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Cilia-dependent GLI processing in neural crest cells is required for tongue development

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ABSTRACT

Ciliopathies are a class of diseases caused by the loss of a ubiquitous, microtubule-based organelle called a primary cilium. Ciliopathies commonly result in defective development of the craniofacial complex, causing midfacial defects, craniosynostosis, micrognathia and aglossia. Herein, we explored how the conditional loss of primary cilia on neural crest cells (*Kif3a$^{f/f}$;Wnt1-Cre)* generated aglossia. On a cellular level, our data revealed that aglossia in *Kif3a$^{f/f}$;Wnt1-Cre* embryos was due to a loss of mesoderm-derived muscle precursors migrating into and surviving in the tongue anlage. To determine the molecular basis for this phenotype, we performed RNA-seq, *in situ* hybridization, qPCR and Western blot analyses. We found that transduction of the Sonic hedgehog (Shh) pathway, rather than other pathways previously implicated in tongue development, was aberrant in *Kif3a$^{f/f}$;Wnt1-Cre* embryos. Despite increased production of full-length GLI2 and GLI3 isoforms, previously identified GLI targets important for mandibular and glossal development (*Foxf1*, *Foxf2*, *Foxd1* and *Foxd2)* were transcriptionally downregulated in *Kif3a$^{f/f}$;Wnt1-Cre* embryos. Genetic removal of GLI activator (GLIA) isoforms in neural crest cells recapitulated the aglossia phenotype and downregulated *Fox* gene expression. Genetic addition of GLIA isoforms in neural crest cells partially rescued the aglossia phenotype and *Fox* gene expression in *Kif3a$^{f/f}$;Wnt1-Cre* embryos. Together, our data suggested that glossal development requires primary cilia-dependent GLIA activity in neural crest cells. Furthermore, these data, in conjunction with our previous work, suggested prominence specific roles for GLI isoforms; with development of the frontonasal prominence relying heavily on the repressor isoform and the development of the mandibular prominence/tongue relying heavily on the activator isoform.

1. Introduction

Primary cilia are ubiquitous microtubule-based cellular projections that are specialized for transducing extracellular signaling cues. Functional disruptions to the primary cilia are associated with a spectrum of complex human genetic disorders known as ciliopathies (Badano et al., 2006; Baker and Beales, 2009; D'Angelo and Franco, 2010; Goetz and Anderson, 2010). Craniofacial dysmorphologies are common characteristics of the ciliopathic disease spectrum. Approximately 30% of known human ciliopathies are primarily characterized by their craniofacial defects (Chang et al., 2015; Tobin et al., 2008; Zaghloul and Brugmann, 2011). Oral malformations, including those affecting the development of the tongue, are among the most common phenotypes present in craniofacial ciliopathies. Ciliopathic conditions such as Oral-facial-digital syndrome, Meckel-Gruber syndrome, and Joubert syndrome frequently present with glossal abnormalities including: an abnormally small tongue (microglossia), bifid or cleft tongue, anterior marginal hamartomas or cysts of the tongue, and tongue tumors (Chang et al., 2015; Gai et al., 2012; Moran-Barroso et al., 1998; Parisi, 2009). Although glossal abnormalities are common occurrences among ciliopathies and have a significant impact on the feeding and speech of patients, the underlying developmental mechanisms that affect glossal development in ciliopathies have not been explored.

The tongue and several other facial structures affected in ciliopathies are derivatives of, or have a substantial contribution from neural crest cells (NCCs). NCCs are a migratory, multipotent cell population that migrate from the dorsal neural tube to populate the facial prominences, including the pharyngeal arches from which the tongue is derived (Noden et al., 1999). Development of the tongue begins with

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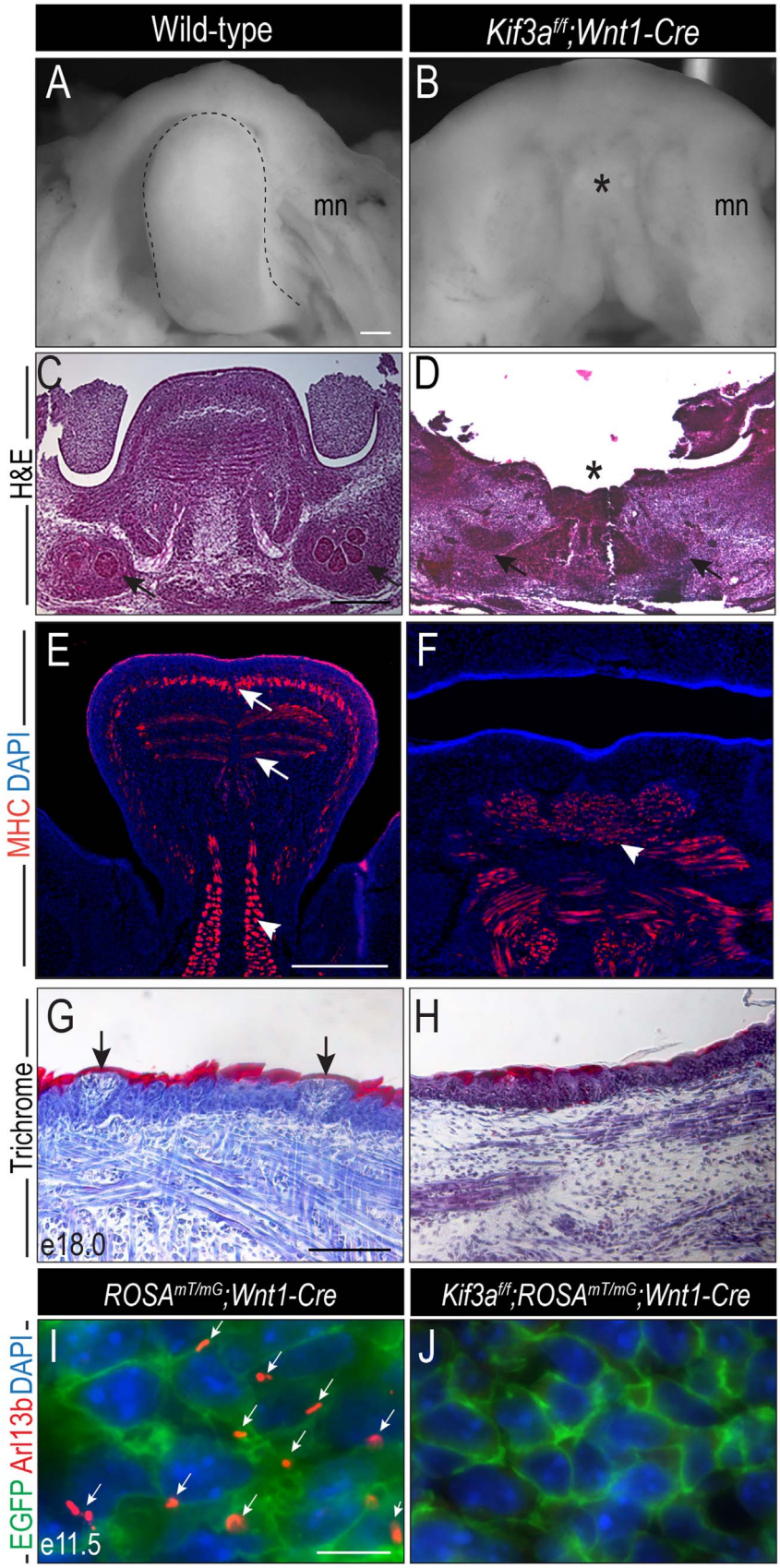

Fig. 1. Loss of primary cilia in NCCs results in aglossia. Dorsal view of the tongue (dotted black lines) or tongue remnant (asterisk) in e13.5 (A) wild-type and (B) *Kif3a$^{f/f}$;Wnt1-Cre* embryos. (C, D) Hematoxylin and eosin (H & E) stained frontal sections of wild-type and *Kif3a$^{f/f}$;Wnt1-Cre* embryos. Black arrows indicate the salivary glands. (E, F) Anti-MHC immunostaining (red) on frontal sections of e13.5 wild-type and *Kif3a$^{f/f}$;Wnt1-Cre* embryos. White arrows indicate intrinsic musculature. White arrow heads mark extrinsic musculature. (G, H) Trichrome staining on e18.0 wild-type and *Kif3a$^{f/f}$;Wnt1-Cre* embryos. Black arrows indicate developing taste buds. (I, J) Anti-Arl13b immunostaining (red) in *ROSA$^{mT/mG}$;Wnt1-Cre* and *Kif3a$^{f/f}$;ROSA$^{mT/mG}$;Wnt1-Cre* embryos. NCCs are marked by EGFP (green). White arrows mark ciliary axonemes (red). Nuclei stained with DAPI counterstain. mn, mandible. Scale bars =(A, B) 500 μm; (C-F) 250 μm; (G, H) 100 μm; (I, J) 10 μm.