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Mini Review

FGF Signaling: Diverse Roles During Cochlear Development

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Abstract

Mammalian inner ear comprises of six sensory organs; cochlea, utricle, saccule, and three semicircular canals. The cochlea contains sensory epithelium known as the organ of Corti which senses sound through mechanosensory hair cells. Mammalian inner ear undergoes series of morphogenesis during development by beginning thickening of ectoderm nearby hindbrain. These events require tight regulation of multiple signaling cascades including FGF, Wnt, Notch and Bmp signaling. In this review, we will discuss the role of newly emerging signaling, FGF signaling, for its roles required for cochlear development.

Introduction

Mammalian ear is composed of the outer, middle and inner ear. Sound generated from outside gathers in the outer ear, travels through the middle ear and transfers to the inner ear. The sensory hair cells in the inner ear then convert mechanical sound vibration to electrical signals and transmit those signals to the brain (1). Inner ear development comprises a series of morphogenic events that are orchestrated by a cascade of tightly regulated molecular cascades (2). During inner ear development, many morphogens play important roles. Fibroblast Growth Factor (FGF) is one of them. FGF signaling plays multiple roles during inner ear development starting as early as embryonic day (E) 8-9 in mouse, when three FGF ligands (FGF3, FGF8 and FGF10) signal to FGFR2 within the otic epithelium for otic placode induction (3). In addition, recent publications indicate the emerging role of FGF signaling during cochlear sensory progenitor proliferation and differentiation (4-7). In this review, we will focus on the various roles of FGF signaling in cochlear development that are identified up-to-date including sensory progenitor proliferation, lateral compartment differentiation, pillar cell differentiation and non-sensory structures development.

FGF signaling

FGF signaling has been implicated in development, metabolism and disease. During vertebrate development, FGFs are widely expressed and regulate multiple diverse processes. FGF family is a group of structurally related polypeptide growth factors. FGFs are typically small ranging about 17-35 kD, secreted, and highly basic protein (8). In mammalian system, there are 22 members of the FGFs that are classified into 7 subfamilies based on their sequence homology and biochemical properties (9). The canonical FGFs are secreted from the cells and bind to

cognate receptors along with heparan sulfate (8, 10). Due to the binding affinity with heparan sulfate, these FGFs function as paracrine or autocrine (11). The hormonal FGFs require novel cofactors, klotho and β-Klotho to bind to their cognate receptors due to lack of binding affinity with heparin sulfate (12, 13). The intracellular FGFs serve as co-factors for voltage gated sodium channels and other molecules (14, 15).

To activate canonical FGF signaling, FGFs bind to their cognate receptors, FGF receptors (FGFR) (8). Binding of FGFs to FGFRs results in receptor dimerization (16, 17). This process is enhanced by heparan sulfate, which forms a tri-molecular complex containing FGF, FGFR and heparin sulfate (8, 18). After dimerization, FGFR auto-phosphorylates to activate itself and phosphorylates intracellular adaptor molecules. FGF/FGFR signaling activates three major downstream signaling pathways: mitogen activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K)/Akt and the phospholipase c-γ (PLC-γ) pathway (19). The most common pathway employed by FGFs is the MAPK pathway. This involves the lipid-anchored docking protein FGFR substrate 2 (FRS2) (20, 21). The two tyrosine auto-phosphorylation sites (Y-653 and Y-654) conduct binding of FRS2 to FGFR. The adaptor protein Grb2 and the protein tyrosine phosphatase Shp2 recognize the FRS2 tyrosine phosphorylation sites and bind (22, 23). Grb2 forms a complex with the guanine nucleotide exchange factor Son of sevenless (SOS) via its SH3 domain (22, 23). Translocation of this complex to the plasma membrane by binding to phosphorylated FRS2 allows SOS to activate Ras by GTP exchange due to its proximity to membrane-bound Ras (21, 24). Once in the active GTP-bound state, Ras interacts with several effector proteins including Raf leading to the activation of the MAPK signaling cascade (21, 24). This cascade leads to phosphorylation of target transcription factors, such as Etv4 and Etv5 (25).

Canonical FGFs are divided by 5 subfamilies and recognize specific cognate receptor isotypes. There are 4 members of FGF receptors in vertebrates and produce many variants using mRNA alternative splicing (26, 27). Among them two major isoforms, the tissue-specific alternative splicing (b and c isoforms) in immunoglobulin (Ig) domain III of FGFRs, have distinct FGFR-binding properties indicating the complexity of FGF signaling (28-33).

The specificity of FGFs for the major splice forms of the FGFRs is critical for both the developmental and pathogenic functions of FGFs. One important observation is that distinct FGFs signal across epithelial-mesenchymal boundaries. For example, in lung development, *Fgf9* is expressed in the outermost layer of the lung, the mesothelium, and has been identified as a key factor that signals to mesenchyme to regulate proliferation, differentiation and the expression of other factors that in turn regulate epithelial development (34, 35). Mesenchymal forms of FGFR1 and 2 have been shown to mediate the FGF9 signal (36). In contrast, *Fgf10* is expressed in mesenchymal cells surrounding the distal tip of the growing epithelium (37). When FGF10 is locally applied to lung explants on beads, lung epithelium expanded toward the source of FGF10 (37, 38). *Fgf10* mutant embryos show lung agenesis due to defects in lung epithelia, indicating that *Fgf10* is an essential regulator of branching morphogenesis (39-41). FGF10 functions through epithelium specific FGFR2, which is also critical for lung branching morphogenesis (41, 42).

In this review, we will overview the role of FGFs and FGFRs during inner ear development and focus on recently emerged role of FGF signaling in cochlear sensory progenitor cells. **Table 1** summarizes phenotypes of inner ear development from the FGFs and FGFRs knock-out mouse lines.

Inner ear development

In vertebrates, the inner ear is comprised of two main functional parts: the cochlea that is responsible for sound detection, and the vestibular system that is dedicated to balance. The development of inner ear involves dramatic morphogenic and patterning events that convert simple thickened epithelium to a complex structure connected to the central nervous system. In mice, the inner ear develops from a bilateral thickening (otic placode) within the ectoderm located adjacent to the hindbrain around E8.5 (43). Induction of otic placode from competent pre-placodal ectoderm has been shown to be mediated by FGF signaling where three FGF ligands (FGF3,8 and 10) signal to FGFR2 within the otic epithelium for otic placode induction (3, 44). One day later, placodal cells invaginate and separate from the surface ectoderm giving rise to the otocyst (43). All cells within the membranous portion of the inner ear are derived from the multipotent progenitor cells initially located within the otocyst (2). Around E10.5, a population of cells delaminates from the ventral region of the otocyst and migrates a short distance ventro-medially. These cells are neuroblasts that will coalesce to form the developing stato-acoustic ganglion (VIII cranial ganglion). Following this event, the spheroidal otocyst undergoes an elaborate series of morphogenic changes resulting in the formation of two main structures: the dorsal vestibular and the ventral cochlear regions (43). During otocyst development, gradients of sonic hedgehog (Shh) and Wnt signaling function to establish positional information across the dorso-ventral axis to confer vestibular and cochlear identities (45, 46).

As the cochlear duct extends and coils, a subset of cells within its ventral aspect begins to develop as the prosensory epithelium (prosensory domain), and these cells become localized to a restricted region of the developing cochlea including a narrow strip that extends along the length

of the cochlear duct (43). Some markers of the prosensory domain include the Jag1 (a Notch ligand), and Sox2 transcription factor (47, 48). Cells within the prosensory domain will give rise to both hair cells and supporting cells within the organ of Corti. In mouse, the prosensory domain cells begin to exit the cell cycle starting from the apex around E12, and a wave of cell cycle exit marks with the expression of $p27^{Kip1}$ then proceeds along the prosensory domain from the apex to the base over the following 48-60 hours (49). Starting at about E13.5, cells in the mid-basal region of the cochlea begin to differentiate to hair cells by expressing the key transcription factor Atoh1, and the region of differentiating cells spreads bidirectionally over the following three days (50). Over the next two weeks, hair cells undergo morphological and biochemical specialization, including the elaboration and polarization of the apical hair bundle stereocilia, the development of mechanosensitivity in hair bundles and the formation of the basal ribbon synapses with neurons of the spiral ganglion (51).

By P0, cochlear sensory epithelium is composed of two types of cells; hair cells and supporting cells. Hair cells are arranged in ordered rows extending the length of the spiral cochlear duct. One row of inner hair cells is located on the medial edge of organ of Corti while three rows of outer hair cells on the lateral edge (43). Supporting cells rest on the basement membrane and send apical projections to the luminal surface. At least five different types of supporting cells are arranged in rows from the outer edge to the inner edge of the organ: Hensen's cells, Deiters' cells, pillar cells; inner phalangeal cells; and border cells. (2).

Cochlear Sensory progenitor cell proliferation

Cochlear sensory progenitor cells start to proliferate beginning E11 (52). Otocysts explanted to ectopic locations *in vivo* and *in vitro* do not develop normally unless some periotic mesenchymal tissues are included in the transplant (53, 54). This indicates that factors in mesenchyme are

required for cochlear growth. We recently identified that FGF9 and FGF20 are expressed at the non-sensory and sensory epithelium of otic vesicle, respectively and send signal to nearby mesenchymal FGFR1 and FGFR2 to promote cochlear sensory progenitor proliferation and subsequent cochlear growth (5). The expression patterns of both *Fgf9* and *Fgf20* and their potential receptors have been previously studied in the developing cochlea and the surrounding mesenchyme. *Fgf9* mRNA is first detected in the otic vesicle epithelium as early as E10.5, specifically in the non-sensory epithelium of the developing cochlear duct in addition to the vestibular components of the inner ear (34). A later study confirmed such expression pattern and showed that *Fgf9* continues to be expressed in the ventral wall of the developing cochlear duct that will give rise to the Reissner's membrane (55). We utilized an *Fgf9-βGal* reporter allele to monitor *Fgf9* expression using βGal activity and antibody analyses showing that there is no overlap between FGF9 and Sox2 expression domains at E11.5 consistent with previously mentioned findings that *Fgf9* is expressed in non-sensory epithelium (56).

As for *Fgf20,* utilizing an *Fgf20-βGal* reporter allele, βGal activity is detected in the anterio-ventral region of the otic vesicle as early as E10.5. Around E11.5, Fgf20-βGal is expressed within the domain of Sox2-positive sensory progenitor cells (4). *In situ* hybridization for *Fgf20* mRNA showed a wave pattern of expression that spreads from the base (around E13.5) to reach the apex (around E16.5) and then declines in a similar pattern (57). Such expression overlapped with Sox2-expressing sensory epithelium (48). Since both FGF9 and FGF20 belong to the same subfamily which shares the same FGFRs, and the timing of expression within the cochlear epithelium overlaps, they might have functionally redundant roles.

By studying various compound mutants of *Fgf9* and *Fgf20,* we now have better understanding of the redundant role of these FGFs in regulating cochlear progenitor cell proliferation. The

cochlear length of the double mutant is reduced more than half compared to double heterozygous controls (56). Furthermore, double mutant cochlea is significantly shorter than that of the single *Fgf9* or *Fgf20* mutants. Studying the size of the prosensory domain in these mouse models reveals decreased size in double mutant. Quantitation of cell proliferation using phosphor-Histone 3 and EdU labeling of E11.5 embryos shows significant reduction in progenitor cell proliferation (56). Such findings indicate a redundant role of *Fgf9* and *Fgf20* in regulating cochlear length.

Analysis of *Fgf9* mutant mice showed normal length of cochlear duct and normal developing sensory epithelium (55). On the other hand, *Fgf20* mutant mice exhibited a cochlear phenotype with a specific deficiency in the formation of outer hair cells and outer supporting cells, which we will discuss later, in addition to a small but significant (10%) decrease in cochlear length when compared to heterozygote controls (4). Deleting either *Fgf9* or *Fgf20* alone does not cause a proliferation defect, yet lack of both had a significant impact on progenitor proliferation and subsequently cochlear duct length.

For *Fgf9* and *Fgf20* to function, they require FGFR interaction. Expression of both *Fgfr1* and *Fgfr2* have been reported in the periotic mesenchyme of the ventral (cochlear) part of the inner ear between E10.5 and E12.5 (55). Mesenchymal FGFR signaling is shown to be both necessary and sufficient for sensory progenitor cell proliferation (56). FGFR1 and FGFR2 shows another evidence of gene redundancy. Deleting either *Fgfr1* or *Fgfr2* in mesenchymal cells using $Twist2^{Cre}$ results in small but significant decrease of cochlear length compared to control, 7% and 20%, respectively (56). Deletion of both *Fgfr1* and *Fgfr2* from mesenchymal cells reveals shortening of the cochlea by 55% compared to control at E18.5, which is comparable to the loss of *Fgf9* and *Fgf20* (56). The size and the proliferation of the Sox2-positive progenitor domain is

significantly decreased at E12.5 indicating the necessity of mesenchymal *Fgfr1* and *Fgfr2* for sensory progenitor proliferation. Gain-of-function experiment expressing a constitutive FGFR1 tyrosine kinase domain in mesenchymal cells results in increased proliferation of sensory progenitors after induction between E10.5 and E12.5 and a 14% increase in cochlear length upon induction from E10.5 to E14.5. Such results indicate the sufficiency of mesenchymal FGFR signaling for progenitor cell proliferation (56). Taken together, FGF9 and FGF20 redundantly signal to mesenchymal FGFR1 and FGFR2 to regulate auditory sensory progenitor cell proliferation in the developing cochlea. To date, the molecular pathways downstream FGF epithelial-mesenchymal interaction for sensory progenitor cell proliferation are not understood.

Cochlear lateral compartment differentiation

Several independent lines of research suggest that FGF20 signals to FGFR1 within the developing sensory epithelium between E13.5 and E14.5 to regulate the differentiation of outer hair cells and supporting cells (4, 56-59). As mentioned previously, *Fgf20* expression overlaps with Sox2-positive domain within the developing cochlea between E13.5 and 16.5 (57). *Fgfr1* mRNA is detected in the ventral wall of the developing cochlear duct which comprises the future prosensory domain at E11.5 (58). This expression level increases over the following few days and becomes concentrated into a cluster of cells at the lateral aspect of the prosensory domain in the region of the developing outer hair cells around E14-15 (60) then starts to decrease concomitant to cellular maturation around E18 (58). Comparing the expression patterns of *Fgf20* and *Fgfr1*, it is obvious that they are close within the prosensory domain around the time of hair cell differentiation.

Due to early developmental lethality in *Fgfr1* null mutants (61, 62), *Fgfr1* hypomorph or

conditional knockouts were previously utilized as a loss-of-function models. Study of cochleae of hypomorphic *Fgfr1* alleles revealed a phenotype ranging from missing third row of outer hair cells throughout the length of cochlear duct to interrupted sensory epithelium with gaps showing no signs of differentiation of hair cells or supporting cells which was more evident towards the apex of the cochlea (58). Conditional deletion of *Fgfr1* in the sensory epithelium resulted in a similar yet more severe phenotype compared to hypomorph. Different Cre drivers were used to regionally inactivate *Fgfr1* in the epithelium of the developing inner ear at different time points; whole otic epithelium at E9.5 using $Foxg1^{cre}$ (56, 58), or $Six1enh21^{cre}$ (59), sensory progenitor population at E10.5 using $Fgf20^{cre}$ (56) and cochlear duct epithelium around E12.5 using $Emx2^{cre}$ (59). A consistent phenotype observed in all these mouse models is the perturbed organ of Corti with patches of sensory epithelium mainly comprised of inner hair cells and supporting cells separated by gaps of undifferentiated cells. Such phenotype is more severe in the apical half than the basal half of the cochlea. The number of differentiated outer hair cells is significantly reduced compared to control (56, 58, 59). Furthermore, in vitro experiments treating cochlear explant cultures from E14 to E16 with a small molecule inhibitor of FGFRs (SU5402) photocopies the phenotype observed in *Fgfr1* conditional knockout experiments with a significant reduction in the number of outer hair cells and supporting cells (57). It is concluded that FGFR1 signaling is required for the differentiation of outer hair cells and supporting cells during cochlear development.

Analyses of the cochlear length, progenitor cell proliferation and prosensory domain size in *Fgfr1* conditional knock out mice exhibit variability across studies. Pirvola *et* al. reported a reduction in the proliferation rate in the ventral wall of the cochlear duct between E12 to E15.5 and a slight decrease in cochlear length at birth in $Fgfr1^{f/f}$:: $Foxg1^{Cre}$ (58). Ono et al. reported a

41–49% decrease in cochlear length in *Fgfr1*$^{f/f}$*:: Six1enh21*^{Cre}, and *Fgfr1*$^{f/f}$*::Emx2*^{Cre} conditional knockout mice at E18.5 (59). The number of proliferating cells within the developing cochlea was reduced in *Fgfr1*$^{f/f}$*:: Six1enh21*^{Cre} but not in *Fgfr1*$^{f/f}$*::Emx2*^{Cre} at E12.5 (59). We reported a 9% decrease in cochlear length in *Fgfr1*$^{-/f}$*::Foxg1*$^{Cre/+}$ and a 19% decrease of cochlear length in *Fgfr1*$^{-/f}$*::Fgf20*$^{Cre/+}$ mice at E18.5 that were not associated with a proliferation defect at E12.5 (56). The size of the Sox2-positive sensory progenitor domain in both mouse models was not affected at E14.5. Such variability across studies could be attributed to mouse models with different backgrounds, different timing of inactivation of *Fgfr1* or different methods of analyses.

FGF20 has been suggested by multiple studies to be the ligand for FGFR1 that is responsible for outer hair cell and supporting cell differentiation (4, 57). Both *in vivo* and *in vitro Fgf20* loss of function studies have revealed such role in cochlear development. *In vitro* studies culturing cochlear explants at E12-13 then treating cultures with antibody against FGF20 using varying concentrations lead to significant dose-dependent reduction of hair cell and supporting cell numbers. Such effect is rescued by the addition of recombinant FGF20 protein (57). Although the effect of *Fgf20* loss of function is evident on hair cell development, the study didn't show whether this effect is specific to outer hair cells or not. A more recent study utilizing an *in vivo* model shows that at P0, the cochlea of *Fgf20* mutants exhibit a similar phenotype as in *Fgfr1* deletion (4), where the basal region contains only two rows of outer hair cells, and the middle and apical region showed patches of hair cells. Specifically, outer hair cell number is significantly reduced in *Fgf20* mutant cochlea at P4 while inner hair cell number is not affected. Along with the reduction of outer hair cell number, this model also shows a significant reduction in the number of Deiters cells and outer pillar cells (outer supporting cells). In this model, cochleae exhibit normal cell proliferation within the prosensory domain indicating that the

reduced number of outer hair cells is due to a differentiation defect rather than a proliferation defect. Treating E13.5-14.5 *Fgf20* mutant cochlear explants with exogenous FGF9 (has same biochemical activity as FGF20 *in vitro*) shows rescue of cochlear phenotype (4). Altogether, there is much evidence that FGF20/FGFR1 signaling is required for lateral compartment differentiation.

Pillar cell differentiation

During post-mitotic stages (around E16.5), FGF8 signals from the inner hair cells to FGFR3 in supporting cells to regulate pillar cell differentiation (63-65). The expression of *Fgfr3* is detected around E16 in a band of cells extending along the length of the cochlear duct in the region that will develop as the pillar cells, outer hair cells, and Deiters cells. Such expression become restricted to pillar cells by P0 (64, 66). *Fgf8* is exclusively expressed in inner hair cells and starts as early as E16 within the basal turn of the cochlea. Such close expression pattern for *Fgf8* and *Fgfr3* triggered studies to investigate the potential ligand-receptor interaction in pillar cell development. Deletion of *Fgfr3* leads to defects in pillar cell development. In *Fgfr3* mutants, two rows of undifferentiated cells are observed in the region between the row of inner hair cells and the first row of outer hair cells that corresponds to the region of pillar cells (63). As in *Fgfr3* mutant mice, pillar cell development is disrupted in E13.5-14.5 cochlear explants exposed to the FGFR inhibitor SU5402 for 18 hours in vitro (64). Gain-of-function experiment treating cochlear explants with FGF2 (a ligand for FGFR3) led to a dose-dependent increase in the number of cells that developed as pillar cells (64), suggesting that FGFR3 activation is sufficient to commit progenitor cells to pillar cell fate. Research demonstrates that FGF8 expressed by the inner hair cells signals to the FGFR3 in the sensory epithelium and regulates the development of pillar cells. Loss-of-function experiments either through deletion of *Fgf8* or inhibition of binding

between *Fgf8* and *Fgfr3* leads to defects in pillar cells development, whereas overexpression of FGF8 or exogenous FGFR3 activation by adding FGF17 induces ectopic pillar cell formation at the expense of outer hair cell development (65).

In humans, a gain-of-function mutation in *FGFR3* causes Muenke syndrome which comprises hearing loss. The mutation is within the extracellular domain of *FGFR3* and increases its binding affinity for certain FGFs. Mouse model of the Muenke syndrome exhibits transformation of two rows of Deiters cells to two rows of pillar cells yielding to four rows of pillar cells and one row of Deiters cells (67). A recent study investigating the underlying molecular mechanisms of this transformation found that such switch occurs sequentially between E17.5 and P3. Surprisingly, supporting cell fate transformation was not rescued by reducing *Fgf8*. Instead, *Fgf10* inhibition was sufficient for rescue of cochlear structure and function. It is concluded that the Muenke syndrome mutation changes the specificity of FGFR3b and FGFR3c such that both acquire responsiveness to FGF10 that normally binds to FGFR1b and FGFR2b (7).

Non-sensory domain development

Reciprocal epithelium-mesenchyme signaling is a fundamental process for the morphogenesis of multiple organs. In inner ear, FGF9 expressed in non-sensory domain of otic epithelium signals to the FGFR1 and FGFR2 (IIIc spliceform) in the surrounding mesenchyme between E11.5 and E13.5 to regulate mesenchymal cell proliferation and subsequent condensation (55). Inner ears of *Fgf9* null mice show hypoplastic vestibular component of the otic capsule and absent semicircular ducts mainly due to reduced proliferation of the prechondrogenic mesenchyme causing capsular hypoplasticity (55). Although the length of the cochlear duct and the structure of cochlear sensory epithelium was not affected in *Fgf9* null mutants at birth, cochlear mesenchyme shows failure in the development of scala vestibule (55). It is concluded that

epithelial FGF9 signaling is required for stimulating growth and remodeling of the otic mesenchyme.

A recent study suggests that FGF10 expressed in otic epithelium signals to FGFR2b expressed in adjacent epithelial regions to induce Reissner's membrane and outer sulcus development between E12.5- E15.5 (68). *Fgf10* is expressed in a sensory- competent domain early in cochlear development, eventually becoming restricted to Kolliker's organ (44), while *Fgfr2b* is expressed in the entire non-sensory domain of the otic epithelium, including the prospective Reissner's membrane, stria vascularis and outer sulcus (68). Examining the cochlea of *Fgf10* mutants at E18.5, cochlear ducts appeared to lack Reissner's membrane and most of the outer sulcus. Immunostaining for specific markers for these two regions confirmed the phenotype. Since FGFR2b receptors are expressed in these two regions, it is suggested that FGF10 signal to FGFR2b receptors to induce these two non-sensory domains, Reissner's membrane and the outer sulcus.

In human, heterozygous mutations in *FGF10* cause LADD (lacrimo-auriculo-dento-digital) syndrome, and 55% of LADD syndrome subjects has hearing loss (69). LADD syndrome is caused by heterozygous mutations in *FGFR2* and *FGFR3* that reduce FGF signaling activity.

Conclusion and future perspective

It is quite evident that FGF signaling plays diverse roles during cochlear development in a context-dependent manner. Different members of the FGF family function either individually or redundantly to regulate progenitor cell number, mediate sensory epithelial patterning, and induce specification and differentiation of different cell populations within the developing cochlea. Developmental defects result from aberrant activity of FGF signaling pathway confirm the

importance of such pathway during different stages of cochlear development. However, there is a few discrepancies among published data that need to be addressed.

The phenotype severity of loss of *Fgf20 in vivo* is less than that observed upon treating cochlear explants with FGF20 antibody that exhibit more hair cell loss (4, 57). Possible explanations would be 1) different genetic background, 2) artifact of the explant, or 3) existence of other FGF functioning with FGF20 to promote lateral compartment differentiation. Our recent experiments show that treatment of *Fgf20* null cochlear explant with FGFR inhibitor decreases number of hair cells compared to *Fgf20* null untreated explant (unpublished). This suggests that there might be other FGF functioning with fGF20 to promote cochlear lateral compartment differentiation.

Another unsolved question is the splice variant of FGFR1 to which FGF20 binds for regulating lateral compartment differentiation. FGF20 belongs to FGF9 subfamily that comprises FGF9, FGF16 and FGF20. They bind to c splice variants of FGFR1, FGFR2, and both b and c splice variant of FGFR3 (Zhang et al, 2006). The phenotype of *Fgf20* mutant mice recapitulates epithelial *Fgfr1* deletion mutants (Privola et al, 2002), yet *Fgfr1b* mutant mice are viable and do not have gross defects (70). In addition, number of hair cells from *Fgfr1b* mutant cochlea is comparable to control (unpublished). This raises possibility that FGFR1C might be expressed in the cochlear sensory epithelium and receives signal from FGF20 to regulate lateral compartment differentiation. Indeed, FGFR2C is expressed in developing lung epithelium and receive signals from FGF9 (71). Therefore, further study is required to better understand the mechanism by which FGF20 regulates cochlear sensory differentiation.

Given the different roles FGF signaling plays during cochlear sensory epithelial development, it is a potential pathway that can be manipulated for hair cell regeneration. Evidence of FGF signaling role in regeneration comes from chicken and zebrafish models that are capable of

spontaneously regenerating their sensory epithelium. Transcriptional profiling of regenerating chicken cochlear sensory epithelia after ototoxic injury revealed that *Fgf20* expression is low initially during the proliferative phase of regenerating supporting cells, then increase later when proliferation stops (72). Such finding indicates a role of FGF20 in the differentiation of proliferating supporting cells during hair cell regeneration, which is comparable to the role of FGF20 in lateral compartment differentiation during normal development. For mammalian hair cell regeneration, a possible way to utilize FGF20 might be first inducing proliferation of supporting cells using Wnt signaling then applying FGF20 to guide hair cell differentiation in cochlear explants with hair cell insult.

Since the mammalian cochlea lacks the capability to regenerate hair cells after damage, the ultimate goal for understanding the role of FGF signaling as well as other signaling pathways is to manipulate these pathways to induce hair cell regeneration. A learned lesson from the diverse roles of FGF signaling is that the context is the main determinant of FGF signaling function. Such concept must be taken into consideration for utilizing this signaling pathway to induce hair cell regeneration.

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Figure legend

Figure 1. A schematic model showing diverse roles of FGF signaling in cochlear development. In the E11.5 cochlea, FGF9 expressed in non-sensory epithelium and FGF20 in sensory epithelium signal to FGFR1 and FGFR2 within the surrounding mesenchyme to regulate cochlear progenitor proliferation. Around E14.5-E15.5, FGF20 expressed within the Sox2 prosensory domain signal to FGFR1 expressed in the lateral edge of the prosensory domain to regulate the differentiation of outer hair cells and supporting cells. Around E16.5, FGF8 expressed by inner hair cells signal to FGFR3 in outer hair cells and supporting cells to regulate the pillar cell differentiation.

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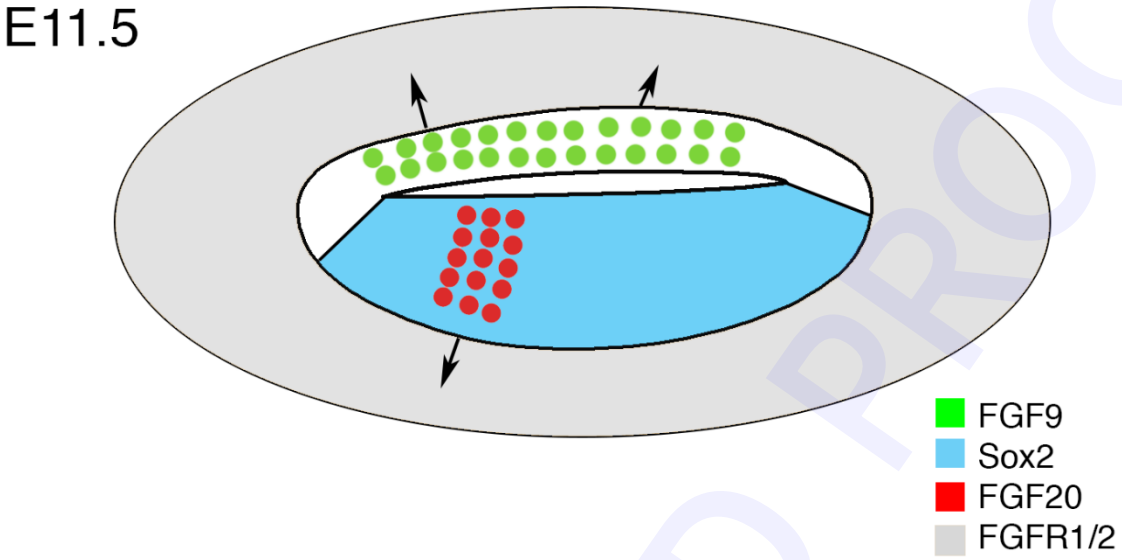

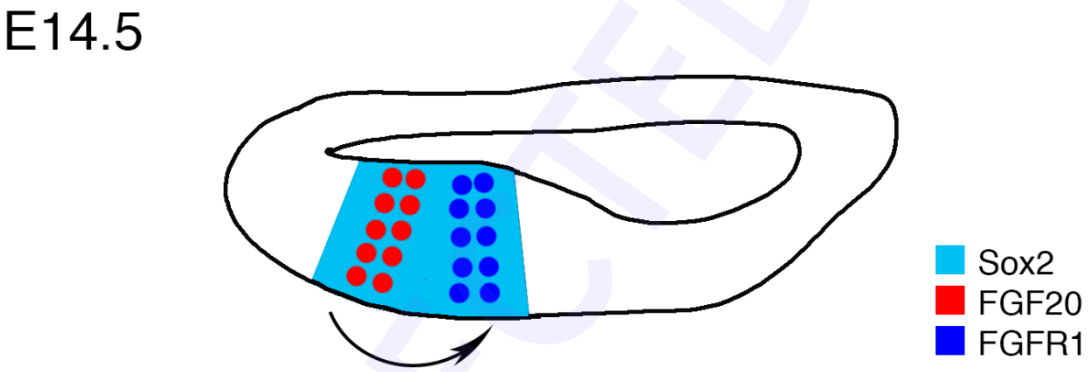

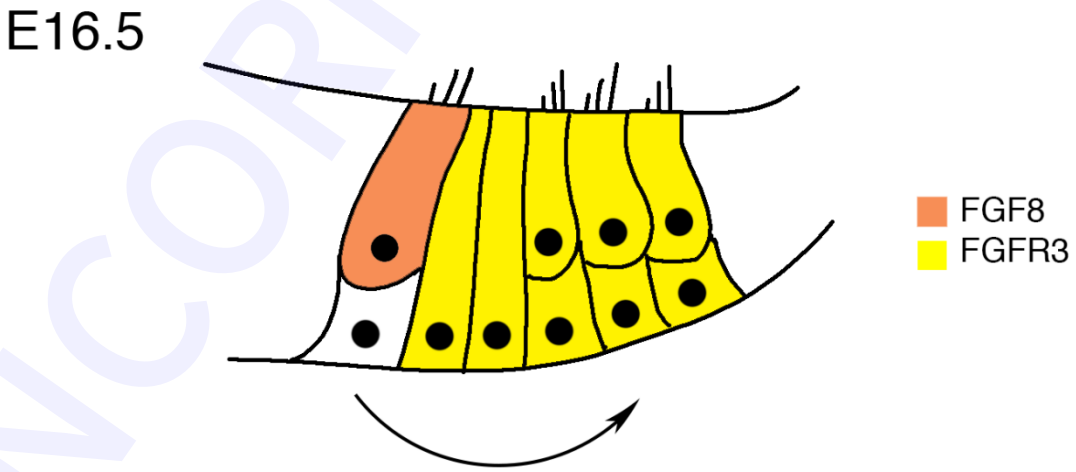

Table 1. phenotypes of FGF mutation in mouse

Gene	Type of mutation	Phenotype	Ref
Fgf3	Double conventional mutation with *Fgf10*	Failure of otic vesicle formation	(73-75)
Fgf8	Conditional mutation with *Foxg1^{Cre}*	Decrease of pillar cells	(65)
Fgf9	Conventional mutation	Decrease of periotic mesenchyme proliferation	(55)
	Double conventional mutation with *Fgf20*	Decrease of cochlear sensory progenitor proliferation	(56)
Fgf10	Conventional mutation	Agenesis of posterior vestibular tissue	(68, 76, 77)
	Double conventional mutation with *Fgf3*	Failure of otic vesicle formation	(73-75)
Fgf20	Conventional mutation	Decrease of cochlear lateral compartment differentiation	(4)
	Double conventional mutation with *Fgf9*	Decrease of cochlear sensory progenitor proliferation	(56)
Fgfr1	Hypomorph, Conditional mutation with *Foxg1^{Cre}*, *Six1enh21^{Cre}*, *Emx2^{Cre}* and *Fgf20^{Cre}*	Decrease of cochlear lateral compartment differentiation	(56, 58, 59)
	Double conditional deletion with *Fgfr2* with *Twist2^{Cre}*	Decrease of sensory progenitor proliferation	(56)
Fgfr2	IIIC isoform specific mutation	Failure otocyst morphogenesis	(78)
	Double conditional deletion with *Fgfr1* with *Twist2^{Cre}*	Decrease of sensory progenitor proliferation	(56)
Fgfr3	Conventional mutation	Loss of pillar cell and increase of outer hair cells	(63, 79)