

Inductive and Morphogenetic Responses of the Inner Ear to FGF Signaling

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Rec date: May 23, 2015, Acc date: May 23, 2015, Pub date: May 25, 2015

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Editorial

Inner ear development entails a series of sequential inductive tissue interactions where one tissue signals to direct the development of adjacent tissue[s]. Inductive signals from mesodermal, endodermal, and neuroectodermal origin control multiple aspects of early inner ear formation. Signals arising from the endoderm and mesoderm that underlie the presumptive otic field in the cephalic surface ectoderm control induction of the otic placode from this region [1,2], whereas signals emanating from the neuroepithelium of the hindbrain direct invagination of the otic placode to form the otic vesicle. Subsequent morphogenesis of the otic vesicle into the elaborate three-dimensional inner ear occurs in response to yet another set of inductive interactions, this time between the epithelium of the otic vesicle, from which the cochlear and vestibular anlagen form, and the surrounding periotic mesenchyme. Thus all tissue layers in and around the otic region are involved in early inner ear development, and the likely molecular signals leading to these interactions are discussed below.

Several members of the fibroblast growth factor (FGF) family from various tissue sources act together to direct different stages of otic vesicle formation [3]. The expression pattern of Fgf3 in the hindbrain adjacent to the developing otic placode [4] originally implicated Fgf3 as a likely candidate in otic induction. Overexpression of Fgf3 in the hindbrain of chicken embryos at a timepoint coincident with invagination of the otic placode resulted in ectopic formation of otic vesicles [2], whereas siRNA blockade of Fgf3 at this developmental stage led to partially invaginated placodes or failure to close the otic pits [2]. Thus in the chicken, Fgf3 is both sufficient and necessary for invagination of the otic placode to form the otic vesicle.

However rather than there being any particular “master regulator” of early inner ear development, it has become clear that several FGFs are redundantly required to direct proper formation of the inner ear. Expression patterns of Fgf3 and other FGF ligands (or Fgf genes) partially overlap during inner ear formation, underscoring the importance of defining compensatory relationships between FGFs during this process. Besides FGF3, detection of Fgf10 expression in the hindbrain neuroepithelium coincides both temporally and spatially with development of the otic placode and vesicle [3], supporting its function as an inductive hindbrain-derived signal. Overexpression of Fgf10 in the anterior hindbrain of the mouse leads to the formation of ectopic vesicles that express markers confirming an early otic character, although the complete repertoire of otic marker expression was not obtained [3]. Overexpression of Fgf3 shows a more limited capacity to act as a hindbrain-derived inductive signal. Interestingly, mice homozygous for null mutation of either the Fgf3 [5] or Fgf10 [6] genes revealed unaltered induction of the otic vesicle, and development of seemingly normal albeit smaller otic vesicles. However, otic vesicle formation is severely disturbed in mouse

embryos doubly mutant for Fgf3 and Fgf10, evidenced in the most affected cases by diminished vesicle size, severe reduction or absence of otic markers, ventral shift of the otic vesicle and absence of the cochleovestibular ganglion (see below) [3]. Taken together, these findings suggest that Fgf3 is redundantly required with Fgf10 to elicit a program of early inner ear formation.

Ectopic expression of FGF10 in the hindbrain is sufficient to rescue the otic defects resulting from double mutation of Fgf3 and Fgf10 [3] and interestingly, induces expression of yet another FGF ligand, Fgf8 in the hindbrain, a site where Fgf8 is not normally expressed in the mouse [2]. During the early stages of inner ear development, Fgf8 is detected in the preplacodal surface ectoderm, underlying mesoderm, and pharyngeal endoderm [2,7,8,9,10] but is absent from the hindbrain. However, early inner ear formation is not affected in Fgf8 mutant mice [2,9], suggesting that Fgf8 is not uniquely required and may act redundantly with other FGF family members. In both zebrafish [11,12] and mouse embryos, double mutation of Fgf3 and Fgf8 during placode induction results in either absence of formation of otic vesicles with a loss of expression of otic markers or in extremely abnormal vesicles that are not able to undergo normal morphogenesis [2].

In the chick, ablation of endoderm prior to otic induction results in hypoplastic or absent otic placodes, indicating that this tissue is required for otic induction. Fgf8 is expressed in the endoderm underlying the otic-inducing mesoderm, and is both necessary and sufficient for expression of Fgf19 in the mesodermal tissue [9]. This has been demonstrated by reduced expression of Fgf19 and subsequent inhibition of otic placode formation in response to siRNA knockdown of Fgf8; further, the rescue of the otic marker Pax2 by Fgf19 in chick otic explants together supports FGF19 as a downstream otic effector of the FGF8 signal. Interestingly Fgf19, which is initially expressed in paraxial mesoderm and subsequently in the endoderm [13], induces the chick neural ectoderm to express Fgf3 as well as additional signals leading to otic induction [9]. Thus a cascade of FGF signals governs otic formation. The expression pattern of Fgf15, the murine ortholog of Fgf19, and the absence of overt otic anomalies in Fgf15 null mutant mice, however, does not support a role for Fgf15 in otic induction in this species [14].

The role of FGF2 in inner ear formation is less clearly defined. In *Xenopus* FGF2 can induce ectopic otic vesicles, however analysis of otic marker expression was limited to the Wnt3a gene [15]. Whereas implantation of FGF2-soaked beads in the chick induced ectopic cells expressing a limited, but not complete program of otic markers [16], misexpression of Fgf2 using retroviruses failed to show any obvious effect on inner ear development [17]. This could be due to different levels of the ligand supplied by the two modes of delivery or a species difference. Although Fgf2 is expressed in the mouse otic placode,

inactivation of the mouse gene revealed no overt morphological changes nor molecular changes that were assayed. Taken together, these findings suggest that the different responses to FGFs during inner ear development may either be species specific [18] or reflect differences intrinsic to experimental design [16].

Although our understanding of the role of FGFs in inner ear development has thus far largely focused on otic induction by hindbrain-derived signaling, there are subsequent developmental events in which particular FGFs also clearly participate. A number of FGFs are expressed within the otic epithelium itself that elicit different developmental roles. Fgf3 and Fgf10 are expressed in the antero-ventral otocyst, in a region from where sensory cells and neuroblasts originate [19,20]. It is therefore not surprising that the vestibulo-acoustic ganglion is reduced in size in Fgf3 mouse mutants [5,21] and defects in vestibular sensory neurons and hair cells are produced following Fgf10 mutation [22]. Thus within the mouse, Fgf3 and Fgf10 are also required in a non-redundant manner for proper development within the otic epithelium as well as the earlier hindbrain-signalling events outlined above [23]. In addition, epithelial-derived FGF8 and FGF20, expressed in the presumptive epithelial domain of the organ of Corti, function in the specification of distinct cell types, in particular pillar cells, one of the supporting cells found in the developing auditory organ [24,25,26]. Similar to the mouse, in the chick, Fgf3 and Fgf10 function in normal development of the semicircular canals [22,27], whereas Fgfs 2, 8, 10 and 19 influence different aspects of vestibulo-cochlear neuronal development [28,16].

Formation of the surrounding cartilaginous otic capsule from periotic mesenchyme is also dependent on interactions with otic epithelial-derived signals. In vitro studies show that when substituted for otic epithelium, FGF2 induces a chondrogenic response, albeit limited, in cultured periotic mesenchyme. However when added in combination with TGF β , a full chondrogenic response, comparable to that which occurs in the presence of its inductive tissue (otic epithelium) is evoked [29]. Furthermore, epithelial-induced chondrogenesis can be inhibited in vitro by antibody blockade of these signaling molecules. Taken together, these studies reveal that FGF2, acting in concert with TGF β , is both necessary and sufficient to initiate otic capsule chondrogenesis.

Acting alone, FGF3 endogenous to the otic epithelium can also induce chondrogenesis in the developing otic capsule [30]. Interestingly, Fgf3 is downregulated in the otic vesicle by exposure to levels of retinoic acid (RA) that can cause inner ear malformations and capsule defects. Fgf3 is therefore a target of RA signaling that may also be involved in capsule chondrogenesis [31,32,33]. Expression of Fgf10 is similarly modified within the otic epithelium by aberrant RA exposure [31]. When cultures of periotic mesenchyme containing otic epithelium are treated with high doses of RA, chondrogenesis is suppressed and levels of Fgf3 and Fgf10 are concomitantly reduced. Otic capsule chondrogenesis can, however, be rescued by supplementation of cultures with a combination of FGF3 and FGF10 ligands, suggesting that these FGFs are indeed subject to control through RA signaling. Furthermore, recent studies show that the transcription factor GATA3 is a direct upstream regulator of Fgf10 that activates inner ear transcription [23].

In summary, multiple FGF ligands from various embryonic tissue sources act in concert to direct proper formation of the inner ear. Signaling by FGFs are required at several stages of inner ear development, initially for induction of the otic placode/vesicle but also subsequently to direct patterning and morphogenesis of the

developing otic vesicle, capsule and vestibulo-acoustic ganglion to form the complex three-dimensional inner ear.

References

1. Groves AK, Bronner-Fraser M (2000) Competence, specification and commitment in otic placode induction. *Development* 127: 3489-3499.
2. Zelarayan LC, Vendrell V, Alvarez Y, Domínguez-Frutos E, Theil T, et al. (2007) Differential requirements for FGF3, FGF8 and FGF10 during inner ear development. *Dev Biol* 308: 379-391.
3. Alvarez Y, Alonso MT, Vendrell V, Zelarayan LC, Chamero P, et al. (2003) Requirements for FGF3 and FGF10 during inner ear formation. *Development* 130: 6329-6338.
4. Wilkinson DG, Peters G, Dickson C, McMahon AP (1988) Expression of the FGF-related proto-oncogene int-2 during gastrulation and neurulation in the mouse. *EMBO J* 7: 691-695.
5. Mansour SL, Goddard JM, Capecci MR (1993) Mice homozygous for a targeted disruption of the proto-oncogene int-2 have developmental defects in the tail and inner ear. *Development* 117: 13-28.
6. Ohuchi H, Hori Y, Yamasaki M, Harada H, Sekine K, et al. (2000) FGF10 acts as a major ligand for FGF receptor 2 IIb in mouse multi-organ development. *Biochem Biophys Res Commun* 277: 643-649.
7. Mahmood R, Mason IJ, Morriss-Kay GM (1996) Expression of Fgf-3 in relation to hindbrain segmentation, otic pit position and pharyngeal arch morphology in normal and retinoic acid-exposed mouse embryos. *Anat Embryol (Berl)* 194: 13-22.
8. McKay IJ, Lewis J, Lumsden A (1996) The role of FGF-3 in early inner ear development: an analysis in normal and kreisler mutant mice. *Dev Biol* 174: 370-378.
9. Ladher RK, Wright TJ, Moon AM, Mansour SL, Schoenwolf GC (2005) FGF8 initiates inner ear induction in chick and mouse. *Genes Dev* 19: 603-613.
10. Crossley PH, Martin GR (1995) The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. *Development* 121: 439-451.
11. Phillips BT, Bolding K, Riley BB (2001) Zebrafish fgf3 and fgf8 encode redundant functions required for otic placode induction. *Dev Biol* 235: 351-365.
12. Léger S, Brand M (2002) Fgf8 and Fgf3 are required for zebrafish ear placode induction, maintenance and inner ear patterning. *Mech Dev* 119: 91-108.
13. Ladher RK, Anakwe KU, Gurney AL, Schoenwolf GC, Francis-West PH (2000) Identification of synergistic signals initiating inner ear development. *Science* 290: 1965-1967.
14. Wright TJ, Ladher R, McWhirter J, Murre C, Schoenwolf GC, et al. (2004) Mouse FGF15 is the ortholog of human and chick FGF19, but is not uniquely required for otic induction. *Dev Biol* 269: 264-275.
15. Lombardo A, Slack JM (1998) Postgastrulation effects of fibroblast growth factor on *Xenopus* development. *Dev Dyn* 212: 75-85.
16. Adamska M, Herbrand H, Adamski M, Krüger M, Braun T, et al. (2001) FGFs control the patterning of the inner ear but are not able to induce the full ear program. *Mech Dev* 109: 303-313.
17. Vendrell V, Carnicero E, Giraldez F, Alonso MT, Schimmang T (2000) Induction of inner ear fate by FGF3. *Development* 127: 2011-2019.
18. Fekete DM (2000) Ear rings: FGF3 involvement comes full circle. *Trends Neurosci* 23: 332.
19. Fekete DM (1996) Cell fate specification in the inner ear. *Curr Opin Neurobiol* 6: 533-541.
20. Fekete DM, Wu DK (2002) Revisiting cell fate specification in the inner ear. *Curr Opin Neurobiol* 12: 35-42.
21. Hatch EP, Noyes CA, Wang X, Wright TJ, Mansour SL (2007) Fgf3 is required for dorsal patterning and morphogenesis of the inner ear epithelium. *Development* 134: 3615-3625.

22. Pauley S, Wright TJ, Pirvola U, Ornitz D, Beisel K, et al. (2003) Expression and function of FGF10 in mammalian inner ear development. *Dev Dyn* 227: 203-215.
23. Economou A, Datta P, Georgiadis V, Cadot S, Frenz D, et al. (2013) Gata3 directly regulates early inner ear expression of Fgf10. *Dev Biol* 374: 210-222.
24. Jacques BE, Montcouquiol ME, Layman EM, Lewandoski M, Kelley MW (2007) Fgf8 induces pillar cell fate and regulates cellular patterning in the mammalian cochlea. *Development* 134: 3021-3029.
25. Hayashi T, Ray CA, Bermingham-McDonogh O (2008) Fgf20 is required for sensory epithelial specification in the developing cochlea. *J Neurosci* 28: 5991-5999.
26. Huh SH, Jones J, Warchol ME, Ornitz DM (2012) Differentiation of the lateral compartment of the cochlea requires a temporally restricted FGF20 signal. *PLoS Biol* 10: e1001231.
27. Chang W, Brigande JV, Fekete DM, Wu DK (2004) The development of semicircular canals in the inner ear: role of FGFs in sensory cristae. *Development* 131: 4201-4211.
28. Fantetti KN, Fekete DM (2012) Members of the BMP, Shh, and FGF morphogen families promote chicken statoacoustic ganglion neurite outgrowth and neuron survival in vitro. *Dev Neurobiol* 72: 1213-1228.
29. Frenz DA, Liu W, Williams JD, Hatcher V, Galinovic-Schwartz V, et al. (1994) Induction of chondrogenesis: requirement for synergistic interaction of basic fibroblast growth factor and transforming growth factor-beta. *Development* 120:415-424.
30. Frenz DA, Liu W (1998) Role of FGF3 in otic capsule chondrogenesis in vitro: an antisense oligonucleotide approach. *Growth Factors* 15: 173-182.
31. Liu W, Levi G, Shanske A, Frenz DA (2008) Retinoic acid-induced inner ear teratogenesis caused by defective Fgf3/Fgf10-dependent Dlx5 signaling. *Birth Defects Res B Dev Reprod Toxicol* 83: 134-144.
32. Frenz DA, Liu W, Cvekl A, Xie Q, Wassef L, et al. (2010) Retinoid signaling in inner ear development: A "Goldilocks" phenomenon. *Am J Med Genet A* 152A: 2947-2961.
33. Cadot S, Frenz D, Maconochie M (2012) A novel method for retinoic acid administration reveals differential and dose-dependent downregulation of Fgf3 in the developing inner ear and anterior CNS. *Dev Dyn* 241: 741-758.