Advanced Review

Compartmentalization of the foregut tube: developmental origins of the trachea and esophagus

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The mammalian trachea and esophagus share a common embryonic origin. They arise by compartmentalization of a single foregut tube, composed of foregut endoderm (FGE) and surrounding mesenchyme, around midgestation. Aberrant compartmentalization is thought to lead to relatively common human birth defects, such as esophageal atresia (EA) and tracheoesophageal fistula (EA/TEF), which can prevent or disrupt a newborn infant's ability to feed and breathe. Despite its relevance to human health, morphogenesis of the anterior foregut is still poorly understood. In this article, we provide a comprehensive review of trachea and esophagus formation from a common precursor, including the embryonic origin of the FGE, current models for foregut morphogenesis, relevant human birth defects, insights from rodent models, and the emerging picture of the mechanisms underlying normal and abnormal foregut compartmentalization. Recent research suggests that a number of intercellular signaling pathways and several intracellular effectors are essential for correct formation of the trachea and esophagus. Different types of defects in the formation of either ventral or dorsal foregut tissues can disrupt compartmentalization in rodent models. This implies that EA/TEF defects in humans may also arise by multiple mechanisms. Although our understanding of foregut compartmentalization is growing rapidly, it is still incomplete. Future research should focus on synthesizing detailed information gleaned from both human patients and rodent models to further our understanding of this enigmatic process.

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INTRODUCTION

The trachea and esophagus derive from a single primordial tube yet quickly become structurally and functionally distinct vital organs. The trachea conducts air exchange between the lungs and the external environment, whereas the muscular esophagus pumps food and liquids from the mouth to the stomach. Defects in proper development of the trachea and esophagus, ranging from communications (fistulas) between the two to an absence of one or the other, profoundly disrupt feeding and breathing and are thus urgent surgical crises for the newborn infant.

Given its importance to physiology and morbidity, the manner by which the trachea and esophagus are formed is a critical issue to understand. Surprisingly, little is known about this process, but experimental animal models are revealing its nature. At early postimplantation stages, the embryonic development of rodents and humans is strikingly similar, and development of the foregut appears to be quite conserved.[1] The initial bifurcation of the common endodermal foregut tube occurs at midgestation, when lung buds bulge ventrally at a point just caudal to the pharynx and dorsal to the septating heart tube (Figure 1(a)). As the lung buds

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undergo elongation and branching morphogenesis to form the luminal architecture of the lungs, the foregut tube between the lung buds and the future larynx becomes compartmentalized into the trachea and esophagus. Each undergoes stereotypic patterns of endodermal and mesenchymal patterning and differentiation to generate the functional organs (Figure 1(c) and (d)). The tracheal mesenchyme must develop into C-shaped cartilage rings ventrally and the trachealis muscle dorsally. Its epithelium must become pseudostratified and correctly differentiated. The esophageal mesenchyme in turn must develop into smooth muscle, and the epithelium must become stratified. For these events to happen correctly, the single primitive foregut tube must first become two parallel tubes; this involves a process of compartmentalization that is surprisingly complex and remains poorly understood. In this article, we trace the common origins of the trachea and esophagus and review key advances and remaining challenges in our understanding of the developmental underpinnings of foregut compartmentalization.

NORMAL FOREGUT DEVELOPMENT IN MAMMALS

Formation of Foregut Precursors: Gastrulation to Gut Tube

Lineage tracing studies in the mouse have revealed the embryonic origins of foregut endodermal domains (Figures 2 and 3). Before gastrulation, the murine embryo consists of epiblast nestled in a 'cup' of primitive endoderm. The precursors to the foregut tissue are located in the posterior epiblast (Figure 2(a)), and as the primitive streak forms, these cells will move through it, acquire an endoderm or mesoderm fate, and begin to migrate anteriorly[2–8] (Figure 2(b)). As the anterior definitive endoderm (ADE) cells migrate further anterior, the ventral foregut endoderm (vFGE) precursors precede the dorsal foregut endoderm (dFGE) precursors (Figure 2(c)). The midline of the vFGE arises from the prechordal plate,[5,9] which itself forms the rostral terminus of the gut tube. The midline of the dFGE appears to arise primarily from the midline cells of the head process (the medial ridge of cells between the node and the prechordal plate), perhaps with a contribution from the node in more posterior regions.[10] The node also gives rise to trunk notochord.[2,11] The endoderm that becomes the lateral portions of the foregut tube arises from the ADE lateral to the midline, again with more ventral tissue arising from more anterior points, as diagrammed[5,8,9] in Figures 2 and 3. The origin of the accompanying foregut mesenchyme has not been explicitly studied, but generally its precursors are within the splanchnic mesoderm progenitors that are also migrating anteriorly during these stages. By early somite stages, the foregut precursors are in place, situated rostral to the anterior intestinal portal and dorsal to the developing heart tube[5,8] (Figures 1(a) and 3).

The anterior foregut tube forms as a result of the rostral folding-over and axial growth of the embryo, bringing the heart precursors to the ventral midline over a pocket of endoderm. The region of the endodermal tube that will compartmentalize into the trachea and esophagus is the segment adjacent to the heart. Immediately, caudal to these organs arise the lungs and stomach, respectively, which are also foregut derivatives. Further posterior, the midgut tube is formed via ventral closure of the embryo during turning morphogenesis, bringing the edges of the ventral endoderm to fuse at the midline.

Resolution of the Notochord from the dFGE

At headfold stages, the cells at the anterior midline contribute to both notochord and dFGE progenitors. Consequently, the precursors of trunk notochord cells at early somite stages are embedded within the dFGE. Between E8.25 and E9.5, the notochord cells resolve from the endoderm in a poorly understood process. The structural milestones of notochord resolution were best described in a histological study by Jurand.[13] First, the cells at the midline invaginate toward the neural tube and then form a rosette. This structure attaches to the floorplate of the neural tube and separates from the dFGE in an anterior–posterior wave, as the distance between the neural tube and foregut tube increases and the space fills with mesenchymal cells (Figure 2(h)–(k)). The cellular mechanisms behind this morphogenesis are largely uninvestigated, but it has been hypothesized that improper notochord resolution might impede later compartmentalization of the foregut tube.[14–17]

Current Models of Foregut Compartmentalization

By the time notochord resolution is complete, lung buds have begun to form on the ventral foregut (approximately E9.75 in mouse). The point at which this occurs is just caudal to the pharynx, specifically pharyngeal arch 6, and dorsal to the looped heart tube. From this point, the rostral foregut tube must resolve into the trachea and esophagus. While it is

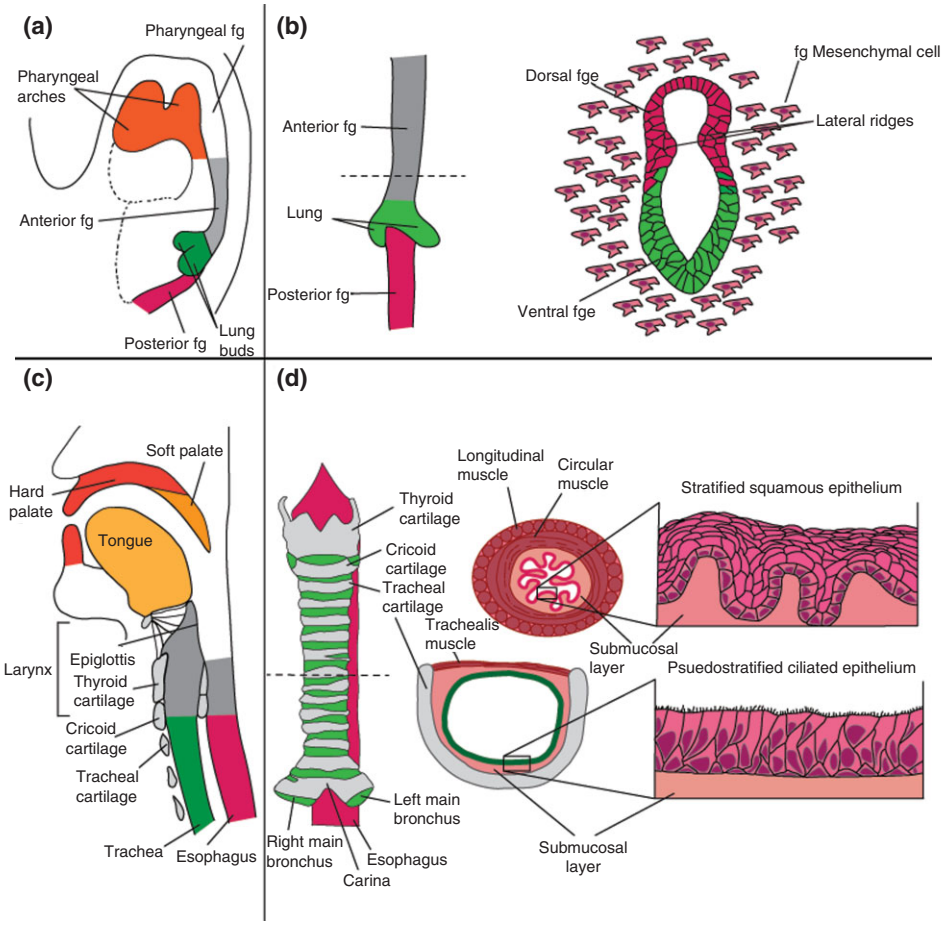

FIGURE 1 | Diagrammatic views of the normal anterior foregut. (a) Sideview of a midgestation embryo showing the anterior primitive foregut (fg) as a single tube with lung buds (green) emerging from the ventral foregut endoderm. (b) A 'whole-mount' view of an isolated foregut just before compartmentalization. A transverse section through such a foregut at the level of the dashed line shows the surrounding mesenchyme and foregut endoderm. In the septation model, the lateral ridges will meet to divide the dorsal (pink) and ventral (green) foregut endoderm (fge) in to the esophagus and trachea, respectively. (c) Sideview of an adult showing the most anterior part of the foregut. The epiglottis provides the normal barrier between the trachea and esophagus, blocking the trachea during swallowing to prevent aspiration of food and liquid. (d) Front view of the isolated trachea and esophagus. The dashed line marks the location of the transverse section to the right, depicting the differentiated structures of each tube. Enlarged views at the far right show the different cellular arrangements of the developed epithelia.

relatively easy to envision the process of dichotomous branching that generates the lungs, it is far more difficult to visualize the emergence of the *parallel* trachea and esophagus from a single tube. As direct imaging of foregut compartmentalization as it happens in a living embryo has yet to be reported, the underlying mechanisms are still an area of debate and investigation.

Currently, there are three distinct models for the formation of the trachea and esophagus from a common primordial tube: (1) outgrowth, (2) mesenchymal 'watershed', and (3) septation.[18–23] In the outgrowth model (Figure 4), the trachea simply buds off the foregut, with the tracheal bud elongating to form the respiratory tube from larynx to lungs.[18,19] In this scenario, the common foregut tube *per se* would then develop into the esophagus, with the ventral outgrowth forming the trachea. At first blush, this mechanism seems reasonable, considering that many other gut-derived organs bud from the gut tube.[24–26] However, many experimental findings seem at odds with this possibility. For example, if the trachea grows out of the foregut tube, one would expect significantly more proliferation in the emerging tracheal endoderm as compared to the esophagus, but this has not been reported. Instead, the early expression of respiratory genes in the ventral half of the early FGE suggests that the entire ventral half of the rostral foregut tube will give rise to the trachea as well as the lungs[27] (Figures 1 and 4).

An alternative to the outgrowth model is the mesenchymal 'watershed' model. In this scheme

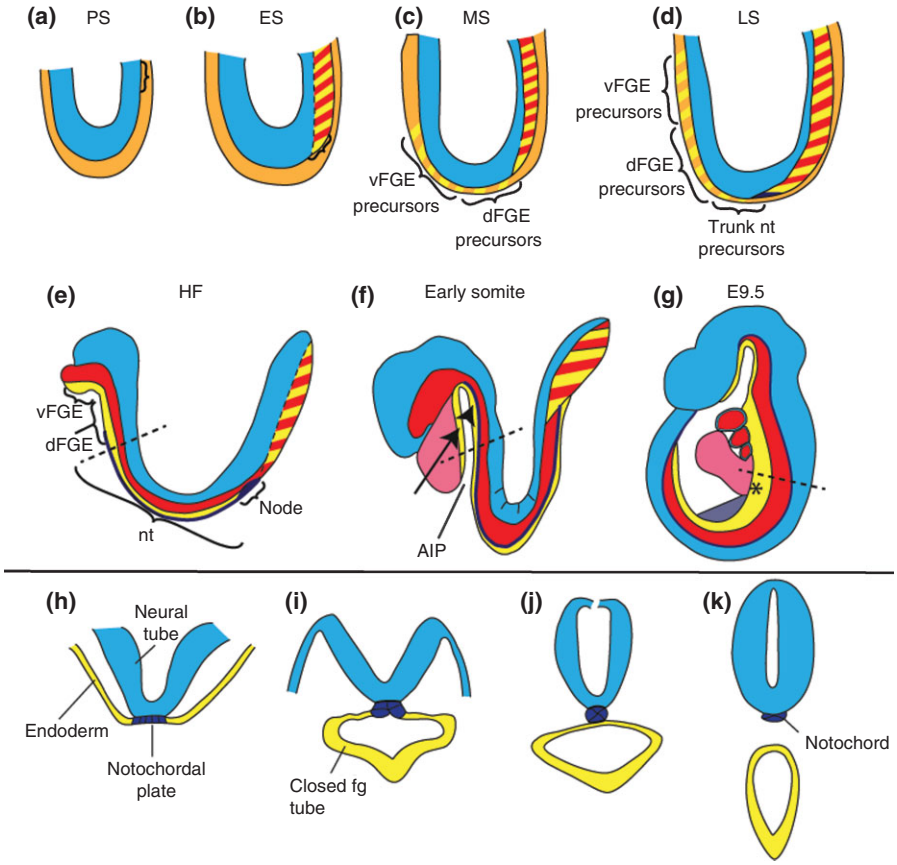

FIGURE 2 | Early development of the anterior definitive endoderm and notochord. (a) Precursors of the anterior definitive endoderm (bracket) reside in the posterior epiblast (blue) at prestreak (PS) stages, just before gastrulation. (b) After passing through the anterior primitive streak (yellow and red stripes) at the early streak (ES) stage, foregut endoderm precursors migrate anteriorly, displacing visceral endoderm (orange) by intercalation.[12] (c) At mid-streak (MS) stage, the ventral foregut endoderm (vFGE) precursors precede those of the dorsal foregut endoderm (dFGE). (d) By the late streak (LS) stage, the node (purple), the origin of trunk notochord, is forming just posterior to the dFGE precursors. (e) At early headfold (EHF) stage, the vFGE precursors are at the most anterior region of the embryo and cells from the node have become embedded within the dFGE at the midline as presumptive notochord (nt), seen in cross section in panel (h). (f) By early somite stages the FGE precursors (arrow, vFGE; arrowhead, dFGE) are all rostral to the anterior intestinal portal (AIP), and the notochord is resolving from the dFGE [cross-section panel (i)]. (g) At E9.5, the gut tube is fully closed [asterisk (*) represents future site of lung bud formation], and the notochord is completely resolved from the endoderm. (h–k) Cross sections of embryos shown above to depict foregut and notochord morphology (information compiled from Ref[2–8,13]).

(Figure 4), the mesenchyme that initially lies at the junction of the emerging lung buds and the foregut tube acts as a fixed wedge or 'watershed', and the growing foregut tube is displaced to either side of it as new tissue is added to the nascent trachea or esophagus.[21] This model allows for similar levels of proliferation throughout the growing foregut.

Importantly, neither the outgrowth nor the watershed models involve shortening of the foregut tube rostral to the point of lung bud emergence. Ioannides et al.[28] measured the length of the divided and undivided mouse foregut at intervals during compartmentalization and found that in normal embryos the absolute length of the undivided portion does, in fact, decrease. Such results support a third model, septation. Here, a septum forms at the lung buds as they emerge from the vFGE (Figure 4). The septum then moves rostrally, dividing the dorsal and ventral portions of the foregut tube into the esophagus and trachea, respectively.[22] Although this model has been accepted widely in the field for many years,[23] Sasaki et al.[21] were unable to find any 'evidence of a septum' using computer software to reconstruct histological sections into three-dimensional models. Nonetheless, in the undivided foregut, many investigators have referred to lateral ridges of foregut epithelium that appear to grow together, essentially forming a transient septum at the point of contact (Figure 1) as the two distinct tubes form. In this model, the transient point of contact would progress rostrally from the level of the lung buds to the larynx. Overall, given that there

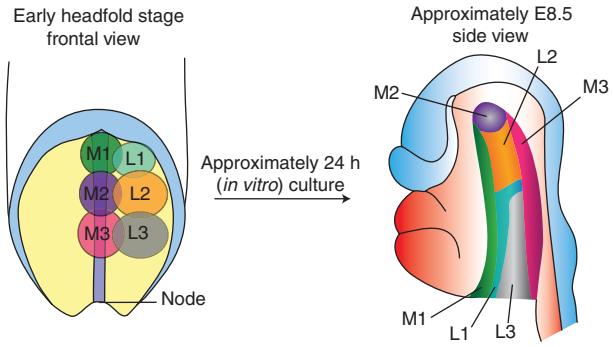

FIGURE 3 | Fate map of the anterior foregut endoderm from early headfold to midsomite stages. This figure summarizes findings from experiments in which anterior definitive endoderm (ADE) cells were fate mapped at the early headfold (EHF) stage and assessed after 24 h of culture.[5,8,9] The color code shows where the descendents are found at E8.5 for a given domain of EHF endoderm cells. Cells at the midline of the EHF embryo (M1–M3) end up at the midline of the foregut tube. M1 becomes largely medial ventral foregut endoderm (vFGE), M2 contributes to the terminus of the anterior FGE, and M3 becomes medial dorsal foregut endoderm (dFGE). L1–L3 domains contribute mostly to regions just lateral to their medial counterparts.

is a large amount of growth occurring during the compartmentalization process, none of these models may be absolutely accurate, but more work is needed in any case to determine the actual morphogenetic process(es).

FOREGUT COMPARTMENTALIZATION DEFECTS IN HUMANS

A large variety of birth defects involve aberrant foregut compartmentalization or morphogenesis of the trachea or esophagus. Relatively rare defects include laryngotracheoesophageal (LTE) clefts (large, continuous regions of communication between the larynx, trachea, and esophagus), tracheal atresia, isolated esophageal stenosis, and isolated tracheo- (or bronchio-) esophageal fistulas[29–33] (Figure 5). The more common types of congenital foregut defects include EA, and these occur at about 1 in 3500 human births.[32] Gross[34] separated the variations of EA into four subtypes (A–D) depending on the presence and location of an accompanying TEF (Figure 5). By far, the most common is Type C, which consists of proximal EA with distal TEF; for example, it accounted for 86.5% of 1058 reported cases of EA in a large study.[35] Such defects were first reported in the literature as early as 1670,[36] but during the early decades of the 20th century they gained significant attention as birth defects that could be repaired with new surgical techniques.[36–38] Until 1944, the

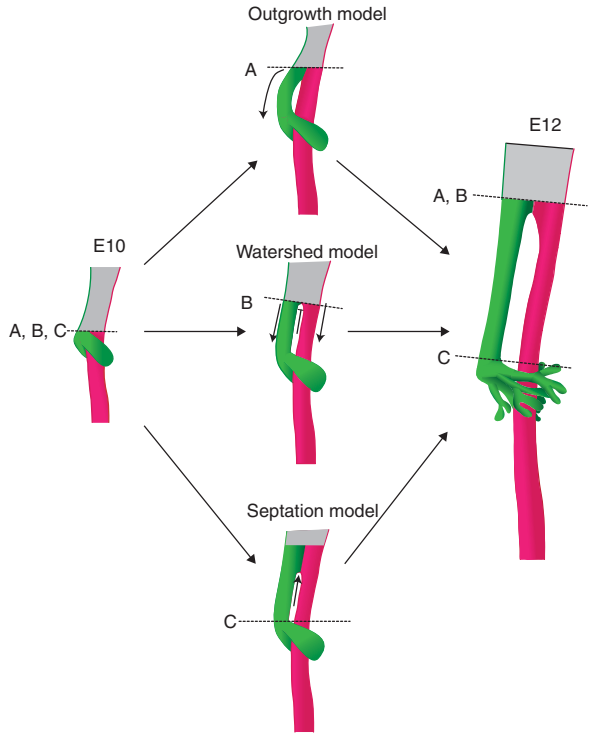

FIGURE 4 | Three models for foregut compartmentalization. At E10 in the mouse, the anterior foregut endoderm is a single tube with nascent ventral lung buds at the level of dashed line 'A, B, and C'. The 'outgrowth' model (top) states that the trachea grows rapidly out of the single foregut tube following the lung buds; dashed line (the original level of the lung buds) 'A' stays at the level of tracheal/esophageal divergence.[18,19] The 'watershed' model (middle) suggests that both the esophagus and trachea elongate and separation is maintained by mesenchyme just caudal to the point of divergence; dashed line 'B' remains at the level of tracheal/esophageal divergence.[20] In the 'septation' model (bottom) the lateral ridges (Figure 3) form a septum that travels up the foregut, dividing the ventral and dorsal domains into the trachea and esophagus, respectively; dashed line 'C' remains a the level of the main bronchi.[21,22] By E12, when compartmentalization is largely complete, the original location of the lung buds (dashed lines A, B, and C) is at the caudal larynx in the case of the 'outgrowth' or 'watershed' model but at the level of the main bronchi in the case of 'septation'. Pink, esophagus/alimentary; green, trachea/respiratory.

mortality rate of infants with EA was 100%. Now it is <10% and death is usually attributable to other associated congenital anomalies.[39] Despite these successes, individuals with surgically corrected foregut anomalies typically endure gastric and/or pulmonary complications throughout life.[40]

Defects in Other Organs Are Often Associated with EA/TEF

The occurrence of foregut compartmentalization defects has been associated with a number of syndromes and malformations, as listed in Table 1.[41]

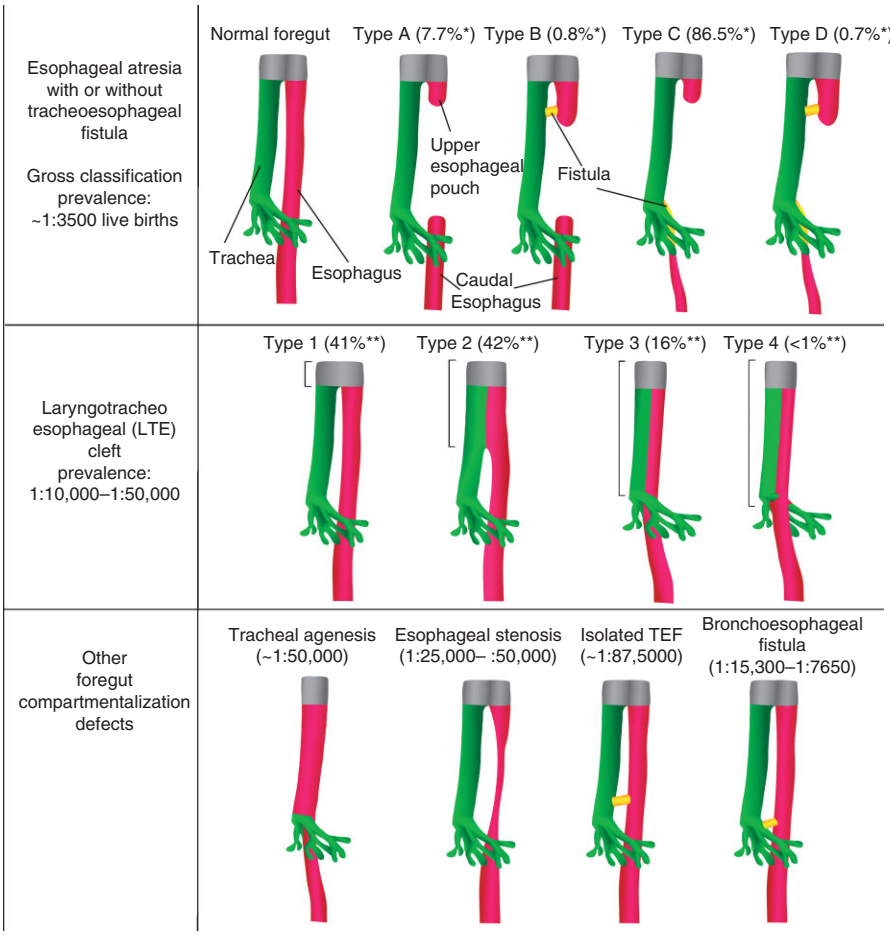

FIGURE 5 | The spectrum of human foregut compartmentalization anomalies. (Top) Three-dimensional representations of Gross Types A–D morphological classifications of esophageal atresia (EA), as evidenced by an upper esophageal pouch, with or without fistula. Type A: isolated EA. Type B: EA with proximal tracheoesophageal fistula (TEF). Type C: EA with distal TEF. Type D: EA with proximal and distal TEF.[32] *Percent incidence among cases of EA/TEF (including H-type isolated fistula).[35] (Middle) Laryngotracheoesophageal (LTE) clefts are relatively rare malformations that involve large continuous regions of communication between the larynx or trachea and esophagus.[29] The extent of the communication in each type is illustrated by brackets. **Percent incidence among cases of LTE clefts.[29] (Bottom left–bottom right) Tracheal agenesis involves the absence of a trachea where lungs sometimes arise from the esophagus;[30] Esophageal stenosis (narrowing) occurs both on its own (two-third of cases) and in combination with EA;[31] Isolated (H-type) TEF;[35] Bronchoesophageal fistula is rare and usually occurs with EA.[33]

Overall, EA/TEF defects are accompanied by other abnormalities in various organ systems 48% of the time, with congenital heart defects and other gastrointestinal anomalies being most common.[35] For example, the VATER/VACTERL association has a 24% incidence of EA/TEF.[42] This association has a broad involvement of malformations of gut derivatives, suggesting a general endodermal problem. The association of EA/TEF with heart malformations likely reflects that foregut compartmentalization and key events in cardiac development are happening in very close proximity (on either side of the ventral splanchnic mesoderm) and in the same temporal window. Thus, it is likely that malfunctions of a common genetic pathway and/or a common precursor tissue might jointly impact development of these organs.

Genes and Pathways Linked to Human EA/TEF

Only a few single genes or pathways have been linked thus far to human EA/TEF (Table 1). In particular, Feingold syndrome involves mutations in *MYCN* (formerly known as *NMYC*) and is likely the most frequent cause of familial EA/TEF, which occurs in 40% of Feingold syndrome patients.[53] CHARGE syndrome patients also present with a 10% incidence of EA/TEF. Sixty percent of CHARGE syndrome patients are haploinsuffient for the chromodomain helicase DNA-binding (*CHD7*) gene.[45] Finally, loss-of-function mutations in *SOX2* have been found in individuals with anophthalmia-esophageal-genital (AEG) syndrome.[1] Unfortunately, the vast majority

TABLE 1 | Human Genes, Syndromes, Associations, and Chromosomal Aberrations Associated with Foregut Compartmentalization Anomalies

Condition	Associated Loci	Prevalence of GI Atresia, TEF, and Laryngeal Clefts	Additional Comments
Feingold syndrome	*MYCN*	30–40% EA/TEF[41]	Most common cause of familial GI atresias[41]
CHARGE syndrome	*CHD7*	10% EA/TEF[41]	
AEG syndrome	*SOX2*	100% EA (OMIM)	EA is a basic diagnostic feature of this syndrome
Pallister–Hall syndrome	*GLI3*	Rare laryngeal clefts in severely affected patients (OMIM)	
Opitz syndrome	*MID1* (X-linked) *TBX1**	44% EA/TEF[43]	
Fanconi anemia	*FANCA* *FANCC* *BRCA2* *FANCD2* *FANCG* *FANCB*	14% GI anomalies[41]	Associated with VACTERL anomalies[41]
VACTERL association	FOX GENE CLUSTER *HOXD13* *ZIC3* *PTEN*[44]	~24% EA/TEF ~42% GI atresias[45]	
Goldenhar syndrome	Heterogeneous (OMIM)	Sporadic; potentially underreported[46]	
17q22q23.3 deletion	*NOG**	4/5 reported individuals with EA/TEF[47]	Mutations in *NOG* don't necessarily cause EA/TEF[47]
Distal 13q deletion	*ZIC2**	Very rare[48]	Associated with VACTERL anomalies[48]
Trisomy 13 (Patau syndrome)	—	Very rare EA/TEF[49,50]	
Trisomy 18 (Edwards syndrome)	—	13%[51]	
Trisomy 21 (Downs syndrome)	—	0.5–1% EA[52]	

*genes of interest in the affected region.

(90%) of infants with EA/TEF do not fit into a defined syndrome or association,[41] and this apparent lack of a common cause likely means that EA/TEF can arise by multiple mechanisms. In an attempt to better understand what those mechanisms might be, researchers have turned to animal models.

THE ADRIAMYCIN RODENT MODEL FOR THE PATHOGENESIS OF FOREGUT MALFORMATIONS

Evaluation of the potential teratogenic effects of the anticancer drug Adriamycin (doxorubicin) revealed a teratogenic effect in the rat when administered early in gestation, inducing a high incidence of EA, intestinal atresia, and TEF, in addition to other anomalies.[54] Subsequently, Adriamycin was optimized for use in the rat as a teratogenic model of EA/TEF.[55] Administration of Adriamycin to pregnant dams on gestational days 8 and 9 (E6.5–7.5 mouse, E13–18 human—see Table 2 for an embryo stage comparison) resulted in a 41.2% incidence of EA with or without TEF.[55] The most common type of foregut malformation upon Adriamycin treatment is Gross Type C (~90%), and other combinations of EAs, tracheal atresias, and TEFs occur at much lower rates (<3%)[56] (Figure 6(c) and (d)). Adriamycin treated rats/mice also display defects in other tissues and organs that closely represent the VATER/VACTERL association. These include cardiovascular defects, vertebral defects, various gut atresias, tracheomalacia, anorectal anomalies, and renal anomalies.[55–63] Because of the striking similarity to human birth defects, maternal Adriamycin administration to rats and mice has been a widely studied model for the developmental biology of EA/TEF.

TABLE 2 | Comparison of Human, Rat, and Mouse Development by Stage and Days Post Fertilzation (dfp)

DD/Somite #	Mouse dpf	Theiler Stage	Rat dpf	Witschi Stage	Human dpf	Carnegie Stage
PS	6	9a	7.75	11	~13	5
ES	6.5	9b	8.5	12	~17	6
MS	6.75	10a	8.5	12	~17	6
LS-OB	7	10b	8.5	12	~18	6
OB-EB	7.25	10c	9	13	~19	7
EB	7.5	11a	9	13	~19	7
LB	7.5	11b	9	13	~23	8
EHF	7.75	11c	9	13	~25	9
LHF	7.75	11d	9	13	~26	9
1–4	8	12a	9.5	14	~27	9
5–7	8.25	12b	10	15	~28	10
8–12	8.5	13	10	15	~28	10
13–20	9	14	10.5	16	~29	11
21–29	9.5	15	11	17–18	~30	12
30–34	10	16	11.5	19–20	~32	13
35–39	10.5	17	12	21–23	~33	14
40–44	11	18	12.5	24–26	~36	15
45–47	11.5	19	13	27	~39	16

DD, Downs and Davies stages; PS, prestreak stage; ES, early streak stage; MS, mid-streak stage; LS, late streak stage; OB, no allantoic bud stage; EB, early allantoic bud stage; LB late allantoic bud stage; EHF, early headfold stage; LHF, late headfold stage.

Adriamycin is an anthracycline antibiotic and is thought to act as a chemotherapy agent via intercalating into DNA, inducing DNA-damage through DNA topisomerase II, causing free-radical formation, and ultimately inducing apoptosis.[75,76] Accordingly, an early hypothesis was that Adriamycin causes decreased cell proliferation and/or excessive cell death in the developing embryo, leading to EA/TEF and other fetal malformations. No clear-cut spatiotemporal domains of proliferation have been associated as yet with normal foregut compartmentalization or with its anomalies in the Adriamycin model, but further research is required to address this possibility.[77] In contrast, investigators have found consistently that there are normally high levels of apoptosis in the lateral ridges of foreguts of untreated embryos as they undergo compartmentalization. In Adriamycin-treated embryos, these levels are significantly reduced.[28,78–80] Therefore, it has been supposed that loss of cell death might contribute to EA/TEF. However, Ioannides et al.[28] recently showed that mouse mutants whose cells are unable to undergo apoptosis (*Apaf1* null mutants) nevertheless form proper esophageal and tracheal tubes, suggesting that regulation of apoptosis is not a critical parameter of foregut compartmentalization.

A high percentage of Adriamycin-exposed embryos display notochord abnormalities, in which the notochord remains sporadically attached to the gut endoderm and is abnormally large and disorganized[58] (Figure 7). In one study, among rat embryos that had either abnormality at E13, 31% had only abnormal notochords, 18% had only EA/TEF, and 50% had both.[15] Multiple investigators have suspected that the notochord abnormalities are important in the pathogenesis of EA/TEF, and perhaps also in the entire VATER/VACTERL association.[14–17,28,80–82] One model is that attachment of the notochord to the endoderm, coupled with the differential growth of each, causes traction on the foregut resulting in atresia[14] (Figure 7). A second potential explanation is that close proximity of the notochord to the endoderm results in the exposure of the endoderm to excessive levels of signaling from the notochord, and this mispatterns the foregut before compartmentalization.[16,80] Interestingly, Gillick et al.[81] and Merei[82] also looked at the coincidence of notochord abnormalities and other gut atresias, and discovered that midgut and hindgut atresias were present wherever the notochord was disrupted.

There is also evidence from the Adriamycin model to suggest that dorsal/ventral patterning of

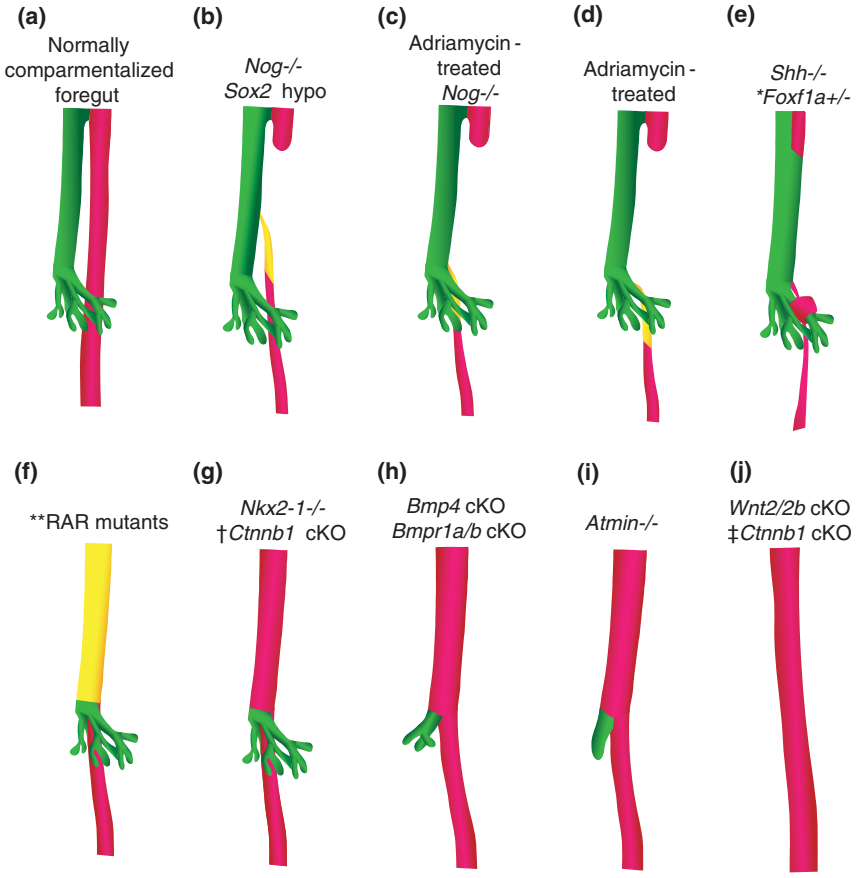

FIGURE 6 | Three-dimensional representation of rodent model foregut compartmentalization defects. Green shading represents respiratory tract tubes, pink shading represents digestive tract tubes, and yellow shading represents tubes with mixed respiratory and digestive characteristics. (a) Normally compartmentalized foregut. (b) Foregut defect found in *Nog* null and *Sox2* hypomorphic mutants, resembling Gross Type C esophageal atresia/tracheoesophageal fistula (EA/TEF) with a high distal fistula.[64,65] (c) Defect found in Adriamycin-treated, $Nog^{-/-}$, and $Gli2^{-/-}$, $Gli3^{-/-}$ animals. This phenotype resembles Gross Type C EA/TEF with a low distal fistula arising from the carina.[55,64,66] (d) Defect found in Adriamycin-treated animals; resembles Gross Type C EA/TEF with a fistula arising from the left main bronchus.[55] (e) Defect found in $Shh^{-/-}$ and $Foxf1a^{+/-}$ mutants; the rostal foregut is improperly partitioned into the trachea and esophagus.[67,68] In $Shh^{-/-}$ (*but perhaps not in $Foxf1a^{+/-}$) mutants, the fistula arises from the level of the carina, merges with the left lobe of the lung forming a cyst-like structure, and remerges to connect to the stomach. (f) Defect found in *RAR* mutants. There is no compartmentalization of the foregut, and the identity of the foregut tube is not known**; may resemble tracheal agenesis or Type 3 laryngotracheoesophageal (LTE) cleft.[69] (g) Defect found in $Nkx2\text{-}1^{-/-}$, *Ctnnb1* cKO† ($Foxg1^{tm1(cre)Skm}$; $Ctnnb1^{tm2.1Kem/-}$) conditional knock-out (cKO), and *Bmpr1a/b* cKO ($Shh^{tm1(EGFP/cre)Cjt}$;$Bmpr1a^{tm2.1Bhr/tm2.2Bhr}$; $Bmpr1b^{tm1Kml}$). There is no compartmentalization of the foregut, which has lost all/most respiratory specification (essentially tracheal agenesis) and varying degrees of abnormal lung development.[67,70] (h) Defect found in *Bmp4* ventral foregut cKO mutants ($Shh^{tm1(EGFP/cre)Cjt}$; $Bmp4^{tm3.1Blh/tm2Blh}$); resembles tracheal agenesis.[67,71] (i) Defect found in $Atmin^{-/-}$ mutants; resembles tracheal agenesis with low degree of lung development.[72] (j) Defect found in *Wnt2/2b* double-knockout mutants and *Ctnnb1* cKO‡ ($Shh^{tm1(EGFP/cre)Cjt}$; $Ctnnb1^{tm2.1Kem}$); resembles tracheal agenesis with no lung development.[73,74]

the foregut tube is abnormal in embryos with EA/TEF. For example, Ioannides et al.[84] showed abnormal expression of *Shh*, a gene important for foregut development, in treated foreguts (see below). In normal embryos *Shh* is expressed in the vFGE before compartmentalization, then switches to the dFGE (esophagus) during compartmentalization; but in Adriamycin-treated embryos, *Shh* expression is diffuse throughout the FGE and never shifts dramatically. Moreover, the caudal esophagus of Adriamycin-exposed embryos with EA/TEF also expresses *Nkx2-1* (formerly known as *Ttf-1*), which is normally expressed only in the thyroid, trachea, and lungs.[85,86]

Consistent with patterning irregularities, the caudal esophagus (fistula tract) of Adriamycin-exposed embryos with EA/TEF sometimes contains pseudostratified respiratory epithelium, which variably transitions to stratified squamous epithelium nearer to the stomach.[56] Additionally, cartilage nodules have been observed on the wall of the fistula tract.[56] As it was always assumed that this tube was

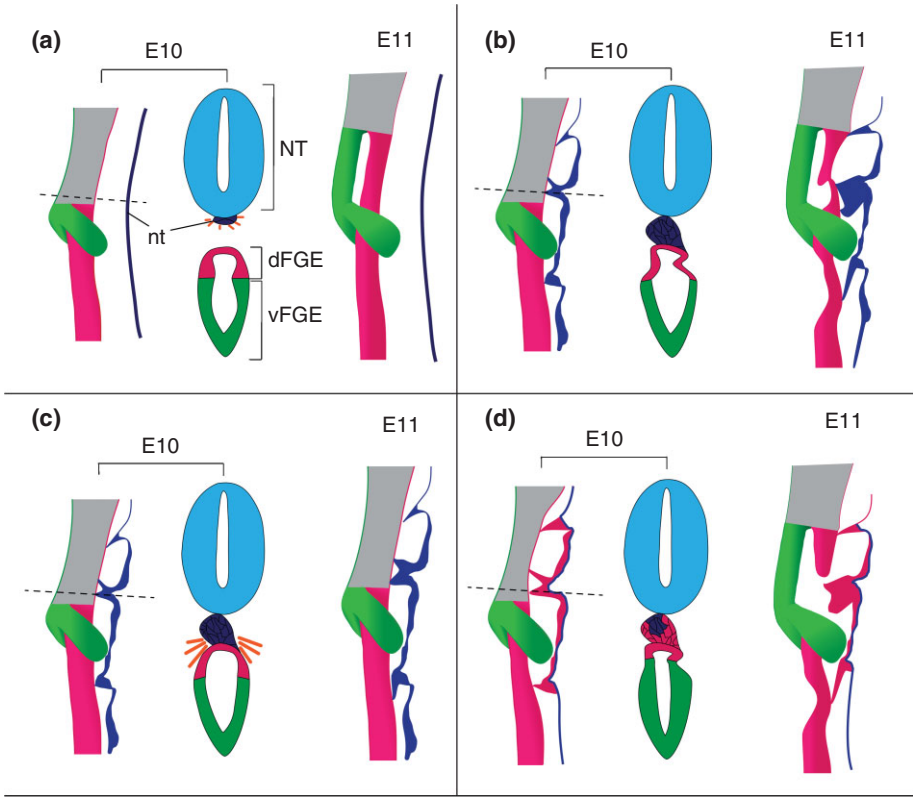

FIGURE 7 | Models for how abnormal development of the notochord might influence foregut compartmentalization. In these diagrams, the following color code is used: gray, uncompartmentalized region of foregut endoderm (FGE); green, respiratory/vFGE; pink, digestive/dFGE; blue, neural tube (NT); dark purple, notochord (nt); orange lines, Hh signals emanating from notochord. (a) Normal foregut and notochord before (E10) and during (E11) foregut compartmentalization in the mouse. A transverse section shows the FGE, notochord, and neural tube at E10. (b) Improper resolution of the notochord from dorsal endoderm causes the notochord to remain tethered to the endoderm. As the foregut and notochord may grow rostrocaudally at different rates, tension between the notochord and foregut could distort the foregut to the point of causing discontinuity/atresia.[14] (c) Improper resolution of the notochord causes large regions of *Shh*-expressing notochord to be in close proximity to the foregut, potentially disrupting patterning and morphogenesis cues and leading to complete compartmentalization failure, as shown here, or EA/TEF (not shown).[16,17,65,80] (d) Improper resolution of the notochord from endoderm causes cells that normally become dorsal foregut endoderm (dFGE) to remain associated with the resulting 'notochord structure', which then contains both notochord and dFGE cells. This leaves the dFGE with too few cells to form an esophagus upon septation.[83]

the caudal portion of the esophagus, the discovery that at least the upper portion had tracheal characteristics came as some surprise. Nevertheless, this finding further supports the validity of the Adriamycin model, because the 'caudal esophagus' of human patients often has respiratory characteristics.[87–89] A potential explanation is that the fistula tract is actually derived from the trachea as a third bronchus, that then fuses with the stomach; in this scenario, the esophagus is completely absent.[85] The origin of the upper esophageal pouch has also been called into question. Beasley et al.[90] reported that there is no evidence of an upper esophageal pouch until as late as E15.25 in the Adriamycin-treated rat, and suggested that it actually arises independently from the epithelium of the pharynx. All of these observations bring into question the mechanisms behind normal foregut compartmentalization and how Adriamycin might act to disrupt it to produce EA/TEF.

MOUSE MOLECULAR GENETIC MODELS FOR FOREGUT COMPARTMENTALIZATION AND ITS DEFECTS

Nkx2-1 and Sox2: Key Factors and Domain Markers for the Anterior Foregut Endoderm

Two transcription factors, *Nkx2-1* and *Sox2*, have been identified as markers and essential developmental factors for dorsoventral regions within the anterior FGE. The respiratory epithelial factor *Nkx2-1* is expressed specifically in the vFGE before foregut compartmentalization and is essential for this

process.[70] In addition, it directly activates the promoters of a several respiratory-specific genes and is essential for differentiation of various cell types in the trachea and lungs.[91,92] *Nkx2-1* null mice die shortly after birth because of respiratory failure,[93] with short, dilated 'tracheas' that connect the pharyngeal region to the stomach and from which the lung buds emerge. The phenotype is described as being similar to 'complete TEF' in humans[93] (Figure 6(g)). In the most anterior part of the common foregut tube, there is evidence of both esophageal and tracheal character, including disorganized ventral cartilage. The most posterior part of the foregut is apparently esophageal. Despite some tracheal characteristics, the common foregut tube of *Nkx2-1* null embryos is indeed 'dorsalized', with high levels of expression of the esophageal markers SOX2 and TCP1 (formerly known as P63) and the presence of circumferential smooth muscle up to nearly the most rostral regions of the foregut.[64]

SOX2 is an HMG-domain transcriptional regulator with evolutionarily conserved expression in the foregut epithelium (high in the esophagus and low in the trachea), and linked to EA in humans (see Genes and Pathways Linked to Human EA/TEF).[1] *Sox2* expression is enriched in the dFGE before compartmentalization.[1,64] Because complete loss-of-function in the mouse results in pregastrulation death of the embryo,[94] its role in esophageal development has remained poorly understood. Use of hypomorphic alleles has shown that it plays roles in both foregut compartmentalization and differentiation. Que et al.[64] showed that embryos with a hypomorphic and a null allele of *Sox2* had a 60% penetrance of EA/TEF (Figure 6(b)). Further characterization of the phenotype revealed that the fistula tract had tracheal characteristics, including the presence of tracheal cartilage and expression of *Nkx2-1*. Significantly, in the 40% of mutants without EA/TEF, the esophagus never expressed *Nkx2-1*. The same group also used *Nkx2-5*$^{tm1(cre)Rjs}$ (*Nkx2-5Cre*) to delete *Sox2* specifically in the vFGE.[95] While only 10% of these embryos had EA/TEF, 60% had short tracheas with long main bronchi and disorganized tracheal cartilage. It has recently been shown that *Sox2* expression is repressed by bone morphogenetic protein (BMP) signaling in the ventral anterior foregut to allow induction of respiratory fate by ventral WNT signaling.[71]

WNT Signaling and Respiratory Specification of the Foregut Epithelium

WNTs are secreted glycoproteins with diverse roles in multiple organ systems during development, homeostasis, and disease.[96,97] Briefly, the canonical pathway involves the stabilization of β-catenin (*Ctnnb1*) in the cytoplasm and its eventual translocation into the nucleus where it binds transcriptional repressor TCF/LEF and initiates target gene transcription.[98] The canonical WNT pathway is known to play important roles in the specification and patterning of gut-derived tissues.[73,74,99–103] *Wnt2* and *2b* are expressed in the ventral foregut mesenchyme and signal to the vFGE.[74] While *Wnt2* null mice have severe lung hypoplasia, *Wnt2/2b* double null mutants have complete lung/trachea agenesis, and *Nkx2-1* is absent from the respiratory primordium[74] (Figure 6(j)). Deletion of *Ctnnb1* throughout the foregut epithelium and mesenchyme resulted in an overall shortening and failed compartmentalization of the foregut[67] (Figure 6(g)). Similarly, use of two different Cre drivers to delete *Ctnnb1* in the vFGE resulted in tracheal agenesis (with[67] or without[73] lung buds) with a loss of *Nkx2-1* expression specifically in the respiratory domain of the foregut, which instead expressed *Sox2*[73] (Figure 6(j)). Conversely, constitutive activation of *Ctnnb1* in the anterior vFGE resulted in significant expansion of the *Nkx2-1*-positive respiratory domain, including the upper stomach epithelium and most of the presumptive esophagus.[73,74] Thus, WNT/β-catenin signaling is necessary and sufficient to induce respiratory cell fate in anterior FGE, as long as *Sox2* is appropriately repressed by BMP signaling.[71]

BMP Signaling and Its Antagonism

The BMP signaling pathway is another major developmental pathway important for the correct compartmentalization of the foregut. BMP4 and BMP7 are the predominate BMPs expressed in the foregut region from E8.5 to E11.5.[65,104] The action of BMP ligands from the ventral foregut mesenchyme upon the FGE is functionally counteracted by the dorsal expression and secretion of BMP antagonists, noggin (*Nog*), and chordin (*Chrd*).[65,105,106] It is reduction of BMP antagonism that causes phenotypes resembling EA/TEF. *Nog* null mutants have a 75% incidence of EA/TEF and at least a 66% incidence of notochord abnormalities. Specifically, the EA/TEF is Gross Type C with the fistula arising somewhere between the rostral atresia and main bronchi,[65,83] as illustrated in Figure 6(b) and (c).

In many ways, the *Nog* null foregut and notochord phenotypes seem to resemble those of the Adriamycin model (see above). There is evidence that the fistula tract has tracheal characteristics, namely cartilage nodules and *Nkx2-1* expression.[65] The common foregut tube also expresses *Nkx2-1* and has

normal tracheal cartilage rings suggesting that, in this model, the esophagus is effectively missing. This could be due to mispatterning of the dorsoventral foregut boundaries, or because of physical loss of dFGE. Que et al.[65] showed that at E9.5, *Shh* expression in the FGE appears expanded to encompass the dorsal foregut. They also showed an apparent dorsal shift/expansion of *Foxf1a* (formerly known as *Foxf1)* expression in the FGE and mesenchyme, and of *Nkx2-1* in the endoderm. However, Li et al.[83] pointed out that while there is certainly a reduction in *Nkx2-1*-negative dorsal endoderm, the *Nkx2-1*-positive ventral domain is not actually larger. This supports the hypothesis that dFGE is deficient. They proposed an intriguing model in which failure of proper notochord resolution from the dFGE results in a large disorganized notochord, as well as a reduced dorsal foregut (Figure 7(d)). They suggested that this reduction leaves an inadequate amount of tissue to create an esophagus when the foregut undergoes septation, resulting in either severe esophageal stenosis (seen in 6/33 mutants[83]) or EA/TEF. In support of their model, they showed that the notochord in *Nog* mutants contains some cells that do not express the notochordal maker, *brachyury (T)*.

While disruption of BMP antagonists causes foregut compartmentalization phenotypes like EA/TEF, loss of *Bmp4* or *Bmpr1a/b* from the ventral foregut results in tracheal agenesis.[67,71] The *Bmp4* conditional KO phenotype, at a gross scale, looks similar to some descriptions of EA/TEF at early stages. There is a single tube connecting the pharynx with the stomach, and from which the lung buds emerge. However, there is no evidence of an upper esophageal pouch, and no 'fistula', but rather a short, rudimentary trachea emerging from the ventral foregut from which the main bronchi emerge. Evidence that this is, in fact, tracheal agenesis comes from the identity of the contiguous foregut tube. It does not express *Nkx2-1*, but instead expresses esophageal marker *Pax9*. Furthermore, its surrounding mesenchyme resembles that of an esophagus rather than a trachea. The authors showed that the phenotype might be due to significantly decreased proliferation of both the ventral mesenchyme and epithelium at E9.5. Additionally, immunohistochemistry for phosphorylated MAPK1/3 (formerly known as ERK1/2) showed that there is a loss of ventral RAS/MAPK pathway activation at E9.25, but it is not known exactly how, or if, this loss contributes to the phenotype. Loss of BMP receptors 1A and 1B from the ventral endoderm results in a similar phenotype with reduction of *Nkx2-1* and expansion of dorsal markers *Sox2* and *Tcp1*. Importantly, when BMP signaling is prevented in the anterior foregut, activation of WNT signaling is no longer sufficient to induce respiratory fate.[71]

Hedgehog Signaling

Hedgehog (Hh) intercellular signaling was one of the earliest signaling pathways to be associated with foregut compartmentalization. Briefly, when Sonic hedgehog (SHH) ligand binds its receptor, patched, repression of smoothened is relieved and target gene transcription is mediated through GLI1,2, and 3.[107] *Shh* is expressed in the ventral FGE and signals to the ventral endoderm and mesoderm until foregut compartmentalization, at which point expression shifts to the nascent esophageal epithelium. At E11.5, *Shh* null mice have a single foregut tube that connects to the stomach, and from which the under-developed lung buds emerge. From the level of the lung buds to the stomach, the foregut tube is dilated and appears to allow large spaces of communication between the developing bronchial lumen, the foregut tube, and the stomach. By E17.5, the overall structure of the foregut appears distorted and complex. Anteriorly, there are places where the 'esophagus' and 'trachea' are just barely partitioned, but the morphology of these tubes and their associated mesenchyme is severely abnormal. Near the lungs, there is no distinct esophagus, but epithelium similar to the mucosal lining of the stomach is present in a distinct region of the lung epithelium. It appears almost as if what was supposed to be esophageal epithelium was not partitioned correctly and became incorporated into the lung. From this mucosal epithelium, a tube arises from the lung to join with the stomach[108] (Figure 6(e)).

The GLI transcription factors are also required for foregut development. Whereas *Gli2* null mice have relatively mild lung defects and a hypoplastic trachea and esophagus, $Gli2^{-/-};Gli3^{+/-}$ mice have a severe lung phenotype and delayed separation of the foregut tube into the trachea and esophagus.[66] $Gli2^{-/-};Gli3^{-/-}$ mice show no separation of the foregut tube into the trachea and esophagus (Figure 6(c)). This results in a phenotype that closely resembles EA/TEF, with an upper esophageal pouch and lung buds arising from the single foregut tube. Interestingly, at E9.5, the primitive foregut tube appears quite small, as compared to wildtype, which may suggest that an earlier endodermal defect precedes the failure of foregut compartmentalization. This notion is supported by the reduction in *Foxa2* expression in the foregut of E9.5 $Gli2^{-/-};Gli3^{-/-}$ embryos.[66]

Foxf1a is a target of Hh signaling in the mouse foregut, as shown by its local up-regulation in tissue culture when SHH-coated beads are present.[68] While

Foxf1a$^{-/-}$ embryos are severely deformed and die around E9.5, *Foxf1a*$^{+/-}$ embryos survive until birth. They display a foregut phenotype very similar to that of *Shh*$^{-/-}$ and *Gli2*$^{-/-}$;*Gli3*$^{-/-}$.[68] The investigators describe the esophagus as 'frequently merging with the trachea' and sometimes ending rostrally in an EA (Figure 6(e)). No bronchoesophageal communications were reported; suggesting that haploinsufficiency of *Foxf1* is not as severe as loss of *Shh*.[68] Recently, human *FOXF1* has been identified in a microdeletion (16q24.1) associated with human EA/TEF and VACTERL association.[44]

Retinoic Acid Signaling

Retinoic acid (RA) is a derivative of Vitamin A, and is crucial for the development of multiple organ systems as well as the early embryonic body plan.[109,110] While EA/TEF has not been linked with fetal vitamin A deficiency in humans, mice genetically deficient in RA signaling can display foregut compartmentalization defects.[69,111–114] In the mouse, there are three retinoic acid receptor (RAR) genes, *Rara, Rarb, and Rarg* (formerly known as α, β, and γ). Each of the RARs generates multiple transcripts through alternative splicing, and their isoforms have unique expression patterns throughout development.[115] Mice lacking the RARA1 and all of the RARB isoforms have an undivided foregut, as do mice lacking all of the RARA isoforms and RARB2[69,111] (Figure 6(f)). The investigators described the phenotype as a developmental 'arrest' of foregut compartmentalization, and noted that the common foregut tube had columnar ciliated epithelium. Additionally, these mutants (and other RAR family mutants) have 'disorganized' tracheal cartilage rings.[69,111] The RA-synthesizing retinaldehyde dehydrogenase 2 (*Aldh1a2*, formerly known as *Raldh2*) has also been deleted in the mouse and is embryonic lethal at E10.5 because of severe cardiac defects.[116] When the embryos are partially rescued with a short dose of RA, they can survive up to birth and display 'incomplete' foregut compartmentalization in addition to other phenotypes. Importantly, this study also showed that although the embryos have abnormal lung and foregut development, the expression levels/patterns of genes important for anterior foregut development (*Shh*, *Foxa2*, and *Nkx2-1*) are not disrupted.[114] This suggests that RA signaling acts in parallel to, or downstream of, these signals during lung and trachea development.

The Atmin Mutant Mouse

Recently, a new mouse mutation with a role in foregut compartmentalization has been identified. To better understand the role of *Atmin* (formerly known as *Asciz*), a DNA damage response gene, *in vivo*; Jurado et al.[72] made a targeted knockout. Surprisingly, loss of *Atmin* had a late embryonic lethality phenotype that was not rescued by loss of *Trp53* (formerly known as *p53)*. In *Atmin* nulls, a single tube connects the pharynx with the stomach. The lungs are completely absent, and a short tracheal nub is apparent on the ventral side at the level of wild type lung buds (Figure 6(i)). These phenotypes suggest complete pulmonary agenesis with an arrest of tracheoesophageal compartmentalization. Immunohistochemistry revealed that the short portion of trachea expressed NKX2-1, but had reduced levels of SOX2 and TCP1 at E11.5. The esophagus appeared normal and histological analysis showed normal muscular structure at E18.5.[72]

CONCLUSION

The Emerging Picture of Foregut Comparmentalization

Our collective knowledge about foregut compartmentalization has grown substantially over the last 15 years. Recently, it has become possible to synthesize this knowledge into a broader view of how this tissue develops correctly and incorrectly. The variety of foregut phenotypes observed in the mouse models points toward two tentative conclusions: (1) foregut compartmentalization defects arise by multiple mechanisms and (2) correct foregut compartmentalization relies on the interaction of multiple developmental signaling pathways.

The Importance of Ventral Patterning

It is now clear that correct dorsal/ventral patterning of the primitive foregut tube is essential for correct compartmentalization (Figure 6(f)–(j)). Loss of the ventral foregut/respiratory marker *Nkx2-1* results in failure of compartmentalization, and a single foregut tube with 'dorsal' characteristics.[64,70] Similarly, loss of WNT-signaling in the ventral foregut in *Wnt2/2b* null or *Ctnnb1* conditional ablations results in loss of the respiratory marker *Nkx2-1* and a phenotype that resembles that of *Nkx2-1* nulls.[67,73,74] Although we have no information regarding D/V patterning of the primitive foregut tube in the *Atmin* null mutant, the fact that the single tube at later stages does not express *Nkx2-1* suggests that it may fall into this category, as well.[72] Finally, BMP signaling in the ventral foregut is also required for development of a trachea as shown by the *Bmp4* and *Bmpr1a/1b* tissue-specific knockouts.[67,71] Recently, Domyan et al.[71] showed the nature of the interaction between the BMP and WNT

pathways in promoting respiratory identity. BMP signaling is required to repress the expression of *Sox2* in the ventral foregut. Without BMP signaling, *Sox2* expression is increased ventrally, and WNT signaling can no longer induce respiratory fate. Despite varying degrees of lung bud development in these mutants, the compartmentalization process appears unable to initiate without correctly patterned vFGE.

The Potential Causes and Consequences of dFGE Reduction

Loss of respiratory specification could account for several of the observed compartmentalization phenotypes in mouse. However, it does not appear to account for all of them (Figure 6(b)–(e)). In particular, *Nog*$^{-/-}$ and Adriamycin-treated mice show ventral expression of *Nkx2-1* in the primitive foregut tube.[28,65,83] Each of these models also has an accompanying notochord defect. Many investigators have provided hypotheses about the significance of the notochord phenotype, and these are represented by three potential mechanisms that are not mutually exclusive: (1) The presence of a large *Shh*-expressing notochord in close proximity to the dorsal endoderm (and, therefore, a local increase in Hh-signaling) disrupts subsequent patterning and morphogenesis of the foregut;[16,17,65,80] (2) Abnormal attachment of the notochord to the dFGE causes traction on the elongating foregut tube and distorts the tissue to cause EA[14] (Figure 7(c)); and (3) Abnormal notochord resolution results in physical loss of dFGE, reducing the size of the tissue that must form an esophagus upon septation and causing EA[83] (Figure 7(c)). In the case of *Nog*$^{-/-}$ mutants, a reduction of dFGE has been documented.[83]

Descriptions of Some Model Phenotypes Remain Incomplete

There is scant information in the literature regarding the state of the primitive foregut tube in the some of the mouse models. In particular, there is no information about dorsoventral patterning or the state of the notochord in RAR, *Foxf1a*$^{+/-}$, and *Gli2;Gli3* mutants. We do know that *Shh*$^{-/-}$ mutants have only a few dorsal cells that are *Nkx2-1*-negative at E10.5.[28] This may suggest that the uncompartmentalized foregut of older embryos is essentially a trachea, and the esophagus has been lost. Although we have so little information about the early foreguts of these mutants, we can still draw informative parallels between some of the mutant phenotypes. For instance, most of these phenotypes have what could be called 'true fistulas' rather than simply an uncompartmentalized foregut (Figure 6(b)–(e)). These fistulas arise from a variety of locations (rostral to the carina, at the carina, or from the left main bronchus). In some cases, they are reported to be esophageal in character, but in others they display some distinctly tracheal characteristics. It has been suggested that in certain instances, the initial insult is EA and that an outgrowth of the trachea reestablishes a connection between the rostral foregut and the stomach. There is some evidence for this possibility in the Adriamycin model, but in other models it has been completely unexplored.[85] Alternatively, if the foregut does septate, a fistula might arise wherever there is just enough dorsally patterned tissue to form an esophagus. Both instances suggest that such phenotypes result from a reduction in dorsal/esophageal tissue by either physical loss or mispatterning. Accordingly, an upper esophageal pouch (suggesting EA) has been found only in mutants that do not have an obvious loss of ventral patterning (Figure 6(b)–(d)). In the case of *Shh*$^{-/-}$ and *Foxf1a*$^{+/-}$ mutants, the upper esophagus may be more closely associated with the trachea (Figure 6(e)), and in RAR mutants an upper esophageal pouch has not been described.

Human Birth Defects and Model Phenotypes

When we compare the foregut phenotypes of rodent models with defects found in humans, we can make some loose comparisons. First, several of the models display defects in other organs that also often accompany human EA/TEF (not described in this review). Second, we can place the model phenotypes into clinical categories. For example, models in which ventral patterning is disrupted tend to display defects resembling Type 3 LTE clefts.[67,69–72,74] Models in which there is an upper esophageal pouch and a 'true fistula' tend to display Gross Type C EA/TEF, and some (Adriamycin, *Nog*$^{-/-}$) also display other types of EA/TEF at low frequencies. The fact that we can make these comparisons is encouraging and suggests that etiological research in rodent models is very applicable to humans.

Compartmentalization Mechanisms Are Still Unknown

While we have learned a great deal about the genetic pathways that are important for compartmentalization of the foregut, the mechanisms behind the actual process are still a mystery. The septation model has been largely accepted in the field for over a century, but has never been formally proven. The Adriamycin model triggered other hypotheses including the watershed model, which remains the most reasonable alternative. Importantly, Ioannides et al.[28] were able to show that the absolute length of

the uncompartmentalized foregut actually decreases as the entire foregut is growing in length, supporting the septation model. The most potentially informative experiment, to live-image compartmentalization of the foregut in organ culture, has not been reported.

FUTURE DIRECTIONS IN THE FIELD

Over the past several decades we have made significant progress in our understanding of normal foregut compartmentalization and of compartmentalization defects. Our knowledge will not be complete, however, without a full description of the cellular and genetic mechanisms behind these processes. Therefore, there are several important immediate goals for the future. First, we must definitively observe whether the foregut does or does not septate. Only then can we be justified in pursuing the cellular mechanisms behind a process of septation. How might the lateral mesenchyme and epithelium change to divide the foregut? One could imagine a process of partial epithelial-mesenchymal transition that induces changes in cell-cell adhesion as well as remodeling of the basement membrane surrounding the dividing foregut. Our second goal should be to better-characterize our current models in terms of the information we now have. For example, how is proliferation altered in models with or without TEF? Is dorsoventral patterning disrupted in the RAR mutant model? How do more complex phenotypes such as $Shh^{-/-}$ and $Foxf1a^{+/-}$ progress through development? Are there notochord defects in models other than Adriamycin and $Nog^{-/-}$? Related to this, medical professionals should attempt to better characterize foregut compartmentalization defects in patients with respect to the features of rodent models, and to point out features that are still unique to humans. This will also contribute to the third goal, which is to use our knowledge about potentially important pathways to help guide investigations that will determine causal genetic anomalies in human patients. Though multiple genes have been associated with foregut compartmentalization defects in humans, the mechanism(s) by which many of these mutations lead to such defects has not been determined. Furthermore, in the vast majority of cases, human foregut compartmentalization defects have an unknown genetic or environmental cause. By taking a multifaceted and collaborative approach that involves basic research, genetic studies, and clinical applications, the next decades will witness another great leap toward solving the mystery of foregut compartmentalization and its anomalies.

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