

# Resident Cellular Components of the Lung

## Developmental Aspects

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In a recent workshop organized by the NIH-NHLBI, investigators working on different aspects of lung biology met to discuss recent progress regarding the origin, development, and characterization of the various cell lineages present in the lung in both normal and disease states. The workshop was entitled "Resident Cellular Components of the Human Lung: Current Knowledge and Goals for Research on Cell Phenotyping and Function." In this article we will highlight some of the developmental aspects of the lung discussed at the meeting. We will review information about developmental signals that are possibly reactivated during lung regeneration/repair and disease processes, and we will pose the questions and challenges viewed to be relevant to further advance the field.

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Lung development in mammals encompasses both prenatal and postnatal life (1, 2). Early during embryonic life, the foregut endoderm is specified into domains that will give rise to organs, such as the thyroid, lung, liver, and pancreas. Once respiratory cell fate has been established, the tracheal and lung primordia form, and the lung subsequently develops into a tree-like system of epithelial tubules and vascular structures that ultimately becomes the airways and the alveoli. During development the endoderm will differentiate into the multiple resident epithelial cell lineages, while the mesoderm will give rise to structures such as the vascular components of the lung, airway smooth muscle, lymphatics, tracheal cartilage, and pleura.

The abundance and localization of the distinct cell types lining the lung vary among species. Tracheal-bronchial glands arise from the underlying airways in late gestation, and mature postnatally. The number, size, and composition of tracheal-bronchial glands are highly variable among various mammalian species. Lung maturation is associated with sacculization and alveolarization to form the gas exchange region required for postnatal respiration. Alveolar epithelial cells undergo marked biochemical, morphological, and functional changes in the latter 1/3 of gestation in most mammals. Alveolar saccules are lined by type I cells that form an extensive gas exchange area with pulmonary capillaries, thereby mediating efficient gas exchange. Alveolar type II cells compose 5–10% of the alveolar surface, but are critical for production of surfactant lipids and proteins that are required for lung function at birth. Alveolarization occurs primarily in the postnatal period in both mouse and human lungs.

### RESPIRATORY CELL FATE SPECIFICATION IN THE DEVELOPING FOREGUT ENDODERM

When and how respiratory cell fate is initially established in the foregut endoderm is still unclear and a matter of debate. Early lineage tracing studies in the mouse support the concept that the peripheral (intrapulmonary) respiratory epithelium, except for neuroepithelial cells, arise from a commonly marked progenitor cell that is distinct from other endodermal cells as early as Embryonic Day (E)7 to 8. Intrapulmonary, ciliated, nonciliated (Clara), goblet, type II, and type I cells share this common lineage, while distinct subsets of cells contribute primarily to proximal airway epithelia lined by basal, ciliated, and non-ciliated cells of the trachea and main bronchi (3).

Commitment to lung cell lineage is marked by the expression of the transcription factor *Titf1* (Thyroid transcription factor 1, *Ttf1*, *Nkx2.1*) early in embryonic development, well before the appearance of definitive lung buds. In the mouse, respiratory progenitors are initially identified as a group of *Titf1*-expressing cells in the prospective lung field of the foregut endoderm at around E9 (4, 5). Although expression of this gene in the foregut has been reported by RT-PCR earlier than at this stage (6), it is unclear whether *Titf1* signals originate solely from the lung, since *Titf1* is expressed by both thyroid and lung primordia.

Genetic studies show that *Titf1* is critical for the development of distal lung progenitors (7). *Titf1*-null mice do form lungs, but these are highly abnormal, with airways expressing no specific marker of lung cell fate, such as surfactant protein genes (5, 7, 8). However, it is still unclear whether *Titf1* is needed to specify lung cell fate or whether it simply marks the initial respiratory lineage. In the thyroid, where *Titf1* is also expressed from the earliest developmental stages, it serves rather as a survival factor for the early thyroid progenitors (9). Definitive insights into the role of *Titf1* in lung specification would require knowledge of other early markers of respiratory progenitors in the foregut. A major limitation in the field is the fact that *Titf1* is currently the only early marker of lung cell fate available for these types of studies.

Another report suggests a critical role for signaling by fibroblast growth factors (Fgf) in specification of the lung lineage. Serls and coworkers (6) have shown that increasing threshold of Fgfs emanating from the heart, at a stage when heart and foregut are in close proximity, specifies initially the liver and then the lung.

Previous studies in vitamin A–deficient animal models have shown that disruption of retinoic acid (RA) signaling may lead to lung agenesis (10). This raises the possibility that in the absence of RA signaling, lung progenitor cells may not be specified or fail to expand. Studies on a genetic model of RA deficiency in which the foregut has never been exposed to endogenous RA showed that RA is actually not required for lung specification (11). These studies show that RA controls Tgf- $\beta$  signaling in the foregut and allows expression of Fgf10, a fibroblast growth factor that is critical for expansion of lung progenitors and for formation of primary lung buds (12). There is also evidence from studies in the *Drosophila* that Notch

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signaling is required for proximal-distal patterning of the respiratory tract (13).

### MOLECULAR MECHANISMS OF EXPANSION OF LUNG PROGENITOR CELLS DURING ORGAN DEVELOPMENT

Little is known about how the initial respiratory lineages are expanded and patterned to form different regions of the respiratory tract. In the E9.5 mouse embryo, Fgf10 is expressed locally in the foregut mesoderm at the prospective lung field and activates Fgfr2b signaling in a subset of *Titf1*-expressing cells, expanding this population to form primary lung buds (reviewed in Reference 1). Fgf10-null mice have tracheae but do not form lungs (14). Although the entire *Titf1* field is able to respond to Fgf10, only the lung progenitors depend on Fgf10 for survival. The reasons for this are currently not well understood. The selective dependence of Fgf10 for survival of the lung (but not tracheal) progenitors suggests an Fgf-dependent mechanism of segregation of respiratory progenitors that occurs during formation of the lung primordium.

Attaining proper size of the lung depends on mechanisms that balance proliferation and differentiation of the various cell lineages. There is evidence that Fgf10 is necessary to generate a proper number of progenitor cells in certain developing organs. For example, in Fgf10-null mice, the pancreas or stomach are much reduced in size (hypoplastic) (15, 16). Fgf10 is expressed throughout lung development (17). Interestingly, lungs from a recently reported Fgf10 hypomorphic mouse are hypoplastic (18). During subsequent stages, other signals contribute to expand the population of distal epithelial progenitor cells at the bud tips, while maintaining them relatively undifferentiated. Among these are the proto-oncogene *Nmyc* and the canonical Wnt pathway. Selective disruption of *Nmyc* in distal lung epithelial cells in mutant mice leads to local premature differentiation and inhibits cell proliferation (19). Genetic studies suggest that expression of *Nmyc* and *Bmp4* (bone morphogenetic protein-4) are under the control of the Wnt pathway in the distal lung epithelium (20). There is also evidence that during expansion of distal lung progenitors Fgf10 controls expression of key components of the Bmp pathway, including receptor (*Bmpr1*), ligand (*Bmp4*), and cathepsin H (*Ctsh*), a cysteine protease that promotes *Bmp4* degradation (21–23). The studies above help to identify molecular interactions potentially critical for the development of lung progenitors and ultimately help to define their niche.

### FACTORS THAT DEFINE SPECIFIC CELL FATES DURING LUNG DEVELOPMENT

Distinct signaling and transcriptional programs regulate epithelial cell differentiation along the proximal-distal axis of the lung (reviewed in Reference 2).

Conducting airways are lined primarily by distinct epithelial cell types, including ciliated, nonciliated columnar, goblet, and basal cells.

Differentiation of neuroendocrine cells occurs relatively early in lung morphogenesis and is influenced by transcriptional pathways regulated by MASH and GFI-1 (growth factor independent 1) (reviewed in Reference 2). Neuroendocrine cells are derived from cell lineages that are apparently distinct from other respiratory epithelial cell types present early in embryonic development. In contrast, most nonciliated, ciliated, and goblet cells share common lineages and progenitor relationships during lung morphogenesis (3). Differentiation of conducting airway cells is dependent upon *Foxa2*, *Titf1*, and  $\beta$ -catenin present early in lung morphogenesis. Thereafter, airway differentiation is further influenced by various transcription factors, including *Sox* and *Ets*

family members, *Foxj1*, and Kruppel-like factor 5 (*Klf5*), which are variably expressed in the developing and mature lung (2).

Transcriptional networks, including *Titf1*, *Nfatc3*, *Nf-1*, *C/EBP $\alpha$* , *Foxa2*, *Etv5* (an *Ets* family member), and *Gata6*, influence both sacculization and alveolarization. They control differentiation of alveolar epithelial cells and all are critical for perinatal surfactant function and for normal formation of the alveolar region (2). Sacculization, alveolarization, and vascularization of the peripheral lung are dependent on many of the transcription factors involved in lung maturation (for example *Titf1*) that influence the expression of factors that may influence pulmonary vascularization (8). Expression of *Foxf1*, a transcription factor expressed in the developing splanchnic mesenchyme, is critical for pulmonary vasculogenesis, regulating a number of genes that control mesenchymal differentiation and blood vessel formation (24). Pulmonary vasculogenesis is directed, in part, by paracrine signaling from the respiratory epithelium to vascular cell precursors in the lung mesenchyme. Continued expression of some of these transcription factors occurs in the mature lung, their expression being dynamically regulated during proliferation and transdifferentiation that is required for repair of the respiratory epithelium after lung injury (25).

### TRANSIENT VERSUS PERMANENT FEATURES IN DIFFERENTIATING LUNG CELLS: REPROGRAMMING CELL FATES

Classical tissue recombinant studies in organ cultures suggest that there is a great deal of plasticity in the epithelium during development, and there is current evidence that this may be also true for the mature organ. In embryonic tissues, for example, lung-specific genes can be induced in kidney epithelium by recombination of lung mesenchyme and kidney epithelium (26). This raises questions about the extent to which cell fates are sealed during organogenesis, and whether and how cell fates can be reprogrammed. In studies with embryonic lung explants, engraftment of distal lung mesenchyme along the trachea converts proximal airway cells into differentiated type II epithelial cells, demonstrating the remarkable plasticity of early respiratory progenitors (27). Similarly, reprogramming of lung epithelial cells has been reported in mice that express a constitutively active  $\beta$ -catenin–*Lef* fusion construct in putative distal epithelial lung progenitors. During normal development Wnt– $\beta$ -catenin signaling in the lung epithelium is required to regulate proximal-distal cell fates as airways branch (28). However, in the presence of constitutively increased Wnt canonical, the lung epithelium of these mice transdifferentiates into intestinal type (29). This model raises the possibility that, even at a relatively later stage, progenitor cells of the lung can still be reprogrammed to transdifferentiate into cells from another foregut-derived structure. These observations also raise the possibility that, during development, specific cell fates require different levels of activation of certain signals such as canonical Wnt, abnormally high levels of Wnt signaling being compatible with intestinal rather than a lung program.

### DIVERSE EPITHELIAL CELL NICHES IN THE MATURE LUNG

The mature mammalian lung is remarkably nonproliferative, with the slow rates of mitosis, and prolonged survival of resident cells. Nevertheless, after infection, resection, inflammation, or toxicant exposure, the lung is capable of rapid and extensive proliferation of various epithelial cell types. In the alveolar region, type II epithelial cells proliferate and differentiate rapidly into type I cells, as well as replace injured epithelial

cells after exposure to various pathogens and toxicants. In conducting airways, numerous cell types, including subsets of nonciliated columnar cells and basal cells, can proliferate. These progenitor cells or their progeny differentiate into other respiratory epithelial cell types, including Clara cells, goblet cells, and ciliated cells. The respiratory epithelium is capable of rapid squamous metaplasia, proliferation, and migration to replace the injured epithelial surfaces (30–33).

A number of experiments support the concept that discrete anatomic regions harbor progenitor cells that are relatively protected from injury (reviewed in References 34–36). Progenitor or “stem cells” in these regions may have unique capacities for both self renewal and for provision of progenitor cells capable of proliferation and migration to repair the injured respiratory epithelium. Basal cells, cells lining the necks of tracheal-bronchial glands, toxicant-resistant nonciliated cells (Clara cells) residing near neuroepithelial bodies (NEBs), and cells within the bronchoalveolar duct junction (BADJ cells) have been proposed as uniquely capable of serving as progenitor cells after extensive epithelial cell injury (32, 37). The signaling and transcriptional programs mediating quiescence and stem and progenitor cell behavior in the respiratory epithelium are of considerable interest to understanding the pathogenesis of both acute and chronic lung disorders and pulmonary tumorigenesis.

#### **TO WHICH EXTENT CAN EARLY DEVELOPMENTAL PROGRAMS BE RECAPITULATED?**

Studies in disease states or during injury-repair of the lung often reveal changes in cell behavior and gene expression that can be reminiscent of that described in specific developmental processes of the lung. For example, during bud formation *Fgf10* and *Bmp4* are sequentially induced in the developing lung as part of a mechanism that presumably controls epithelial growth (reviewed in Reference 1). Interestingly, in the adult lung *Fgf10* and *Bmp4* appear to be coordinately expressed during hyperoxia injury repair. Hyperoxia induces a hyperproliferative response in type II cells, which is accompanied by up-regulation of *Fgf10* followed by a peak in *Bmp4* coincident with the decrease in cell proliferation (38).

During development, *Fgf10* acts as a survival factor for the distal lung progenitors. In the adult mouse, exogenous FGF10 decreases asbestos-induced alveolar epithelial cell DNA damage and apoptosis, in part by mechanisms involving MEK/ERK-dependent signaling (39). Nevertheless, aberrant *Fgf10* signaling has been associated with tumorigenesis. There is evidence that FGF10 acts as a proto-oncogene in mouse mammary tumors and that *Fgf10* is overexpressed in a subset of human breast cancers (40). Transgenic mice expressing *Fgf10* in the lung epithelium show multiple pulmonary tumors (41). Moreover, targets of *Fgf10* expressed in epithelial progenitors of the embryonic lung and other organs that depend on *Fgf10*-*Fgfr2b* signaling for survival are also found in cancer (22). Understanding the context in which these signals work will likely provide useful insights into the programs involved in lung cell plasticity, repair, and tumorigenesis.

#### **ABNORMAL CELLULAR PROGRAMS IN LUNG INJURY-REPAIR AND CANCER**

The respiratory tract represents an extensive surface area that is continually exposed to pathogens, particles, and toxicants that can influence respiratory epithelial cell proliferation and differentiation. Innate host defense systems protect the lung, and maintain pulmonary homeostasis after injury via complex

mechanisms that include mucociliary clearance, fluid and electrolyte regulation, and the synthesis and secretion of a variety of host defense molecules by the respiratory epithelium. Changes in differentiation and function of the respiratory epithelium associated with acute and chronic injury, in turn, can influence the pathogenesis of many chronic and acute lung diseases. For example, goblet cell hyperplasia is associated with asthma, chronic obstructive lung disease, and cystic fibrosis. Likewise, recurrent exposure to endotoxin, pathogens, and other toxicants (e.g., cigarette smoke) is associated with metaplasia of the respiratory epithelium or goblet cell hyperplasia. Goblet cell metaplasia occurs from nonciliated and, perhaps, ciliated cells and is associated with the loss of *Foxa2*. Increased Spdef (SAM-pointed domain Ets-like factor), an Ets family transcription factor, is sufficient to cause goblet cell differentiation in the respiratory epithelium (42). Acute and chronic lung injury also causes squamous cell metaplasia that perturbs mucociliary clearance and host defense processes in the lung. Increased cell turnover associated with metaplasia may be an important antecedent to pulmonary carcinogenesis. Abnormalities in multiple signaling and transcriptional pathways, including ras, Pten, Myc, Egfr, and  $\beta$ -catenin are associated with pulmonary tumorigenesis and represent potential targets for therapeutic interventions (recently reviewed in Reference 43). Since many of the transcriptional programs influencing lung morphogenesis and differentiation are shared with those expressed during repair, the precise control of proliferation and differentiation must be exerted to maintain normal lung structure.

Hyperproliferation and metaplasia of the respiratory epithelium are associated with high levels of expression of many of the transcription factors influencing lung morphogenesis and repair, and it is intriguing to speculate that some of these processes are involved in pulmonary carcinogenesis. Lung cancer cells often express many of the cell markers and share histological and ultrastructural characteristics of more normal respiratory epithelial cells. For example, expression of *Titf1* is useful in distinguishing metastatic adenocarcinomas that arise from the lung rather than breast, pancreas, and other organs (44). Recent studies demonstrate the high frequency of an amplification of 16q, a region containing the *TTF-1* gene in human pulmonary carcinomas (45). Differentiated cell markers and mRNA expression profiling are useful in distinguishing adenocarcinomas from squamous or small cell carcinomas. The maintenance of differentiated cell features and the variability of epithelial cell differentiation seen in various lung tumors may indicate their derivation from so-called “tumor stem cells” or, alternatively, oncogenic transformation of the many distinct subsets of progenitor cells present in the lung.

#### **CONCLUDING REMARKS AND QUESTIONS: WHAT WE DON'T KNOW AND NEED TO KNOW**

The respiratory epithelium consists of diverse cell types whose numbers, localization, and function vary dynamically during lung morphogenesis and after acute and chronic lung injury. The signaling and transcriptional pathways controlling respiratory epithelial function, differentiation, and proliferation provide a framework for investigation and the elucidation of abnormalities in repair of the lung that underlie many acute and chronic lung diseases, as well as lung cancer.

Progress in critical areas of lung biology has been limited by the restricted availability of markers of the various lung cellular components and by the poor understanding of the mechanisms by which lung cells acquire and maintain their phenotype. In the lung this is further complicated by the great diversity of cell types and the dynamic changes in tissue structure and cell fate

that occur during development and disease. Over the years, a panel of markers has been developed to identify some differentiated cellular profiles both in the developing and the adult lung. However, little is still known about the molecular profile of the multipotent progenitor cells that reside at the various germ layers and regions of the lung as the respiratory tract forms. Similarly, few cell-specific markers or cell surface markers are available to investigate issues related to the biology of putative progenitor/stem cell niches in the adult lung. In most cases analysis of these cells involves isolation procedures that disrupt their interactions with neighbor cells and may alter their original molecular signature. Markers of hematopoietic lineage have been used as surrogate markers of progenitor/stem cells, but current data question this use. Prospective global surveys of specific regions of the developing and mature lung would be useful to characterize different lung cell niches. However, feasibility of these studies is challenged by the limiting amount of material collected and the detection sensitivity of the methodologies currently available. Most of the available global surveys of the lung are limited to mRNA profiling, and these are largely of poorly defined mixed cell populations. Thus, potentially critical aspects of lung biology related to post-transcriptional and post-translational events are understudied, and could be revealed by an integrated analysis of the transcriptome, proteome, and glycome of the lung. Moreover, little is known about the molecular features of hyperplastic and metaplastic lesions of the human lung and the relationship of these expression profiles to that of the normal or developing lung tissue. Characterizing these lesions would help to identify regulatory networks potentially involved in injury-repair programs of the lung. Initiatives that address one or multiple aspects of the problems described above would:

- a. Develop strategies and technologies for sampling and molecular characterization of single cell or groups of cells and their microenvironment in the developing or adult lung.
- b. Miniaturize assays and develop sensitive methods for large-scale analyses and validation of mRNA, protein, and carbohydrates that minimize the requirement of large number of samples/tissues.
- c. Characterize and compare global patterns of gene/protein expression at critical developmental stages in murine and human lungs using multidisciplinary approaches.
- d. Characterize the molecular signature of reactive, nonneoplastic lesions, including metaplasias and hyperplasias in archival/fresh specimens from human biopsies, and establish potential relationships with original tissues.
- e. Develop methodologies to recognize, isolate, and lineage-trace lung progenitor cells in animal models through different developmental stages and in the adult during injury repair.
- f. Develop and characterize biologically significant model systems that reproduce specific microenvironments of the embryonic or adult lung, which can provide insights into niche-specific signaling events.
- g. Identify cell-specific and cell surface markers useful for the identification and purification of distinct pulmonary cell types.
- h. Develop useful genetic tools for the introduction, deletion, and mutation of genes in various pulmonary cell types in the mouse and other species.

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