

Development of the pulmonary vasculature: Current understanding and concepts for the future

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ABSTRACT

The pulmonary circulation is a highly specialized vascular bed that physically and functionally connects the heart and the lungs. The interdependence of these two organs is illustrated in embryonic development, when the lung endoderm protrudes into the surrounding mesoderm as the heart tube elongates and folds into structurally distinct chambers. The pulmonary vascular precursors then undergo highly stereotyped cellular maturation and patterning to form a multilayered vascular network that parallels the airways and links the arterial and venous poles of the heart. Upon the first breath, the mature pulmonary circulation is poised to receive the entire cardiac output for efficient gas exchange, and deliver oxygenated blood to the systemic circulation. Disruption of this developmental process can result in congenital defects such as the syndrome tetralogy of Fallot, or differentiation defects leading to persistent pulmonary hypertension of the newborn. Prior studies into the role of angiogenesis and vasculogenesis in pulmonary vascular development have not clearly yielded the identity of pulmonary vascular precursors, or the signals coordinating vascular maturation. We outline key questions on pulmonary vascular development that consider the role of heart-lung interaction in promoting the differentiation and patterning of the pulmonary vasculature.

Key Words: vascular, progenitor, fate mapping

COORDINATED DEVELOPMENT BETWEEN THE HEART AND THE LUNG

The mammalian vascular network is composed of two major circulations, systemic and pulmonary, that are connected in series and separated by the four-chambered heart. While the pulmonary vasculature shares similar histological features with its systemic counterpart, they differ drastically in their physiology, function, and anatomic relationship to the heart. Unlike all other vascular beds, the pulmonary circulation facilitates gas exchange with the ambient air via direct conduits to and from the heart. This process is accomplished using a complex endothelial-epithelial alveolar gas exchange unit in the periphery of the respiratory system. The heart-lung vascular connection continues to grow and remodel in utero as these two organs codevelop in close coordination.

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Cardiac morphogenesis occurs prior to lung development, as the cardiac mesoderm condenses into a primary heart tube with an arterial and a venous pole at opposite ends.^[1] As the cardiac mesoderm undergoes asymmetric looping to form distinct chambers, it also envelops the ventral aspect of the foregut endoderm that will give rise to esophagus and lungs. The process of looping morphogenesis brings the venous pole ventral to the foregut endoderm.^[2] This mesoderm-endoderm interaction is crucial to lung development, as the venous pole mesoderm specifies Nkx 2.1+ lung progenitors in the ventral foregut endoderm via Wnt paracrine signaling.^[3] The endoderm closest to the venous pole then buds from the foregut to give rise to the future trachea as it begins to separate from the esophagus along the ventral-dorsal axis.^[4]

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ANGIOGENESIS VERSUS VASCULOGENESIS AS MODELS OF PULMONARY VASCULAR DEVELOPMENT

Serial histology of mouse embryos demonstrates the presence of a primitive endothelial plexus surrounding the newly formed airway at E9.5 day at the very beginning of lung development.^[5] Vascular connection between the heart and the lung has been described as early as 34 day gestation in human^[6] and E10.5 day in mouse.^[7] Early studies of pulmonary vascular development described both vasculogenic and angiogenic processes depending on the methodologies utilized. Vascular casting demonstrating the appearance of central sprouting vessels was taken as evidence of central angiogenesis concurrent with peripheral vasculogenesis in the developing lung.^[8,9] In contrast, use of endothelial-specific reporter demonstrated differentiating endothelial network that connected the heart and the lungs prior to patent lumen formation, suggesting vasculogenesis as the primary process.^[7]

Despite the numerous proposed models of angiogenesis versus vasculogenesis in pulmonary vascular development, the identity of pulmonary vascular precursors remains elusive due to technical limitations. Studies of pulmonary vascular development thus far have mostly depended on histological analysis of differentiated endothelial cells using static methods. While these studies provide snapshots of endothelial maturation around the lung and heart, they do not address the origin of the endothelial nor the mural cells (*e.g.*, vascular smooth muscle and pericyte) that make up the pulmonary circulation. Previous studies have not isolated the progenitor population that gives rise to the mature pulmonary vasculature nor the signals that influence the maturation and patterning of vascular progenitors. This is due, in part, to the lack of genetic tools available for sophisticated cell lineage tracing, along with the failure to consider vascular development in the context of heart-lung crosstalk as they develop in close proximity. To better understand the origin of the pulmonary vasculature, we have identified the following key questions to address in future studies.

WHAT IS THE CELLULAR ORIGIN OF THE PULMONARY VASCULATURE?

The pulmonary vasculature has been described as appearing *de novo* within the mesoderm ventral and lateral to the foregut endoderm,^[5] suggesting that this mesoderm pool gives rise to the future pulmonary vasculature as lung develops from the foregut. The application of genetic cell lineage tracing using Cre-loxP mouse lines has extensively

mapped the various mesoderm domains that give rise to the heart;^[1] however, no study to date has established the mesodermal origin of the pulmonary vasculature. Given the temporal and spatial proximity of cardiac and pulmonary vascular development, as well as histological similarities between the two compartments,^[10] it is likely that a common, multipotent mesoderm precursor exists that gives rise to both the heart and the pulmonary vasculature. Temporal-specific genetic fate mapping of known cardiac progenitors combined with clonal analysis will be required to confirm that cardiogenic mesoderm gives rise to the pulmonary vasculature. Furthermore, these experiments will define the cellular hierarchy and clonal relationship between different pulmonary vascular compartments (*e.g.*, arterial vs. venous, smooth muscle vs. endothelium).

WHAT ARE THE SIGNALS THAT PROMOTE PULMONARY VASCULAR DEVELOPMENT?

Given that prior studies have suggested the presence of a vascular network prior to the morphological formation of the lung, future studies will be critical in understanding whether paracrine signals from the early anterior foregut promote pulmonary vascular development. Several paracrine pathways are active in this region of the foregut with many signaling ligands expressed in the anterior foregut endoderm including Shh, Bmp, and Wnt.^[11] All of these pathways are critical for respiratory development, but little is understood about their importance in lung vascular development. Our previous work has implicated Wnt signaling in promoting vascular smooth muscle development and integrity through the action of the Wnt7b ligand.^[12] Other studies have also shown an important role for both Bmp and Shh signaling in smooth muscle development in the lung.^[13,14] Interestingly, in contrast to Wnt7b expression, Shh expression precedes the formation of the trachea and lungs^[3] suggesting that it may play a pivotal role in the early formation of the pulmonary vasculature. The use of temporally and spatially specific deletion methods, including Cre-loxP mouse lines, will be required to fully understand how these pathways act individually as well as cooperatively to promote the vascular connection between the heart and the lung.

SUMMARY AND FUTURE DIRECTION

Advances in imaging as well as cell fate mapping will be required to understanding how the pulmonary vasculature forms, and how much of its development is derived from the early cardiac mesoderm. The ability to accurately image the entire developing mouse embryos up to E10.5 for vascular

markers including CD31 (PECAM)^[15] will provide a key road map of how the pulmonary vasculature develops at its earliest stages. Coupled with techniques such as optical projection tomography and high-resolution magnetic resonance imaging, our understanding of the morphological processes that promote early investment of the respiratory organ with a complex vasculature will be greatly increased.

In conjunction with new and improved imaging techniques, the use of novel as well as existing inducible fate-mapping tools in mice will provide a sophisticated analysis of cardiopulmonary vascular development. Inducible Cre-loxP systems that mark specific domains of the early mesoderm, including those encompassing the lateral splanchnic and cardiac mesoderm adjacent to the developing lung, will provide an accurate spatial and temporal map of the mesoderm progenitors that contribute toward the cardiopulmonary vasculature. Using inducible Cre-loxP systems combined with stochastic, multicolor reporter line,^[16] clonal analysis can be performed to determine whether single mesodermal progenitors can generate multiple pulmonary vascular lineages including endothelium, smooth muscle, pericytes, and other mesenchymal derivatives such as airway smooth muscle and pulmonary fibroblasts.

Finally, the proper identification of pulmonary vascular precursors will enhance our understanding of paracrine signaling pathways active in the foregut and lung endoderm to promote cardiopulmonary vascular formation. Many studies have pointed to the importance of the early endoderm in patterning vascular formation in the lung as well as other tissues.^[17] Using novel Cre-loxP models to activate or inactivate different signaling pathways within vascular precursors, researchers can determine the cell autonomous and noncell autonomous effects of endoderm-derived signaling ligands on pulmonary vascular differentiation and patterning.

Ultimately, understanding the role of cardiac mesoderm-lung endoderm interaction in pulmonary vascular development would provide mechanistic insights into the congenital cardiopulmonary diseases where vascular patterning and differentiation are perturbed. Furthermore, deciphering

the signaling pathways necessary for pulmonary vascular development could potentially shed light on mechanisms involved in vascular regeneration and remodeling in adult lung diseases.

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