Acta Odontologica Scandinavica, 2011; 69: 360–366

informa
healthcare

ORIGINAL ARTICLE

Developmentally regulated expression of intracellular Fgf11-13, hormone-like Fgf15 and canonical Fgf16, -17 and -20 mRNAs in the developing mouse molar tooth

PÄIVI KETTUNEN, TOMASZ FURMANEK, RAJIB CHAULAGAIN, INGER HALS KVINNSLAND & KEIJO LUUKKO

Section of Anatomy and Cell Biology, Department of Biomedicine, University of Bergen, Bergen, Norway

Abstract

Objective. To investigate and compare the cellular expression of non-secreted Fgf11-14 and secreted Fgf15-18 and -20 mRNAs during tooth formation. ***Materials and methods.*** mRNA expression was analyzed from the morphological initiation of the mouse mandibular first molar development to the onset of crown calcification using sectional *in situ* hybridization. ***Results.*** This study found distinct, differentially regulated expression patterns for the *Fgf11-13, -15-17* and *-20,* in particular in the epithelial-mesenchymal interface, whereas *Fgf14* and *18* mRNAs were not detected. *Fgf11, -15, -16, -17* and *-20* were seen in the epithelium, whereas *Fgf12* and *-13* signals were restricted to the mesenchymal tissue component of the tooth. *Fgf11* was observed in the putative epithelial signaling areas, the tertiary enamel knots and enamel free areas of the calcifying crown. *Fgf15, Fgf17* and *-20* were transiently colocalized in the thickened dental epithelium at E11.5. Later *Fgf15* and *-20* were exclusively expressed in the epithelial enamel knot signaling centers. In contrast, *Fgf13* was present in the dental mesenchyme including odontoblasts cell lineage, whereas *Fgf12* appeared transiently in the preodontoblasts. ***Conclusions.*** The expression of the *Fgf11-13, -15, -17* and *-20* in the epithelial signaling centers and/or epithelial–mesenchymal interfaces at key stages of the tooth formation suggest important functions in odontogenesis. Future analyses of the transgenic mice will help elucidate *in vivo* functions of the studied Fgfs during odontogenesis and whether any of the functions of the tooth expressed epithelial and mesenchymal Fgfs of different sub-families are redundant.

Key Words: *tooth, organogenesis, tissue interactions, fibroblast growth factor, in situ hybridization*

Introduction

The tooth, in particular the mouse mandibular first molar one, is an excellent model system for analyzing molecular mechanisms regulating organ formation [1–4]. Morphologically, tooth formation starts as a thickening of the oral epithelium. During the subsequent bud, cap and bell stages, the shape of the tooth is established by epithelial folding morphogenesis. Subsequently the shape of the tooth crown is finalized by secretion of the enamel and dentin by amelo- and odontoblasts, respectively.

Tooth formation including crown mophogenesis and differentiation of the tooth-specific odonto- and ameloblasts is controlled by the reciprocal, instructive interactions between the tooth-forming ectodermal epithelium and neural crest derived ectomesenchyme [5,6]. Molecules belonging to conserved families such as Wnt (wingless), canonical Fgf- (fibroblast growth factor), Bmp- (bone morphogenetic protein) and Hedgehog-families mediate epithelial-mesenchyme signaling [1,7]. Signaling pathways are integrated into complex networks at different levels and are modulated by specific inhibitors and activators [7] such as the Fgf-signaling inhibiting Sproyty-family of proteins [8]. Many of the key signals are specifically expressed in three consecutive primary (PEK), secondary (SEK) and tertiary (TEK) epithelial enamel knot signaling centers, the successive activity of which appears to be of crucial importance for molar crown morphogenesis and determination of its final shape [9–12].

Correspondence: Paivi Kettunen, Section of Anatomy and Cell Biology, Department of Biomedicine, University of Bergen, Jonas Lies vei 91, 5009 Bergen, Norway. Tel: +47 55 58 63 64. Fax: +47 55 58 63 60. E-mail: paivi.kettunen@biomed.uib.no

(Received 28 November 2010; revised 17 January 2011; accepted 6 February 2011)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.3109/00016357.2011.568968

Fgfs (fibroblast growth factor) form a large family of conserved signaling proteins with multiple, essential developmental functions in neural induction as well as organ patterning and morphogenesis [13,14]. Defected FGF signaling underlies various human developmental defects and disorders including many craniofacial ones [15]. To date, 22 (Fgf 1–23) Fgfs have been reported in mouse and man. Fgf15 is a mouse ortholog of human FGF19 [13,14]. Based on the mediation of their biological responses and on the mechanisms of how they are released from cells the mammalian Fgf family have been divided into three sub-families, namely the intracellular (intracrine) *Fgf11/12/13/14* (iFgfs), the hormone-like (endocrine) *Fgf15/21/23* (hFgfs) and the canonical (paracrine) (cFgf) *Fgf1/2/5*, *Fgf3/4/6*, *Fgf7/10/22*, *Fgf8/17/18* and *Fgf9/16/20* sub-families [14]. cFgfs are secreted extracellular signaling proteins that bind and activate cell surface tyrosine kinase Fgf receptors (Fgfrs) with heparin/heparan sulfate as a cofactor and act as autocrine/paracrine signals. They have been shown to control the development of various organs and tissues [14–16]. Similarly, eFgfs are secreted and mediate their biological responses via Fgfrs, but they bind to Fgfrs and heparin/heparan sulfate with very low affinity, making it possible for them to function in an endocrine manner [14]. iFgfs are not secreted and they function as intracellular proteins in a Fgfr-independent manner [14].

Fgf signaling is essential for tooth formation. Many cFgfs and Fgfrs are expressed in the developing tooth in a developmentally regulated manner. cFgfs have been shown to mediate epithelial and mesenchymal tissue interactions and regulate various cellular functions such as regulation gene expression, cell proliferation, apoptosis and dental stem cells as well as controlling dental patterning, morphogenesis and calcification [17–28]. Recently Fgfr2, which is essential for tooth morphogenesis, was demonstrated to mediate tissue interactions integrating tooth formation and innervation [29].

Earlier several Fgfs have been reported in the tooth germ [17,18,30,31] and many of them have been shown to serve important functions in odontogenesis [17,18,30,31]. The aim of the study was to investigate mRNA expression of non-secreted Fgf11–14 and secreted Fgf15–18 and -20 during early tooth development. The analysis was performed from the morphological initiation of the mouse mandibular first molar development (embryonic day (E) 11.5) until the onset of crown calcification (day 3–5 post-natally (PN)) using radioactive *in situ* hybridization.

Materials and methods

Preparation of tissues

The use of the mice was approved by the Animal Welfare Committee of the Department of Biomedicine at the University of Bergen under the surveillance of the Norwegian Animal Research Authority. The NMRI mice were mated overnight and the appearance of a vaginal plug was taken as day 0.5 of embryonic development (E0.5). The adult animals were killed by cervical dislocation and the embryos and pups by decapitation. The developmental stage of the tooth germs was further confirmed by morphological criteria from the tissue sections. The tissues were fixed in 4% PFA (paraformaldehyde). The E14.5-PN5 tissues were decalcified in 12.5% EDTA (ethylenediaminetetraacetic acid) and 2.5% PFA in phosphate-buffered saline (PBS). The tissues were paraffin embedded and cut into 7 μm sections.

Generation of Fgf cDNAs for in situ hybridization

The Fgf cDNAs were generated by RT-PCR from total RNA isolated from E10–E13 mouse heads and subcloned into pGEM-T Easy Vector (Promega Corporation, Madison, WI). The details of the construction of the cDNAs are available upon request.

In situ hybridization

In situ hybridization on sections was performed as described [12,17]. Briefly, the ^{35}S-UTP-labeled antisense and sense cRNA probes were generated by *in vitro* transcription. No specific signal was detected in sections hybridized with the control sense probes (not shown). Bright- and dark-field images of the *in situ* sections were photographed using a Zeiss Axioskop 2 microscope (Carl Zeiss Jena GmbH, Jena, Germany) and a SPOT Insight digital camera (Diagnostic Instruments, Sterling Heights, MI). The figure plates were prepared with Adobe Photoshop 6.0 program (Adobe Systems, San Jose, CA).

Three-dimensional reconstruction of gene expression

Three-dimensional (3D) reconstruction of the gene expression in the developing tooth germs was generated from 10 μm serial frontal bright and dark field images. The processing of the images was done using custom-made scripts and programs made with Java Advanced Imaging and Java 3D (Sun Microsystems, Menlo Park, CA). 3D reconstruction was rendered with perspective camera view in Visualization Toolkit (Kitware, Inc., Clifton Park, NY, USA). About 70% transparent 3D surface was generated using the Marching Cubes function in the Visualization Toolkit from the outlines of the jaw epithelium. Hybridization signal with intensity over 190 was considered to represent positive gene expression. The sections were median-filtered to reduce the background signal in the 3D-image.

Results

In situ hybridization analysis revealed that *Fgf11-13*, *Fgf15-17* and *-20* showed distinct, differentially regulated expression patterns, in particular at the interface of epithelial-mesenchymal tissues, while *Fgf14* and *18* mRNAs were not detected in the tooth germ proper. Fgf18 transcripts were seen in the oral mesenchyme next to the dental and vestibular lamina for E16.5 molar (Figure 1F).

Comparison of Fgf expression during early stages of molar formation

At the morphological onset of tooth formation (E11.5) the molar tooth germ is visible as an epitelial thickening. At this stage, Fgf15, -17 and -20 were colocalized in the dental epithelium, whereas Fgf13 was seen in the underlying presumptive dental mesenchyme (Figures 1C, 2A1, B1 and 3B1). At E12.5 when the tooth germ was at the early bud stage, Fgf17 transcripts displayed restricted expression in the dental epithelium while Fgf13 expression continued in the underlying mesenchyme (Figures 1D and 3B2). Fgf15 expression, however, had shifted

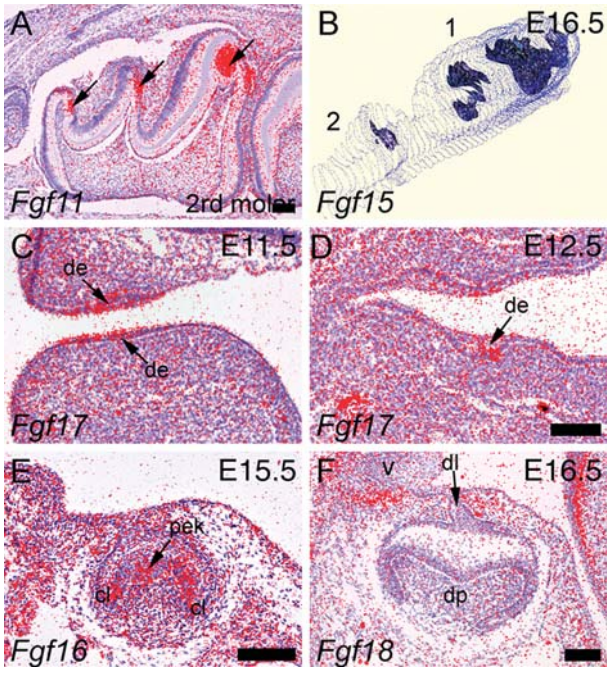

Figure 1. Localization of Fgf11 (A), -15 (B), -17 (C, D), -16 and -18 mRNAs during embryonic (E) (B–F) and 5-day post-natal (A) development of the mandibular first (B–F) and second (A) molar tooth germs. mRNA expression (red color in A, C–F) and blue (B) is detected by radioactive *in situ* hybridization in sagittal (A) and frontal (C–F) sections. Arrows in (A) show *Fgf11* expression in the ameloblasts of the enamel-free areas. (B) Three-dimensional (3D) computer reconstruction of the E16.5 first (1) and second (2) molars showing Fgf15 mRNA expression in the dental papilla mesenchyme. Dental epithelium (blue color) is outlined marking the epithelial–mesenchymal interface in each serial section. The expression of Fgf15 in the epithelial enamel knots is not shown in the model (for epithelial expression see Figure 2A5). Abbreviations: cl, cervical loop; de, dental epithelium; dl, dental lamina; dp, dental papilla mesenchyme; pek, primary enamel knot; v, vestibular lamina. Scale bars: 100 μm. Bar in (D) applies to (C, D).

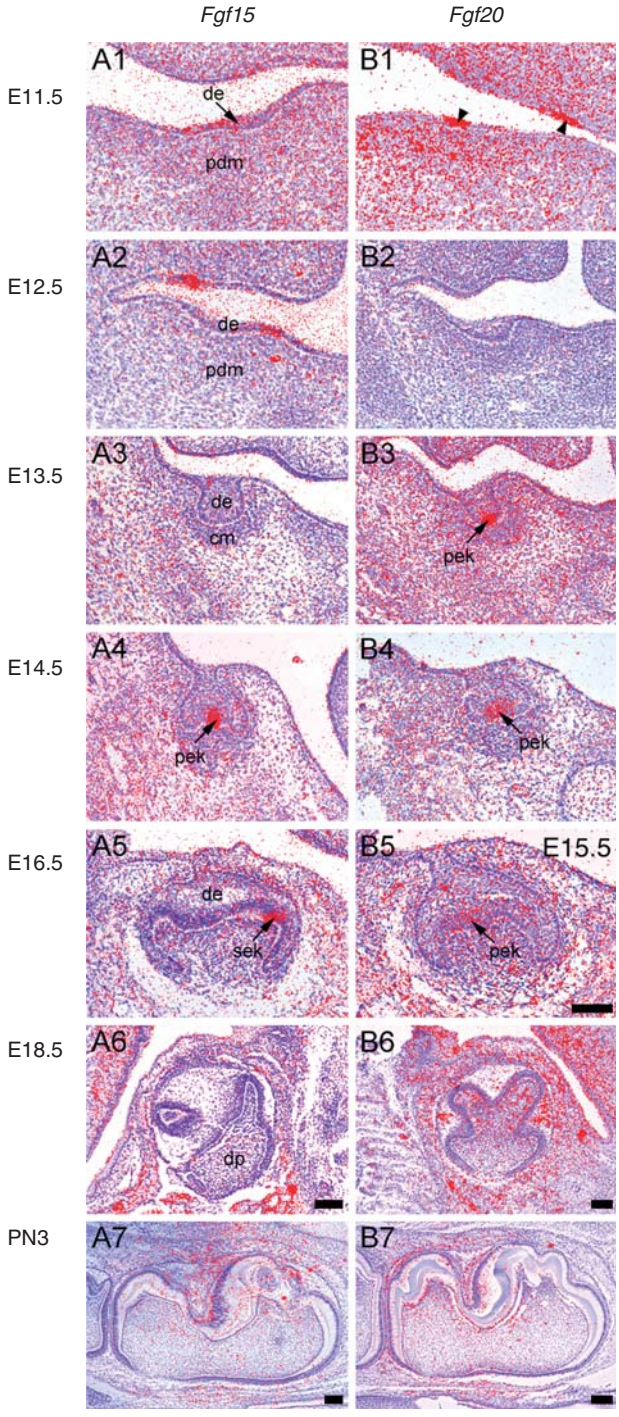

Figure 2. Localization of Fg15 and -20 mRNAs during embryonic (E) (A1–B6) and post-natal (PN) (A7, B7) development of the mandibular first molar tooth germ. mRNA expression (red color) is detected by radioactive *in situ* hybridization in frontal (A1–B6) and sagittal (A7, B7) sections. Arrowhead in B1 indicates *Fgf20* expression in the dental epithelium of the upper and lower jaw. Abbreviations: cm, condensed dental mesenchyme; de, dental epithelium; dm, dental mesenchyme; dp, dental papilla mesenchyme; pek, primary enamel knot; pdm, presumptive dental mesenchyme; sek, secondary enamel knot. Scale bars: 100 μm in B5 applies to A1–B5; 100 μm in A6–B7.

to the oral epithelium at the lingual side of the epithelial bud (Figure 2A2).

Comparison of Fgf expression during molar morphogenesis

At E13.5 the dental epithelium had formed a bud and the dental mesenchyme had condensed around it. At this stage the primary enamel knot (PEK) is located at the tip of the epithelial bud [32]. Of note, the secreted Fgf20 was exclusively colocated in the signaling center (Figure 2B3). While Fgf17 mRNAs were down-regulated from the tooth, Fgf13 expression continued in the condensed dental mesenchyme. In addition, the peridental mesenchyme, including the developing, tooth supporting alveolar bone showed Fgf13 transcripts (Figure 3B3). In contrast, Fgf12 expression was mostly confined to the developing mandibular bone at this stage (Figure 3A3).

One day later (E14.5) the dental epithelium had reached the cap stage. The PEK is now observable in the middle part of the anterior-posterior axis of the enamel organ as a distinct torpedo-shaped epithelial cell cluster [10]. The secreted Fgf15 and -20 were exclusively expressed in the signaling center (Figures 2A4 and B4). Fgf13 mRNA expression continued in the dental mesenchyme and was now evident in the dental papilla and the enamel organ surrounding the dental follicle (Figure 3B4). Like *Fgf12*, they were also present in the peridental mesenchyme (Figures 3A4 and B4). A weak *Fgf16* signal was seen in the inner dental epithelium including the cervical loops and PEK at E15.5 stage (Figure 1E). At later stages, little if any expression of *Fgf16* was detectable in the inner dental epithelium.

The molar cusp formation is evident when the secondary enamel knots (SEK) gradually appear at the sites of future cusp tips [10]. At the early bell stage (E16.5) Fgf15 was specifically expressed in the SEKs. Moreover, transcripts were also dynamically expressed in the coronal part of the dental papilla mesenchyme, especially at the cusp tips (Figure 2A5), as visualized by 3D reconstruction (Figure 1B). As in earlier stages, Fgf12 and -13 expressions continued in the mesenchymal tissue components of the tooth, the dental papilla and follicle (Figures 3A5, B5 and B6).

Comparison of Fgf expressions during molar crown calcification

The final shape of tooth crown becomes determined after differentiation of odonto- and ameloblasts, which secrete dentin and enamel, respectively. In the mouse molar, however, the tips of the cusps and the transverse connecting lophs of the parallel cusps are not covered by enamel. This is proposed to be regulated by the signaling of the tertiary enamel

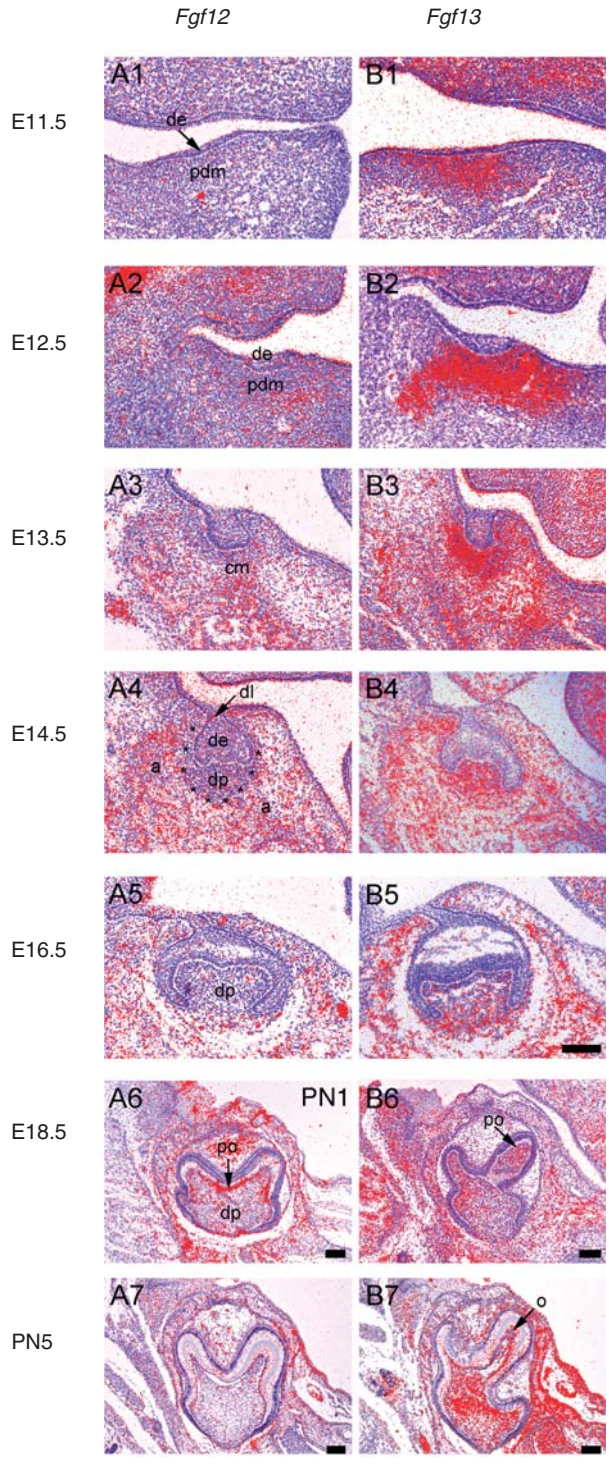

Figure 3. Localization of Fgf12, and -13 mRNAs during embryonic (E) A1–B5 and B6 and post-natal (PN) (A7–B7) development of the mandibular first molar tooth germ. mRNA expression (red color) is detected by radioactive *in situ* hybridization in frontal sections (A7). The apparent expression of Fgf12 and -13 the formation bone next to the tooth germ suggest a role for intracellular FGF signaling in the development of the tooth supporting alveolar bone. Abbreviations: cm, condensed dental mesenchyme; de, dental epithelium; dl, dental lamina; dp, dental papilla mesenchyme; po, preodontoblasts; pdm, presumptive dental mesenchyme. Asterisks in A4 indicate the mesenchymal dental follicle surrounding the dental epithelium. Scale bars: 100 μm in B5, applies to A1–B5; 100 μm A6–B7.

knot (TEK) and the enamel free signaling areas (EFAs), respectively [12]. *Fgf11* was exclusively observed in the cells of the enamel-free areas next to TEK and EFA (Figure 1A). Besides the developing alveolar bone, *Fgf12* was transiently expressed in the differentiating odontoblasts (Figure 3A6). Fgf13 transcripts were evident in the E18 dental papilla including the preodontoblasts (Figure 3B6). Later, during crown calcification the expression of *Fgf13* continued in the dental pulp, including the odontoblasts (Figure 1B7), whereas Fgf12 transcripts were not observed in the odontoblasts secreting dentin, as shown for PN5 molar (Figure 3A7).

Discussion

Fgf signaling serves critical functions during odontogenesis. In this descriptive study we have systematically compared, for the first time, the presence and cellular localization of the intracellular Fgf11-14 and secreted Fgf15-18, -20 mRNAs during tooth formation. We found distinct expression patterns for the intracellular *Fgf11-13*, hormone-like *Fgf15* and canonical *Fgf17* and *-20*, while Fgf14 and 18 mRNAs were not detected in the tooth germ proper.

Fgfs show dynamic expression mainly in either epithelial or mesenchymal tissue component

Whereas *Fgf11*, *-15*, *-17* and *-20* were seen in the dental epithelium the expression of *Fgf12* and *-13* was restricted to the mesenchymal tissue components. This, in line with the earlier findings, shows that individual Fgfs are predominantly expressed in either epithelial or mesenchymal tissue component and often show overlapping expression patterns [17,18,21–23,33]. The distinct expression domains of the observed Fgfs suggest that they are differentially regulated.

There is emerging data that, besides the canonical Fgfs, the hormone-like Fgfs and intracellular Fgfs serve developmental functions. Hormone-like Fgfs acts locally as a differentiation factors in embryos [34] and, for instance FGF15/19, regulate cardiac outflow and brain development [35–37]. Although the developmental importance of the intracellular Fgf sub-family has remained mostly unknown, Fgf13 has been shown to control neural differentiation in *Xenopus* and over-expression experiments suggest involvement in chick limb development [38,39]. Fgf14 knockout mice suffer from neurological disorders [40]. Odontogenic functions for the tooth expressed endocrine and intracellular Fgfs are supported by their expression patterns. The tooth expressed iFgfs and hFgfs showed spatio-temporally changing expression domains, especially in sites of signaling centers and/or the epithelial–mesenchymal interface, where signaling controlling odontogenesis takes place.

Possible roles for Fgfs in tooth initiation

The early dental epithelium expresses signaling molecules of different families that signals to the adjoining mesenchyme and activate expression of genes essential for tooth formation [7,27]. Canonical Fgf signalling, as shown for Fgf8, is essential for the molar formation [17,24,27]. Fgf8, like the observed canonical Fgf17 and -20, is expressed in the early dental epithelium, signals to the underlying mesenchyme and controls the expression of genes essential for tooth formation [41–43]. The expression of *Fgf17* and *-20* and their *Fgfr1c* signaling receptor in the thickened dental epithelium and underlying mesenchyme, respectively [33,44], suggest that, like Fgf8, Fgf17 and -20 may mediate epithelial signaling to the mesenchyme and regulate early tooth formation.

We also observed endocrine *Fgf15* in the presumptive dental epithelium. It has been shown earlier that the rostral patterning center of the developing brain, like the early dental epithelium, expresses *Fgf8* and *Fgf15*, which have parallel differential signaling properties [36]. Fgf15 represses proliferation and promotes neural differentiation, whereas Fgf8 has opposing effects [36,37]. Moreover, their expression is controlled by Shh, which is also expressed in the early dental epithelium [36,37,45–47]. Thus, it is tempting to speculate that Fgf15, like Fgf18, functions in the early stages of tooth formation and that its expression is regulated by Shh. Intracellular Fgf13, which was expressed in the dental mesenchyme including the odontoblast cell lineage at all stages studied, may exert autocrine in the mesenchyme. Fgf13 regulates neural differentiation in early developing *Xenopus* [38] and may, therefore, serve similar functions during odontogenesis.

Possible roles for Fgfs in molar crown morphogenesis

The enamel knots are clusters of non-proliferative epithelial cells which, by exerting paracine signaling to the adjoining epithelial and mesenchymal cells, control the molar crown shape [9,10]. The PEK appears in the tip of the dental epithelium at the bud stage and later it is present in the cap stage tooth germ [10,32]. In the molar tooth, it is followed by the appearance of the SEKs in the future cusp tips at the bell stage [10]. The PEK and SEK cells specifically express number of key signaling molecules of different families [10,17,18,32]. That secreted *Fgf20* was specifically expressed in both PEK and its receptor, *Fgfr1c*, in the adjacent dental epithelial and mesenchymal tissues [33]. This suggests that Fgf20 may act as a paracrine epithelial-mesenchymal signal involved

in tooth morphogenesis and that its function may be redundant with other enamel knot-expressed canonical Fgfs such as Fgf3-4 and -9, which for instance promote cell proliferation [17,18]. Intracellular *Fgf15* was also present in both PEK and SEK. Because Fgf15 signaling receptors are not located in the signaling centers [33,44] it may, like Fgf20, excert paracrine signaling to the adjacent dental epithelium and mesenchyme [33,44]. Interestingly, available data of the function of Fgf15 suggests that it appears to promote gene expression and cell differentiation [35,36,48].

Dual role for Fgf signaling in crown calcification

The final molar shape is determined when the tooth-specific odonto- and ameloblasts secrete hard-tissues, dentin and enamel, respectively, in the crown. The differentiation of the tooth-specific cells is regulated by epithelial–mesenchymal interactions between the dental papilla and inner dental epithelium cells and involves signaling molecules [49,50]. There is evidence for a role of Fgf signaling in dentin and enamal formation. For instance, Fgf1 and Fgf2 can act synergistically with Tgf-beta1 and Igf-I to strengthen their inductive effects on odontoblast differentiation *in vitro* [51]. Moreover, targeted genetic inactivation of *Fgfr1* in ameloblasts results in abnormal enamel formation [28]. We found that Fgf15 transcripts were present in the secondary enamel knots and in adjacent dental papilla mesenchyme at the cusp tips prior to the odontoblast differentiation. Because the differentiation of the odontoblasts starts from the tips of the cusps [52] it is tempting to propose that Fgf15 may be involved in the initiation of the odontoblast differentiation. The expression of *Fgf12* and *Fgf13* was seen in the pre-odontoblasts and in the odontoblast cell lineage including the secreting odontoblasts, respectively. This suggests a putative autocrine function for the intracellular Fgfs in determination and differentiation of the odontoblast cell lineage and possibly in maintenance of the odontoblast function. In contrast to *Fgf12* and *-13*, *Fgf11* signal was exclusively observed in the epithelial tissue component, namely in the ameloblasts of the enamel-free areas of the calcifying crown. The inhibitory paracrine signaling from the adjacent TEK and EFA signaling area are proposed to control the establishment of the enamel-free areas at the cusp tips and in the transverse connecting lophs of the parallel cusps in the mouse molar [12]. Thus, intracellular i*Fgf11* may, by exerting autocrine signaling, control the signaling functions of the TEK and EFA and thereby control the formation of the enamel-free areas and the final crown shape.

In conclusion, our results here show dynamic expression patters for *Fgf11-13*, *-15*, *-17* and *-20* during odontogenesis. Their presence in the epithelial signaling centers and/or epithelial–mesenchymal interface suggests that they may serve important signaling functions during tooth formation. Future investigations of transgenic mice will help to elucidate *in vivo* functions of the studied Fgfs during odontogenesis and whether their functions are redundant.

Acknowledgements

Ms Kjellfrid Haukanes is acknowledged for skillful technical assistance. This study has been supported by a grant from the Norwegian Cancer Society.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Cobourne MT, Sharpe PT. Making up the numbers: the molecular control of mammalian dental formula. Semin Cell Dev Biol 2010;21:314–24.
[2] Thesleff I. The genetic basis of tooth development and dental defects. Am J Med Genet A 2006;140:2530–5.
[3] Lesot H, Brook AH. Epithelial histogenesis during tooth development. Arch Oral Biol 2009;54(Suppl 1):25–33.
[4] Luukko K, Moe K, Sijaona A, Furmanek T, Hals K, Vinnsland I, Midtbo M, et al. Secondary induction and the development of tooth nerve supply. Ann Anat 2008;190: 178–87.
[5] Jernvall J, Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev 2000; 92:19–29.
[6] Cobourne MT, Sharpe PT. Tooth and jaw: molecular mechanisms of patterning in the first branchial arch. Arch Oral Biol 2003;48:1–14.
[7] 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.
[8] Klein OD, Minowada G, Peterkova R, Kangas A, Yu BD, Lesot H, et al. Sprouty genes control diastema tooth development via bidirectional antagonism of epithelial-mesenchymal FGF signaling. Dev Cell 2006;11:181–90.
[9] Thesleff I, Jernvall J. The enamel knot: a putative signaling center regulating tooth development. Cold Spring Harb Symp Quant Biol 1997;62:257–67.
[10] 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.
[11] Coin R, Lesot H, Vonesch JL, Haikel Y, Ruch JV. Aspects of cell proliferation kinetics of the inner dental epithelium during mouse molar and incisor morphogenesis: a reappraisal of the role of the enamel knot area. Int J Dev Biol 1999;43:261–7.
[12] Luukko K, Loes S, Furmanek T, Fjeld K, Kvinnsland IH, Kettunen P. Identification of a novel putative signaling center, the tertiary enamel knot in the postnatal mouse molar tooth. Mech Dev 2003;120:270–6.
[13] Itoh N, Ornitz DM. Evolution of the Fgf and Fgfr gene families. Trends Genet 2004;20:563–9.
[14] Itoh N, Ornitz DM. Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. J Biochem 2011;149:121–30.
[15] Nie X, Luukko K, Kettunen P. FGF signalling in craniofacial development and developmental disorders. Oral Dis 2006;12: 102–11.

[16] Itoh N, Ornitz DM. Functional evolutionary history of the mouse Fgf gene family. Dev Dyn 2008;237: 18–27.
[17] 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.
[18] Kettunen P, Laurikkala J, Itaranta P, Vainio S, Itoh N, Thesleff I. Associations of FGF-3 and FGF-10 with signaling networks regulating tooth morphogenesis. Dev Dyn 2000; 219:322–32.
[19] 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.
[20] Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I. Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. J Cell Biol 1999;147:105–20.
[21] Dassule HR, McMahon AP. Analysis of epithelial-mesenchymal interactions in the initial morphogenesis of the mammalian tooth. Dev Biol 1998;202:215–27.
[22] Sarkar L, Sharpe PT. Expression of Wnt signalling pathway genes during tooth development. Mech Dev 1999;85: 197–200.
[23] Nadiri A, Kuchler-Bopp S, Haikel Y, Lesot H. Immunolocalization of BMP-2/-4, FGF-4, and WNT10b in the developing mouse first lower molar. J Histochem Cytochem 2004; 52:103–12.
[24] 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.
[25] 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.
[26] Ohuchi H, Hori Y, Yamasaki M, Harada H, Sekine K, Kato S, 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.
[27] Tucker A, Sharpe P. The cutting-edge of mammalian development; how the embryo makes teeth. Nature Rev Gen 2004; 5:499–508.
[28] Takamori K, Hosokawa R, Xu X, Deng X, Bringas P, Jr, Chai Y. Epithelial fibroblast growth factor receptor 1 regulates enamel formation. J Dent Res 2008;87:238–43.
[29] Kettunen P, Spencer-Dene B, Furmanek T, Kvinnsland IH, Dickson C, Thesleff I, et al. Fgfr2b mediated epithelial-mesenchymal interactions coordinate tooth morphogenesis and dental trigeminal axon patterning. Mech Dev 2007; 124:868–83.
[30] Cam Y, Neumann MR, Oliver L, Raulais D, Janet T, Ruch JV. Immunolocalization of acidic and basic fibroblast growth factors during mouse odontogenesis. Int J Dev Biol 1992;36:381–9.
[31] Onishi T, Umemura S, Shintani S, Ooshima T. Phex mutation causes overexpression of FGF23 in teeth. Arch Oral Biol 2008;53:99–104.
[32] Vaahtokari A, Aberg T, Jernvall J, Keranen S, Thesleff I. The enamel knot as a signaling center in the developing mouse tooth. Mech Dev 1996;54:39–43.
[33] 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.
[34] Itoh N. Hormone-like (endocrine) Fgfs: their evolutionary history and roles in development, metabolism, and disease. Cell Tissue Res 2010;342:1–11.
[35] Vincentz JW, McWhirter JR, Murre C, Baldini A, Furuta Y. Fgf15 is required for proper morphogenesis of the mouse cardiac outflow tract. Genesis 2005;4:192–201.
[36] Borello U, Cobos I, Long JE, McWhirter JR, Murre C, Rubenstein JL. FGF15 promotes neurogenesis and opposes FGF8 function during neocortical development. Neural Dev 2008;3:17.
[37] Ishibashi M, McMahon AP. A sonic hedgehog-dependent signaling relay regulates growth of diencephalic and mesencephalic primordia in the early mouse embryo. Development 2002;129:4807–19.
[38] Nishimoto S, Nishida E. Fibroblast growth factor 13 is essential for neural differentiation in Xenopus early embryonic development. J Biol Chem 2007;282:24255–61.
[39] Munoz-Sanjuan I, Simandl BK, Fallon JF, Nathans J. Expression of chicken fibroblast growth factor homologous factor (FHF)-1 and of differentially spliced isoforms of FHF-2-during development and involvement of FHF-2 in chicken limb development. Development 1999;126:409–21.
[40] Wang Q, Bardgett ME, Wong M, Wozniak DF, Lou J, McNeil BD, et al. Ataxia and paroxysmal dyskinesia in mice lacking axonally transported FGF14. Neuron 2002;35: 25–38.
[41] Bei M, Maas R. FGFs and BMP4 induce both Msx1-independent and Msx1-dependent signaling pathways in early tooth development. Development 1998;125: 4325–33.
[42] 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.
[43] Tucker AS, Matthews KL, Sharpe PT. Transformation of tooth type induced by inhibition of BMP signaling. Science 1998;282:1136–8.
[44] Mason I. Initiation to end point: the multiple roles of fibroblast growth factors in neural development. Nat Rev Neurosci 2007;8:583–96.
[45] Cobourne MT, Hardcastle Z, Sharpe PT. Sonic hedgehog regulates epithelial proliferation and cell survival in the developing tooth germ. J Dent Res 2001;80:1974–9.
[46] Sarkar L, Cobourne M, Naylor S, Smalley M, Dale T, Sharpe PT. Wnt/Shh interactions regulate ectodermal boundary formation during mammalian tooth development. Proc Natl Acad Sci USA 2000;97:4520–4.
[47] Gritli-Linde A, Bei M, Maas R, Zhang XM, Linde A, McMahon AP. Shh signaling within the dental epithelium is necessary for cell proliferation, growth and polarization. Development 2002;129:5323–37.
[48] Wright TJ, Ladher R, McWhirter J, Murre C, Schoenwolf GC, Mansour SL. Mouse FGF15 is the ortholog of human and chick FGF19, but is not uniquely required for otic induction. Dev Biol 2004;269:264–75.
[49] Thesleff I, Hurmerinta K. Tissue interactions in tooth development. Differentiation 1981;18:75–88.
[50] Lesot H, Lisi S, Peterkova R, Peterka M, Mitolo V, Ruch JV. Epigenetic signals during odontoblast differentiation. Adv Dent Res 2001;15:8–13.
[51] Martin A, Unda FJ, Begue-Kirn C, Ruch JV, Arechaga J. Effects of aFGF, bFGF, TGFbeta1 and IGF-I on odontoblast differentiation in vitro. Eur J Oral Sci 1998;106(Suppl 1): 117–21.
[52] Thesleff I, Keranen S, Jernvall J. Enamel knots as signaling centers linking tooth morphogenesis and odontoblast differentiation. Adv Dent Res 2001;15:14–18.