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NATIONAL HEART, LUNG, AND BLOOD INSTITUTE WORKSHOP

Molecular Determinants of Lung Development

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Abstract

Development of the pulmonary system is essential for terrestrial life. The molecular pathways that regulate this complex process are beginning to be defined, and such knowledge is critical to our understanding of congenital and acquired lung diseases. A recent workshop was convened by the National Heart, Lung, and Blood Institute to discuss the developmental principles that regulate the formation of the pulmonary system. Emerging evidence suggests that key developmental pathways not only regulate proper formation of the pulmonary system but are also reactivated upon postnatal injury and repair and in the pathogenesis of human lung diseases. Molecular understanding of early lung development has also led to new advances in areas such as generation of lung epithelium from pluripotent stem cells. The workshop was organized into four different topics, including early lung cell fate and morphogenesis, mechanisms of lung cell differentiation, tissue interactions in lung development, and environmental impact on early lung development. Critical points were raised, including the importance of epigenetic regulation of lung gene expression, the dearth of knowledge on important mesenchymal lineages within the lung, and the interaction between the developing pulmonary and cardiovascular system. This manuscript describes the summary of the discussion along with general recommendations to overcome the gaps in knowledge in lung developmental biology.

Keywords: lung development; lung cell fate; lung cell differentiation; tissue interaction; environmental impact

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The pulmonary system develops from a series of complex events that occur during prenatal and postnatal life. These events involve coordinated growth and differentiation of the epithelial and mesenchymal components of the immature lung to form the bronchial tree and alveoli; the pulmonary, bronchial, and lymphatic vasculatures; the nerves; and the pleura (1).

The basic mechanisms that regulate lung development and the link with human lung disease are among the most challenging and exciting areas of scientific inquiry. Increasing evidence has linked pregnancy complications that potentially disrupt important developmental pathways to human lung disease. Heterozygous mutation in thyroid transcription factor 1 (or Nkx2.1), an early marker and regulator of lung epithelial cell fate, is associated with perinatal respiratory distress and lethality (2, 3). Alterations in the Notch pathway, which is critical for airway epithelial and vascular development, have been found in patients with COPD, cancer, and pulmonary hypertension (4–9). Similarly, mutations or altered expression of components of the Wnt pathway, essential for lung development, correlate with increased lung tumor formation (10). Uteroplacental insufficiency disrupts epigenetic mechanisms required for proper expression of peroxisome proliferator–activated receptor γ, leading to defects in lung maturation and epithelial–mesenchymal interactions (11–13).

Research on lung development in the past decades has identified key transcription regulators, signaling pathways, and environmental factors in this process. Besides informing on molecules potentially involved in lung disease pathogenesis, this knowledge is having a major impact in the nascent field of lung progenitor–stem cell biology and regenerative medicine. There is an increasing need to reinforce new research on the connection between basic developmental studies and improving human lung health.

The Division of Lung Diseases, National Heart, Lung, and Blood Institute convened a workshop on September 7 and 8, 2011 to identify key knowledge gaps and priority areas in lung development research and to make recommendations for future research directions. The workshop sought to facilitate communication among diverse research groups, including scientists outside of the lung field, to encourage novel and systematic approaches to solve fundamental questions and to identify opportunities for advancing lung development research.

Summary of Discussions

Early Cell Fate and Morphogenesis

This session focused on early lung cell fate decisions and commitment and strategies to generate useful lung cell types for basic research and eventual therapeutic use.

The early lung endoderm can be identified before lung bud formation by the expression of the transcription factor Nkx2.1 in the ventral anterior foregut. However, this gene is also present in the thyroid primordium, and so far no early markers specific to lung endoderm have been reported, making the study of these early events difficult. Signaling pathways, including Wnt and BMP signaling, are important in early lung endoderm specification (14, 15). However, little is understood about the molecular mechanisms that promote lung progenitors versus other organ-specific endoderm progenitors in the foregut. Roles of epigenetic mechanisms, including noncoding RNAs and chromatin remodeling factors, in modulating the specificity and plasticity of early tissue specific progenitors in the lung need to be compared with findings from other tissues (16). The early events in lung specification and development can be positively reactivated in postnatal regeneration and adversely reactivated in disease (17). Whether such changes are causative or correlative is not well understood. Further investigation into the role of developmental signaling pathways in adult injury and repair will help identify potential novel therapeutic targets to improve lung regeneration and disease outcomes. The lung codevelops with the cardiovascular system to provide the intimate connection between the vasculature and airways, which is required for blood oxygenation. Little is known about the signals coordinating the codevelopment.

Research priorities are as follows. (*1*) Define the connection between developmental defects and human lung disease: Test whether pathways known to promote lung cell proliferation, progenitor expansion, and differentiation are involved in pediatric and adult lung diseases. Determine whether defects in early lung morphogenesis, such as defective tracheal-esophageal septation (fistulas) and/or defects in branching, relate to susceptibility to human disease. (*2*) Elucidate the role of epigenetic regulation in lung development and regeneration: Identify the changes in the epigenome between normal and altered lung development in mouse and human models. Use mouse genetic models and human cell models to test whether modulation of epigenetic pathways can alter development of specific lung cell lineages and affect repair and regeneration. (*3*) Apply knowledge of lung developmental pathways in generation of lung cell lineages from pluripotent stem cells: Recapitulate the developmental processes of lung specification and differentiation to promote cell type–specific differentiation in embryonic stem/induced pluripotent stem cells. (*4*) Identify mechanisms underlying the codevelopment of the pulmonary and cardiovascular systems: Define the codevelopment in animal models, including cell fate analysis. Determine the contribution and cell fates of structural components and supporting cell lineages.

Mechanisms of Lung Cell Differentiation

The respiratory epithelium harbors a variety of cellular phenotypes throughout the tracheobronchial tree and alveoli. The distribution and balance of these epithelial cell types differ along the proximal–distal axis as a function of the regional microenvironment. Little is known about the roles of distinct microenvironments (niches) and their regional differences in cellular and extracellular matrix (ECM) composition in promoting multipotent progenitor states or cell lineage differentiation in lung development and regeneration (18).

The mesenchymal compartment displays even greater complexity, encompassing a diverse population of endothelial cells, smooth muscle cells, ECM-producing fibroblasts, and cartilage-associated cells, among others. There is limited information on the origin and fate of these various mesenchymal cell types, on how they influence epithelial cell differentiation, and on their behavior during lung injury and repair.

Much progress has been made in the understanding of the molecular regulation of airway branching. However, the precise mechanisms that result in formation of the saccules and alveoli remain elusive. The complexity of these events, including their potential dependence on physical forces and local vascular blood flow, has been difficult to model in culture.

The early stages in the differentiation of airway progenitors are poorly understood and should be facilitated with the discovery of novel markers for specific airway lineage fate. Little is known about mechanisms of commitment to ciliated, secretory, or neuroendocrine cell fate and their role in lung epithelial regeneration. A relationship between primary and motile ciliogenesis has

been recently proposed (19) and may provide insights into basic mechanisms of ciliogenesis potentially applicable to lung disease and repair. Recent studies highlight the importance of a tight control of mitotic spindle orientation and planar cell polarity in the developing lung (20, 21). Studies are required to better define how changes in cyto-architecture organization, cell adhesion, and cell–cell interactions influence lung epithelial cell fate in development and in response to injury.

Research priorities are as follows. (*1*) Develop animal or cell culture models of alveolar formation: Design and test relevant cell and organ culture models of sacculation, alveolar septation, and type I cell–endothelial interactions that can reproduce the mechanical properties of the early alveolar stage lung. (*2*) Identify lung mesenchymal cell lineages: Develop markers that better delineate pericytes, myofibroblasts, lipofibroblasts, smooth muscle cells, and their precursors. Determine mechanisms of plasticity of mesenchymal phenotypes. Understand how injury affects mesenchymal phenotype or cell fate and potentially influences postnatal lung development and injury repair. (*3*) Determine the role of ECM in epithelial differentiation, injury, and repair: Generate a more comprehensive characterization of the temporal and spatial localization of abundant and rare matrix components. Characterize properties of ECM and microenvironment that promote regional differences in epithelial differentiation in development and injury–repair models. (*4*) Elucidate mechanisms of epithelial differentiation in development and repair after injury: Characterize the differentiation programs of the airway epithelial lineages and mechanism for proper repopulation of the airways after injury. Determine the trophic requirements of this process from other cell types in the lung and in other tissues. Understand the mechanisms of aberrant differentiation in metaplasias and hyperplasias in human lung disease. (*5*) Develop appropriate animal models to study specific aspects of human lung disease: Develop a better understanding of the basic progression, histopathology, and pathophysiology of lung diseases such as bronchopulmonary dysplasia, congenital diaphragmatic hernia, and rare lung diseases. Use animal models and phenotypic screens to identify components of the disease process.

Tissue Interactions in Lung Development

This session covered a variety of topics on tissue and organ interactions. Lung development requires orchestrated patterning of multiple cell and tissue types, including airway epithelium, pulmonary vasculature, lymphatic tissue, and nerves. Emerging data suggest that regeneration of the epithelial and vascular components of the lung is required to reestablish proper lung function after injury. The genetic and epigenetic factors that direct and coordinate the cross-talk among these tissues to ensure proper patterning of the developing lung are largely unknown. Signaling pathways that govern the tissue interaction in patterning the airways with blood vessels, lymphatics, and innervations are also elusive. These pathways may be affected by a variety of cellular and extracellular factors. Roles of major environmental factors, such as oxygenation, metabolic events, immune and inflammatory systems, endogenous progenitor cells, the microbiome, and mechanical factors related to the heart, musculoskeletal system, pleura, and placenta need to be dissected more thoroughly. Lymphatic vessels play key roles in lung inflammation (22). However, the formation and remodeling of lung lymphatics and their influence on lung maturation and function has not been studied extensively. The dynamic interactions of lymphatics, lung vasculature, parenchymal structure, and airway epithelium need to be studied during normal development and at pathogenic conditions.

Additional gaps in knowledge include roles of pulmonary innervation in normal lung development and diseases; pleural biology and potential functions of the pleura in developmental signaling and structural organization of the embryonic lung and during injury, repair, and regeneration; and influence from other tissues such as brain, kidney, pancreas, liver, and gut on lung development. The overall conclusion was that tissue interactions in lung development should be studied because they relate to other aspects of human development and human disease.

Research priorities are as follows. (*1*) Investigate the impact of other tissues in lung development pre- and postnatally: Characterize the reciprocal relationships between patterning and function of the heart and lung in development and disease. Define features of the diaphragm, pleura, and innervations of them that affect lung development. Determine how the placenta affects lung development via growth factors and stem cells. (*2*) Define lung cell patterning, maintenance, and plasticity in early development, postnatal maturation, and adulthood adaptation: Determine the contribution of the microenvironments (matrix), macroenvironment, and cell partnerships and interactions in regulating regional specificity of epithelial, smooth muscle, and endothelial cells. Define the conditions and factors that maintain unique circulations (bronchial, lymphatic pre- and intraacinar). Determine how neurons develop and contribute to lung parenchymal and vascular development. (*3*) Explore the mechanisms that couple the developing alveoli and vasculature in response to mechanotransduction: Characterize the circulating and mechanical factors induced by patterns of breathing, fluid mechanics, and gas exchange in lung development pre- and postnatally. (*4*) Use observations and principles derived from lung developmental studies to inform research in other areas: Study the establishment of host defense in development, maintenance of the unique microbiome, and interactions with the thymus and lymph nodes. Determine the influence of inflammation, immune abnormalities, and macrophage phenotypes on postnatal lung development and predisposition to later human disease (asthma, chronic obstructive pulmonary disease, and pulmonary arterial hypertension).

Environmental Impact on Early Lung Development

Discussions in this session focused on how the maternal and fetal environments influence early developmental events in the lung and the consequences for postnatal life. A focus on early environmental and pulmonary interactions is critical because early insults can alter lung development and processes involved in lung injury and repair.

Environmental insults that alter lung development and predispose to lung disease in postnatal life include intrauterine growth restriction, preterm birth that requires prolonged mechanical ventilation support, exposure to maternal tobacco smoke or nicotine, micronutrient deficiencies (vitamin A), and chronic exposure to biomass fuel smoke (23, 24). These conditions could have generational and transgenerational effects on the susceptibility of pulmonary diseases.

Within larger categories of disease (e.g., chronic lung disease of prematurity, reactive airways disease, and asthma), the effect of environmental factors on subtypes (e.g., intrauterine growth restriction) need to be delineated.

It remains elusive how the macroenvironment affects the pulmonary microenvironment *in utero*. Although the environmental impact likely occurs through epigenetic regulations, specific mechanisms through which environmental factors interact with genes and chromatin to affect alveolarization and predispose to postnatal pulmonary disease need to be identified (25). The epigenetic changes may function as informative molecular biomarkers or may cause disease later in life. One major barrier to study environmental impacts is how to identify the best accessible human samples. This discussion led to topics focused on strengths versus limitations of using accessible tissue/cell samples to assess the impact of environmental insults on lung development and disease and to identify biomarkers of disease or mechanisms of disease pathogenesis.

Research priorities are as follows. (*1*) Understand how the environment factors interact with epigenetics to influence lung development *in utero* and postnatally and later susceptibility to lung injury. (*2*) Conduct specialized investigations in animal models and culture systems of perinatal lung development and injury to demonstrate causality and efficacy of interventions. (*3*) Use insights from animal models and culture systems to ask epidemiological questions about exposures of humans. Identify biomarkers. (*4*) Correlate disease information with data embedded in accessible samples, such as urine, leukocytes in blood, tracheal aspirates, bronchoalveolar lavage fluid, and buccal smears. (*5*) Carry out longitudinal studies that span prenatal (maternal environment before and during pregnancy) and postnatal periods to better characterize maternal risk factors that determine lung size and function. (*6*) Carry out longitudinal physiologic and molecular characterizations to identify subtypes of lung diseases.

Final Workshop Recommendations (Not in Priority Order)

1. Apply knowledge of developmental processes to investigate human lung development and disease and identify developmental principles critically involved in human lung diseases. This will include, but not be limited to, identifying clinical phenotypes that have developmental origins.
2. Develop better experimental models to study the mechanisms of human lung diseases. Use these models and phenotypic screening tools to identify components of the disease process.
3. Develop models and tools specifically to study late lung development, including tracheobronchial, saccular, and alveolar formation; cell interactions; pre- to postnatal transition; postnatal maturation; and adaptability to the impact of environmental exposures.
4. Characterize the development of mesodermal, neural, and other nonendodermal lineages in the lung. Dissect individual lung mesenchymal cell lineages and determine the plasticity of mesenchymal phenotypes in response to injury.
5. Determine how lung blood vessels and lymphatics develop, undergo maturation, are maintained, and respond to or contribute to lung pathology and disease.
6. Study the impact of other organs and tissues (including placenta) on pre- and postnatal lung development. Identify tissue interactions in the human lung from development to disease.
7. Characterize the extracellular matrix and other features of the microenvironment that promote regional differences in lung cellular differentiation during repair and regeneration.
8. Develop strategies for integration of systems biology approaches to decipher the mechanisms of developmental origins of lung diseases.
9. Identify and study appropriate human cells and tissues obtained from developing/fetal/perinatal subjects to help define relevant disease pathways, biomarkers for prediction or treatment, and the relevance of animal and cell culture models. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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