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Review

# Development and stem cells of the esophagus

Yongchun Zhang[a], Ming Jiang[a], Eugene Kim[a], Sijie Lin[a], Kuancan Liu[a,b], Xiaopeng Lan[b], Jianwen Que[a,*]

[a] Division of Digestive and Liver Diseases and Center for Human Development, Department of Medicine, Columbia University, NY 10032, USA
[b] Institute for Laboratory Medicine, Fuzhou General Hospital, PLA, Fuzhou, Fujian 350025, PR China

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ABSTRACT

The esophagus is derived from the anterior portion of the developmental intermediate foregut, a structure that also gives rise to other organs including the trachea, lung, and stomach. Genetic studies have shown that multiple signaling pathways (e.g. Bmp) and transcription factors (e.g. SOX2) are required for the separation of the esophagus from the neighboring respiratory system. Notably, some of these signaling pathways and transcription factors continue to play essential roles in the subsequent morphogenesis of the esophageal epithelium which undergoes a simple columnar-to-stratified squamous conversion. Reactivation of the relevant signaling pathways has also been associated with pathogenesis of esophageal diseases that affect the epithelium and its stem cells in adults. In this review we will summarize these findings. We will also discuss new data regarding the cell-of-origin for the striated and smooth muscles surrounding the esophagus and how they are differentiated from the mesenchyme during development.


**Contents**



*Abbreviations:* EA, esophageal atresia; TEF, tracheoesophageal fistula; LES, lower esophageal sphincter; Bmp, bone morphogenetic protein; Shh, sonic hedgehog; Ihh, Indian hedgehog; MYBPH, myosin binding protein H; ROCK1, Rho kinase 1; sFRPs, secreted Frizzled-related proteins; RA, retinoic acid; Raldh2, retinaldehyde dehydrogenase 2.

* Corresponding author.
*E-mail address:* jq2240@cumc.columbia.edu (J. Que).

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## 1. Introduction

The esophagus serves as a channel to transport food from the mouth to the stomach. Fitting the need, the esophagus is ensheathed by multiple layers of muscles that are essential to generate peristalsis to move food. Within the lumen a thick stratified squamous epithelium is required to sustain the passing of the abrasive raw food, which is facilitated by secretions of the esophageal submucosal glands. During embryonic development, the esophagus and trachea initially share a single-lumen tube at the anterior region of the foregut. As organogenesis proceeds, the anterior foregut separates and generates the trachea ventrally and the esophagus dorsally. This tracheal-esophageal separation occurs at around E9.5–E11.5 in mice and approximately 4–6 weeks of gestation in humans. Although the exact cellular and molecular mechanisms remain elusive, recent studies of mouse genetic models suggest that this separation involves dorsal-ventral patterning of signaling molecules (e.g. Bmps, Wnts) and transcription factors (e.g. SOX2, NKX2.1). Disruption of this expression pattern leads to various anomalies such as esophageal atresia with or without tracheoesophageal fistula (EA/TEF) where the esophagus is closed as a blind end sac proximally, and in a worse scenario the foregut remains as a single-lumen tube [1]. Some of the signaling molecules and transcription factors continue to play essential roles in the subsequent development of the esophagus, especially for the morphogenesis of the epithelium which involves a transition from simple columnar to stratified squamous. These molecules are also required for the maintenance of the epithelium in the adult esophagus. Abnormal activities of relevant signaling pathways or abnormal expression of the transcription factors have been associated with the pathogenesis of several common esophageal diseases including eosinophilic esophagitis, Barrett's esophagus, and even cancer. This review will summarize these findings with a focus on how signaling pathways and transcription factors regulate tracheal-esophageal separation and morphogenesis of the esophageal epithelium. We will also discuss new findings regarding the development of the mesenchyme, especially the formation of the muscle layers in the esophagus.

The anterior foregut exhibits dorsal-ventral differences in terms of gene expression prior to the start of tracheal-esophageal separation. For example, the transcription factor SOX2 is abundantly expressed in the dorsal epithelium [2]. By contrast, NKX2.1 (also known as TTF1) is enriched in the epithelium at the ventral side [3–5]. As discussed in more details below this dorsal-ventral expression of signaling molecules and transcription factors is essential for the specification of the early foregut endoderm into different territories, i.e. respiratory epithelium and esophageal epithelium (Fig. 1). When the esophagus is completely separated from the respiratory system, the epithelium lining the nascent organ is simple columnar. The epithelium promptly starts to stratify and differentiate to form a multiple-layered squamous epithelium that consists of basal and suprabasal cells (Fig. 2). The squamous epithelium is about 4–6 cell thick with a single layer of basal cells in the adult mouse esophagus, while in the human esophagus the squamous epithelium contains 20–30 layer of cells with several layers of basal cells at the bottom [6]. In addition, an acellular layer of keratin is found on the top of the squamous epithelium in rodent esophagus similar to the skin, but this keratin layer is absent in the human esophagus (Fig. 2). On the other hand, human but not rodent esophagus contains abundant submucosal glands that are responsible for producing mucins and bicarbonate etc. [7]. The mechanism leading to the formation of these glands remains unknown due to the lack of animal models.

The mesenchyme enclosing the epithelium is seemingly homogeneous when the esophagus is established from the mouse foregut at E11.5. Similar to the epithelium the mesenchyme is also highly proliferative at this stage. Previous genetic studies have shown that several transcription factors including FOXP1 and FOXP2 are important for the differentiation of the mesenchyme into layers of muscle cells (see review by Jacobs et al. [8]). Many questions remain unanswered, however, including determining the cell-of-origin for the surrounding striated muscle. Recent lineage tracing experiments provide some unexpected results. These findings will be discussed below.

## 2. Establishment of the esophagus from the early foregut

Previously three models have been proposed to describe how the esophagus is separated from the trachea, including: (1) The outgrowth model in which the trachea extends with the lung primordium from the early foregut, (2) the watershed model in which a yet-to-be identified mesenchymal condensation serves as a wedge to split the anterior foregut into the trachea and esophagus, and (3) the septation model where epithelial cells at the dorsal–ventral midline make contact across the midline of the lumen and fuse to form a septum [1]. Recent evidence, however, has suggested that none of these models fully accounts for tracheal-esophageal separation. For example, the outgrowth model predicts that the common tube above the lung budding site will be specified as one part of the esophagus. Nevertheless, characterization studies showed that the expression of the respiratory marker NKX2.1 is present in the ventral common foregut throughout tracheal-esophageal separation [2,9]. According to the watershed model, the common tube above the mesenchymal condensation would remain undivided. Contrary to this prediction, the length of the undivided foregut becomes shorter as separation proceeds [10]. Regarding the septation model, the predicted septum has never been detected by scanning electron microscopy during chicken foregut separation. Instead the authors observed an epithelial saddle at the site where the lung buds initiate, which is continuously present at the location where the trachea and esophagus are splitting [11].

We recently developed a mouse foregut culture system to visualize the separation process and proposed an alternative model termed "splitting and extension model". We used a *SOX2-GFP* knockin allele to monitor the movement of the foregut epithelium and observed an epithelial saddle that is initiated at the lung-esophageal boundary in the E9.5 foregut [1]. The saddle subsequently moves in a caudal-cranial direction to split the trachea and esophagus, while both nascent organs extend caudally (see movie in [1]). The epithelial saddle is composed of cells from the lung and future esophagus (Fig. 3), raising the possibility that abnormal lung development (e.g. branching defects) is associated with abnormal separation of the esophagus from the trachea. It is worth mentioning that up to 72% of surviving adolescents and adults with treated EA/TEF continue to suffer from respiratory problems throughout their lifetime [12–14]. Consistently, lung lobe fusion (horseshoe), agenesis, or hypoplasia with abnormal epithelial differentiation in the airways has been reported in patients with EA/TEF [15]. The etiology and mechanism of EA/TEF formation remains largely unknown. Nevertheless, recent studies with animal models are beginning to provide insight into the dysmorphogenetic processes. Several signaling pathways (e.g. Bmp, Wnt) and transcription factors (e.g. SOX2) have been shown to play important roles in the regulation of tracheal-esophageal separation [2,3,16]. Intriguingly, in these animal models EA/TEF is always accompanied by disturbances in lung development most commonly characterized by lobulation and branching defects [8]. These observations support the hypothesis that tracheal-esophageal separation and lung development are closely linked. It remains unclear however how these developmental processes are connected and which underlying common mechanisms exist. A combination of live