Developmental Biology ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Hedgehog activity controls opening of the primary mouth

Jacqueline M. Tabler [a,b], Trióna G. Bolger [a], John Wallingford [b,c], Karen J. Liu [a,*]

[a] Department of Craniofacial Development and Stem Cell Biology, King's College London, UK
[b] Department of Molecular Biosciences, University of Texas at Austin, USA
[c] Howard Hughes Medical Institute, USA

ARTICLE INFO

Article history:
Received 2 June 2014
Received in revised form
24 September 2014
Accepted 25 September 2014

Keywords:
Hedgehog
Wnt
Primary mouth
Xenopus
Mouse
Buccopharyngeal membrane
Stomodeum
Fibronectin

ABSTRACT

To feed or breathe, the oral opening must connect with the gut. The foregut and oral tissues converge at the primary mouth, forming the buccopharyngeal membrane (BPM), a bilayer epithelium. Failure to form the opening between gut and mouth has significant ramifications, and many craniofacial disorders have been associated with defects in this process. Oral perforation is characterized by dissolution of the BPM, but little is known about this process. In humans, failure to form a continuous mouth opening is associated with mutations in Hedgehog (Hh) pathway members; however, the role of Hh in primary mouth development is untested. Here, we show, using *Xenopus*, that Hh signaling is necessary and sufficient to initiate mouth formation, and that Hh activation is required in a dose-dependent fashion to determine the size of the mouth. This activity lies upstream of the previously demonstrated role for Wnt signal inhibition in oral perforation. We then turn to mouse mutants to establish that SHH and Gli3 are indeed necessary for mammalian mouth development. Our data suggest that Hh-mediated BPM persistence may underlie oral defects in human craniofacial syndromes.

© 2014 Elsevier Inc. All rights reserved.

Introduction

To feed or breathe the oral opening must connect with the gut. The primary mouth marks the location of this interface, and perforation is essential (Dickinson and Sive, 2006; Hardin and Armstrong, 1997; McClay et al., 1992; Poelmann et al., 1985; Soukup et al., 2013; Takahama et al., 1988; Watanabe et al., 1984). Despite the fundamental importance of the primary mouth, little is known about the molecular control of its development. In mammals, the buccopharyngeal membrane (BPM) is hidden internally, behind the expanding facial prominences and is surrounded by the brain and cardiac tissues, making mammalian primary mouth development a challenging process to investigate (Poelmann et al., 1985; Soukup et al., 2013; Theiler, 1969; Waterman, 1977). However, a series of elegant studies have shown that *Xenopus laevis* is a tractable model for understanding primary mouth development (Dickinson and Sive, 2007, 2006, 2009; Jacox et al., 2014; Kennedy and Dickinson, 2014).

In mammals and amphibians the mouth opening forms as a result of contact between invaginating primary mouth ectoderm and foregut endothelium (Fig. 1A) (Dickinson and Sive, 2006, 2009; Soukup et al., 2013; Waterman, 1977, 1985; Waterman and Schoenwolf, 1980). In *Xenopus*, invaginating ectoderm appears as a depression called the stomodeum (Dickinson and Sive, 2006), and this depression deepens as apoptosis and cell intermingling thin the epithelium (Dickinson and Sive, 2006, 2009; Poelmann et al., 1985). The basement membrane (BM) separating foregut endoderm and stomodeal ectoderm dissolves to permit intercalation of the epithelial bilayer and subsequent oral perforation (Dickinson and Sive, 2006, 2009; Soukup et al., 2013; Waterman, 1977, 1985; Waterman and Schoenwolf, 1980) (Fig. 1).

At present, only a single signaling system has been identified as a molecular regulator of primary mouth development. In *Xenopus*, Wnt signal inhibition is necessary for stomodeal specification and perforation (Dickinson and Sive, 2009). Wnt/β-catenin signaling is necessary to promote transcriptional activation of the basement membrane component fibronectin (FN) (Gradl et al., 1999), while Wnt inhibitors Crescent and Frzb-1 are required within the stomodeum for dissolution of the basement membrane separating foregut endoderm and oral ectoderm (Dickinson and Sive, 2009). Loss of either inhibitor results in a small, imperforate primary mouth. Concomitantly, facial Wnt-8 gain-of-function is sufficient to suppress stomodeum formation (Dickinson and Sive, 2009). However, the stomodeal ectoderm becomes unresponsive to Wnts long before perforation, suggesting that Wnts do not directly control mouth opening (Dickinson and Sive, 2009). Therefore, it is necessary to consider other signaling pathways during primary mouth morphogenesis.

* Correspondence to: Department of Craniofacial Development and Stem Cell Biology, King's College London, Floor 27, Tower Wing, Guy's Hospital, SE1 9RT, London, UK. Fax: +44 20 7188 1674.
E-mail address: karen.liu@kcl.ac.uk (K.J. Liu).

http://dx.doi.org/10.1016/j.ydbio.2014.09.029
0012-1606/© 2014 Elsevier Inc. All rights reserved.

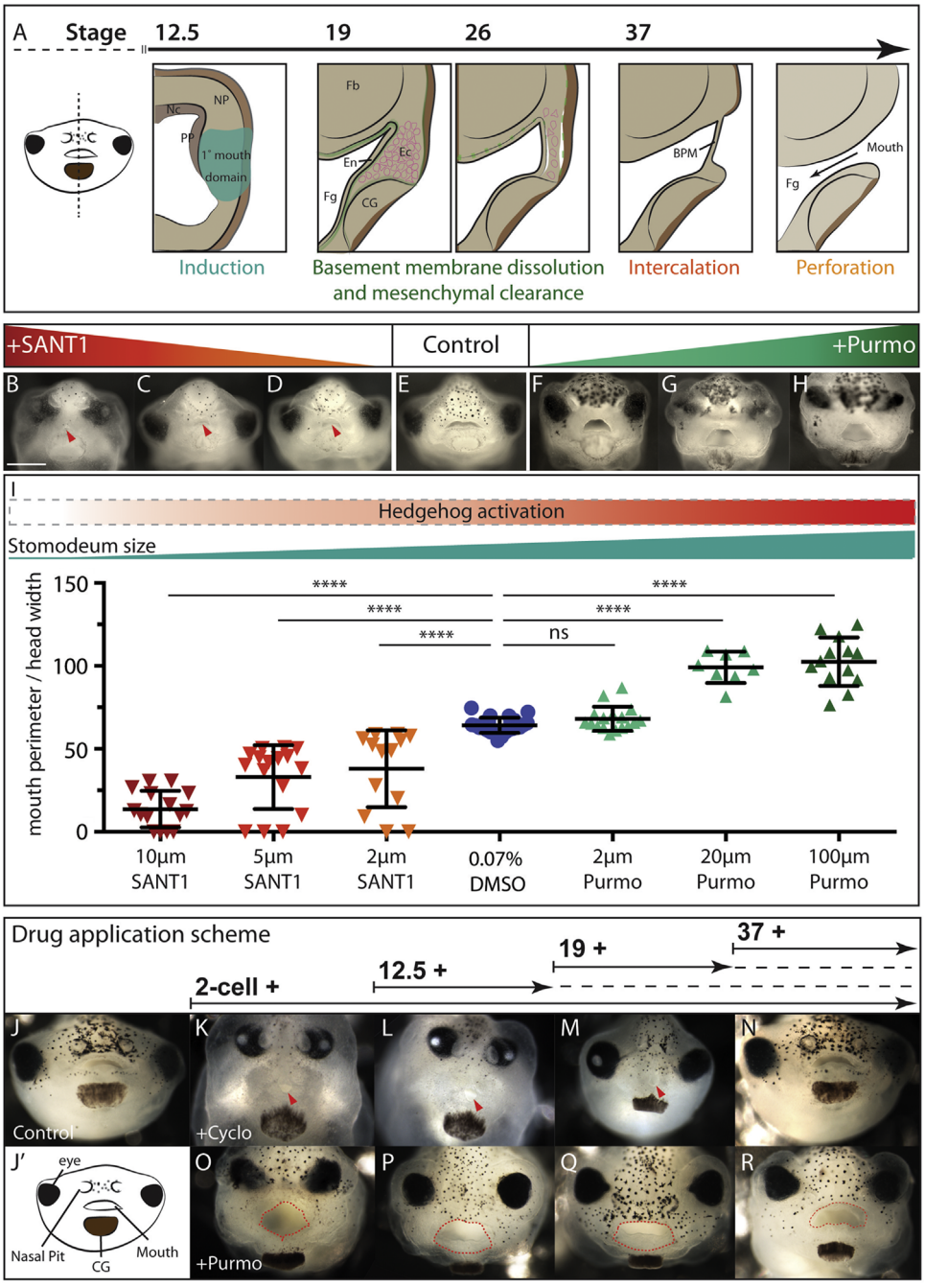

Fig. 1. Hedgehog perturbation affects the size of the oral opening. (A) Schematic illustrating primary mouth development. Frontal view of *Xenopus* tadpole indicates sectional plane for schematics. Stage 12.5, primary mouth induction occurs anterior to the prechordal plate (PP), notochord (Nc) and neural plate (NP). Stage 19 foregut endoderm (Fg) abuts mouth ectoderm (Ec, pink), separated by fibronectin-rich basement membrane (BM, green), between forebrain (Fb) and cement gland (CG). BM dissolves and mesenchymal clearance thins stomodeum (green dashed line indicates BM). Stage 37, buccopharyngeal membrane (BPM) formation. Stage 40, BPM perforation. (B–D) Frontal view of stage 45 tadpoles incubated from 2-cell stage with 10 µM (B), 5 µM (C), or 2 µM SANT1 (D) (B, $n=13/13$, C, $n=16/16$, D, $n=15/15$). Primary mouth is indicated by red arrowhead. (E) Control tadpole, 0.07% DMSO ($n=26/26$). (F–H) Tadpoles incubated with 2 µM (F), 20 µM (G) or 100 µM purmorphamine (H). Increase in mouth size was observed with increasing concentrations of purmorphamine (F, $n=70/70$, G, $n=43/43$, H, $n=154/154$). (I) Quantification of mouth size for 10 µM, 5 µM, or 2 µM SANT1, 0.07% DMSO, 2 µM, 20 µM or 100 µM purmorphamine, where mouth perimeter is normalized to width of the head. $^{****}P<0.001$. Scheme indicating primary mouth size (green) in relationship to Hh activity (red bar). (J) Stage 45 control tadpole. (J′) Facial anatomy schematic. (K–N) Tadpoles incubated with 250 µM cyclopamine from 2-cell stage (K), between stages 12.5–19 (L), 19–37 (M), or from 37 (N). (O–R) Tadpoles treated with 100 µM purmorphamine from the 2-cell stage (O), between stages 12.5–19 (P), 19–37 (Q), or from stage 37 (R).

In mammals, virtually nothing is known about the molecular control of primary mouth formation, but several craniofacial syndromes, including CHARGE, Down, Holzgreve–Wagner–Rehder, Greig cephalopolydactyly syndrome (GCPS) and synostotic syndromes, as well as cleft palate, have been associated with persistent BPM (Kliegman, 2011; DéMurger et al., 2014; Pillai et al., 1990; Verma and Geller, 2009). Notably, Holzgreve–Wagner–Rehder syndrome involves cleft palate and postaxial polydactyly, phenotypes associated with Hh perturbation (Legius et al., 1988). Furthermore, a recent publication reports that GCPS—characterized by mutations in the Hh effector Gli3—caused oral anomalies in all prenatal cases observed, and in one instance a complete absence of the oral opening (DéMurger et al., 2014). We therefore tested the requirements for Hedgehog signaling during primary mouth development. We present data suggesting that Hh signaling is required for BPM dissolution in both *Xenopus* and mouse. Moreover, we show that Hh

Please cite this article as: Tabler, J.M., et al., Hedgehog activity controls opening of the primary mouth. Dev. Biol. (2014), http://dx.doi.org/10.1016/j.ydbio.2014.09.029

Download English Version:

https://daneshyari.com/en/article/10931704

Download Persian Version:

https://daneshyari.com/article/10931704

Daneshyari.com