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The pulmonary mesenchyme directs lung development

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Each of the steps of respiratory system development relies on intricate interactions and coordinated development of the lung epithelium and mesenchyme. In the past, more attention has been paid to the epithelium than the mesenchyme. The mesenchyme is a source of specification and morphogenetic signals as well as a host of surprisingly complex cell lineages that are crucial for normal lung development and function. This review highlights recent research focusing on the mesenchyme that has revealed genetic and epigenetic mechanisms of its development in the context of other cell layers during respiratory lineage specification, branching morphogenesis, epithelial differentiation, lineage distinction, vascular development, and alveolar maturation.

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Introduction

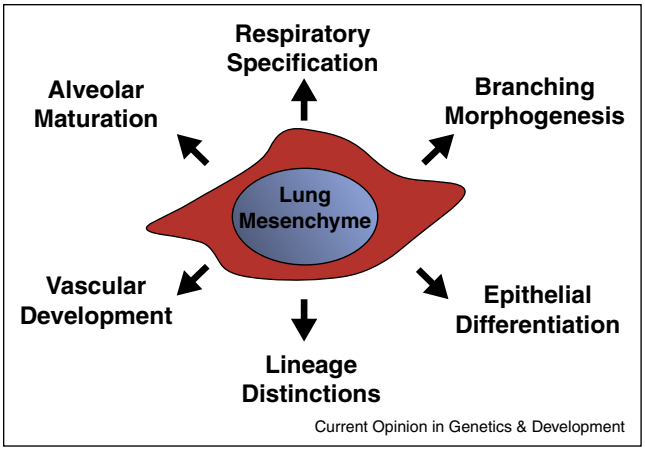
Development of the respiratory system proceeds through a well-described series of steps beginning with division of the anterior common foregut tube into the respiratory endoderm ventrally and the esophagus dorsally. The respiratory tract then undergoes extensive branching to form the proximal conducting airways, followed by distal septation generating the gas exchange units, or alveoli, of the mature lung. These processes are coupled with coordinated differentiation of the airway and distal lung epithelium leading to a regionally specific pattern of cell types. Formation of a functional lung also requires simultaneous development of both the pulmonary vascular system (central systemic circulation) and bronchial vascular system (local lung circulation). The genetic and epigenetic regulation, as well as the specialized intra-cellular, inter-cellular, and extracellular mechanisms responsible for proper development of the respiratory system continue to be elucidated. Each of the steps in lung development is reliant upon inductive cues and reciprocal interactions between the pulmonary epithelium and the surrounding mesenchyme. Loss of or abnormalities in this crucial interaction can lead to severe anatomical and functional defects in the airway and alveoli. Many of the phenotypes associated with such abnormalities result in lethality or severe morbidity in humans and are being investigated in biochemical, cellular, tissue culture, organ explant, and animal models. Despite its importance in directing airway and alveoli development, many aspects of the activities and regulatory mechanisms of the lung mesenchyme are not well understood, a deficit recognized at a recent workshop hosted by the National Heart, Lung, and Blood Institute [1]. In this review, we will discuss recent (primarily within the past 2–3 years) advances in respiratory development, focusing on the role of the lung mesenchyme (Figure 1). For more comprehensive discussions of lung development, please see recently published reviews including [2–7].

The mesenchyme provides crucial signals for respiratory lineage specification

Specification of the respiratory system takes place in the ventral anterior foregut endoderm, as indicated by the expression of *Nkx2-1* (also named *Ttf1*) beginning at embryonic day (E) 8.25 in mice [8–10]. Collective work on respiratory lineage specification implicates the surrounding ventral mesenchyme as a crucial source of signals, including FGF, WNT, BMP, RA, and TGFβ, that direct endodermal expression of *Nkx2-1* in a temporal and spatial context dependent fashion [10–20]. Understanding the specification mechanisms initiated within the lung mesenchyme as well as identifying the specific mediators of mesenchyme–epithelium interactions has been a focus of recent research.

As an example of these signals, combined mesenchymal expression of *Wnt2* and *Wnt2b* has been shown to be required for respiratory lineage specification and the expression of *Nkx2-1* [12]. However, the upstream factors that control the expression of these signals in the mesenchyme are less clear. In *Xenopus*, it was shown that morpholino knockdown of *Osr1* and *Osr2*, a pair of transcription factor genes, led to loss of *Wnt2b* expression in the mesenchyme [21•]. In mice, it was shown that genetic inactivation of *Tbx5* before respiratory specification led to reduced *Wnt2* and loss of *Wnt2b* expression in the mesenchyme and unilateral loss of *Nkx2-1* expression in the prospective pulmonary epithelium [22•]. These data suggest that *Osr1/2* and *Tbx5* are required in the lung mesenchyme for normal *Wnt2* and *Wnt2b* expression and

Figure 1



The lung mesenchyme holds a central position in the formation of a functional lung. A diagram delineating the various topics covered in this review.

subsequent specification of the respiratory foregut epithelium.

Although evidence suggests that specification signals from the mesenchyme can control *Nkx2-1* expression via transcription factors [15], a recent study suggests that they may also act through an epigenetic mechanism [23•]. It was shown that NANCI, a long non-coding RNA, is expressed in the ventral foregut and acts as a positive regulator of *Nkx2-1* expression [23•]. Furthermore, it was found that NANCI is regulated itself by mesenchymal WNT signaling [23•].

Despite these advances, several open questions remain. For example, as each of the signals essential for respiratory specification is also active in other tissues, how they function to only specify the respiratory fate in a regionally constrained manner in the anterior ventral foregut is not understood. In addition, evidence demonstrates that the same signals, for example WNT, can function as either a promoter or inhibitor of the respiratory fate when acting at different time windows of development [12–14]. How these different effects are mediated is not understood. Furthermore, the mechanisms by which fate specification is translated into morphogenesis, that is, budding of the respiratory primordia from the anterior foregut tube, have not been determined.

The mesenchyme provides crucial signals that drive epithelial branching morphogenesis

Following specification and physical separation of the respiratory lineage precursors from the esophagus within the anterior foregut, future conducting airways and alveolar regions are laid down according to a proximal-distal blueprint, through largely stereotypical branching events directed by cues from the adjacent mesenchyme. A cardinal mesenchymal signal that drives branching is FGF10 [24,25]. Its restricted expression in the distal mesenchyme at sites of future branch destination led to the proposal that the local source of FGF10 acts as a chemoattractive cue for directing nascent branches. However, a recent study showed that lungs with ubiquitous over expression of *Fgf10* still formed discrete branches although the pattern is grossly abnormal especially at later developmental stages [26]. These findings together suggest that, in addition to proper regulation of *Fgf10* expression, other constraints, such as heparin sulfate-based modification of signaling activity, may contribute to the construction of a stereotypical branching pattern [27•].

WNT signaling in the mesenchyme is a well-established driver of branching morphogenesis, however the specific WNT ligands and regulatory partners had not been clearly defined. Miller and colleagues showed that mesenchymal *Wnt2* and epithelial *Wnt7b* cooperatively control branching morphogenesis, proximal-distal patterning, and development of distal lung progenitors [28]. To identify upstream activators and regulatory partners of WNT signaling in the mesenchyme, Miller *et al.* conducted an in vitro screen that uncovered important interactions with homeobox transcription factors ESX1, MSX1/2, Nkx5-2 and the sumoylation factor PIAS4 [29]. For example, MSX1/2 were found to enhance canonical WNT signaling in a WNT ligand dependent manner specific to lung mesenchyme, possibly through transcriptional repression of WNT antagonists [29].

Proper development and activity of the mesenchyme requires interactions with both the neighboring epithelium and mesothelium. For example, mesothelial specific deletion of *Fgf9* results in loss of *Wnt2a* expression in the mesenchyme and decreased airway branching [30]. This suggests that activation of mesenchymal WNT/β-catenin signaling is dependent on *Fgf9* expressed in the mesothelium [30].

The lung mesenchyme is also a source of micro-RNAs (miRs) that control lung branching through an epigenetic mechanism. Carraro *et al.* recently showed that *miR-142-3p* acts in the developing lung mesenchyme to regulate lung bud outgrowth and branching morphogenesis [31•]. It functions by promoting the activity of the WNT-FGF feed-forward signaling loop that maintains the lung mesenchyme in an undifferentiated state [31•].

A central question in the coordination between branch growth and patterning is the transition from the branching that forms the airways and the branching that forms the alveoli. Alanis *et al.* recently demonstrated that the airway and alveolar regions are distinguished by two waves of developmental cues that interact to establish a crucial boundary between these domains, the bronchoalveolar