Published in final edited form as:

Dis Esophagus. 2013 ; 26(4): 356–358. doi:10.1111/dote.12055.

Genetic and cellular mechanisms of the formation of Esophageal Atresia and Tracheoesophageal Fistula

Ian J. Jacobs¹ and Jianwen Que^{2,3,*}

¹Department of Biology, University of Rochester, Rochester, NY, 14642

²Department of Biomedical Genetics, University of Rochester, Rochester, NY, 14642

³UR Stem Cell and Regenerative Medicine Institute, Rochester, NY, 14642

Abstract

Foregut separation involves dynamic changes in the activities of signaling pathways and transcription factors. Recent mouse genetic studies demonstrate that some of these pathways interact with each other to form a complex network, leading to a unique dorsal-ventral patterning in the early foregut. In this review we will discuss how this unique dorsal-ventral patterning is set prior to the foregut separation and how disruption of this patterning affects the separation process. We will further discuss the roles of downstream targets of these pathways in regulating separation at cellular and molecular levels. Understanding the mechanism of normal separation process will provide us insights into the pathobiology of a relatively common birth defect Esophageal Atresia (EA) with/without Tracheo-esophageal Fistula (TEF).

Keywords

foregut separation; EA/TEF; Sox2; Bmp

Introduction

The trachea and esophagus are separated from a developmental intermediate called the foregut. This separation process occurs at around 4–5 weeks of human gestation and at embryonic (E) day 9.5–11.5 in mice. Although remaining controversial, previous characterization studies suggest that during separation a tracheoesophageal septation forms by lateral ingrowth of two lateral epithelial folds that eventually fuse in the midline to divide the foregut into the dorsal and ventral side (Gray and Skandalakis, 1972). Subsequently, the ventral side gives rise to the trachea and the dorsal side gives rise to the esophagus. Failed foregut separation leads to the formation of Esophageal Atresia with/without Tracheoesophageal Fistula (EA/TEF) which affects one in 3500 newborns. Surprisingly, although EA/TEF is relatively common and requires immediate surgical intervention, the pathobiology of this birth defect remains largely unknown. Studies in several mouse genetic models have just begun to shed some important insights into the underlying mechanisms.

* Author for correspondence: Jianwen_Que@urmc.rochester.edu, Department of Biomedical Genetics, University of Rochester, 601 Elmwood Ave., Box 633, Rochester, NY 14642, Phone: 585-273-4125, Fax: 585-273-1450.

Foregut separation involves dynamic changes in cell shape, adhesion between cells accompanied by extensive proliferation and differentiation of epithelial and mesenchymal compartments. It is believed that these cellular events are coordinated by a complex network of signaling molecules and transcription factors. Accordingly, several signaling pathways including Sonic Hedgehog (Shh), Retinoid Acid (RA) and Wnt have been shown to regulate foregut separation (Goss et al., 2009; Harris-Johnson et al., 2009; Litingtung et al., 1998; Mendelsohn et al., 1994). In addition, we and others recently showed that Bone morphogenetic protein (Bmp) pathway also plays important roles for the morphogenesis of the early foregut (Li et al., 2007; Que et al., 2006). In this review we will summarize these recent findings and propose a model in which a dorsal-ventral patterning of signaling molecules and their downstream transcription factors is critical for the separation of the foregut into the trachea and esophagus, and disruption of the patterning often results in the formation of EA/TEF.

Dorsal-ventral patterning of signaling molecules in the unseparated foregut

The initial understanding of foregut separation largely came from early studies of the respiratory system. Genetic mutations leading to lung defects often affect the separation of the trachea from the foregut tube (Goss et al., 2009; Harris-Johnson et al., 2009; Que et al., 2006). Further analysis revealed that the activities of multiple signaling pathways are different between the dorsal and ventral sides of the foregut prior to the separation. For example, the Wnt, Bmp and Shh signaling pathways are activated in the ventral side only (Goss et al., 2009; Harris-Johnson et al., 2009; Litingtung et al., 1998; Que et al., 2006). Accordingly, downstream components of these pathways are enriched in the ventral side of the unseparated foregut tube. By contrast, inhibitors of the relevant pathways such as Noggin, a Bmp inhibitor, are preferentially expressed at the dorsal side (Li et al., 2007; Que et al., 2006). Genetic deletion of the relevant signaling activators or inhibitors disrupts the dorsal-ventral balancing and leads to the formation of EA/TEF (Li et al., 2007; Que et al., 2006). In the case of the Wnt pathway, simultaneous removal of ligands Wnt2 and 2b abrogates Wnt signaling and results in the failure of the specification of the trachea and lung (Goss et al., 2009). In line with these findings, conditional deletion of Wnt signaling mediator β -catenin in *Shh-Cre; \beta*-catenin^{loxP/loxP} mutants also leads to the formation of EA/TEF (Harris-Johnson et al., 2009). Similarly, inhibition of Bmp signaling in the ventral foregut by deleting *Bmp4* affects the formation of trachea (Li et al., 2008). Conversely, loss of *Noggin* which is preferentially expressed in the dorsal epithelium leads to ectopic Bmp activity in this region and results in defects in the separation. Of note is that the TEF formed in *Noggin* null mutants shares some common features with the trachea: the lining epithelial cells are simple columnar and express Nkx2.1, a transcription factor specifically marking respiratory epithelium (Que et al., 2006).

Transcription factors differentially expressed in the dorsal and ventral side of the foregut regulate foregut separation and specify the respective epithelial identity

Prior to foregut separation, the epithelial cells at the ventral side are tightly packed into a pseudostratified structure with the longer cellular axis facing the lumen. By contrast, the epithelial cells lining the dorsal foregut are loosely connected and the cell shape is flat and elongated. These morphological differences are accompanied by different gene expression. Through extensive immunohistochemistry and in situ hybridization we have identified several genes that are differentially expressed in the dorsal and ventral foregut. *Sox2*, one of these genes, is preferentially expressed in the epithelium of the dorsal foregut and plays important roles in the separation of the foregut and differentiation of esophageal epithelium.

Sox2 is a transcription factor belonging to the Sry (sex-determining region on the Y chromosome)-related family which is characterized by a high mobility (HMG) DNA binding motif. *Sox2* is important for the self-renewal of embryonic stem cells and stem cells in the tongue and trachea (Okubo et al., 2006; Que et al., 2009; Ura et al., 2011). Deletion of *Sox2* results in peri-implantation lethality (Avilion et al., 2003). Interestingly, genetic association study has linked *Sox2* haploinsufficiency to the formation of EA/TEF in patients with AEG syndrome (Williamson et al., 2006). However, loss of one copy of *Sox2* does not lead to EA/TEF in the *Sox2*^{GFP/+} mouse embryos and requires further reduction of *Sox2* protein levels to 30% of wildtype in the *Sox2*^{GFP/COND} mutant, which harbors a null allele (*Sox2*^{GFP}) and a hypomorphic allele (*Sox2*^{COND}). Significantly, the epithelial cells lining the TEF express high levels of tracheal epithelial marker *Nkx2.1*, suggesting that these cells have been specified towards a respiratory cell fate (Que et al., 2007). Conversely, the epithelial cells lining the TEF of the *Nkx2.1* null mutants express high levels of *Sox2* (Que et al., 2007). Together these results support a model in which a dorsal-ventral patterning of the transcription factors is critical for the separation of the foregut and subsequent morphogenesis of the trachea and esophagus.

Conclusion and future directions

Although the exact cellular mechanisms underling the formation of EA/TEF remain to be determined, genetic studies in mouse models have begun to provide some insights into the molecules that regulate the foregut morphogenesis. These studies have shown that the dorsal-ventral patterning of the signaling molecules and transcription factors in the foregut prior to separation is important for the subsequent separation. Of note is that in Adriamycin-treated rodents EA/TEF develops with similar perturbation of signaling activities (Hajduk et al., 2011; Ioannides et al., 2010; Spilde et al., 2004), although it remains to be investigated whether the patterning of the transcription factors has similarly been disrupted. In the future coupling whole genome sequencing with the Adriamycin model could potentially provide new information into genes that are involved in the regulation of foregut separation.

Acknowledgement

We apologize that due to space constraints many relevant references could not be cited. We thank members of our laboratory and Dr. Brigid Hogan, Department of Cell Biology at Duke University for helpful discussion. Research in the Que lab is partly supported by NIH K99/R00 Independent Pathway Award DK082650 and March of Dimes Basil O'Connor Starter Scholar Research Award.

References

- Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R. Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* 2003; 17:126–140. [PubMed: 12514105]
- Goss AM, Tian Y, Tsukiyama T, Cohen ED, Zhou D, Lu MM, Yamaguchi TP, Morrissey EE. Wnt2/2b and beta-catenin signaling are necessary and sufficient to specify lung progenitors in the foregut. *Dev Cell.* 2009; 17:290–298. [PubMed: 19686689]
- Gray, SW.; Skandalakis, JE. The esophagus. In: Gray, SW.; Skandalakis, JE., editors. *Embryology for Surgeons*. 1st ed.. Philadelphia, PA: Saunders; 1972. p. 63-99. *Journal of the Medical Association of Georgia*
- Hajduk P, Sato H, Puri P, Murphy P. Abnormal notochord branching is associated with foregut malformations in the adriamycin treated mouse model. *PLoS One.* 2011; 6:e27635. [PubMed: 22132119]
- Harris-Johnson KS, Domyan ET, Vezina CM, Sun X. beta-Catenin promotes respiratory progenitor identity in mouse foregut. *Proc Natl Acad Sci U S A.* 2009; 106:16287–16292. [PubMed: 19805295]
- Ioannides AS, Massa V, Ferraro E, Cecconi F, Spitz L, Henderson DJ, Copp AJ. Foregut separation and tracheo-oesophageal malformations: the role of tracheal outgrowth, dorso-ventral patterning and programmed cell death. *Dev Biol.* 2010; 337:351–362. [PubMed: 19913007]
- Li Y, Gordon J, Manley NR, Litingtung Y, Chiang C. Bmp4 is required for tracheal formation: a novel mouse model for tracheal agenesis. *Dev Biol.* 2008; 322:145–155. [PubMed: 18692041]
- Li Y, Litingtung Y, Ten Dijke P, Chiang C. Aberrant Bmp signaling and notochord delamination in the pathogenesis of esophageal atresia. *Dev Dyn.* 2007; 236:746–754. [PubMed: 17260385]
- Litingtung Y, Lei L, Westphal H, Chiang C. Sonic hedgehog is essential to foregut development. *Nat Genet.* 1998; 20:58–61. [PubMed: 9731532]
- Mendelsohn C, Lohnes D, Decimo D, Lufkin T, LeMeur M, Chambon P, Mark M. Function of the retinoic acid receptors (RARs) during development (II). Multiple abnormalities at various stages of organogenesis in RAR double mutants. *Development.* 1994; 120:2749–2771. [PubMed: 7607068]
- Okubo T, Pevny LH, Hogan BL. Sox2 is required for development of taste bud sensory cells. *Genes & Development.* 2006; 20:2654–2659. [PubMed: 17015430]
- Que J, Choi M, Ziel JW, Klingensmith J, Hogan BL. Morphogenesis of the trachea and esophagus: current players and new roles for noggin and Bmps. *Differentiation.* 2006; 74:422–437. [PubMed: 16916379]
- Que J, Luo X, Schwartz RJ, Hogan BL. Multiple roles for Sox2 in the developing and adult mouse trachea. *Development.* 2009; 136:1899–1907. [PubMed: 19403656]
- Que J, Okubo T, Goldenring JR, Nam KT, Kurotani R, Morrissey EE, Taranova O, Pevny LH, Hogan BL. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. *Development.* 2007; 134:2521–2531. [PubMed: 17522155]
- Spilde TL, Bhatia AM, Mehta SS, Hembree MJ, Preuett BL, Ostlie DJ, Prasad K, Li Z, Snyder CL, Gittes GK. Aberrant fibroblast growth factor receptor 2 signalling in esophageal atresia with tracheoesophageal fistula. *Journal of pediatric surgery.* 2004; 39:537–539. [PubMed: 15065023]
- Ura H, Murakami K, Akagi T, Kinoshita K, Yamaguchi S, Masui S, Niwa H, Koide H, Yokota T. Eed/Sox2 regulatory loop controls ES cell self-renewal through histone methylation and acetylation. *EMBO J.* 2011; 30:2190–2204. [PubMed: 21540835]

Williamson KA, Hever AM, Rainger J, Rogers RC, Magee A, Fiedler Z, Keng WT, Sharkey FH, McGill N, Hill CJ, Schneider A, Messina M, Turnpenny PD, Fantes JA, van Heyningen V, FitzPatrick DR. Mutations in SOX2 cause anophthalmia-esophageal-genital (AEG) syndrome. *Human molecular genetics*. 2006; 15:1413–1422. [PubMed: 16543359]