentiate into ameloblasts that secrete enamel matrix, forming the hard tissues of the tooth crown, dentin and enamel [6]. It is thought that FGFs from

the EK induce the differentiation of odontoblasts [96, 97]. At this stage, FGF3 and FGF10 are expressed in the mesenchyme. When dental papilla cells differentiate into odontoblasts, the expression of *Fgf3* and *Fgf10* is downregulated [55].

FGF signaling also plays an important role in the differentiation of ameloblasts. *Fgf4* and *Fgf9* are expressed in the inner enamel epithelium (IEE) [56], *Fgf2* is expressed in supporting cells called the stellate reticulum and *Fgfr1* and *Fgfr2IIIb* are expressed in the ameloblasts at the bell stage. Inactivation of *Fgfr1* in the epithelium resulted in dysfunctional ameloblasts that produced disorganized enamel [98]. Overexpression of *Fgf2* in cultured embryonic molars resulted in decreased expression of amelogenin, whereas inhibition of FGF2 increased amelogenin expression and enamel formation [99]. *Tbx1*, which encodes a transcription factor, is expressed in the dental epithelium; addition of FGF2 and FGF4 in tooth cultures induces *Tbx1* expression, and *Tbx1* expression is decreased in $Fgfr2^{-/-}$ mice [100]. Furthermore, *Tbx1* is necessary for ameloblast differentiation, as incisors from $Tbx1^{-/-}$ mice cultured in vitro lack ameloblasts and do not form enamel [101]. Interestingly, Ras superfamily members that are downstream of FGFs play a role in amelogenesis, including Rac, a GTPase involved in cytoskeletal remodeling. Conditional inactivation of *Rac1* in the epithelium in mouse results in ameloblasts that express decreased levels of amelogenin and have loose attachment to the secreted enamel matrix, resulting in hypo-mineralized enamel [102].

Increasing FGF signaling by decreasing Sprouty gene expression results not only in formation of supernumerary teeth but also in ectopic enamel formation [57]. In $Spry2^{+/-};Spry4^{-/-}$ mice, ameloblasts differentiate on the lingual aspect of the incisor and form ectopic enamel [103, 104]. Mice with increased signaling of HRas, which lies downstream of FGFs, had disorganized, hypo-mineralized enamel, and inhibiting the MAPK pathway rescued this phenotype [105].

FGFs regulate adult stem cells in the continuously growing mouse incisor

Rodent incisors grow continuously throughout the life of the animal, and the cervical loop, located in the proximal end of the incisor, is the niche that houses the dental stem cells [1]. Continuous incisor growth is counterbalanced by abrasion, which in rodent incisors is facilitated by absence of enamel on the lingual surface. This lack of lingual enamel is due to the absence of ameloblasts on that side [106]. Asymmetric abrasion not only maintains the incisor length but also generates a sharp tip (Fig. 2a), and a number of signaling factors and adhesion proteins have

been implicated in the maintenance of the continuous growth of the incisor. Histologically, the cervical loop contains several cell types: inner enamel epithelium (IEE), outer enamel epithelium (OEE), stellate reticulum (SR), stratum intermedium (SI), transit-amplifying (T-A) and differentiated ameloblasts. There is an additional group of more tightly condensed cells located between the SR and OEE [107] (Fig. 2b, c), and the function of this cell type is not clear.

Fgf10 is expressed in the mesenchyme that surrounds the epithelium of the apical part of the cervical loop, as well as in the mesenchyme underlying the inner enamel epithelium. *Fgf10* deletion leads to morphologically abnormal formation of the cervical loop and hypoplasia of the incisor [108]. Deletion of *Fgf10* causes decreased proliferation of ameloblast progenitor cells in the cervical loop, suggesting that *Fgf10* is essential for the maintenance and proliferation of the progenitor cells in the niche. *Fgf3* is asymmetrically expressed in the mesenchyme, with higher levels in the labial cervical loop, where *Fgf3* expression underlies the T-A cells. In the absence of *Fgf3*, the lower incisor lacks enamel, and the incisors are thin and frequently break. In $Fgf3^{-/-};Fgf10^{+/-}$ mice, the enamel layer is either very thin or missing in the incisors, and the labial cervical loop is not fully formed [70].

In order for stem cells to proliferate and differentiate in the cervical loop, FGFs must downregulate E-cadherin expression in the stem cells so that they can move out of the niche, proliferate and become T-A cells, which differentiate into more mature ameloblasts. E-cadherin is not downregulated in the T-A region in $Fgf3^{-/-};Fgf10^{+/-}$ mice, and cell proliferation is dramatically decreased in this region [107]. In contrast, $Spry2^{+/-};Spry4^{-/-}$ have ectopic *Fgf3* expression in the lingual mesenchyme, which is associated with the formation of lingual E-cadherin negative T-A cells and ameloblasts [103, 107].

Unlike *Fgf3* and *Fgf10*, which are expressed in the mesenchyme, *Fgf9* expression persists in the epithelium of the cervical loop in mouse [56]. Deletion of *Fgf9* results in a smaller labial cervical loop, and the region of *Shh* expression extends toward a more posterior position in the labial cervical loop [62]. Ectopic FGF9 significantly downregulates the expression of *Shh* mRNA in incisor explants [62]. Since *Shh* expression in the T-A region is required for ameloblast differentiation [109], *Fgf9* may protect progenitors from exposure to the *Shh* signal and keep them in an undifferentiated state in the cervical loop.

Signaling by FGF10 and FGF9 is mediated by FGFR2b. Conditional deletion of or decreased signaling through *Fgfr2b* results in lack of ameloblasts and enamel, suppressed *Shh* expression and decreased cellular proliferation [110, 111], which further supports the hypothesis that FGF9 regulates the proliferation and differentiation of the
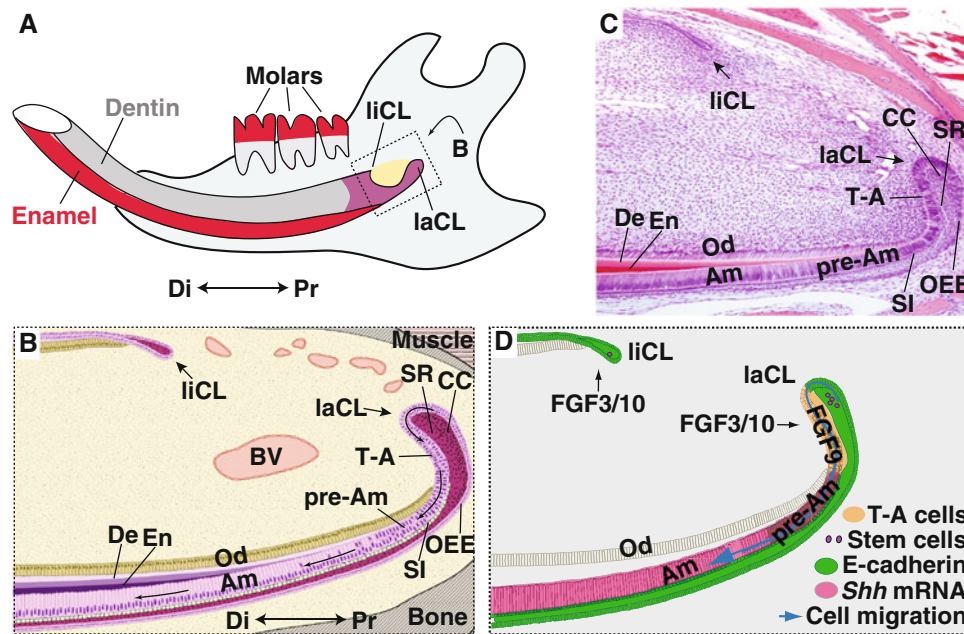
Fig. 2 FGF signaling regulates the behavior of stem cells in the mouse incisor. **a** Schematic diagram of an adult mouse hemimandible with three molars and an incisor containing the lingual (liCL) and labial (laCL) cervical loops at the proximal end. Enamel, secreted by ameloblasts, is present only on the labial surface of the incisor. Dentin, produced by odontoblasts, is deposited on both the labial and lingual surfaces. Schematic diagram (**b**) and image of H&E stained (**c**) sagittal sections of the adult mouse incisor showing the various cell types present. The dental epithelial stem cells reside in the stellate reticulum (SR), condensed cell (CC) or outer enamel epithelium (OEE) regions of the labial cervical loop (laCL). The epithelial stem cells differentiate to form proliferating progenitors, called transit-amplifying (T-A) cells. T-A cells give rise to the pre-ameloblasts (pAm) that differentiate into ameloblasts (Am). The stratum intermedium (SI), which is a single layer of cells that subtends the ameloblasts, also arises from stem cells. *BV*, blood vessel; *Di*, distal; *De*, dentin; *En*, enamel; *liCL*, lingual cervical loop; *Od*, odontoblasts; *Pr*, proximal. **d** FGF signaling regulates dental stem cell maintenance, proliferation and differentiation. FGF3 and FGF10 signals regulate the expression of E-cadherin protein and cell proliferation in the CLs. FGF9 is present in the T-A region to maintain a low level of *Shh* expression in the progenitor cells and avoid their premature cell differentiation in the laCL

progenitor cells in the cervical loop (Fig. 2d). Recently, expression of additional FGF molecules was discovered in the mouse incisor, including *Fgf1, Fgf7, Fgf16, Fgf17, Fgf18* and *Fgf21* [54]. However, the influence of these FGFs on stem cell behavior is not yet known.

Consequences of FGF signaling dysregulation in human tooth development

Dysregulation of FGF signaling can have profound consequences on human tooth development, including tooth agenesis and enamel defects (Fig. 3). Several syndromes are caused by mutations in FGFRs, including the Apert, Crouzon, and Pfeiffer craniosynostosis syndromes. Apert syndrome (OMIM #101200) is characterized by craniosynostosis, midface hypoplasia, and syndactyly of the hands and feet, commonly with bony fusion, and it is caused by gain of function mutations in *FGFR2* [112]. Similarly, Crouzon syndrome (OMIM #123500) is caused by mutations in *FGFR2* and characterized by craniosynostosis and secondary effects on craniofacial structures including hypertelorism, parrot-beaked nose, short upper lip, maxillary hypoplasia, and mandible prognathism [113]. Pfeiffer syndrome (OMIM #101600) is caused by mutations in both *FGFR1* and *FGFR2* and characterized by craniosynostosis, resulting in hypertelorism, short nose, and midface hypoplasia, cutaneous syndactyly of the hands and feet, and short and broad fingers and toes [114, 115]. Little is known about the dental phenotype of Pfeiffer syndrome, but Apert and Crouzon syndrome patients have hypodontia, most commonly of the third molar, maxillary lateral incisor, and mandibular second premolar [116, 117].

Lacrimo-auriculo-dento-digital (LADD; OMIM #149730) syndrome is an autosomal dominant congenital disorder characterized by aplasia, atresia or hypoplasia of lacrimal and salivary glands, cup-shaped ears, hearing loss, and digital anomalies. LADD is caused by heterozygous missense mutations in *FGFR2*, *FGFR3*, and *FGF10* that are thought to result in loss of function [118–120]. Individuals with LADD present with varying dental anomalies, including missing teeth, peg shaped teeth, and enamel hypoplasia [121]. Autosomal recessive congenital deafness with labyrinthine aplasia, microtia, and microdontia (LAMM; OMIM #610706) is

Fig. 3 Syndromes caused by mutations in genes encoding FGFs, FGFRs and their downstream effectors have distinct dental characteristics. Diagram of the FGF signaling pathway with *dashed lines* connecting syndromes to the protein encoded by the causative gene. Although these syndromes are caused by mutations in the same pathway, they have distinct dental characteristics, described in the text. (LADD, Lacrimo-auriculo-dento-digital; LAMM, Autosomal recessive congenital deafness with labyrinthine aplasia, microtia, and microdontia; CFC, Cardio-facio-cutaneous; CS, Costello; TS, Tuberous sclerosis)
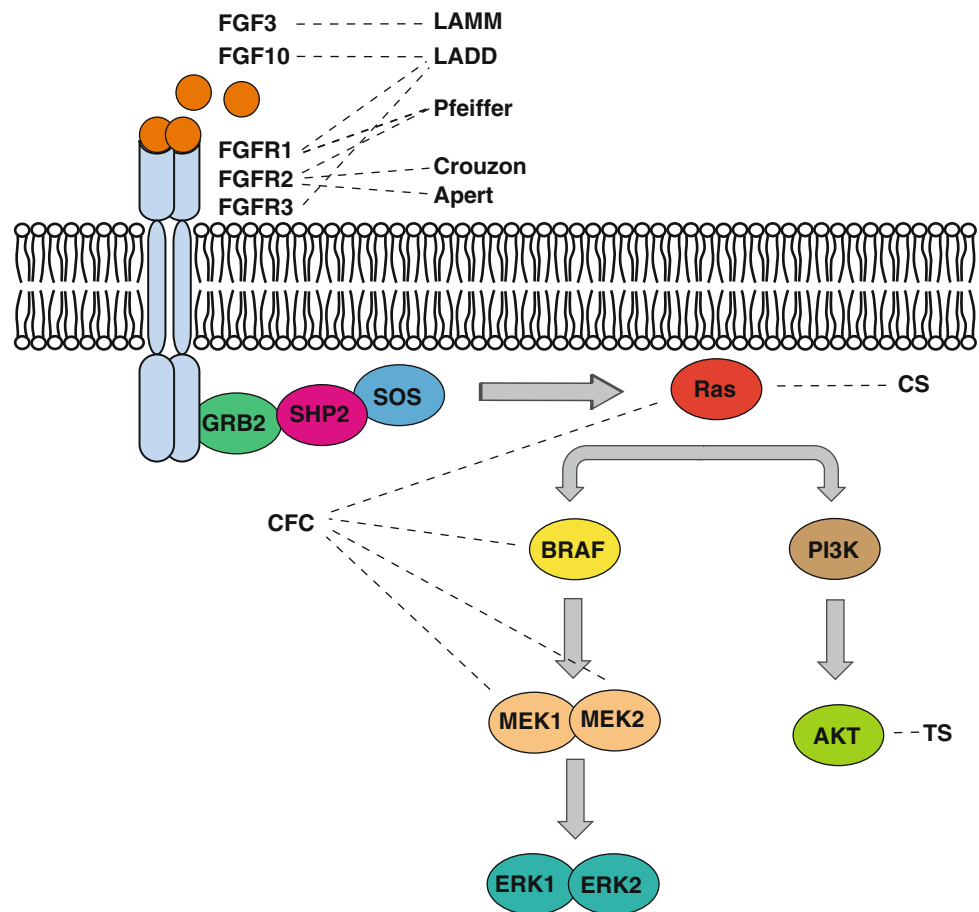
similarly characterized by malformed or missing inner ear structures, malformed external ear, and small, peg shaped teeth. This condition is caused by homozygous or compound heterozygous mutations in *FGF3* [122–125].

Increasing signaling downstream of FGFs also causes disruption in enamel formation in humans. A group of syndromes termed the RASopathies are caused by activating mutations in the Ras pathway. Two of these syndromes are Cardio-facio-cutaneous syndrome (CFC; OMIM #115150) and Costello syndrome (CS; OMIM #218040), and both CS and CFC are characterized by craniofacial dysmorphia, ectodermal abnormalities, congenital heart defects, growth delay, and neurocognitive deficits [126, 127]. In addition, CS individuals present with musculoskeletal anomalies [128, 129]. Nearly all CS patients have a heterozygous, de novo germline mutation in *HRAS* that results in a constitutively active Ras protein [130], whereas CFC is caused by activating mutations in genes encoding proteins downstream of Ras: BRAF, MAP2K1, MAP2K2, and KRAS [131, 132]. Interestingly, even though both CS and CFC individuals have activated Ras signaling, their tooth number, shape, and morphology are normal. However, whereas the enamel of CFC individuals appeared normal [133], CS individuals had

hypo-mineralized enamel. Likewise, mice with increased HRas signaling had disorganized, hypo-mineralized enamel [105]. Tuberous sclerosis (TS; OMIM #191100) is caused by mutations in *TSC1* or *TSC2*, which encode proteins that function downstream of AKT, and gingival fibromas and enamel pitting of the maxillary incisors and canines have been reported in this syndrome [134]. Thus, dysregulation of FGF signaling can dramatically affect tooth formation, resulting in tooth agenesis or anomalies in tooth morphology and enamel structure. It is intriguing that mutations in the same pathway can have different manifestations in tooth development, highlighting the complexity of Ras signaling and its effectors downstream of FGFs.

Conclusion

Here, we have summarized some of the important roles of FGF signaling in tooth development. To date, expression of 12 of the 22 FGF family members has been reported in teeth, and in many cases these ligands play important roles from initiation of tooth development through formation of mineralized tissues. Specifically in rodents, FGFs are

important for maintenance of stem cell pools fueling the continuously growing incisor throughout the life of the animal. The tooth provides an excellent model to gain further insight into the transduction and regulation of FGF signaling in developmental and stem cell biology. Insights from studies in the tooth will be applicable to other organ systems and may help to lay the foundation for development of pharmaceuticals that treat dysregulation of FGF signaling in patients.

References

1. Harada H, Kettunen P, Jung HS, et al. Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. J Cell Biol. 1999;147:105–20.
2. Mina M, Kollar EJ. The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. Arch Oral Biol. 1987;32:123–7.
3. 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(Suppl):155–69.
4. Jernvall J, Kettunen P, Karavanova I, Martin LB, Thesleff I. Evidence for the role of the enamel knot as a control center in mammalian tooth cusp formation: non-dividing cells express growth stimulating Fgf-4 gene. Int J Dev Biol. 1994;38:463–9.
5. Vaahtokari A, Aberg T, Thesleff I. Apoptosis in the developing tooth: association with an embryonic signaling center and suppression by EGF and FGF-4. Development. 1996;122:121–9.
6. Thesleff I, Hurmerinta K. Tissue interactions in tooth development. Differentiation. 1981;18:75–88.
7. Crossley PH, Martinez S, Martin GR. Midbrain development induced by FGF8 in the chick embryo. Nature. 1996;380:66–8.
8. Crossley PH, Minowada G, MacArthur CA, Martin GR. Roles for FGF8 in the induction, initiation, and maintenance of chick limb development. Cell. 1996;84:127–36.
9. Christen B, Slack JM. FGF-8 is associated with anteroposterior patterning and limb regeneration in Xenopus. Dev Biol. 1997;192:455–66.
10. Vogel A, Rodriguez C, Izpisua-Belmonte JC. Involvement of FGF-8 in initiation, outgrowth and patterning of the vertebrate limb. Development. 1996;122:1737–50.
11. Phillips BT, Bolding K, Riley BB. Zebrafish fgf3 and fgf8 encode redundant functions required for otic placode induction. Dev Biol. 2001;235:351–65.
12. Slack JM, Darlington BG, Heath JK, Godsave SF. Mesoderm induction in early Xenopus embryos by heparin-binding growth factors. Nature. 1987;326:197–200.
13. Sutherland D, Samakovlis C, Krasnow MA. Branchless encodes a Drosophila FGF homolog that controls tracheal cell migration and the pattern of branching. Cell. 1996;87:1091–101.
14. Feldman B, Poueymirou W, Papaioannou VE, DeChiara TM, Goldfarb M. Requirement of FGF-4 for postimplantation mouse development. Science. 1995;267:246–9.
15. Ornitz DM, Itoh N. Fibroblast growth factors. Genome Biol. 2001;2(REVIEWS3005).
16. Itoh N, Ornitz DM. Evolution of the Fgf and Fgfr gene families. Trends Genet. 2004;20:563–9.
17. Popovici C, Roubin R, Coulier F, Birnbaum D. An evolutionary history of the FGF superfamily. BioEssays. 2005;27:849–57.
18. Goldfarb M. Fibroblast growth factor homologous factors: evolution, structure, and function. Cytokine Growth Factor Rev. 2005;16:215–20.
19. Metzger RJ, Krasnow MA. Genetic control of branching morphogenesis. Science. 1999;284:1635–9.
20. Hoffman MP, Kidder BL, Steinberg ZL, et al. Gene expression profiles of mouse submandibular gland development: FGFR1 regulates branching morphogenesis in vitro through BMP- and FGF-dependent mechanisms. Development. 2002;129:5767–78.
21. Mailleux AA, Spencer-Dene B, Dillon C, et al. Role of FGF10/FGFR2b signaling during mammary gland development in the mouse embryo. Development. 2002;129:53–60.
22. Mariani FV, Ahn CP, Martin GR. Genetic evidence that FGFs have an instructive role in limb proximal-distal patterning. Nature. 2008;453:401–5.
23. Jukkola T, Lahti L, Naserke T, Wurst W, Partanen J. FGF regulated gene-expression and neuronal differentiation in the developing midbrain-hindbrain region. Dev Biol. 2006;297:141–57.
24. Werner S, Duan DS, de Vries C, et al. Differential splicing in the extracellular region of fibroblast growth factor receptor 1 generates receptor variants with different ligand-binding specificities. Mol Cell Biol. 1992;12:82–8.
25. Chellaiah AT, McEwen DG, Werner S, Xu J, Ornitz DM. Fibroblast growth factor receptor (FGFR) 3. Alternative splicing in immunoglobulin-like domain III creates a receptor highly specific for acidic FGF/FGF-1. J Biol Chem. 1994;269:11620–7.
26. Johnson DE, Lu J, Chen H, Werner S, Williams LT. The human fibroblast growth factor receptor genes: a common structural arrangement underlies the mechanisms for generating receptor forms that differ in their third immunoglobulin domain. Mol Cell Biol. 1991;11:4627–34.
27. Vainikka S, Partanen J, Bellosta P, et al. Fibroblast growth factor receptor-4 shows novel features in genomic structure, ligand binding and signal transduction. EMBO J. 1992;11:4273–80.
28. Duan DS, Werner S, Williams LT. A naturally occurring secreted form of fibroblast growth factor (FGF) receptor 1 binds basic FGF in preference over acidic FGF. J Biol Chem. 1992;267:16076–80.
29. Avivi A, Yayon A, Givol D. A novel form of FGF receptor-3 using an alternative exon in the immunoglobulin domain III. FEBS Lett. 1993;330:249–52.
30. Orr-Urtreger A, Bedford MT, Burakova T, et al. Developmental localization of the splicing alternatives of fibroblast growth factor receptor-2 (FGFR2). Dev Biol. 1993;158:475–86.
31. Alarid ET, Rubin JS, Young P, et al. Keratinocyte growth factor functions in epithelial induction during seminal vesicle development. Proc Natl Acad Sci USA. 1994;91:1074–8.
32. Yan G, Fukabori Y, McBride G, Nikolaropolous S, McKeehan WL. Exon switching and activation of stromal and embryonic fibroblast growth factor (FGF)-FGF receptor genes in prostate epithelial cells accompany stromal independence and malignancy. Mol Cell Biol. 1993;13:4513–22.
33. Gilbert E, Del Gatto F, Champion-Arnaud P, Gesnel MC, Breathnach R. Control of BEK and K-SAM splice sites in alternative splicing of the fibroblast growth factor receptor 2 pre-mRNA. Mol Cell Biol. 1993;13:5461–8.
34. Eswarakumar VP, Lax I, Schlessinger J. Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev. 2005;16:139–49.
35. Clark SG, Stern MJ, Horvitz HR. C. elegans cell-signalling gene sem-5 encodes a protein with SH2 and SH3 domains. Nature. 1992;356:340–4.

36. Li W, Nishimura R, Kashishian A, et al. A new function for a phosphotyrosine phosphatase: linking GRB2-Sos to a receptor tyrosine kinase. Mol Cell Biol. 1994;14:509–17.

37. Saxton TM, Henkemeyer M, Gasca S, et al. Abnormal mesoderm patterning in mouse embryos mutant for the SH2 tyrosine phosphatase Shp-2. EMBO J. 1997;16:2352–64.

38. Hadari YR, Kouhara H, Lax I, Schlessinger J. Binding of Shp2 tyrosine phosphatase to FRS2 is essential for fibroblast growth factor-induced PC12 cell differentiation. Mol Cell Biol. 1998;18:3966–73.

39. Kouhara H, Hadari YR, Spivak-Kroizman T, et al. A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway. Cell. 1997;89:693–702.

40. Lowenstein EJ, Daly RJ, Batzer AG, et al. The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. Cell. 1992;70:431–42.

41. Lebreton S, Boissel L, Moreau J. Control of embryonic Xenopus morphogenesis by a Ral-GDS/Xral branch of the Ras signalling pathway. J Cell Sci. 2003;116:4651–62.

42. Wilson R, Vogelsang E, Leptin M. FGF signalling and the mechanism of mesoderm spreading in Drosophila embryos. Development. 2005;132:491–501.

43. Chambard JC, Lefloch R, Pouyssegur J, Lenormand P. ERK implication in cell cycle regulation. Biochim Biophys Acta. 2007;1773:1299–310.

44. Pacold ME, Suire S, Perisic O, et al. Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase gamma. Cell. 2000;103:931–43.

45. Rodriguez-Viciana P, Warne PH, Dhand R, et al. Phosphatidylinositol-3-OH kinase as a direct target of Ras. Nature. 1994;370:527–32.

46. Castellano E, Downward J. RAS interaction with PI3K: more than just another effector pathway. Genes Cancer. 2011;2:261–74.

47. Habets GG, Scholtes EH, Zuydgeest D, et al. Identification of an invasion-inducing gene, Tiam-1, that encodes a protein with homology to GDP-GTP exchangers for Rho-like proteins. Cell. 1994;77:537–49.

48. Michiels F, Habets GG, Stam JC, van der Kammen RA, Collard JG. A role for Rac in Tiam1-induced membrane ruffling and invasion. Nature. 1995;375:338–40.

49. Ferro E, Trabalzini L. RalGDS family members couple Ras to Ral signalling and that's not all. Cell Signal. 2010;22:1804–10.

50. Hanafusa H, Torii S, Yasunaga T, Nishida E. Sprouty1 and Sprouty2 provide a control mechanism for the Ras/MAPK signalling pathway. Nat Cell Biol. 2002;4:850–8.

51. Kim HJ, Bar-Sagi D. Modulation of signalling by Sprouty: a developing story. Nat Rev Mol Cell Biol. 2004;5:441–50.

52. Sasaki A, Taketomi T, Kato R, et al. Mammalian Sprouty4 suppresses Ras-independent ERK activation by binding to Raf1. Nat Cell Biol. 2003;5:427–32.

53. Kettunen P, Karavanova I, Thesleff I. Responsiveness of developing dental tissues to fibroblast growth factors: expression of splicing alternatives of FGFR1, -2, -3, and of FGFR4; and stimulation of cell proliferation by FGF-2, -4, -8, and -9. Dev Genet. 1998;22:374–85.

54. Porntaveetus T, Otsuka-Tanaka Y, Basson MA, et al. Expression of fibroblast growth factors (Fgfs) in murine tooth development. J Anat. 2011;218:534–43.

55. Kettunen P, Laurikkala J, Itaranta P, et al. Associations of FGF-3 and FGF-10 with signaling networks regulating tooth morphogenesis. Dev Dyn. 2000;219:322–32.

56. 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;211:256–68.

57. Klein OD, Minowada G, Peterkova R, et al. Sprouty genes control diastema tooth development via bidirectional antagonism of epithelial-mesenchymal FGF signaling. Dev Cell. 2006;11:181–90.

58. Ferguson CA, Tucker AS, Sharpe PT. Temporospatial cell interactions regulating mandibular and maxillary arch patterning. Development. 2000;127:403–12.

59. 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;90:247–55.

60. Trumpp A, Depew MJ, Rubenstein JL, Bishop JM, Martin GR. Cre-mediated gene inactivation demonstrates that FGF8 is required for cell survival and patterning of the first branchial arch. Genes Dev. 1999;13:3136–48.

61. Yokohama-Tamaki T, Ohshima H, Fujiwara N, et al. Cessation of Fgf10 signaling, resulting in a defective dental epithelial stem cell compartment, leads to the transition from crown to root formation. Development. 2006;133:1359–66.

62. Kurosaka H, Islam MN, Kuremoto K, et al. Core binding factor beta functions in the maintenance of stem cells and orchestrates continuous proliferation and differentiation in mouse incisors. Stem Cells. 2011;29:1792–803.

63. Haara O, Harjunmaa E, Lindfors PH, et al. Ectodysplasin regulates activator-inhibitor balance in murine tooth development through Fgf20 signaling. Development. 2012;139:3189–99.

64. Tucker AS, Yamada G, Grigoriou M, Pachnis V, Sharpe PT. Fgf-8 determines rostral-caudal polarity in the first branchial arch. Development. 1999;126:51–61.

65. Ohuchi H, Hori Y, Yamasaki M, et al. FGF10 acts as a major ligand for FGF receptor 2 IIIb in mouse multi-organ development. Biochem Biophys Res Commun. 2000;277:643–9.

66. Kettunen P, Spencer-Dene B, Furmanek T, et al. Fgfr2b mediated epithelial-mesenchymal interactions coordinate tooth morphogenesis and dental trigeminal axon patterning. Mech Dev. 2007;124:868–83.

67. De Moerlooze L, Spencer-Dene B, Revest JM, et al. An important role for the IIIb isoform of fibroblast growth factor receptor 2 (FGFR2) in mesenchymal-epithelial signalling during mouse organogenesis. Development. 2000;127:483–92.

68. Hosokawa R, Deng X, Takamori K, et al. Epithelial-specific requirement of FGFR2 signaling during tooth and palate development. J Exp Zool B Mol Dev Evol. 2009;312B:343–50.

69. Harada H, Toyono T, Toyoshima K, et al. FGF10 maintains stem cell compartment in developing mouse incisors. Development. 2002;129:1533–41.

70. Wang XP, Suomalainen M, Felszeghy S, et al. An integrated gene regulatory network controls stem cell proliferation in teeth. PLoS Biol. 2007;5:e159.

71. D'Souza RN, Aberg T, Gaikwad J, et al. Cbfa1 is required for epithelial-mesenchymal interactions regulating tooth development in mice. Development. 1999;126:2911–20.

72. Satokata I, Maas R. Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. Nat Genet. 1994;6:348–56.

73. St Amand TR, Zhang Y, Semina EV, et al. Antagonistic signals between BMP4 and FGF8 define the expression of Pitx1 and Pitx2 in mouse tooth-forming anlage. Dev Biol. 2000;217:323–32.

74. Lu MF, Pressman C, Dyer R, Johnson RL, Martin JF. Function of Rieger syndrome gene in left-right asymmetry and craniofacial development. Nature. 1999;401:276–8.

75. Lin CR, Kioussi C, O'Connell S, et al. Pitx2 regulates lung asymmetry, cardiac positioning and pituitary and tooth morphogenesis. Nature. 1999;401:279–82.

76. van Genderen C, Okamura RM, Farinas I, et al. Development of several organs that require inductive epithelial-mesenchymal

interactions is impaired in LEF-1-deficient mice. Genes Dev. 1994;8:2691–703.

77. 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;16:3173–85.

78. Tummers M, Thesleff I. The importance of signal pathway modulation in all aspects of tooth development. J Exp Zool B Mol Dev Evol. 2009;312B:309–19.

79. Jernvall J, Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev. 2000;92:19–29.

80. Salazar-Ciudad I, Jernvall J. A gene network model accounting for development and evolution of mammalian teeth. Proc Natl Acad Sci USA. 2002;99:8116–20.

81. Salazar-Ciudad I, Jernvall J. A computational model of teeth and the developmental origins of morphological variation. Nature. 2010;464:583–6.

82. Pispa J, Jung HS, Jernvall J, et al. Cusp patterning defect in Tabby mouse teeth and its partial rescue by FGF. Dev Biol. 1999;216:521–34.

83. Ohazama A, Courtney JM, Tucker AS, et al. Traf6 is essential for murine tooth cusp morphogenesis. Dev Dyn. 2004;229:131–5.

84. Dassule HR, Lewis P, Bei M, Maas R, McMahon AP. Sonic hedgehog regulates growth and morphogenesis of the tooth. Development. 2000;127:4775–85.

85. Harjunmaa E, Kallonen A, Voutilainen M, et al. On the difficulty of increasing dental complexity. Nature. 2012;483:324–7.

86. Jarvinen E, Salazar-Ciudad I, Birchmeier W, et al. Continuous tooth generation in mouse is induced by activated epithelial Wnt/beta-catenin signaling. Proc Natl Acad Sci USA. 2006;103:18627–32.

87. Liu F, Chu EY, Watt B, et al. Wnt/beta-catenin signaling directs multiple stages of tooth morphogenesis. Dev Biol. 2008;313:210–24.

88. Wang XP, Suomalainen M, Jorgez CJ, et al. Modulation of activin/bone morphogenetic protein signaling by follistatin is required for the morphogenesis of mouse molar teeth. Dev Dyn. 2004;231:98–108.

89. Jernvall J, Aberg T, Kettunen P, Keranen 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;125:161–9.

90. Aberg T, Wang XP, Kim JH, et al. Runx2 mediates FGF signaling from epithelium to mesenchyme during tooth morphogenesis. Dev Biol. 2004;270:76–93.

91. Peterkova R, Peterka M, Viriot L, Lesot H. Development of the vestigial tooth primordia as part of mouse odontogenesis. Connect Tissue Res. 2002;43:120–8.

92. Prochazka J, Pantalacci S, Churava S, et al. Patterning by heritage in mouse molar row development. Proc Natl Acad Sci USA. 2010;107:15497–502.

93. Tureckova J, Lesot H, Vonesch JL, et al. Apoptosis is involved in the disappearance of the diastemal dental primordia in mouse embryo. Int J Dev Biol. 1996;40:483–9.

94. Mustonen T, Pispa J, Mikkola ML, et al. Stimulation of ectodermal organ development by Ectodysplasin-A1. Dev Biol. 2003;259:123–36.

95. Charles C, Hovorakova M, Ahn Y, et al. Regulation of tooth number by fine-tuning levels of receptor-tyrosine kinase signaling. Development. 2011;138:4063–73.

96. Thesleff I, Keranen S, Jernvall J. Enamel knots as signaling centers linking tooth morphogenesis and odontoblast differentiation. Adv Dent Res. 2001;15:14–8.

97. Tompkins K. Molecular mechanisms of cytodifferentiation in mammalian tooth development. Connect Tissue Res. 2006;47:111–8.

98. Takamori K, Hosokawa R, Xu X, et al. Epithelial fibroblast growth factor receptor 1 regulates enamel formation. J Dent Res. 2008;87:238–43.

99. Tsuboi T, Mizutani S, Nakano M, Hirukawa K, Togari A. Fgf-2 regulates enamel and dentine formation in mouse tooth germ. Calcif Tissue Int. 2003;73:496–501.

100. Mitsiadis TA, Tucker AS, De Bari C, Cobourne MT, Rice DP. A regulatory relationship between Tbx1 and FGF signaling during tooth morphogenesis and ameloblast lineage determination. Dev Biol. 2008;320:39–48.

101. Caton J, Luder HU, Zoupa M, et al. Enamel-free teeth: Tbx1 deletion affects amelogenesis in rodent incisors. Dev Biol. 2009;328:493–505.

102. Huang Z, Kim J, Lacruz RS, et al. Epithelial-specific knockout of the Rac1 gene leads to enamel defects. Eur J Oral Sci. 2011;119(Suppl 1):168–76.

103. Klein OD, Lyons DB, Balooch G, et al. An FGF signaling loop sustains the generation of differentiated progeny from stem cells in mouse incisors. Development. 2008;135:377–85.

104. Boran T, Peterkova R, Lesot H, et al. Temporal analysis of ectopic enamel production in incisors from sprouty mutant mice. J Exp Zool B Mol Dev Evol. 2009;312B:473–85.

105. Goodwin AF, Tidyman WE, Jheon AH, et al. Abnormal Ras signaling in costello syndrome (CS) negatively regulates enamel formation. Hum Mol Genet. 2013 [Epub ahead of print].

106. Smith CE, Warshawsky H. Histological and three dimensional organization of the odontogenic organ in the lower incisor of 100 gram rats. Am J Anat. 1975;142:403–29.

107. Li CY, Cha W, Luder HU, et al. E-cadherin regulates the behavior and fate of epithelial stem cells and their progeny in the mouse incisor. Dev Biol. 2012;366:357–66.

108. Harada H, Toyono T, Toyoshima K, Ohuchi H. FGF10 maintains stem cell population during mouse incisor development. Connect Tissue Res. 2002;43:201–4.

109. Seidel K, Ahn CP, Lyons D, et al. Hedgehog signaling regulates the generation of ameloblast progenitors in the continuously growing mouse incisor. Development. 2010;137:3753–61.

110. Parsa S, Kuremoto K, Seidel K, et al. Signaling by FGFR2b controls the regenerative capacity of adult mouse incisors. Development. 2010;137:3743–52.

111. Lin Y, Cheng YS, Qin C, et al. FGFR2 in the dental epithelium is essential for development and maintenance of the maxillary cervical loop, a stem cell niche in mouse incisors. Dev Dyn. 2009;238:324–30.

112. Wilkie AO, Slaney SF, Oldridge M, et al. Apert syndrome results from localized mutations of FGFR2 and is allelic with Crouzon syndrome. Nat Genet. 1995;9:165–72.

113. Reardon W, Winter RM, Rutland P, et al. Mutations in the fibroblast growth factor receptor 2 gene cause Crouzon syndrome. Nat Genet. 1994;8:98–103.

114. Muenke M, Schell U, Hehr A, et al. A common mutation in the fibroblast growth factor receptor 1 gene in Pfeiffer syndrome. Nat Genet. 1994;8:269–74.

115. Lajeunie E, Ma HW, Bonaventure J, et al. FGFR2 mutations in Pfeiffer syndrome. Nat Genet. 1995;9:108.

116. Stavropoulos D, Bartzela T, Bronkhorst E, Mohlin B, Hagberg C. Dental agenesis patterns of permanent teeth in Apert syndrome. Eur J Oral Sci. 2011;119:198–203.

117. Reitsma JH, Ongkosuwito EM, van Wijk AJ, Prahl-Andersen B. Patterns of tooth agenesis in patients with the syndrome of crouzon or apert. Cleft Palate Craniofac J. 2012 [Epub ahead of print].

118. Rohmann E, Brunner HG, Kayserili H, et al. Mutations in different components of FGF signaling in LADD syndrome. Nat Genet. 2006;38:414–7.

119. Milunsky JM, Zhao G, Maher TA, Colby R, Everman DB. LADD syndrome is caused by FGF10 mutations. Clin Genet. 2006;69:349–54.
120. Shams I, Rohmann E, Eswarakumar VP, et al. Lacrimo-auriculo-dento-digital syndrome is caused by reduced activity of the fibroblast growth factor 10 (FGF10)-FGF receptor 2 signaling pathway. Mol Cell Biol. 2007;27:6903–12.
121. Guven Y, Rosti RO, Tuna EB, Kayserili H, Aktoren O. Oro-dental findings of a family with lacrimo-auriculo-dento digital (LADD) syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;106:e33–44.
122. Tekin M, Hismi BO, Fitoz S, et al. Homozygous mutations in fibroblast growth factor 3 are associated with a new form of syndromic deafness characterized by inner ear agenesis, microtia, and microdontia. Am J Hum Genet. 2007;80:338–44.
123. Tekin M, Ozturkmen Akay H, Fitoz S, et al. Homozygous FGF3 mutations result in congenital deafness with inner ear agenesis, microtia, and microdontia. Clin Genet. 2008;73:554–65.
124. Alsmadi O, Meyer BF, Alkuraya F, et al. Syndromic congenital sensorineural deafness, microtia and microdontia resulting from a novel homoallelic mutation in fibroblast growth factor 3 (FGF3). Eur J Hum Genet. 2009;17:14–21.
125. Sensi A, Ceruti S, Trevisi P, et al. LAMM syndrome with middle ear dysplasia associated with compound heterozygosity for FGF3 mutations. Am J Med Genet A. 2011;155A:1096–101.
126. Roberts A, Allanson J, Jadico SK, et al. The cardiofaciocutaneous syndrome. J Med Genet. 2006;43:833–42.
127. Rauen KA. HRAS and the Costello syndrome. Clin Genet. 2007;71:101–8.
128. Tidyman WE, Lee HS, Rauen KA. Skeletal muscle pathology in Costello and cardio-facio-cutaneous syndromes: developmental consequences of germline Ras/MAPK activation on myogenesis. Am J Med Genet C Semin Med Genet. 2011;157C:104–14.
129. Stevenson DA, Allen S, Tidyman WE, et al. Peripheral muscle weakness in RASopathies. Muscle Nerve. 2012;46:394–9.
130. Aoki Y, Niihori T, Kawame H, et al. Germline mutations in HRAS proto-oncogene cause Costello syndrome. Nat Genet. 2005;37:1038–40.
131. Niihori T, Aoki Y, Narumi Y, et al. Germline KRAS and BRAF mutations in cardio-facio-cutaneous syndrome. Nat Genet. 2006;38:294–6.
132. Rodriguez-Viciana P, Tetsu O, Tidyman WE, et al. Germline mutations in genes within the MAPK pathway cause cardio-facio-cutaneous syndrome. Science. 2006;311:1287–90.
133. Goodwin AF, Oberoi S, Landan M, et al. Craniofacial and dental development in cardio-facio-cutaneous syndrome: the importance of Ras signaling homeostasis. Clin Genet. 2013;83:539–44.
134. Schwartz RA, Fernandez G, Kotulska K, Jozwiak S. Tuberous sclerosis complex: advances in diagnosis, genetics, and management. J Am Acad Dermatol. 2007;57:189–202.