

# Roles of FGF8 subfamily in embryogenesis and oral-maxillofacial diseases (Review)

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**Abstract.** Fibroblast growth factors (FGFs) are diffusible polypeptides released by a variety of cell types. FGF8 subfamily members regulate embryonic development processes through controlling progenitor cell growth and differentiation, and are also functional in adults in tissue repair to maintain tissue homeostasis. FGF8 family members exhibit unique binding affinities with FGF receptors and tissue distribution patterns. Increasing evidence suggests that, by regulating multiple cellular signaling pathways, alterations in the FGF8 subfamily are involved in craniofacial development, odontogenesis, tongue development and salivary gland branching morphogenesis. Aberrant FGF signaling transduction, caused by mutations as well as abnormal expression or isoform splicing, plays an important role in the development of oral diseases. Targeting FGF8 subfamily members provides a new promising strategy for the treatment of oral diseases. The aim of this review was to summarize the aberrant regulations of FGF8 subfamily members and their potential implications in oral-maxillofacial diseases.

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## 1. Introduction

The mammalian fibroblast growth factor (FGF) family includes 22 members, which can be subdivided into six subgroups based on sequence similarities, biochemical properties and evolutionary relationships (1). FGF8, FGF17 and FGF18 are classified as the FGF8 subfamily, since these three proteins share 60-80% amino acid sequence identities and similar receptor-binding properties (2,3).

FGF8 subfamily members are highly conserved between humans and mice (Fig. 1). FGF8, reported as an androgen-induced growth factor, was originally isolated from the conditioned medium of the mouse mammary carcinoma cell line SC-3 (4,5). Human FGF8 isoform a and b show 100% similarity in protein sequence with murine FGF8 isoform a and b, respectively (6). However, human FGF8e and FGF8f isoforms share 98% identity with corresponding murine isoforms (7). Both FGF17 and FGF18 were originally isolated from mouse embryos. Similar to FGF8, 98.6% and 99% homology has been identified between human and murine FGF17 and FGF18, respectively (6,8).

It is documented that FGF8 and FGF17 are expressed in the heart, limb, kidney and central nervous system (CNS), and in craniomaxillofacial development, whereas FGF18 is expressed in the cartilage and palate. The pivotal roles of FGFs and their binding receptors (FGF receptors, FGFRs) in development and pathogenic processes are thought to be due to the increasing functional diversities of the signaling systems enabled by them (9).

All FGFs share a conserved structure, including an internal core region encompassing 120-125 amino acids, which is essential for binding to FGFR, as well as a carboxy-terminal and an amino-terminal (Fig. 1) (10). FGF8 subfamily members have a hydrophobic amino-terminal (~22 amino acids), which is a typical cleavable signal peptide (11). Interestingly, the amino-terminal sequences of FGF17 and FGF18 are more closely conserved to each other, than to FGF8 (6). Variations in the N-terminal tails of FGF8 subfamily members may play a role in their distinct biological functions (12). The core region structure of all FGFs is a conserved  $\beta$ -trefoil fold consisting of 12 antiparallel  $\beta$ -strands ( $\beta$ 1- $\beta$ 12). However, the alternatively spliced  $\beta$ F and  $\beta$ G strands of FGF8 subfamily members harbor the primary receptor binding sequences and give them binding affinities to specific FGFRs (13,14).

The complexity of FGF8 mRNA splicing was reported previously. The first exon of FGF8 gene in mice contains at least four different coding sequences that can be alternatively spliced to produce eight secreted isoforms (a-h). However, there are only four isoforms (a, b, e and f) in humans because of a terminator sequence in exon 1B of the FGF8 gene. The shortest FGF8 isoform, FGF8a, contains the core region, which is conserved in all FGF8 isoforms. All other FGF8 isoforms have an extra sequence, which is different for each isoform, flanking the common core region (15). Nevertheless, FGF8 variants share identical reading frames and the same peptide sequence in their carboxy-terminal domains (5,16). None of the alternative-splicing events alters the sequence of the conserved FGF core domain (11).

Human FGF17 gene codes for two isoforms: FGF17a and FGF17b. FGF17a is not thought to be involved in FGF signal transduction, since FGFRs are not activated in NIH3T3 cells treated with FGF17a (17).

## 2. Signal transduction

*FGF8 receptors and signal transduction.* FGF8 subfamily members share a similar receptor binding specificity. They exhibit high binding affinities with IIIc variants of FGFR1-3 and the non-spliced FGFR4 (3), while only a weak affinity is observed with IIIb variants (13,18). Furthermore, it has been demonstrated that FGF8 and FGF17 have a higher binding affinity with FGFR3c and FGFR4, and a weaker binding affinity with FGFR1c. Likewise, FGF18 preferentially binds to FGFR3c rather than FGFR2c (3).

Binding with FGF ligands leads to a conformational shift in the FGFR that activates the intracellular kinase domain, resulting in intermolecular transphosphorylation of the tyrosine kinase domains and the intracellular tail. FGFR substrate 2 (FRS2) and phospholipase C $\gamma$  (PLC $\gamma$ ), which are key adaptor proteins phosphorylated by FGFRs, are then recruited to the FGFR intracellular tail, phosphorylated by FGFRs, and in turn initiate intracellular signal cascades. The FRS2/Ras/mitogen-activated protein kinase (MAPK) pathway, PLC $\gamma$ /Ca<sup>2+</sup> pathway, and phosphoinositide-3-kinase (PI3K)/AKT pathway are considered to be major downstream effector pathways of the FGF8 subfamily (Fig. 2) (19).

*Ras/MAPK pathway.* FGFRs can directly phosphorylate several tyrosine sites on the FRS2 protein after binding with FGFs (20). These phosphorylation sites are recognized by the adapter protein growth factor receptor-bound protein 2 (Grb2), a small adaptor molecule, which forms a complex with the guanine nucleotide exchange factor Son of Sevenless (SOS) via its SH3 domain, allowing SOS to activate Ras. Activation of Ras triggers a series of downstream effector proteins, including Raf, MAPK kinase (MEK) and MAPK, and the latter finally enter the nucleus and phosphorylates transcription factors, including c-Myc and activation protein-1 (21,22).

*PI3K/Akt pathway.* Besides SOS, Grb2 is able to recruit the adaptor protein Grb2-associated binding protein 1 (Grb1) to the complex, once Grb2 is activated by FGFR-phosphorylated FRS2 $\alpha$ . This leads to activation of PI3K, which eventually activates AKT (2,23).

*PLC $\gamma$ /Ca<sup>2+</sup> pathway.* The PLC $\gamma$ /Ca<sup>2+</sup> pathway is activated when PLC $\gamma$  directly binds to an autophosphorylated tyrosine in the C-terminal tail of FGFRs, resulting in PLC $\gamma$  phosphorylation and activation. Activated PLC $\gamma$  hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) to produce two second messengers: Phosphatidylinositol-3,4,5-triphosphate (PIP3) and diacylglycerol (DAG). PIP3, in turn, stimulates the release of intracellular calcium, while DAG activates calcium-dependent downstream signaling of protein kinase C (23,24).

Several other pathways are also found to be activated by FGFRs, such as the signal transducer and activator of transcription signaling pathway, the Wnt signaling pathway associated with tooth development (25), and the Sonic hedgehog (Shh) signaling pathway involved in the palate (26). These pathways interact with other growth factor pathways, forming a network that regulates cell behavior (Fig. 3).

## 3. Regulation of cell phenotype by FGF8 subfamily members

Previous studies have suggested that FGFs play important roles in morphogenesis, angiogenesis and development of a variety of cells. FGFs bind to FGFRs with different affinities, eliciting a wide range of biological responses, including cellular proliferation, differentiation, migration, adhesion and survival (Fig. 4) (18,23,27).

## 4. Epithelial-to-mesenchymal transition in oral-maxillofacial development

Due to the critical role of FGF8 and FGF18 in embryo development, germline inactivation of FGF8 and FGF18 genes causes embryonic or perinatal death in mice and the loss of all embryonic mesoderm and endoderm-derived structures (3,5,28). FGF17 knockout mice are viable but exhibit impaired hindbrain development, suggesting that FGF17 may play a less critical role than FGF8 or FGF18 (29,30).

*FGF8 subfamily members in craniofacial development.* The mechanisms responsible for craniofacial development are partially understood (31). In mammals, the first branchial arch (BA1) develops a number of craniofacial skeletal elements, including teeth, the mandible and maxilla, the lateral skull wall, and parts of the tongue and other soft-tissue derivatives (32).

FGF8 is considered to be an epithelial cell-originating factor that regulates gene expression when patterning of the mandibular mesenchyme occurs during BA1 development (Fig. 4) (32,33). FGF8 is involved in the development of a large proximal region of the BA1 primordium, either promoting mesenchymal cell survival or inducing BA1 morphogenesis (32). It has been identified that FGF8 positively regulates Sprouty gene expression in the BA1. A reduced level of Sprouty homolog 2 (Spry2) in mutant BA1 mesenchyme may partially compensate for reduced Ras activity due to the inactivation of FGF8 by depressing other receptor tyrosine kinase pathways normally inhibited by Sprouty, and thereby promote cell survival (22,32). FGF8 is expressed in the outmost lateral regions of each pharyngeal pouch and exhibits a high level of expression in the overlying surface ectoderm of the mandibular and maxillary prominences. It is reported

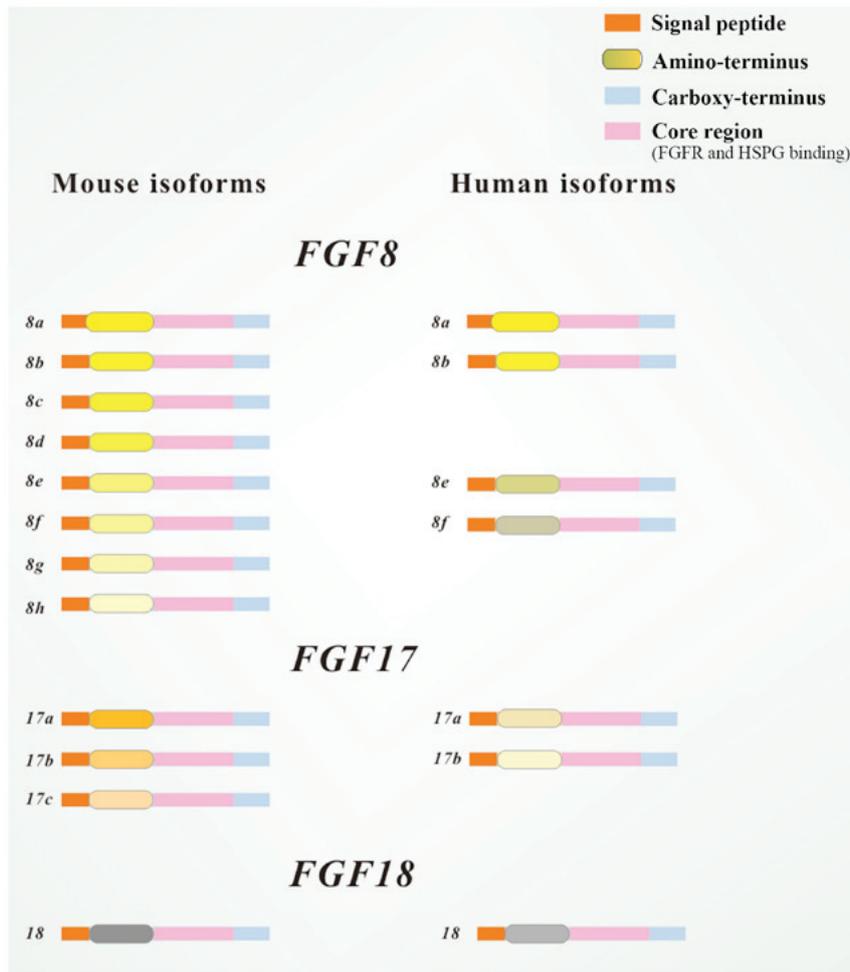


Figure 1. Structure of isoforms of FGF8 subfamily members. All peptides contain an FGFR and HSPG binding site in the core region and a cleavable secreted signal sequence in the amino-terminal (marked in different colors). Human isoforms are very similar to their murine counterparts in both the N- and C-terminus. Mouse FGF8 has eight isoforms: FGF8a, FGF8b, FGF8c, FGF8d, FGF8e, FGF8f, FGF8g and FGF8h; human FGF8 has only four isoforms: FGF8a, FGF8b, FGF8e and FGF8f. Mouse FGF17 has three isoforms: FGF17a, FGF17b and FGF17c; human FGF17 has only two isoforms: FGF17a and FGF17b. FGF, fibroblast growth factor; FGFR, FGF receptor; HSPG, heparan sulfate proteoglycan.

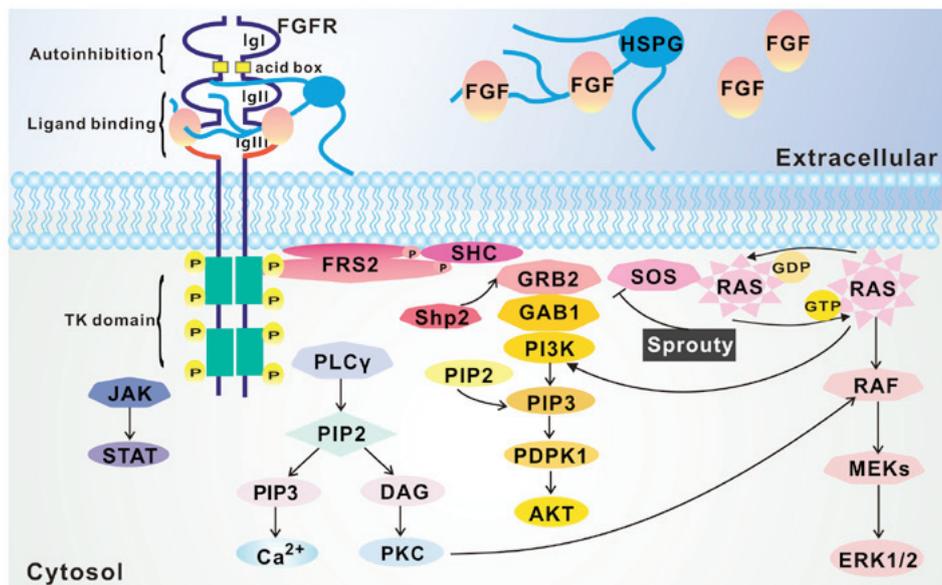


Figure 2. FGF signaling transduction. FGFs form a 2:2:2 complex with FGFR and HSPG. The FGF-FGFR binding is stabilized by the interaction with HSPG, thus leading to receptor dimerization and autotransphosphorylation. After receptor activation, two of the signaling branches, PI3K/AKT and RAS/MAPK pathways, are initiated via FRS2. FGFR signals are also transduced to the DAG-PKC and PIP3-Ca<sup>2+</sup>-releasing signaling branches via PLC $\gamma$ . FGF, fibroblast growth factor; FGFR, FGF receptor; HSPG, heparan sulfate proteoglycan.

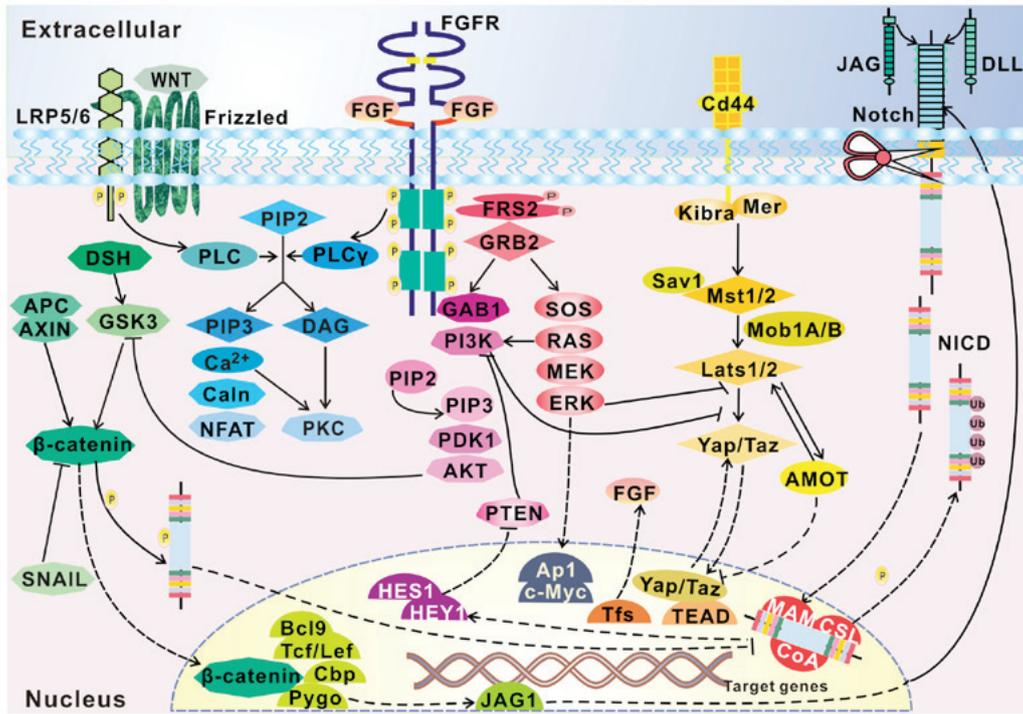


Figure 3. Crosstalk between the FGF signaling pathway and other signaling pathways. Upon binding with FGFs, FGFRs activate downstream effectors, including PLCγ, FRS2, Ras/MAPK and PI3K/Akt, leading to subsequent activation of the Wnt signaling pathway, Hippo signaling pathway and Notch signal pathway. FGF, fibroblast growth factor; FGFR, FGF receptor.

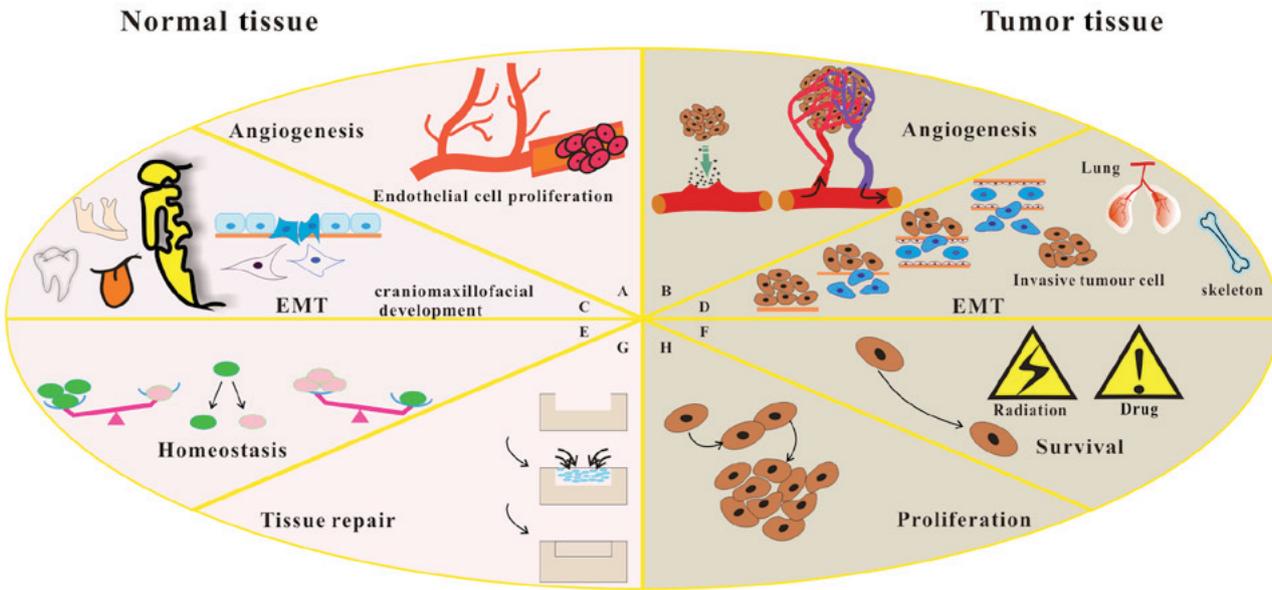


Figure 4. FGF8 subfamily in the regulation of normal tissue homeostasis and tumor development. In normal or tumor tissues, FGF8 subfamily members regulate (A and B) endothelial cell proliferation, (C and D) EMT, (E) tissue homeostasis, (F) cell survival, (G) tissue repair and (H) cell proliferation. FGF, fibroblast growth factor; EMT, epithelial-mesenchymal transition.

that FGF8 mutations affect signaling in the BA1 ectoderm and result in failure in developing BA1 structures (34). Newborn mice harboring these mutants lack most BA1-derived structures, except those that develop from the distalmost region of BA1, including mandibular incisors (32).

The signal transduction pathways activated by FGF8 are implicated in early steps of craniofacial development (28). FGF8, -17 and -18 have been demonstrated to be key regulators in craniofacial structure formations, including tooth, palate,

mandible and salivary gland (35,36). Thus, FGF8 subfamily members may play different roles in different developmental contexts (22,32).

*Regulating odontogenesis.* In the initial stages of tooth development, FGF signaling is involved in the dental epithelium and the invagination of the dental epithelium into the underlying mesenchyme (37). FGF8 is expressed in both the epithelium and mesenchyme, promoting initiation and patterning of tooth

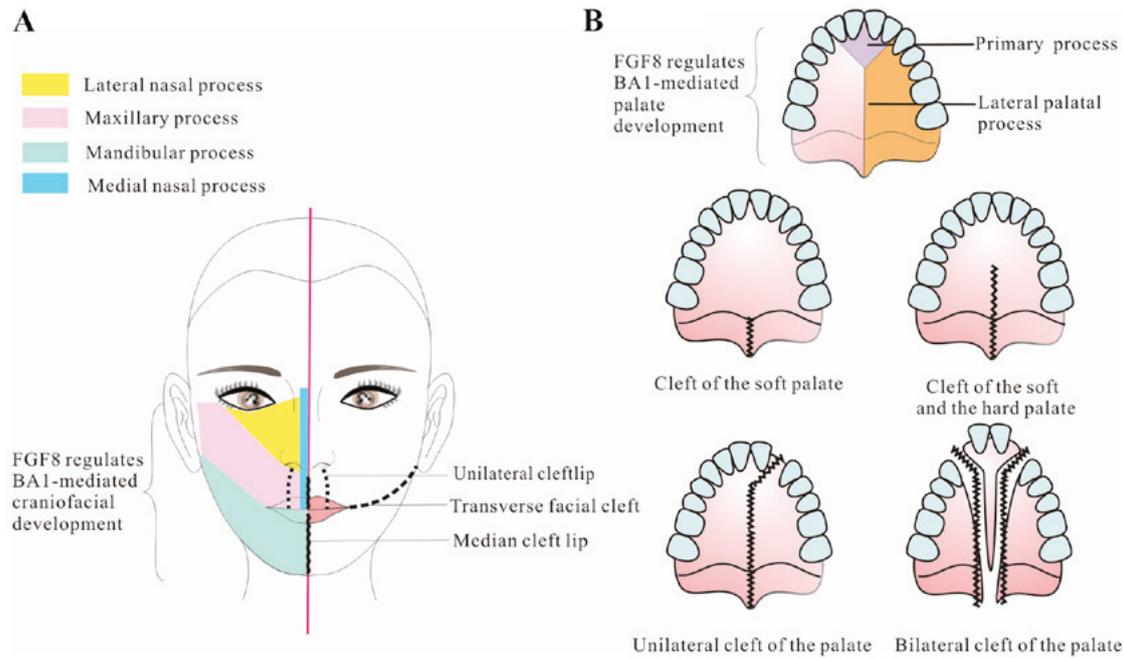


Figure 5. Aberrant regulation of the FGF8 subfamily in oral-maxillofacial diseases. Dysregulation of FGF8 subfamily members disrupts BA1-mediated craniofacial development, leading to maxillofacial abnormalities, including (A) cleft lip and (B) cleft palate. FGF, fibroblast growth factor; BA1, first branchial arch.

morphogenesis (38). FGF17 is expressed in the epithelium, while FGF18 is expressed in the mesenchyme (28,38,39).

The expression of pituitary homeobox 2 (Pitx2), a marker for the dental lamina band, is restricted to the dental epithelium of both molars and incisors throughout odontogenesis (37,40,41). Bone morphogenetic protein 4 (BMP4) and FGF8 may act as feedback regulators to control Pitx2 expression during early odontogenesis. FGF8 positively regulates Pitx2 expression and BMP4 exerts the opposite effect (40,42). In the absence of FGF8, Pitx2 expression in the oral epithelium is diminished, thereby affecting tooth development (43,44). In addition, by activating the dental mesenchymal markers Barx1 and Pax9 in the mesenchyme, the coordination of FGF8 and BMP4 signaling pathways determines not only the proto-molar or proto-incisor fate of mandibular mesenchymal cells, but also the tooth emergence sites by regulating the migration of the tooth germinal cells (37,42,45). Conditional FGF8 gene knockout in the ectoderm leads to significantly decreased expression of Barx1 and Pax9 in the presumptive molar region, and thus molar teeth are not formed and rudimentary incisors develop instead (37,46-52).

FGF18 is mainly expressed in the mesenchyme. As the epithelium within the dental lamina continues to form the epithelial bud, FGF18 continues to be expressed in the mesenchyme. In the incisor at the bell stage, FGF18 expression is detected in the mesenchyme of the cervical loop. FGF18 expression is also detected at the buccal side of the mesenchyme at the lamina stage. In addition, FGF18 is involved in the regulation of odontoblasts in the latter stages of molar tooth development and may regulate dentin matrix formation and/or mineralization in both the crown and root formation (37,39).

**FGF8 subfamily members in tongue development.** FGF8 and FGF18 are associated with the generation and early morphogenesis of the tongue (53). For rodents, the formation

of the tongue begins at E11, when two lateral tongue buds derive from the branchial arch (35,54). These two buds merge to shape into the tongue primordium at E11.5, and cell proliferation is followed by cell differentiation in both the local epithelium and mesenchyme, causing the rapid enlargement of the tongue (55,56). Tongue epithelium differentiation begins at E13, initiated from a single circumvallate papilla and numerous fungiform papillae, which is followed by the differentiation of filiform papillae and foliate papillae at approximately E15 (54). Both FGF8 and FGF18 are expressed in the tongue epithelium at relatively constant levels during early tongue development, whereas FGF17 is undetectable (18). FGF8 is strongly expressed in the dorsal tongue epithelium and also more diffusely in the underlying tongue mesenchyme. However, FGF8 expression is downregulated before morphologically detectable gustatory papillae initiation (53), suggesting that FGF8 might be less important during these stages. A similar expression pattern is found in FGF18 during tongue development. FGF18 is highly expressed in the lingual margin of the anterior half of the tongue, and weakly expressed in the posterior half. Like in other organs, FGF18 is thought to stimulate proliferation of both mesenchymal and epithelial cells during tongue development (35).

**Regulation of skeletal development.** Mandibular development from BA1 is regulated by the interaction of the mesenchyme with the endoderm and ectoderm. FGF8 expression is spatially restricted to the ectoderm precursors of the proximal mandible and its expression is regulated by adjacent endoderm-derived signals (17). FGF8 is pivotal for the survival and migration of mesenchymal cells in BA1. FGF8 overexpression due to ectopic activation in CNC-derived mesenchymal cells inhibits tissue differentiation and organogenesis in the craniofacial region, instead of impairing migration. Therefore, FGF8 expression does not affect the odontogenic fate of the

CNC-derived mesenchymal cells (46). If the expression of FGF8 is conditionally lost in the ectoderm of BA1, the arch is markedly reduced in size, resulting in almost complete loss of the BA1-derived skeletal structures and tooth agenesis (28).

The expression of FGF18 persists in mesenchymal cells and osteoblasts during mandibular bone development. FGF18 is thought to positively regulate osteogenesis and negatively regulate chondrogenesis (18,57-59). However, FGF17 expression is only observed in the maxilla (17).

*FGF8 subfamily members in salivary gland branching morphogenesis.* The development of the embryonic submandibular salivary gland (SMG) begins with an invagination of the original oral epithelium into undifferentiated mandibular mesenchyme, which requires epithelial-mesenchymal transition. FGF8 and its cognate receptor, FGFR-2c (IIIc), are essential for branching morphogenesis (26,60,61).

FGF8 hypomorphic mice, which have defective FGF8 throughout embryogenesis, develop hypoplastic SMGs. FGF8 ectoderm conditional mutants, with silenced FGF8 expression in the BA1 ectoderm, exhibit ontogenic arrest followed by involution and eventually disappear at E18.5. SMG aplasia in FGF8 conditional mutants indicates that FGF8 signaling is essential for salivary gland branching morphogenesis. Notably, the presence of an initial SMG bud is observed in FGF8 mutants, suggesting that the initial bud formation is independent of FGF8 (61).

### 5. Aberrant regulation of FGF8 subfamily members in oral-maxillofacial diseases

During early craniomaxillofacial morphogenesis, an important role of the FGF8 subfamily is to control epithelial-mesenchymal transition (EMT) (5). Errors during this complex process can cause craniofacial anomalies (Fig. 5). Interestingly, EMT is also implicated in the progression of invasive metastasis of malignant tumor cells (62). For instance, FGF8 is reactivated and overexpressed in ovarian, breast and prostate cancers, and further promotes tumor invasion and metastasis (5,63,64).

The subsequent sections will focus on the aberrant signaling of FGF8 subfamily resulting in diseases of the oral and maxillofacial regions.

*FGF8 subfamily members and cleft lip and/or palate (CLP).* CLP is among the most commonly observed congenital malformations. CLP can affect feeding, speech, hearing and dental function. Although the condition can be surgically repaired to different degrees, lots of patients maybe still suffer lifelong psychosocial effects from the malformation (65).

During the final stages of palatogenesis, two palate shelves undergo mesenchyme proliferation, expansion and elevation, then dissolution of the epithelium and midline fusion. Failure to undergo any of these processes results in a palatal cleft (Fig. 5) (66).

Genome-wide analysis has suggested that FGF8 mutations are involved in the development of CLP. For instance, D73H missense mutation in the FGF8 gene was found in a CLP patient. Neither parent of the patient had the variant allele, suggesting that the mutation arose in the patient. Structural analysis revealed that D73 is located in the docking domain

for binding with FGFR2 IIIc, and the side chain of D73 creates three hydrogen bonds connecting with the FGFR. This mutation reduces the binding affinity of FGF8 with its cognate receptors, probably by both destabilizing the conformation of the N-terminus of FGF8 and subsequently eliminating hydrogen bonding with receptors (30,67). The FGF8 D73H mutation is the first disease-associated mutation in the coding region of FGF8. The patient with this mutation appeared to have non-syndromic CLP with no additional phenotypes (67).

T-box 1 (Tbx1) and transcription factor activator protein 2 (Tfap2) are FGF8-associated transcriptional mediators of developmental abnormalities that control palatal elongation and elevation. In Tbx1-null mice, FGF8 expression is markedly downregulated in the palate epithelium (68). Mutations in the Tfap2a gene induce upregulated FGF8 expression, resulting in cleft palate by changing growth and morphogenesis. Reducing FGF8 expression compensates these morphogenic changes, and decreases the incidence of cleft palate in mice. Thus, it is reasonable to infer that loss of Tbx1 and Tfap2 results in aberrant proliferation and apoptosis in palatal cells, probably through altering FGF8 expression (65).

By contrast, CLP in mice caused by enhanced cell apoptosis in the malformed mandible and tongue may be associated with the prominent decrease of FGF8 expression in the ectoderm (25,65). In addition, Sp8 is the zinc finger transcription factor gene, which is considered as a strong candidate gene for causing non-syndromic CLP (69). Reduced expression of murine FGF8 and FGF17 in the anterior neural ridge and olfactory pit signaling centers caused by mutations of Sp8 could also lead to CLP (70).

Cleft palate is also observed in FGF18 null mice, but the mechanisms remain unclear (65). Shh signaling is reported to play a key role in palate development by regulating the expression of Foxf1 and Foxf2 of the Fox family transcription factors (26,71,72). Foxf1 and Foxf2 regulate Shh transduction and repress FGF18 expression in the palatal mesenchyme during the palatal shelf growth. Notably, Foxf2 mutants exhibit decreased Shh expression but enhanced FGF18 expression. These results suggest a Shh-Foxf-FGF18-Shh negative feedback loop in the reciprocal epithelial-mesenchymal signaling molecular network controlling palatogenesis (26,58,72).

*FGF8 subfamily members and ciliopathies.* Ciliopathies are a wide class of human disorders commonly characterized by craniofacial dysmorphology. The most frequent craniofacial phenotype in ciliopathies is a high arched palate with a prominent median groove, but with the roof of the mouth remaining intact across the midline. The disease is associated with secondary dental anomalies, including postnatal gingival swelling and molar crowding, which greatly influence speech and quality of life. Tabler *et al* (73) reported that the primary cause of ciliopathic high arched palate is excessive neural crest cells producing an enlarged BA1 and maxillary hyperplasia in early embryogenesis.

Fuzzy (Fuz) is a central regulator of vertebrate ciliogenesis (74). Ciliopathic Fuz mutant mice indicate that the mechanistic basis of this phenotype originates from dysregulated Gli processing, which in turn leads to craniofacial FGF8 overexpression. More specifically, loss of Gli3 can result in increased cranial FGF8 gene transcription, resulting in a

dramatic increase in FGF signaling. Notably, at E9.5 in Fuz mutants, FGF8 distribution is significantly expanded, and exhibits a mediolateral expansion within the mandibular and maxillary prominences. This expansion is maintained in mutant maxillae at E10.5. It is believed that excessive FGF signals drive the maxillary hyperplasia that is the basis of the palate defects, and by contrast, downregulation of FGF8 ameliorates the maxillary phenotypes (73,75).

It has been suggested that the most likely reason for craniofacial defects in Fuz mutant mice is increased expression of FGF8. High arched palate is also a common feature of FGF hyperactivation syndromes, raising the new possibilities that clinical diagnosis and treatment of high arched palate should also be reconsidered in this developmental and molecular context (73).

*FGF8 subfamily members and agnathia.* Agnathia is a malformation characterized by agenesis of the mandible. As an isolated abnormality, agnathia is rare, with an estimated incidence of 1 in 70,000 newborns. The abnormality includes ventral microstomia, malpositioning of the external ears and persistence of the buccopharyngeal membrane. These conditions are usually lethal, as the airway cannot be established (34). Mechanistic studies suggest a role of BMP and FGF8 in BA1 patterning, including mandibular development (76,77).

It has been identified that ectodermal FGF8 expression could be either activated or repressed by BMP4 in a dose-dependent fashion, and that high BMP4 levels repress FGF8, while low signaling levels promote FGF8 transcription. Differential effects of BMP4 on FGF8 expression are also observed in the proximal and distal mandible (77,78). FGF8 is known to be expressed in the epithelium of the proximal region and BMP4 in the distal region (78). In the distal mandibular ectoderm, the absence of BMP4 antagonists, chordin and noggin, results in high levels of BMP4, strongly downregulating FGF8 expression and increasing cell death during mandibular outgrowth, thus leading to mandibular hypoplasia (76). On the other hand, a low level of BMP4 is needed to maintain FGF8 expression, which is required for proximal mandible formation. Thus, this concentration and position-dependent relationship between BMP4 and FGF8 is necessary for appropriate proximodistal patterning of the mandible (34).

*FGF8 and odontogenic tumors (OTs).* OTs are a special category of neoplasms discovered exclusively in the jawbones or related soft tissues, stemming from tooth-forming apparatus or their remnants (79). OTs include solid multicystic ameloblastoma (SMA) from epithelial origin, ameloblastic fibroma (AF) from mixed origin, and odontogenic myxoma (OM) from ecto-mesenchymal origin (80).

The pathogenesis of OTs is a complex process, where certain steps are similar to the odontogenic processes. Expression of either tumor initiating factors or tumor progression factors in OTs exhibits a striking resemblance to those expressed during odontogenesis (79). Since FGF8 plays a major role in odontogenesis, it is believed that FGF8 may affect OT development.

FGF8 has been identified to be expressed in both epithelial and mesenchymal tumor tissues to varying degrees, suggesting that it is involved in epithelial and mesenchymal interactions (81). FGF8 is expressed in different odontogenic tumors with a

distinct intensity according to the tumor's properties. Higher expression of FGF in invasive types may suggest a resemblance to proliferative stages of odontogenesis. However, in milder cases, a lower level of expression is observed, which may be parallel with different stages of odontogenesis (36). Swarup *et al* (36) analyzed the expression of FGF8 in OTs and tooth development. Dental organs of various odontogenic stages and 30 OTs, including 10 cases of SMA, 10 cases of AF and 10 cases of OM, were evaluated in both mesenchymal and epithelial tissues by immunohistochemistry. Among all OTs, the epithelial tissue of SMA exhibited the strongest signal for FGF8, whereas the mesenchymal FGF8 signal was highest in OM. However, the overall reactivity for FGF8 in AF was low. Therefore, increased FGF8 expression may exert a marked effect on tumor initiation and progression by inducing odontogenic epithelium changes in SMA. Upregulation of FGF8 was associated with OM and was likely to have induced an aggressive phenotype. Limited data on upregulation of FGF8 expression have been reported in myxoinflammatory fibroblastic sarcoma (82) and other tumors. Thus, FGF8 may be associated with the initiation and progression of OM, but further investigation is required.

*FGF8 subfamily members and oral squamous cell carcinoma (OSCC).* Oral cancer is one of the most common cancer types in the world. More than 90% of oral cancer cases are OSCC, which is associated with severe deformity and high mortality due to poor prognosis and strong potential for metastasis. Despite improvements in treatment, the clinical outcome remains unacceptable, with a 5-year survival rate of approximately 60% over the last decade (83,84).

Low-density lipoprotein receptor-related protein 6 (LRP6) is reported to be an essential Wnt coreceptor for activating the canonical Wnt/ $\beta$ -catenin signaling pathway (85), and FGF8 is a potential downstream gene of the Wnt pathway (86). It was previously demonstrated that FGF8 is present in an LRP6-related protein-protein interaction network by bioinformatics analyses, and that FGF8 expression exerts a positive synergistic effect with its upstream oncogene, LRP6, in OSCC (84).

FGF8 is required for LRP6-induced proliferation in OSCC cell lines (84,87-89). Knockdown of FGF8 by short interfering RNAs significantly inhibits endogenous FGF8 expression, and subsequently abolishes LRP6-induced cell proliferation in OSCC cell lines. Additionally, using FGF8 immunostaining methods, the immunostaining signals of FGF8 are paralleled by LRP6 in corresponding OSCC tissue slides. Furthermore, co-immunofluorescent staining in OSCC tissues revealed that strong FGF8 signals are frequently observed in cells with strong LRP6 signals. Collectively, this suggests that overexpression of LRP6 triggers FGF8 expression in OSCC cell lines, and the two genes act synergistically (84).

Notably, compared with LRP6 expression alone, it has been identified that concurrent expression of FGF8 and LRP6 could be a better predictor of OSCC patient outcome. Patients with high expression levels of both LRP6 and FGF8 exhibit even shorter overall survival time compared with patients with high LRP6 or FGF8 expression alone. Similarly, concurrently low LRP6 and FGF8 expression is associated with a better survival rate compared with low LRP6 or FGF8 expression alone (84). A similar association between simultaneous expression of

LRP6/FGF8 and patient outcome has also been identified in tongue cancer (90).

*Genome-editing technology in FGF8-associated diseases.* The CRISPR-Cas9 system, as the most effective genome-editing tool, has been successfully applied in diverse organisms (91). Disease-causing mutations in FGF8 subfamily members introduced by a CRISPR-Cas9 system have been reported in a lamprey model. CRISPR/Cas9-mediated disruption of FGF8/17/18 in the sea lamprey (*Petromyzon marinus*) produced mutant F0 embryos, with most of the injected embryos developing into complete or partial mutants (92). The ability of the CRISPR-Cas9 system to create large numbers of mutant embryos without inbred lines not only provides new possibilities for studying development in model organisms with life histories that prohibit the generation of mutant lines, but also suggests a potential application in treating human diseases. However, biosecurity risks of this technology, including off-target effects and other unknown side effects, must be carefully estimated before clinical usage. The question thus remains whether CRISPR/Cas9 approaches can be used as a 'silver bullet' solution for the management of FGF8-associated oral diseases (93).

## 6. Conclusions

In recent decades, a large number of studies have described the relevance of the FGF8 subfamily in human embryonic development. Aberrant FGF signaling transduction, caused by activating mutations, increased expression or abnormal isoform splicing is observed in the development of oral-maxillofacial diseases. Abnormal regulation of the FGF8 signaling pathway has also been implicated in the development of oral cancer. Targeting FGF8 subfamily members may provide a novel and promising strategy for the treatment of oral diseases. Further work is still required to illustrate the detailed mechanisms underlying the roles of the FGF8 subfamily in the development of oral disease.

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## Availability of data and materials

Not applicable.

## Authors' contributions

RL and QC contributed to the conception of the study. YH wrote the main part of the manuscript. All authors read and

approved the final manuscript. YY and ST helped perform the analysis with constructive discussion.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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