Molecular Embryology of the Foregut

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The digestive and respiratory systems have different physiological functions (nutrition, food transit, and evacuation for the former; breathing and oxygen supply to the blood for the latter) and are generally considered to be and studied as 2 independent structures. Although at birth they are separated, they both derive from a common and transient developmental structure, the foregut, which is the anterior part of the gastrointestinal (GI) tract (1). The GI tract is a remarkably complex, 3-dimensional, specialized, and vital system that is derived from a simple tube-like structure. The vertebrate GI tract includes the luminal digestive system (ie, esophagus, stomach, intestine, and colon, the ''gut'') and the GI-tract derivatives (ie, thyroid, lungs, liver, and pancreas). The gut is composed of 3 germ layers: mesoderm (which forms the smooth muscle layer), endoderm (which forms the epithelial lining), and ectoderm (which includes the enteric nervous system). The gut develops from 2 invaginations at the anterior (anterior intestinal portal) and posterior (caudal intestinal portal) end of the embryo that elongate and fuse to form a straight tube. The primitive gut tube is initially patterned into 3 broad domains along its anterior–posterior (AP) axis: the fore-, mid-, and hindgut. As they develop, each region of the gut is characterized by unique mesodermal and endodermal morphologies, which can be easily discerned by gross and microscopic examination. Specifically, these tissues show regional differentiation along the AP axis that specifies pharynx, esophagus and stomach (the foregut), small intestine (the midgut), and large intestine (hindgut). This regionalization is maintained throughout life and is essential and necessary for normal gut function. These patterning events are remarkably conserved across species (1), and patterning anomalies are likely to be responsible for many of the human gut malformation syndromes, such as tracheo-esophageal atresia (TE), infantile hypertrophic pyloric stenosis, or anal atresia (1,2).

FOREGUT DEVELOPMENT

Lung and gut are 2 independent systems that originate from 1 common embryonic organ, the foregut. The development of the foregut is not well documented in comparison to that of other parts of the digestive system. The respiratory system originates from the formation of an endodermal diverticulum in the ventral wall of the foregut, whereas the esophagus forms from the foregut dorsal wall (1). Specifically, the foregut endoderm evaginates and pushes the surrounding mesenchyme to form the 2 presumptive lung buds. In avian embryos, these processes manage to form 2 independent and separate endodermal structures, dorsally the esophagus and ventrally the lung buds, in a short time (approximately 10 hours). Later, the lung buds grow caudally, leading to the formation of a temporary TE septum. The appearance of the TE septum is followed first by the expansion of the trachea and second by the final separation of the 2 endodermally derived systems.

MOLECULAR PATHWAYS INVOLVED IN FOREGUT DEVELOPMENT

The cellular events that contribute to foregut specification, lung development, and separation of trachea and esophagus are regulated by different and specific molecular pathways. The regionalization of the different parts of the gut is controlled by the localized expression of different *Hox* genes, which are homeobox-containing transcription factors (3). For example, *Hoxa3* and *Hoxb4* are specifically expressed in the foregut endoderm, whereas *Hoxc5* and *Hoxa13* are expressed more caudally in the midgut and hindgut endoderm, respectively (3,4). Sonic Hedgehog (Shh), a member of the Hedgehog (Hh) family of morphogens, is expressed in the entire endodermal layer but not in the pancreas (3). Moreover, Litingtung et al (5) reported a specific and dynamic expression pattern of *shh* during TE development, with earlier expression and patterning of the ventral foregut by Shh and transient inhibition of *shh* expression in the tracheal endoderm. Others transcription factors also show specific expression profiles in the endoderm, such as the sex-determining region Y–related high-mobility group (HMG) transcription factors *Sox2* (foregut endoderm) and *Sox9* (midgut/hindgut endoderm) (6,7). The homeodomain transcription factor *Nkx2.1* (also called TTF1 or T/EBP) is specifically expressed in the anterior part of the foregut and in the endoderm of the developing trachea but not of the esophagus (8). All of these genes are expressed in the foregut endoderm in a spatially regulated manner (Fig. 1), and therefore their deregulation could be implicated in the genesis of TE malformations.

To investigate their function(s) and requirement(s) during foregut development, these genes have been inactivated in different transgenic mouse lines. For instance, inactivation of *Nkx2.1* is associated with the presence of a common TE lumen, suggesting that NKX2.1 is essential for the development and separation of TE endoderm (10). Inactivation of *shh* in mice, as for *Nkx2.1,* leads to a common TE lumen, demonstrating an essential function of Shh during TE development. Shh is a ligand and activator of the Hh-signaling pathway. During the development of the GI tract, *shh* is localized to the gut endoderm, but it acts on the gut mesenchyme to control endodermal–mesenchymal interactions (3). After processing by the Golgi apparatus, Shh is secreted by endodermal cells and binds to its receptor PATCHED (or PTCH1), which is expressed in the surrounding mesenchymal cells. PATCHED activation is followed by cleavage and activation of the GLI1/2 transcription factors, which regulate various genetic networks and the expression of mesenchymal-specific genes, such as *Ptch1*, *Gli1,* and bone morphogenetic protein 4 (*Bmp4*) (3,7).

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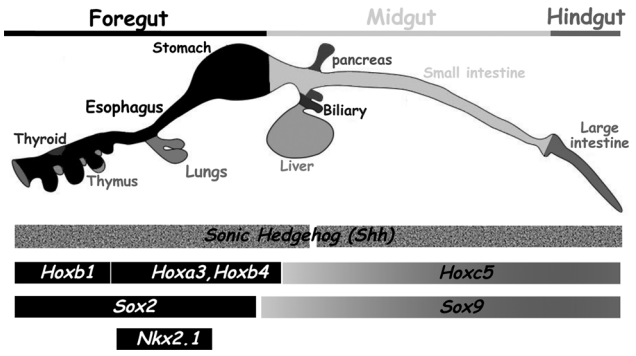

FIGURE 1. Molecular pathways involved in the regionalization of the foregut endoderm. Expression boundaries of selected factors expressed in the endoderm along the antero–posterior axis. The embryonic gastrointestinal (GI) tract is divided into fore-, mid-, and hindgut. *Shh* is expressed in the entire endodermal layer, sparing the pancreas. *Hox* genes, which are homeobox-containing transcription factors, harbor a specific localized expression into the GI endoderm. Sex-determining region Y–related high mobility group transcription factors *Sox2* and *Sox9* present exclusive pattern in GI endoderm. The transcription factor *Nkx2.1* is expressed into the foregut. Adapted from (9).

EPITHELIAL–MESENCHYMAL INTERACTIONS DURING THE FOREGUT DEVELOPMENT

Epithelial–mesenchymal interactions are essential for the development and differentiation of the GI tract (7); however, the contribution of the mesenchyme during the development of the foregut is not often commented upon. Different studies reported a significant mesenchymal condensation at the site of TE separation (11,12). Moreover, in rats, treatment with the teratogenic agent adriamycin reproducibly induces esophageal atresia/TE fistula. Such malformations are associated with lower cellularity and disorder of the surrounding mesenchymal cells already at early stages of foregut misdevelopment (12), supporting the notion of a major involvement of the foregut mesenchyme during foregut development.

As previously stated, the Hh signaling pathway regulates foregut development through epithelial–mesenchymal interactions, and different members of this pathway, such as the transcription factor *Gli2* (13), are expressed in the foregut mesenchyme. To analyze the interplay of the different components of the Hh pathway that are expressed in the foregut mesenchyme, Motoyama et al (14) inactivated *Gli2* in mice and observed the presence of TE malformation associated with alterations of visceral smooth muscle cells. In addition, inactivation of both *Gli2* and *Gli3* caused a stronger phenotype with a common TE structure, demonstrating that inhibition of genes of the Hh pathway, which are normally expressed in the foregut mesenchyme, triggers similar phenotypes as inactivation of the endodermally expressed *shh*.

Recently, the function of the BMP signaling pathway during TE development was investigated. This signaling pathway is activated in the gut mesenchyme following expression and activation of *shh* and *Patch*. While *shh* is localized to the ventral foregut endoderm, *Bmp4* is expressed in the neighboring mesenchyme but not in the dorsal part of the foregut, which will give rise to the esophagus (15) and in which *Noggin,* the inhibitor of the BMP pathway, is expressed. To analyze the function of the BMP pathway during foregut development, Que (15) et al inactivated *Noggin* and observed the presence of a common TE structure. In a rescue experiment in which *Noggin*−/− mice were crossed with *BMP4*+/− mice to decrease the activity of the BMP pathway, the progeny showed normal separation and formation of the trachea and esophagus, supporting the idea that the level of BMP activity is crucial for the development of these 2 structures. These examples demonstrate that the foregut mesenchyme is essential for the correct development of the foregut, and that the mesenchyme could actively participate in the development of these 2 structures.

CONCLUSIONS AND PERSPECTIVES

Candidate factors for foregut development include known pattern formation genes that were first identified in *Drosophila*, such as nuclear homeotic transcription factors (HOX, SOX and NKX factors) and secreted factors (BMP and Hh factors) (3,6,9,16). Genetic evidence from different animal models indicates that these molecules play multiple and crucial roles in the development and septation of the trachea and esophagus. The identification of *NMYC* and chromodomain helicase DNA binding (CDH) *CDH7* mutations in syndromic forms of TE bring us new factors that could be involved during the development of the trachea and esophagus (16). To better understand the molecular basis of human malformations and syndromes, such as TE atresia, it is essential to better dissect normal foregut development at the molecular level and to stimulate translational research between scientists and clinicians.

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