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# FGF and Notch signaling in sensory neuron formation: A multifactorial approach to understanding signaling pathway hierarchy

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ABSTRACT

The ophthalmic trigeminal (opV) placode exclusively gives rise to sensory neurons, making it a good model to study the molecular regulation of sensory neurogenesis. A number of signaling pathways including Wnt, PDGF, FGF, and Notch have been shown to be involved in the process of opV placode cell development. However, the regulatory relationships between these signaling pathways in placode cells are still unknown and have been difficult to study experimentally. Using a novel multifactorial approach in chick embryos that allows for inhibition of FGF throughout the tissue or in individual cells, with simultaneous inactivation of Notch signaling, we investigated the potential interaction between the FGF and Notch signaling pathways in trigeminal sensory neurogenesis. This study builds on prior research describing the individual role of FGF signaling or Notch signaling in opV placode development, where blocking FGF signaling resulted in neurogenesis failure, while blocking Notch signaling resulted in enhanced neurogenesis. Reported here, blocking both pathways simultaneously resulted in a reduction in the number of cells delaminating from the opV placode and undergoing sensory neuron differentiation. Further, Notch inhibition alone did not lead to an increase in the number of cells expressing FGFR4 or in the FGFR4 expression domain, but did result in a highly fragmented basal lamina, which was reversed when blocking FGF signaling. Cumulatively, the results presented here do not support a model of Notch/FGF interdependence, rather that FGF and Notch act in parallel to promote sensory neurogenesis.



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## 1. Introduction

In vertebrates, areas of head ectoderm termed cranial placodes and a subset of neural crest cells give rise to all peripheral sensory neurons of the cranial ganglia (D'Amico-Martel and Noden, 1983). Most neural precursor tissue can give rise to multiple cell types; however, the ophthalmic trigeminal placode (opV) contributes only sensory neurons within the trigeminal ganglion, making it a unique model for the study of sensory neurogenesis (Begbie et al., 2002).

For the past decade, core publications have helped characterize the function of genes and signaling pathways known to be active in opV placode development; Pax3 and FGF, as well as the canonical Wnt and Notch/Delta signaling pathways are all active here (Canning et al., 2008; Dude et al., 2009; Lassiter et al., 2007, 2009, 2010).

### 1.1. *Molecular pathways in sensory neurogenesis*

FGF signaling has been shown to be involved in the development of not only the opV placodes. As examples, localized FGF signaling induces uncommitted ectoderm lateral to the developing hindbrain to develop the vertebrate inner ear (reviewed by Ohyama et al., 2006; Schimmang, 2007; Ladher et al., 2010). Disruption of FGF3 and FGF8 signaling by SU5402, an FGF receptor antagonist, blocks critical steps in the development of the otic placode. In zebrafish it has also been shown that FGF signaling must be active through mid-somitogenesis stages to maintain otic placode cell fate (Leger and Brand, 2002). Additionally, FGF signaling is required for the development of the lens placode in mice (Garcia et al., 2011).

In the opV placode domain in chick, initial expression of *FGFR4* mRNA occurs at the 10 somite stage (ss), shortly after expression of Pax3, an early marker of opV placode cells (Stark et al., 1997). Individual opV placode cells express *FGFR4* transiently just prior to and during delamination, and peak expression occurs concomitantly with peak neurogenesis at the 15–28 ss. *FGFR4* expression is quickly downregulated in each cell coincident with delamination, and is therefore not detected in the condensing trigeminal ganglion (Stark et al., 1997). Studies in which FGF signaling was blocked with the dominant-negative secreted-FGFR4 (sFGFR4) gene fragment prevented cellular delamination from placodal ectoderm to the spot of future ganglion formation, indicating that FGF signaling is required for delamination and differentiation during opV sensory neurogenesis (Lassiter et al., 2009).

In the Notch/Delta signaling pathway, a well-known regulator of cellular and neuronal differentiation, the transmembrane Notch receptor is activated by the membrane-bound Delta or Serrate ligands. The function of Notch signaling can be viewed as a switch that regulates developmental choices (Lewis, 1998). Many studies have characterized the general rule that precocious neuronal differentiation occurs when Notch signaling is blocked while neuronal differentiation is inhibited when Notch signaling is activated (Abelló et al., 2007; Bolos et al., 2007; Daudet et al., 2007; Kageyama et al., 2005; Lewis, 1998; Nelson et al., 2007; Yoon and Gaiano, 2005). In sensory neurogenesis, reduced Notch signaling in the avian dorsal root ganglion resulted in the generation of DRG neurons, while Notch activation prevented neuronal differentiation but permitted glial differentiation in vitro (Wakamatsu et al., 2000). Lassiter et al. (2010) carefully characterized the expression of several Notch signaling pathway components as part of their functional assessment of Notch signaling in trigeminal placode development. For example, Ngn2, which induces the expression of Delta1 (Castro et al., 2006) and is inhibited by the Notch effector gene Hes1 (Shimojo et al., 2008), is first expressed in the opV placode at ~10–11 ss, with more robust expression at ~16 ss, indicating that the timing of Notch downregulation is coincident with peak *FGFR4* expression and neuronal differentiation. In the same study, inhibition of Notch signaling by the gamma-secretase inhibitor DAPT resulted in premature and enhanced neuronal differentiation, while misexpression of the Notch intracellular domain blocked neuronal differentiation. From these results it was concluded that Notch signaling is a primary regulator of the sensory neuron cell fate in the trigeminal placodes, with downregulation of Notch signaling being required for neurogenesis (Lassiter et al., 2010).

### 1.2. *Pathway interactions in development*

Although several components involved in sensory neurogenesis have been identified, a more complete regulatory model of trigeminal sensory neurogenesis, including how these pathways complement one another, has not been formed. This study primarily aims to determine the crosstalk and hierarchy between FGF and Notch signaling in sensory neurogenesis. Even in other well-described developmental systems, this question is somewhat unclear. For example, it has been known for some time that FGF and Notch signaling contribute to somite segmentation (Goldbeter et al., 2007), where it is proposed that these pathways work in coordination to regulate somite formation periodicity through a molecular oscillator known as the segmentation clock. It has been suggested that Notch signaling acts as a regulator switch in somite segmentation, with many of its target genes displaying cyclic expression, while FGF signaling is thought to act upstream and/or parallel to this pathway (Gibb et al., 2010). Consistent with this theory, Notch knockout mice showed a complete disruption of somitogenesis (Ferjentsik et al., 2009). Further, evidence from mutant mice with disrupted FGF signaling resulted in a decrease of the Notch target *Lunatic Fringe*, which suggests that FGF at least in part regulates Notch signaling (Wahl et al., 2007). In contrast, however, inhibition of Notch activity in zebrafish does not completely disrupt somite segmentation, and therefore may be coordinating a Notch-independent oscillator, while FGF target genes control oscillations (Lewis, 2009; Ozbudak and Pourquie, 2008; Kawamura et al., 2005). A recent review highlights a model wherein Hes7 lies at the interface between Notch and FGF signaling in somitogenesis (Harima and Kageyama, 2013). Although the interplay between the FGF and Notch signaling pathways in somitogenesis is becoming clearer, how the information applies to other systems is unknown.

Interactions between FGF and Notch have also been shown in neuroepithelial precursor mouse cells where FGF activates Notch signaling thereby inhibiting neuronal differentiation (Faux et al., 2001). In contrast however, it has been shown that activation of Notch signaling in NIH 3T3 cells suppresses