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Fibroblast growth factor signaling in mammalian tooth development

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Abstract

In this review, we discuss the central role of fibroblast growth factor (FGF) signalling 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.

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Keywords

Fibroblast growth factors; Tooth development; Adult stem cells; Mouse; Human

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 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-phos-phorylation 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.

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; thisoccurs,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 orshape 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-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 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 cranio-synostosis 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 craniosyn-ostosis 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 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].Inaddition,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].Tuberoussclerosis (TS; OMIM #191100)iscausedby 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.

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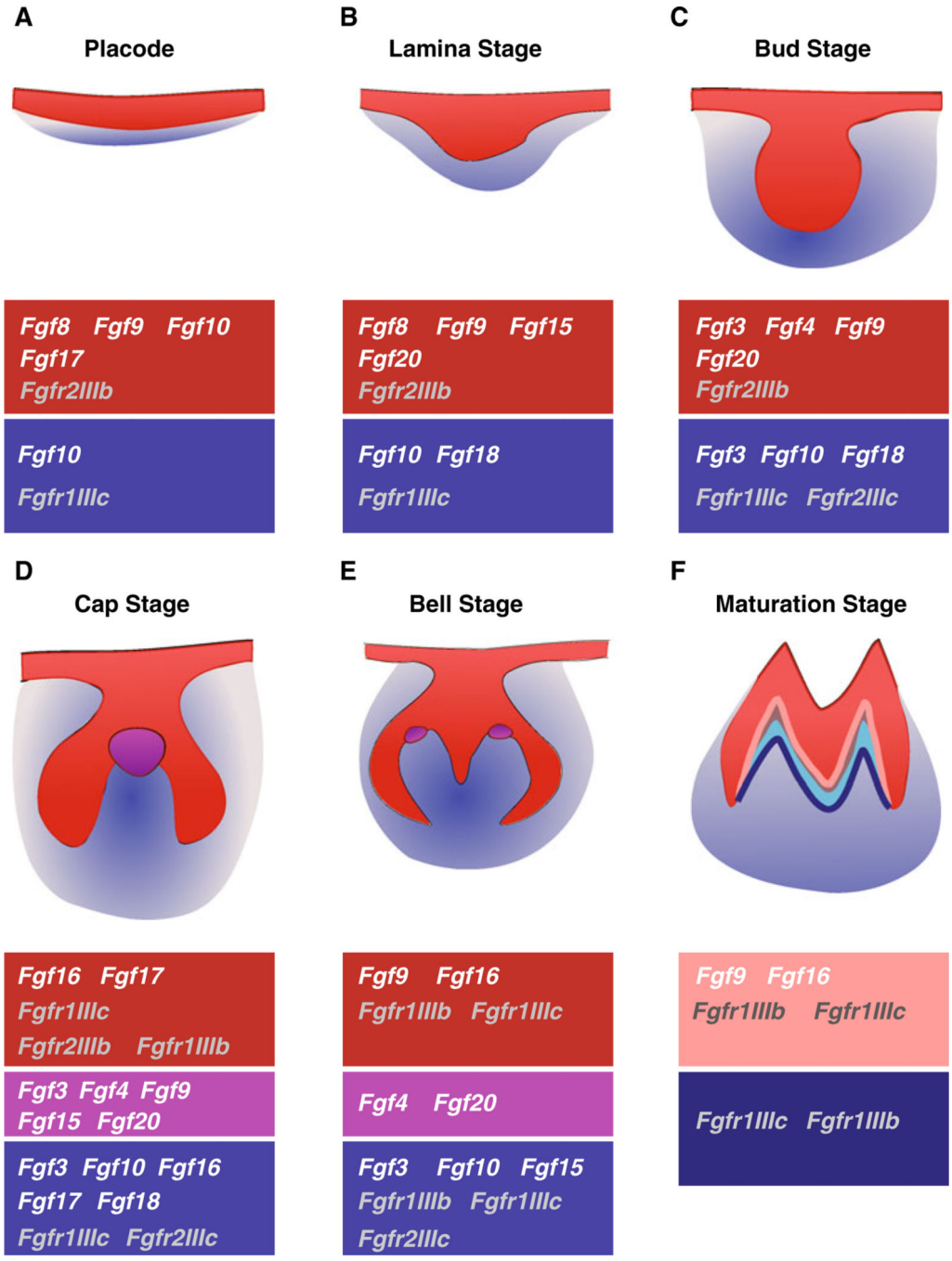

Fig. 1.

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*, F*gf17*, *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*)

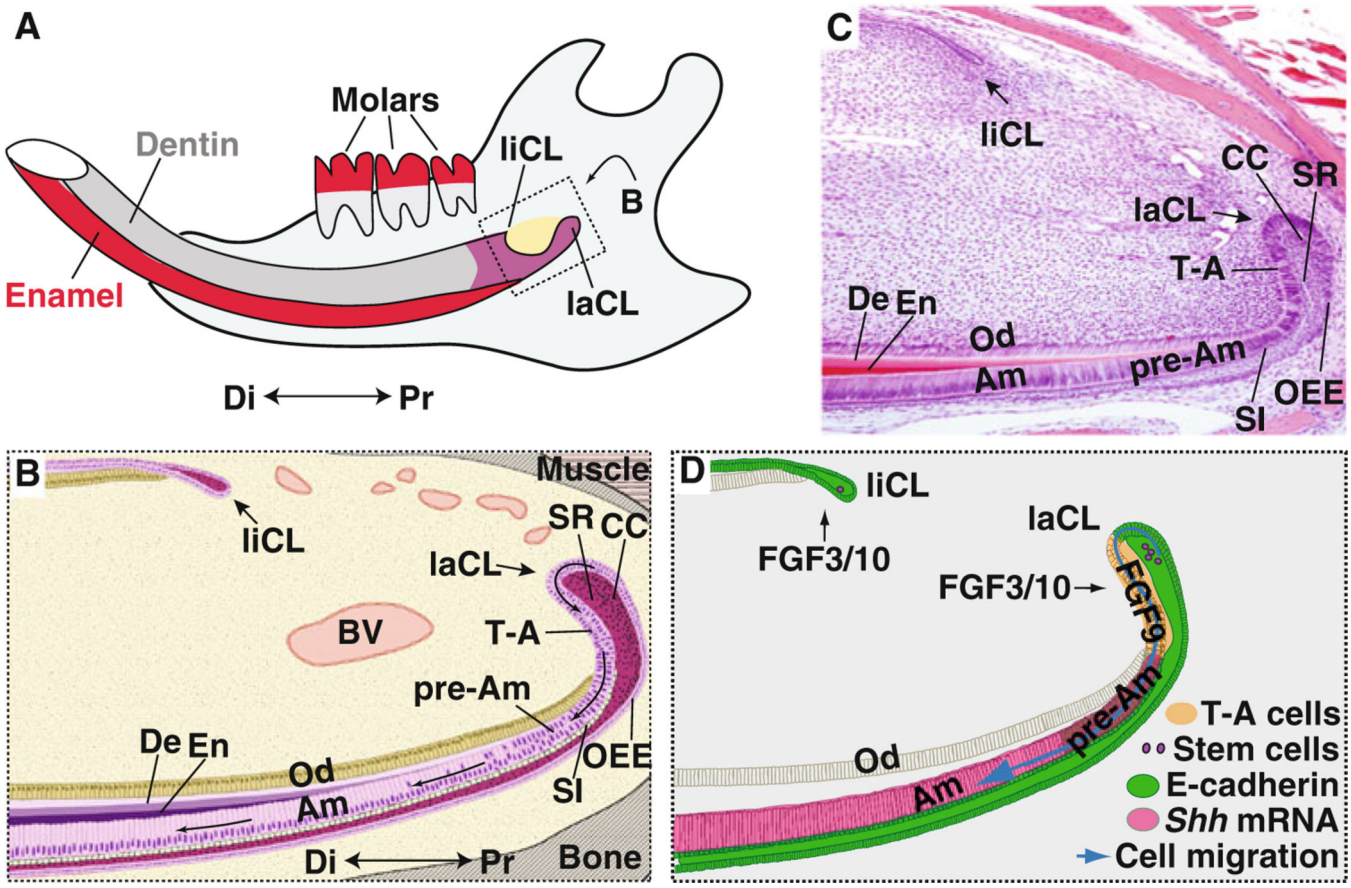

Fig. 2.

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

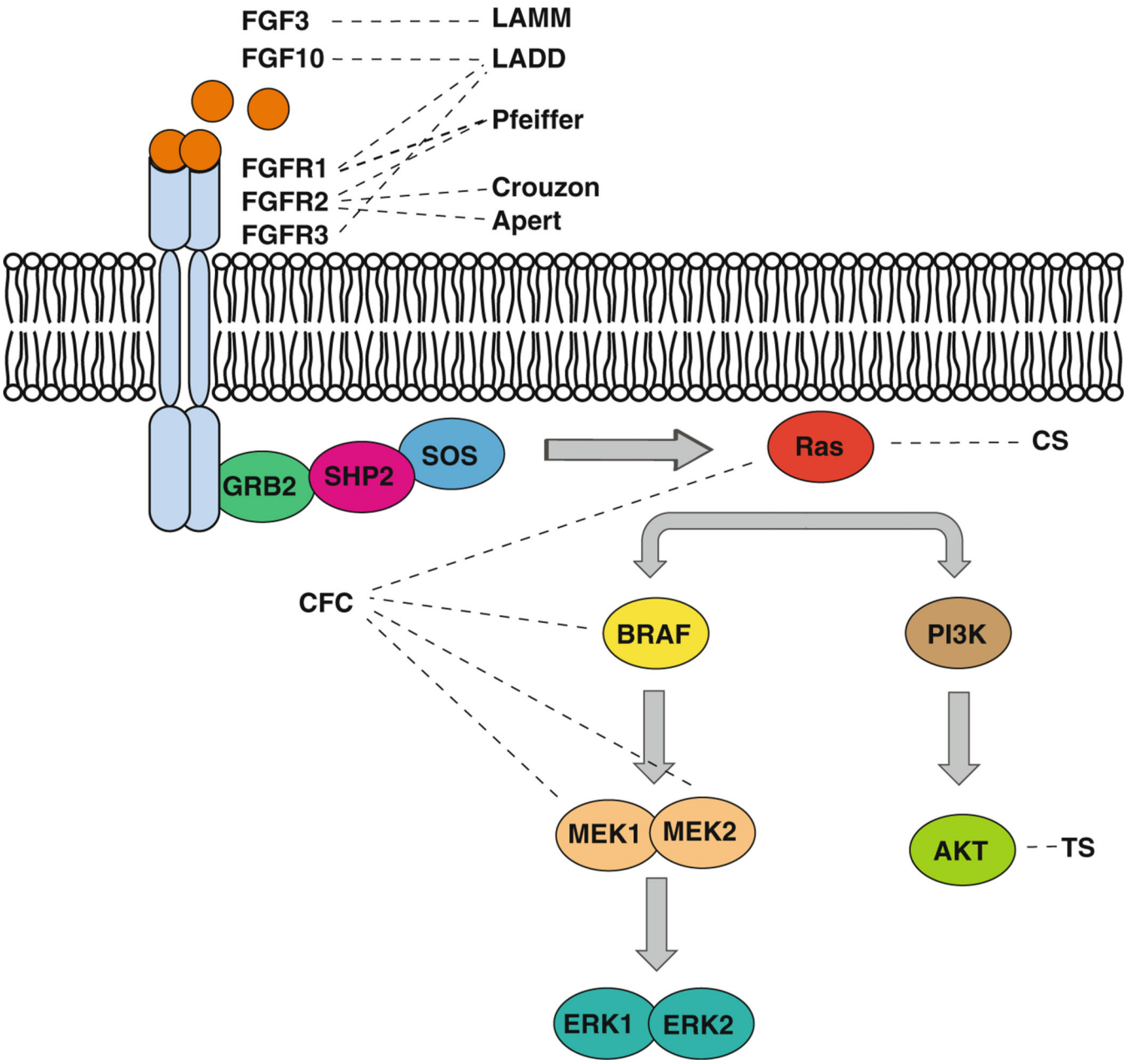

Fig. 3.

Syndromes caused by mutations in genes encoding FGFs, FGFRs and their downstream effectors have distinct dental characteristics. Diagram of the FGF signalling 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-auriculodento-digital; LAMM, Autosomal recessive congenital deafness with labyrinthine aplasia, microtia, and microdontia; CFC, Cardio-facio-cutaneous; CS, Costello; TS, Tuberous sclerosis)

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