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REVIEW ARTICLE

FGF signalling in craniofacial development and developmental disorders

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The Fgf signalling pathway is highly conserved in evolution and plays crucial roles in development. In the craniofacial region, it is involved in almost all structure development from early patterning to growth regulation. In craniofacial skeletogenesis, the Fgf signal pathway plays important roles in suture and synchondrosis regulation. Mutations of FGF receptors relate to syndromatic and non-syndromatic craniosynostosis. The Fgf10/Fgfr2b signal loop is critical for palatogenesis and submandibular gland formation. Perturbation of the Fgf signal is a possible mechanism of palatal cleft. Fgf10 haploinsufficiency has been identified as the cause of autosomal dominant aplasia of lacrimal and salivary glands. The Fgf signal is also a key regulator of tooth formation: in the absence of *Fgfr2b* tooth development is arrested at the bud stage. Fgfr4 has recently been identified as the key signal mediator in myogenesis. In this review, these aspects are discussed in detail with a focus on the most recent advances.
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Craniofacial development is initiated from that of the brain. The neural tube in the most anterior portion balloons into three primary vesicles that will develop into the forebrain (prosencephalon), the midbrain (mesencephalon) and the hindbrain (rhombencephalon) respectively. The hindbrain is segmented along the anterior–posterior axis into compartments, termed rhombomeres, as a consequence of cell lineage restriction by differential activity of regulatory genes. In the lateral ridges of the neural plate a pluripotent cell population is formed, termed the neural crest. Cranial neural crest cells are a multipotent, migratory lineage that gives rise to the majority of the facial structures, including the peripheral nervous system and the skeletons. The emergence of cranial neural crest cells and their contribution to craniofacial structures constitutes an important feature of craniofacial development in vertebrates. This characteristic also makes the vertebrate head a revolutionary novelty (Gans and Northcutt, 1983).

Facial structures are formed by the development and outgrowth of the facial primordia, initially a number of discrete buds surrounding the primitive oral cavity, consisting of neural crest and mesoderm-derived mesenchyme and an epithelial layer of ectoderm and endoderm. Those prominences include a single median frontonasal and paired maxillary and mandibular prominences. The latter are derivatives from the first pair of branchial arches. Those prominences fuse in the midline to form a continuous face in advancing development. Fusion is an important aspect for craniofacial development. Perturbation in this process leads to different types of orofacial cleft, such as cleft lip, oblique facial cleft, lateral facial cleft, mandibular cleft, cleft palate and tongue cleft.

Epithelial–mesenchymal interaction is an important mechanism for initiation of organogenesis in the craniofacial area. Early orofacial epithelium expresses inductive signals to the underlying mesenchyme or ectomesenchyme. The mesenchyme, together with the epithelium, undergoes morphogenesis in response to the inducing signal and feeds back to the epithelium for further development.

These well-orchestrated developmental processes are controlled by an intrinsic signal network. Craniofacial disorders occur when these development events are perturbed by environmental factors or genetic mutations. Many common developmental disorders that afflict human beings, such as orofacial clefts and craniosynostosis, are the result of genetic mutations or have a genetic background. The genetic mechanisms of craniofacial development has begun to be elucidated, with FGF, BMP, SHH and many other developmental signal pathways playing critical roles. The FGF signalling pathway is highlighted by the aetiologic relationship

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of FGF receptor (FGFr) mutations with human craniosynostosis, which is characterized by premature suture fusion (Wilkie, 1997; Nuckolls *et al*, 1999).

FGF is a conserved signal pathway in evolution, composed of more than 23 members. Four distinct FGFrs bind and are activated by FGF ligands. For FGFr1 to FGFr3, two exons, an invariate IIIa and one of IIIb or IIIc, encode the third Ig loop. These alternative mRNA splicings produce each FGFr as two splice variants (*IIIb* and *IIIc*), each with unique ligand binding properties. These receptor isoforms are differently distributed and perform distinct functions during development. The splice variant *IIIb* is mainly expressed in epithelium, while *IIIc* is mainly present in mesenchyme. The roles of Fgf signalling in vertebrate embryogenic craniofacial development have been extensively investigated using mouse, chicken and zebrefish models in the past decade (Table 1). In this review, the roles of FGF in craniofacial development and developmental disorders are discussed.

Fgf signalling in the development and outgrowth of the facial primordia

Fgf signalling is inductive for neural crest formation (Baker and Bronner-Fraser, 1997; Villanueva *et al*, 2002; Monsoro-Burq *et al*, 2003, 2005), it also promotes the formation of chondrocyte lineage in the cranial neural crest (Sarkar *et al*, 2001; Monsoro-Burq *et al*, 2005). In advancing development, Fgf signalling is present in both the epithelia and mesenchyme and mediates the epithelial–mesenchymal interaction involved in almost all structure development. *Fgfr1* and *Fgfr2* are broadly expressed in the facial primordia (Wilke *et al*, 1997; Bachler and Neubuser, 2001). However, targeted deletion of *Fgfr1* or *Fgfr2* results in early embryonic death around gastrulation, preventing analysis of their roles in further development (Deng *et al*, 1994; Yamaguchi *et al*, 1994; Xu *et al*, 1998). Fgf ligands are expressed in redundant and restricted domains in the facial primordia during this period: *Fgf8, -9* and *-10* are intensely expressed at nasal pits, whereas *Fgf3*, *-15* and *-17* expression is restricted to the medial side of the nasal pits (Bachler and Neubuser, 2001). Fgf8 is particularly important in early craniofacial patterning and growth. Ectoderm-expressed Fgf8 induces homeobox gene expression in ectomesenchyme, which is critical for the structure formation within the primordia (Cobourne and Sharpe, 2003). Deletion of Fgf8 also leads to early embryonic death at gastrulation, preventing assessment of its role in further development with this model (Meyers *et al*, 1998; Sun *et al*, 1999). Conditional loss of Fgf8 functions in the ectoderm of the first branchial arch exhibits almost complete loss of the first arch-derived skeletal structures and tooth agenesis in some regions (Trumpp *et al*, 1999). During early facial primordia development, Fgf8 has strong synergistic effects with Shh on chondrogenesis *in vitro* and is sufficient to promote chondrogenesis *in vivo* (Tucker *et al*, 1999; Abzhanov and Tabin, 2004). In the zebrafish embryo, inhibition of *Fgfr* activity following

Table 1 Endogeneous *Fgf*/*Fgfr* expressed during the development of the craniofacial structures and their possible roles in developmental disorders

Facial structures	*Critical Fgf receptors*	*Critical Fgf ligands*	*Common developmental disorders in human beings*	*Roles of Fgf signalling*
Facial primordia	Fgfr1 and Fgfr2 (Wilke *et al*, 1997; Bachler and Neubuser, 2001)	Fgf8, Fgf3, Fgf2 (Richman *et al*, 1997; Bachler and Neubuser, 2001; Walshe and Mason, 2003)	Facial clefts, insufficiency of craniofacial development	Remain to be defined
Skeletogenesis	Fgfr1c, Fgfr2b, Fgfr2c, Fgfr3c (Iseki *et al*, 1999; Rice *et al*, 2003)	Fgf2, Fgf1(Moore *et al*, 2002)	Syndromic and non-syndromic craniosynostosis, Achondrodysplasia, Thanatophoric dysplasia, Hypochondroplasia	FGFr1–3 mutations are identified (OMIM nos: 101200, 123500, 101400, 101600, 187600, 100800, 146000)
Palate	Fgfr2b (De Moerlooze *et al*, 2000; Lee *et al*, 2001; Britto *et al*, 2002; Rice *et al*, 2004)	Fgf10 (Rice *et al*, 2004; Alappat *et al*, 2005)	Cleft palate	FGF10/FGFr2b mutation is suggested as a possible mechanism
Submandibular gland	Fgfr1, Fgfr2b, Fgfr2c (De Moerlooze *et al*, 2000; Hoffman *et al*, 2002; Jaskoll *et al*, 2002)	Fgf10, Fgf8 (Sekine *et al*, 1999; Ohuchi *et al*, 2000; Jaskoll *et al*, 2004)	Aplasia of lacrimal and salivary glands	FGF10 mutation identified (OMIM nos: 180920, 103420); FGF8/FGFr2c mutations as a possible mechanism (Jaskoll *et al*, 2004)
Tooth	Fgfr1-3 (Kettunen *et al*, 1998)	Fgf8, Fgf10, Fgf3, Fgf4, Fgf9 (Kettunen *et al*, 1998, 2000; Kettunen and Thesleff, 1998; Harada *et al*, 2002)	Hypodontia, Oligodontia	Remain to be defined

neural crest emigration from the neural tube results in complete absence of cranial and pharyngeal cartilages (Walshe and Mason, 2003). Moreover, inhibition of both *Fgf3* and *Fgf8* causes complete absence of pharyngeal cartilages and almost complete loss of the neurocranial cartilage, whereas inhibition of *Fgf3* results only in absence of cartilage elements in some pharyngeal arches, implying that they are the main ligands for early chondrogenesis within the facial primordia and branchial arch (Walshe and Mason, 2003). *Fgf2* is also expressed in facial ectoderm. Exogenous Fgf2 and Fgf4 can increase the length of the cartilage rod formed in the frontonasal and mandibular mesenchyme (Richman *et al*, 1997).

Perturbation of the developmental process of the facial primordia leads to various orofacial clefts and insufficiency of facial development. Among facial clefts, cleft lip is a most commonly seen developmental anomaly. Cleft lip is in most of the cases concomitant with cleft palate, but also occurs as isolated deformity, suggesting there are both common and differing molecular basis for their formation. Different mechanisms in their development are also demonstrated in mouse (Liu *et al*, 2005). As will be discussed in the following part, Fgf signalling is critical to palatogenesis. However, its role in the formation of lip and cleft lip remains to be elucidated.

Congenital mandibular hypoplasia is also a common craniofacial anomaly, most frequently resulted from maldevelopment of the first or second branchial arches (Singh and Bartlett, 2005). Most of the cases are associated with human syndromes. The most common is oculo-auriculo-vertebral spectrum (OMIM no. 164210), which includes hemifacial and bifacial microsomia. The next most seen is the mandibulofacial dysostosis or Treacher Collin's syndrome (OMIM no. 154500), in which TCOF1 gene mutation is postulated to be the genetic mechanism. Occasionally, non-syndromic congenital mandibular hypoplasia occurs as a subgroup (Singh and Bartlett, 2005). Many cases are also with facial clefts or microglossia/aglossia (Singh and Bartlett, 2005), implying a possible common mechanism for those deformities in this subgroup. As Fgf signalling is critically expressed in facial primordia development and stimulates cell proliferation in most of cell lineages, it is reasonable to speculate that disruption of this signalling at a critical developmental stage might be a mechanism in some types of facial underdevelopment.

Fgf signalling in craniofacial skeletogenesis and skeletal disorders

The craniofacial skeleton includes the neurocranium and facial bones. These bones are formed through two distinct mechanisms: endochondral ossification and intramembranous ossification. In endochondral ossification a cartilage template is formed first. In the periphery of the template, perichondral mesenchyme cells differentiate into osteoblasts and form bone tissue while the chondrocytes in the cartilage anlagen become hypertrophic. Thereafter the cartilage template is replaced by bone tissue via chondrocyte apoptosis and osteoblast invasion. In this process, chondrocytes in the template exhibit a life cycle of proliferation, differentiation, maturation and apoptosis. Axis and appendicular bones are predominantly formed through endochondral ossification. Bones formed in this manner are also termed endochondral bones. In intramembranous ossification, condensed mesenchyme cells directly differentiate into osteoblasts and form bone tissue without any cartilaginous precursor. Bones that are formed through intramebranous ossification are also called intramembranous bone or membrane bones (Cohen, 2000). Facial bones and cranial vault are mostly formed through intramembranous ossification, whereas the basicranium is formed through endochondral ossification. During intramembranous ossification, fibrous sutures are formed connecting the individual bones and function as growth centres. In endochondral basicranium cartilaginous structures similar to long bone growth plates, termed synchondroses, are developed as growth centres.

Endochondral ossification and intramembranous ossification are integrated in some bones, for example the clavicle, long bones and mandible. The clavicle is partly endochondral bone and partly intramembranous bone. During long bone formation, intramembranous ossification occurs in the perichondral area (Cohen, 2000). In the mandible, the condyle cartilage growth centre and anterior part of Meckel's cartilage contributes to its development through endochondral ossification (Bhaskar *et al*, 1953; Ishizeki *et al*, 1999).

As well as the critical role in early chondrogensis within the facial primordia and branchial arch, the Fgf signal pathway plays important regulatory roles in advancing skeletogenesis. *Fgfr2c* is required for the regulation of osteoblast lineage and normal skeletogenesis. Deletion of *Fgfr2c* or expression of gain function mutated FGFr2c in mice results in multiple skeletal and craniofacial abnormalities (Eswarakumar *et al*, 2002; Yu *et al*, 2003). *Fgfr2b* also regulates craniofacial skeletogenesis: *Fgfr2b* null mice show apparent premature fusion of the suture between the parietal and squamous temporal bones (De Moerlooze *et al*, 2000). Fgfr1 is also a positive regulator for skeletal formation: it has a synergistic effect with Fgfr2 and stimulates osteoblast differentiation (Iseki *et al*, 1999; Hajihosseini *et al*, 2004). Fgfr3, on the contrary, was identified as a negative regulator of chondrogenesis and osteogenesis (Deng *et al*, 1996; Chen *et al*, 1999). The inhibitory role to chondrocyte proliferation is mediated through STAT-1 pathway (Sahni *et al*, 1999). Fgfr3 has also been demonstrated to inhibit expression of *Ihh* and *Bmp4* in proliferating chondrocytes of the growth plate (Naski *et al*, 1998). Disrupting the murine Fgfr3 gene produces severe and progressive bone dysplasia with enhanced and prolonged endochondral bone growth accompanied by expansion of proliferating and hypertrophic chondrocytes within the cartilaginous growth plate (Colvin *et al*, 1996; Deng *et al*, 1996).

In the developing craniofacial skeleton, Fgf/Fgfr signalling is present in both endochondral and intramembranous bones and plays important roles in regulating their development and growth (Britto *et al*, 2001; Moore *et al*, 2002; Ornitz and Marie, 2002; Marie, 2003; Rice *et al*, 2003). *Fgf2*, *Fgfr1* and *Fgfr2* were found in the cranial vault in embryonic development (Kim *et al*, 1998; Johnson *et al*, 2000). Blocking of Fgf2 with neutralized beads prevents osteogenesis at this site (Moore *et al*, 2002). Fgfr1 and Fgfr2 are important in regulating the morphology and patent of craniofacial sutures, acting synergistically with other conserved developmental genes (Kim *et al*, 1998; Johnson *et al*, 2000). Function of the Fgf signal is integrated with that of Twist (Rice *et al*, 2000), which causes craniosynostosis via haploinsufficiency (Wilkie, 1997). FGFrs, on the contrary, cause craniosynostosis through gain-function mutations. The majority of mutations in FGFRs have been found in the third Ig loop or in the linker region between the second and the third Ig loops (Wilkie, 1997; Nuckolls *et al*, 1999). Apert (OMIM no. 101200), Crouzon (OMIM no. 123500) and Saethre-Chotzen (OMIM no. 101400) syndromes are caused by FGFr2 mutations, whereas Pfeiffer syndrome (OMIM no. 101600) is caused by FGFr1 mutations.

In the endochondral basicranium, *Fgfr1*, *-2* and *-3* isoforms are all present during its development (Britto *et al*, 2001; Rice *et al*, 2003). In the synchondrosis, *Fgfr3* is highly present in the proliferating chondrocytes, whereas *Fgfr1c* and *Fgfr2c* are in the perichondral region (Rice *et al*, 2003). Synchondrosis fusion and development deficiency in the basicranium of *Fgfr2c* null mice implies primary anomalies in the basicranium, simultaneously with those of cranial vault. Mutations of FGFr3 lead to thanatophoric dysplasia (TD, OMIM no. 187600), achondroplasia (ACH, OMIM no. 100800) and hypochondroplasia (HCH no. 146000), which mainly afflict the basicranium among the craniofacial skeletons.

Fgf signalling in palatogenesis

The mammalian palate is formed by the union of the primary palate and the secondary palate. The primary palate forms from the posterior protrusion of the fused maxillary prominences, whereas the secondary palate forms from paired lateral maxillary palatal shelves. Formation of the mammalian secondary palate is a multi-step process that includes palatal shelf outgrowth, elevation, fusion and maturation (Ferguson, 1988). In this process, the mesenchyme within the palatal prominences actively proliferates, which is essential for palatal outgrowth and fusion. Palatal fusion is a critical event in palatogenesis. The initiation of palatal fusion is from the anterior area of the secondary palate, subsequently extending rostrally and caudally (Chou *et al*, 2004). The fusion process in the palate is relatively a late and fragile event in comparison with the facial fusion.

The medial edge epithelia (MEE), after fusion, form the midline epithelial seam (MES), which subsequently undergoes transformation or apoptosis and finally disappears. Any disturbance in those processes could lead to palatal cleft. For example, delayed palatal shelf elevation, reduced mesenchyme proliferation and abnormal apoptosis of MES are all possible aetiologies of palatal cleft.

In the secondary palate, the origin, development and regulation of anterior and posterior parts are distinct (Noden, 1983, 1988; Zhang *et al*, 2002). The anterior and posterior parts of the secondary palate also behave differently observed *in vitro* culture system (Chou *et al*, 2004). The anterior part of the secondary palate, together with the primary palate, is exclusively derived from the neural crest (Noden, 1988): its mesenchyme condenses and differentiates into osteoblasts to form intramembranous bones that subsequently fuse in the midline. This part therefore corresponds to the presumptive hard palate. The posterior part of secondary palate, on the contrary, is mainly derived from paraxial mesoderm, which will develop into palatal muscles and form the future soft palate (Noden, 1983, 1988). Cleft may occur only in the posterior palate, while the anterior palate develops normally, suggesting that there might be different mechanisms governing the development of the anterior and posterior parts of the palate.

The well-orchestrated process is under tight genetic control and also sensitive to environmental factors. The genetic mechanism underlying palatal development is beginning to be elucidated. Bmp, Shh, Sox9, Msx, Fgf, Tgf-β3 and Egf signals all play crucial roles in palatogenesis. Disruption of those genes displays cleft palate in mice. The Fgf signal is critical within the genetic hierarchy underlying the developmental process.

The Fgf signal is involved in palatogenesis in multiple stages. Both FGFs and FGFrs were localized in sequential stages of human palatal shelf fusion from palatal shelf elevation to the completion of fusion (Britto *et al*, 2002). In the mouse model, Fgfr1 and Fgfr2 were detected in the epithelium of the developing palatal shelves from the time of their outgrowth from the maxillary processes through completion of fusion *in vivo* and *in vitro* (Lee *et al*, 2001). Expression of both receptors was particularly strong during the phases of MEE fusion and the ultimate dissolution of the MES (Lee *et al*, 2001). Fgfr1 and Fgfr2 are also localized in the lateral palatal mesenchyme (Lee *et al*, 2001). These data suggest that Fgf signalling may play a role in the epithelial–mesenchymal interactions that dictate fusion, ongoing differentiation and maturation of the developing palate.

Convincing evidence was provided by the use of transgenic mice. Deletion of the *Fgfr2b* isoform or *Fgf10* results in cleft palate, suggesting a crucial role for this signal pathway (De Moerlooze *et al*, 2000; Rice *et al*, 2004; Alappat *et al*, 2005). Fgfr2b is a key signal in mediating epithelial–mesenchymal interaction during organogenesis. In the developing palate, epithelial *Fgfr2b* expression was associated with mesenchymal expression of *Fgf10* (Rice *et al*, 2004). These results imply that the Fgf10/Fgfr2b signal pathway, which is crucial to the development of numerous organs, is also used in palatogenesis. Their roles in palatogenesis are specifically investigated by these transgenic mouse

models (Rice *et al*, 2004; Alappat *et al*, 2005). In these mice, cell proliferation was reduced in the palate; Shh and its receptor Patched1 expression was also altered, suggesting that the Fgf10/Fgfr2b pathway regulates Shh expression in the palate, which is mitogenic to the palatal mesenchyme (Rice *et al*, 2004). Fgf10 was also shown to be required for the survival of MEE cells and for normal expression of Jagged2 and Tgfβ3 in the palatal epithelia (Alappat *et al*, 2005).

These data from animal models suggest that genetic determination of the occurrence of cleft palate, and perturbation of the Fgf10/Fgfr2b signal pathway might be a mechanism. Moreover, in many FGFr-related craniosynostosis syndromes cleft palate is also present. For example, in Apert syndrome, palatal clefts occur in 75% of patients (Kreiborg and Cohen, 1992). This highlights the role of Fgf signalling in palatogenesis and supports genetic determination or high genetic background for syndromic cleft palate.

Fgf signalling in submandibular salivary gland development

The submandibular salivary gland (SMG) is a classic developmental model for studying epithelial–mesenchymal interactions, branching morphogenesis and organogenesis. Embryonic SMG morphogenesis is initiated with a thickening of the oral epithelium of the mandibular arch, then undergoing sequential morphological changes including budding, branching and elongation, and finally the luminen is formed. Secretory function is thereafter developed.

The importance of Fgf signalling in SMG development is also demonstrated by *Fgfr2b* and *Fgf10* null mice, which display clear deficiencies or agenesis of the salivary gland (Sekine *et al*, 1999; De Moerlooze *et al*, 2000; Ohuchi *et al*, 2000). *Fgfr2b* null mice exhibit agenesis or dysgenesis of various organs, such as the lung, pituitary gland and salivary glands, which typically undergo budding and branching morphogenesis (De Moerlooze *et al*, 2000). In *Fgf10* null mice absence of thyroid, pituitary and salivary glands was also identified (Sekine *et al*, 1999; Ohuchi *et al*, 2000). These results imply that the Fgf10/Fgfr2b signal pathway is critical for the patterning and initiation of SMG development. Recently, it has also been demonstrated that the Fgf10/Fgfr2b signal loop that regulates branching morphogenesis in lung is also critical in SMG branching (Steinberg *et al*, 2005). Furthermore, the Fgfr1 signal was also shown to play an important role in SMG development: downregulation of Fgfr1 in developing SMG decreases the branching morphogenesis (Hoffman *et al*, 2002). The expression of *Fgf10* in mouse SMG development is shown in Figure 1.

Among the Fgf ligands, besides Fgf10, Fgf8 is also a key signal for the initiation and advancing development of SMG. By studying the hypomorphic and conditionally mutated mice, Fgf8 was found to regulate SMG in a dose-dependent manner (Jaskoll *et al*, 2004). In the absence of *Fgf8*, branching morphogenesis did not occur

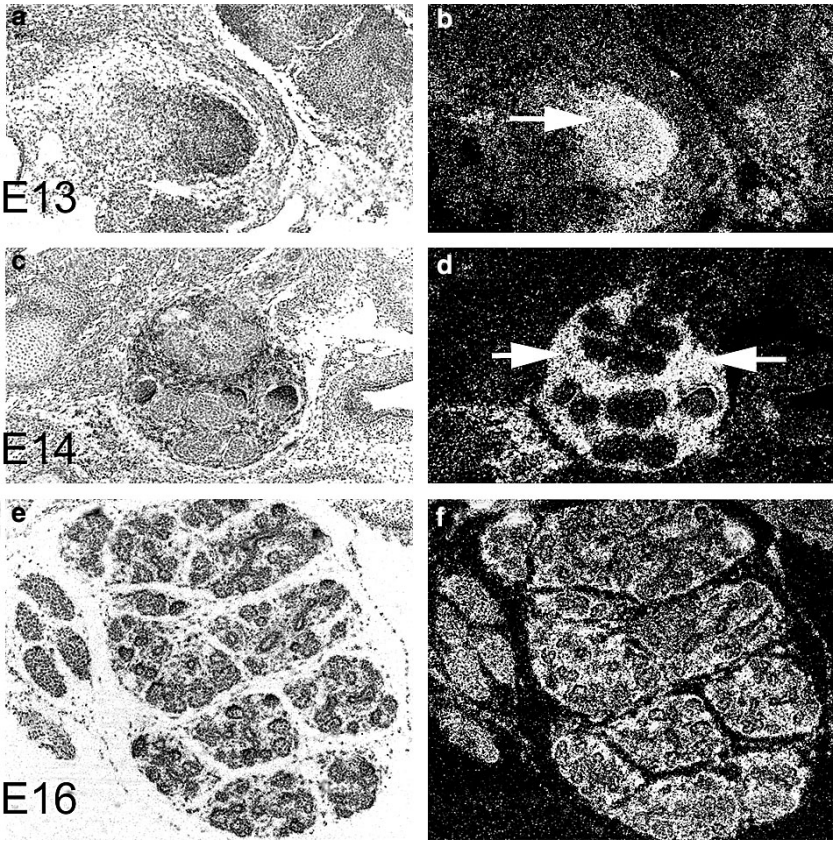

Figure 1 Fgf10 mRNA expression in mouse submandibular gland development detected by radioactive *in situ* hybridization. *Fgf10* is highly expressed in the SMG bud at the early initiation stage (**a** and **b**). Later it is expressed in the mesenchyme in advanced development (**c**–**f**). (**a**), (**c**) and (**e**) are bright field images; (**b**), (**d**) and (**f**) are dark field images. Arrows indicate the expression. E: embryonic days

(Jaskoll *et al*, 2004). It was further shown to regulate Fgf10 and Shh expression in the developmental process: exogenous Fgf10 and Shh supplementation to *Fgf8*-deficient SMG restores the abnormal phenotype towards normal *in vitro* (Jaskoll *et al*, 2004). Fgf8 is one of the preferred ligands for Fgfr2c: in *Fgfr2c* null mice the SMG is also hypoplastic (Jaskoll *et al*, 2002).

Fgf1, *Fgf3* and *Fgf7* are also expressed in the developing SMG. Exogenous Fgfs added to mandibular epithelial rudiments cultured without mesenchyme induces distinct morphogenesis: Fgf7 induces epithelial budding, whereas Fgf10 induces duct elongation (Steinberg *et al*, 2005). On the contrary, Fgf2, -4, -6 and -9 appeared to have no essential roles in SMG development, as implied by studies on phenotype of knockout mice and their expression patterns (Fiore *et al*, 1997; Colvin *et al*, 1999; Jaskoll *et al*, 2004).

Autosomal dominant aplasia of lacrimal and salivary glands (ALSG, OMIM no. 180920 and OMIM no. 103420) is a rare condition characterized by irritable eyes and dryness of the mouth. Recently, haploinsufficiency of FGF10 during a crucial stage of development has been suggested to be the cause of ALSG, as heterozygous mutations were identified in FGF10 in all individuals with ALSG in two extended pedigrees (Entesarian *et al*, 2005). By carefully examination of Fgf10 (+/−) mice, those researchers also found the phenotype of SMG is similar to ALSG (Entesarian *et al*, 2005). In addition, hypoplasia and aplasia of SMG in Fgf8 and Fgfr2c conditional abrogation mice suggest that they might be candidate genes as well (Jaskoll *et al*, 2002, 2004).

Fgf signalling in tooth development

Tooth morphogenesis initiates from thickening of the stomodeal epithelium. This dental lamina gives rise to an epithelium bud in further development. Thereafter the early tooth bud coupled with the underlining mesenchyme undergoes sequential morphological transformation, known as the cap stage and bell stage. Fgf signals play crucial roles in tooth initiation and further development. In particular, Fgf8 is an early ligand expressed during tooth initiation, involving tooth patterning and position determination. In advancing development, the Fgf signal is present in both the epithelium and mesenchyme: expression of *Fgf3*, *-4*, *-9*, *-10,* and *Fgfr1-3* isoforms are developmentally regulated in this process (Jernvall and Thesleff, 2000). The Fgf signal induces *Msx* expression in the early developing stage, which is critical for tooth formation (Bei and Maas, 1998). Recently, it was found that Runx2 mediates the function of Fgf from epithelium to mesenchyme during tooth morphogenesis (Aberg *et al*, 2004). The Fgf signal also directs the epithelial growth and folding. Without the *Fgf* signal, the tooth does not develop beyond the bud stage, as demonstrated by transgenic mouse lacking functional *Fgfr2b* isoforms or over expressing a negative *Fgfr* receptor (Celli *et al*, 1998; De Moerlooze *et al*, 2000).

Fgfs are mitogenic to dental tissues except the enamel knot, which lacks receptor expression (Kettunen *et al*, 2000). Intense expression of *Fgfr1* and *Fgfr2* isoforms in odontoblasts and ameloblasts suggests that the Fgf signal participates in regulation of their differentiation and secretory functions (Kettunen *et al*, 2000). In Figure 2, the expression of *Fgfr1* in developing mouse incisors is shown.

Mouse incisors erupt throughout the lifetime by the renewal of dental epithelium produced from the cervical loop located in the tooth apex. Therefore, the mouse incisor presents an excellent model for the study of stem cell niche formation. Recently, it has been demonstrated that Fgf10 maintains stem cell compartment in

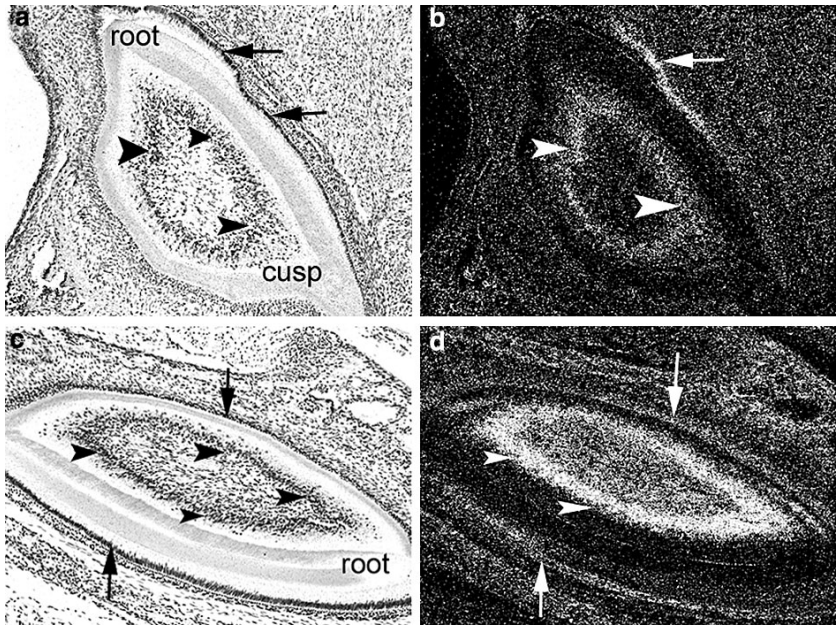

Figure 2 Fgfr1b and Fgfr1c mRNA expression in developing incisors of 5 days postnatal mouse detected by radioactive in situ hybridization. Fgfr1b transcripts are localized in both the odontoblasts and ameloblasts at a moderate level in the upper incisor (**a** and **b**). *Fgfr1c* is intensely expressed in dentine-forming odontoblasts in the lower incisor (**b** and **d**). Arrows indicate the ameloblasts, arrow heads indicates the odontoblasts. (**a**) and (**c**) are bright field images, (**b**) and (**d**) are dark field images

developing incisors in mice (Harada *et al*, 2002). The cervical loop, including the putative stem cell, is not formed in the developing incisors of *Fgf10* null mice, and the incisors in such mice lose the ability to grow continuously (Harada *et al*, 2002).

In humans, the absence of one or more teeth is a common development anomaly. Excluding the third molars, which are absent in about 20% of the population, the incidence of absence of one or more teeth has also been reported to be relatively high. Many studies have reported that the prevalence of hypodontia, congenital absence of one or several permanent teeth without any systemic disorders, varies from 2.6% to 11.3% (Larmour *et al*, 2005). The teeth most often missing are second premolars, upper lateral incisors and lower central incisors. Consequently, this trait is termed incisor–premolar hypodontia (Arte *et al*, 1996). Recently, many candidate genes have been identified, such as MSX1 and PAX9 (Stockton *et al*, 2000; Cobourne and Sharpe, 2003). Even though Fgf signalling is critical for tooth development, there is still no direct evidence for an aetiological relationship between Fgf and hypodontia. On the contrary, in a study carried out to identify the candidate gene through genetic mapping using linkage analyses, FGF3 and FGF4 loci were excluded from possible sites for gene mutation for incisor–molar hypodontia (Arte *et al*, 1996).

Fgf signalling in the development of craniofacial muscle and the muscular tongue

Muscle development typically undergoes a series of processes, including determination, migration, proliferation, differentiation and maturation. These processes are mediated by intrinsic molecular factors, such as Pax9 and myogenic regulatory factors (Amthor *et al*, 1999). Those genes are regulated by some other patterning and developmental genes, such as of the Fgf family. Fgf/Fgfr signalling is generally believed to promote myogenic proliferation but repress myogenic differentiation. Fgfr1 has been shown to be important for skeletal muscle development: altered expression of this gene causes abnormal muscular development (Patel *et al*, 1999; Scata *et al*, 1999; Flanagan-Steet *et al*, 2000). Overexpression of a full-length Fgfr1 increased myocyte proliferation and delayed differentiation; conversely, reduction in functional Fgf signalling by expression of a truncated Fgfr1 decreased proliferation and enhanced differentiation of myocytes (Scata *et al*, 1999). In another study, loss of Fgfr1 signalling reduced skeletal muscle mass and disrupted myofibre organization in the developing limb (Flanagan-Steet *et al*, 2000).

A very recent study has shown that Fgfr4 plays critical roles in mediating the Fgf signal in myogenesis (Marics *et al*, 2002). Inhibition of Fgfr4 leads to a dramatic loss of limb muscles; muscle markers, such as Myf5, MyoD, and the embryonic myosin heavy chain, are affected; and muscle progenitor differentiation is arrested, which can be rapidly reverted by the addition of exogenous Fgf protein (Marics *et al*, 2002). On the contrary, overexpression of Fgf8 in somites promotes Fgfr4 expression and muscle differentiation in this tissue (Marics *et al*, 2002). These results demonstrate that myogenic differentiation is positively controlled by Fgf signalling, a notion that contrasts with the general view that Fgf promotes myoblast proliferation and represses myogenic differentiation. Moreover, Fgfr4 has been identified to be the main receptor expressed and functioned during muscle regeneration, and Fgf6 is likely the key ligand for Fgfr4 during this process (Zhao and Hoffman, 2004).

Craniofacial myogenesis is characterized by its early maturation and intercalation within tissues of neural crest origin. The tongue is a good model for studying craniofacial myogenesis, as it is basically a muscular organ and is easily accessible for experimental procedures. Molecular events of muscular development in tongue also differ from that of other skeletal muscles (Dalrymple *et al*, 1999; Yamane *et al*, 2000). So far, the role of Fgf signalling in tongue muscle development is largely unknown. In our unpublished data, peak expression of Fgfrs and some Fgfs was coincident with the vigorous proliferation period in embryonic development of the tongue. Based on this expression pattern and its mitogenic effect to skeletal muscles at other sites, it is very likely that the Fgf signal is a mitogen for the rapid embryonic expansion of the tongue.

Proper development of the tongue is important for the related structures within the oral cavity. Rapid withdrawal of the tongue in embryogenesis is critical for proper palatogenesis. Delay in this process may disturb proper palatal shelf elevation and hence might lead to cleft palate. Congenital macroglossia is commonly observed in human syndromes and other pathological conditions, such as Down's syndrome, Crouzon syndrome and Beckwith–Wiedemann syndrome (Vogel *et al*, 1986). Anomalies such as microglossia, aglossia and clefted glossia are mostly seen in syndromic and non-syndromic mandibular hypoplasia (Singh and Bartlett, 2005), but also occur as isolated developmental deformities, although the incidence is very rare.

Conclusive remarks

In conclusion, Fgf signalling plays critical roles in craniofacial development, being involved in multi-stage development of almost all structures. Unraveling of the Fgf signal pathway within the genetic cascade for craniofacial development to a great extent expands our understanding of human development disorders in the craniofacial region, and provides the possibility of novel strategies to prevent those disorders. It is also worth noting that, besides craniosynostosis, the role of Fgf signalling in the majority of craniofacial developmental disorders remains to be elucidated. Identification of muted genes from the Fgf/Fgfr family in these disorders is a huge task for future research. However, the complex mechanisms of craniofacial development coupled with intrinsic and extrinsic factors make it a difficult task. The use of mouse models greatly facilitates our advances in craniofacial research and provides important aetiological clues to some developmental disorders, especially

those with a high genetic background. However, it should also be stressed that the phenotype of transgenic modified mice is not identical to what we see in clinical cases. Therefore interpretations of human disorders with information from animal model should be meticulous.

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