Cell Plasticity in Lung Injury and Repair

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In April 2010, a NIH workshop was convened to discuss the current state of understanding of lung cell plasticity, including the responses of epithelial cells to injury, with the objectives of summarizing what is known, what the field needs to know, and how to get there. The proximal stimulus for this workshop is the body of recent evidence suggesting that plasticity is a prominent but incompletely characterized property of lung epithelial cells, and that a focus on understanding this aspect of epithelial cell biology in particular, may be an important window into disease pathobiology and pathogenesis. In addition to their many vital functions in maintaining tissue homeostasis, epithelial cells have emerged as both a central target of disease initiation and an active contributor to disease progression, making a workshop to investigate the role of cell plasticity in lung injury and repair timely. The workshop was organized around four major themes: lung epithelial cell plasticity, signaling control of plasticity, fibroblast plasticity and crosstalk, and translation to human disease. Although this breakdown was recognized to be somewhat artificial, it was felt that this approach would promote cross-fertilization among groups that ordinarily do not communicate and lend itself to the generation of new approaches. The summary reports of individual group discussions below are followed by consensus priorities and recommendations of the workshop participants.

Keywords: epithelial-mesenchymal transition (EMT); idiopathic pulmonary fibrosis; cell lineage

Respiration is dependent on a lung structure that is maintained by interactions among multiple cell types. This diversity of cell lineages adjusts to the mechanical and environmental challenges required for pulmonary function throughout life, providing a physical barrier to microbial pathogens, particles, and toxicants and enhancing host defense processes via mucociliary clearance and production of molecules mediating innate and acquired immunity. Lung structure is further maintained by the

ability of pulmonary cells to proliferate, migrate, and differentiate to regenerate normal lung structures after injury. In spite of the elegance of this multitiered protective system, compromises in lung structure, whether initiated by inherited disorders or by interactions with the environment, are a major cause of morbidity and mortality associated with chronic lung disease. Common pulmonary disorders (e.g., pulmonary fibrosis, chronic obstructive pulmonary disease, asthma, cystic fibrosis, emphysema, bronchopulmonary dysplasia, and bronchiectasis) affect millions of people worldwide. In these disorders, marked changes in lung structure are accompanied by alterations in cell differentiation and function (e.g., terminal metaplasia, dysplasia, hyperplasia and perhaps epithelial-mesenchymal transition of the epithelium, and, in the case of fibroblasts, transition to a myofibroblast phenotype). These alterations are significantly influenced by cell-cell and cell-matrix interactions. Knowledge regarding the cellular and molecular processes maintaining normal lung cell homeostasis and mediating abnormal lung repair associated with chronic lung diseases will provide the framework for the development of strategies to prevent, diagnose, and treat pulmonary disorders that remain a significant health burden throughout the world. It was a testimony to the vitality and fluid nature of this field that there was no easy consensus among the participants on the definition of cell plasticity itself. Yet it was largely agreed that cell plasticity could be defined, at least for the purposes of the workshop, as “the ability of some cells to switch from one specific program of gene expression (phenotype) to another in response to specific signals from the environment in a regulated fashion. The changes may be reversible or irreversible and likely but not necessarily involve changes in cell shape, adhesion, and proliferation.” It was recognized that regulated phenotypic transitions may occur in a number of cell types besides epithelial cells, including endothelial cells and mesenchymal cells. However, so as not to dilute the focus of the workshop, discussion was largely limited to phenotypic transitions of epithelial cells and fibroblasts and cross-talk between these two cell types. In addition, one of the major limitations to advancing the field has been a lack of good models for pulmonary injury and fibrosis that involve epithelial injury independent of inflammation and that would more closely reflect human disease. Thus, discussion of alternate approaches to develop improved models of fibrosis was also a focus.

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Although it was recognized that many of the processes being discussed were relevant to other diseases, such as chronic obstructive pulmonary disease, to maintain the focus of the workshop, discussions of injury models were largely directed toward fibrosis.

GROUP 1: LUNG EPITHELIAL CELL PLASTICITY

Early Definitions of Lung Epithelial Lineages

The many specialized epithelial cells of the lung have long been defined by two inextricably linked properties: structure and function. This has led to definitions of lung epithelial cells that are based on their location and physical appearance, such as ciliated, goblet, serous, and basal cells of the conducting airways and type 1 and 2 epithelial cells of the alveoli. Classic microscopy studies using thymidine labeling to detect cell proliferation examined lung sections in the steady state or after injury (1–9). These studies defined subsets of airway and alveolar epithelial cells that exhibited the capacity to proliferate after injury. In addition, some labeled cells changed their shape, implying an alteration to the differentiated phenotype of the epithelial cells (10, 11). Developmental biologists established that multiple types of lung epithelial cells arose from the definitive endoderm, with lung epithelial progenitors being first detected in the primitive anterior-ventral foregut of the embryo and identified by expression of the transcription factor *Nkx2.1* (*Titf1*) (12). The concept that a limited number of lung epithelial cell types were specified during embryonic development and more or less retained their committed differentiated state throughout life became conventional wisdom.

New Paradigms in Epithelial Biology Initiate Debate over Lung Epithelial Plasticity

A number of advances in molecular and stem cell biology have begun to challenge this rigid paradigm, suggesting that much more diversity and fluidity may be present in various lung epithelial compartments (13–16). In instances where lung epithelia have appeared to derive from or turn into cellular phenotypes that were viewed as being a departure from the normal expected biology of that cell, the somewhat vague term “plasticity” was used to imply that a cell’s program was pushed or pulled away from its natural homeostatic or developmental state by signaling mechanisms that are poorly understood. Because the diseased or injured lung frequently displays histology that is apparently disordered, metaplastic, or filled with mobilized cells, many investigators speculate that the genetic programs and lineage relationships present in the epithelial cells in these tissues may similarly deviate from normal. Whether these programs indicate cellular plasticity or the recapitulation of normal developmental programs is an active area of study that can best be advanced by the generation of new molecular tools, markers, lineage tags, and bioinformatics approaches.

Emerging Technologies Allow Evaluation of Putative Heterogeneity and Plasticity in Epithelial Cells

Evolving technologies have also recently challenged rigid definitions of sets of epithelial cells. For example, the ability to track cells *in vivo* and over time after injury, with lineage tags or multiple marker genes, has suggested that no single marker can adequately define one type of lung epithelial cell. It is likely that significant heterogeneity and plasticity exist in each type of lung epithelial subset (17, 18). Furthermore, the advent of modern flow cytometry has allowed increasing fractionation of cells previously thought to contain only a single cell type, revealing heterogeneity in these

cell subsets, based on gene expression, proliferative capacity, cell surface markers, or *in vitro* differentiation repertoire (19, 20).

Regarding lung epithelial cell biology, the recent discoveries in reprogramming have challenged investigators to acknowledge that extreme alterations in lineage, epigenetic memory, and differentiation repertoire are at least feasible in the laboratory dish in any cell type. The untested question becomes whether these alterations, such as reprogramming or cellular plasticity, ever happen naturally *in vivo* or whether some favorable resetting of cellular states can be engineered to treat lung epithelia damaged by injury or disease.

Alterations in Cell Fate: Epigenetic Memory of Lineage and Plasticity

Plasticity rests upon the assumption that the cell has a definable “genetic program” (such as a transcriptome) in one state and a memory of that program. This memory of lineage and cell state is generally considered to be the epigenetic state of the cell, which is heritable, but it also can shift as the cell makes decisions regarding fate under the influence of a variety of stimuli.

New Meets Old: Lung Microscopy Revisited with New Markers, Genetic Tags, and Molecular Biology

Sophisticated imaging and the capacity to generate an increasingly diverse tool box of inducible genetic lineage tagging approaches now provides the seminal opportunity to again use the microscope to define the lineage relationships of parent and progeny during normal development as well as during disease and after injury. Induced genetic marking of certain lung cells or their niches will also allow study of micro- or macroenvironments and supporting niche cells that are increasingly described in other organ systems but virtually unstudied in lung biology.

Recommendations

1. Fate mapping of lung epithelial and mesenchymal cells at high resolution during development, injury, and repair. Development of additional lung cell-specific transcriptional drivers useful for gene addition, deletion, lineage analysis, and purification of various pulmonary cell types from normal and diseased lung.
2. Establishment of new cellular definitions that take into account heterogeneity and complexity of lung epithelial cells previously defined by only one marker gene. This would require development of (1) panels of cell-specific markers useful for the identification and purification of multiple lung cell types and (2) highly specific reagents (e.g., antibodies, mRNAs, and surface markers) useful for the identification of pulmonary cells from various mammalian species.
3. Translation of lung epithelial subtype definitions described in animal studies to human lung to assess whether similar lung epithelial phenotypes exist in the normal or diseased human lung.
4. Defining the genetic and epigenetic programs of purified sets of lung epithelial cells in the normal and diseased lung as well as at key time points in development. This will necessitate banking of mRNA, miRNA, epigenetic, proteomic, and transcriptional data and the formation of associated data warehouses and bioinformatics infrastructures. Particular attention should be focused on the key molecules (e.g., DNA methyltransferases, histone methyltransferases and deacetylases, and polycombs) that

regulate epigenetic changes because these changes likely determine heritable memory of cellular lineage.

5. Generation of *in vitro* model systems that recapitulate developmental milestones and mechanisms controlling cell fate decisions potentially using (1) immortalized normal and disease-specific pulmonary cell lines; (2) novel induced pluripotent stem cell and embryonic stem cell systems; and (3) *in vitro* and *in vivo* cell and organ culture systems, bioengineered scaffolds, and organoids.
6. Development of improved functional assays for assessing differentiated cell function for specific pulmonary cell types.

GROUP 2: SIGNALING CONTROL OF PLASTICITY

Transcriptional, Posttranscriptional, and Epigenetic Regulation of Gene Expression

Effective repair and regeneration of the lung after injury to the conducting airways or gas exchange apparatus requires precise control of progenitor cell fate. Thus, it is essential to identify the progenitor population(s) generating the cells and matrix comprising the effectively repaired lung and to understand how their proliferation and differentiation are regulated. Based on data imported from development, regeneration, and cancer, we review here knowledge about progenitor cell identity and control in the lung. We discuss the idea that mechanisms regulating a prototypical change in differentiated state—the epithelial–mesenchymal transition (EMT)—may reveal the multilevel linkage between signaling, gene expression, and phenotype.

Lessons from Development

The Wnt signaling network as a paradigm for the control of cell fate. Wnt signaling plays a critical role in specification and differentiation of early lung endoderm progenitors. The pathway appears to have a more profound effect on distal than on proximal lung development (21–23). These findings, along with data showing that Wnt signaling is important for tissue regeneration, suggest that this pathway may regulate lung epithelial regeneration and repair, especially in the distal or alveolar epithelium.

Wnt signaling may affect multiple cellular processes that are potentially antagonistic during tissue repair (e.g., progenitor expansion and fibrosis). Studies of this pathway need to include a more thorough understanding of how signaling networks integrate and control the response of adult lung epithelium to external injury and disease, especially with respect to epithelial–mesenchymal cross-talk. This should include studies exploring the integration of multiple signaling pathways, including FGF, BMP, Notch, and Shh, during injury and regeneration coupled to an expanded repertoire of injury and regeneration models.

Progenitor potential of basal cells in airways disease. Small airways of the human lung are sites of significant pathology. They are approximately 1.0 to 1.5 mm in internal diameter, the same diameter as mouse trachea and primary bronchi that are known to use p63⁺ basal cells as stem cells. Mouse airway basal cells that can be identified as p63⁺, Ngfr⁺, Krt5/14⁺, P-cadherin⁺, T1α⁺, and Egfr⁺ are multipotent stem cells that can self-renew and give rise to secretory and ciliated cells. Studies show that human basal cells are also multipotent stem cells that can generate surface epithelium and submucosal glands. However, we lack a rigorous comparison of human and mouse basal cells.

Although there is a paucity of studies on basal cells and their distribution in human lung disease, their capability as progenitors provides motivation to understand their potential role in hyperplasia, squamous metaplasia, and mucus metaplasia. We do not know whether aberrant basal cell function contrib-

utes to defective airway remodeling in human respiratory diseases. Defects, if present, may be intrinsic to basal cells or result from abnormalities in signaling from the niche, including stroma, endothelial cells, neighboring epithelial cells, and immune cells. Advances in this area will require injury–repair models in the mouse designed to study the role of basal cells in airway pathology together with ready access to well curated pathological samples of human airways disease.

Lessons from Cancer Biology

Weinberg and Polyak have championed the concept that epithelial tumors are populated by a small subset of tumor cells with stem cell features (26). In murine breast models, CD44⁺/CD24[−]/lin[−] marks tumor-initiating cells that have mesenchymal features, the capacity to form mammospheres *in vitro*, and stem-like gene expression profiles enriched in Wnt-, Notch-, and EMT-related genes (24, 25). Furthermore, microRNAs involved in EMT regulation (miR-200, miR-335, miR-10b, and miR-9) are differentially expressed in breast tumors and correlate with poor prognosis (26, 27).

Although far less developed than the published work on breast cancer, studies on lung cancer report evidence of tumor cell populations with stem-like properties, including asymmetric cell division, polarized sphere formation in 3-D cultures, slow cell division and prolonged quiescence, prominent expression of drug efflux pumps, resistance to cytotoxic agents, and stem-like gene expression profiles (28–31). The cell surface markers that distinguish these cells from the larger tumor cell population have not been fully studied, but by far the most commonly cited marker of these cells is CD133 (28).

Lessons learned from lung tumor cells should be applied to normal lung progenitor cells with caution because, unlike normal lung progenitors, tumor cells have aberrant chromosomes, somatic mutations, abnormal cellular architecture, and altered expression of surface receptors. However, there are important parallels between lung cancer cells and nonmalignant progenitors residing in a diseased lung. Both share an “activated” microenvironment that is often hypoxic, fibrotic, inflammatory, angiogenic, and enriched in cytokines and growth factors.

Lessons from Lung Injury

Evidence of epithelial plasticity appears after many airway and parenchymal lung injuries, ranging from metaplasia and transdifferentiation in the major airways with viral infections to hyperplasia and potentially EMT in the lung parenchyma during fibrogenesis (13, 32, 33). It is not known whether only a small subset of preexisting, relatively undifferentiated cells accounts for regeneration and EMT after injury or whether most or all differentiated alveolar epithelial cells dedifferentiate, proliferate, or are reprogrammed toward different cell lineages. These are critical gaps in our understanding of lung repair.

The epithelial potential for proliferation and drastic phenotypic change after injury has elicited considerable interest in the underlying signaling mechanisms that drive epithelial responses to injury. Lung injury superimposes on regulated, epithelial cell TGF-β1, signaling additional insults that may alter the outcome. Persistent inflammation results not only in persistence of fibrin- and fibronectin-rich matrices in the lung that accentuate repair but also potentially the persistence of proinflammatory proteases, chemokines, and cytokines (e.g., IL-6 and IL-13) that foster ongoing TGF-β1 activation and fibrosis (34, 35). A second complicating insult is local hypoxia, in part from alveolar edema or collapse (36). Hypoxia results in accumulation of hypoxia inducible factor (HIF)-1α and

epithelial reprogramming that resembles that of TGF- β 1 signaling (37). Collectively, these insults challenge the self-regulating repair mechanisms of the distal lung and perpetuate a profibrotic state. The specific roles of alveolar collapse and edema and the resulting hypoxia and HIF signaling in lung fibrogenesis are not known.

Recommendations

1. Establish the role of specific progenitor cells in repair and regeneration of the airways and gas exchange apparatus when the process is effective.
2. Determine the hierarchy and identity of progenitor cells that function when repair and regeneration of the airways or gas exchange apparatus fails.
3. Characterize the relative importance of extrinsic signals versus cell autonomous functions.
4. Delineate key signaling pathways (e.g., Wnts, Notch, and FGFs) and transcriptional regulators of cell fate decisions (and plasticity if verified) in lung epithelial subsets.
5. Identify critical parallels and differences between the circuitry of cancer and fibrosis.
6. Determine relevance of cell types identified as progenitors in mouse to human lung disease by comparison with carefully curated banks of human tissue.
7. Analyze interactions between TGF- β 1 and β -catenin signaling pathways in lung regeneration and fibrosis as a prelude to drug trials targeting these pathways.
8. Establish the role of epigenetic and other posttranscriptional alterations in gene expression accompanying changes in state triggered by Wnt or TGF- β 1.

GROUP 3: FIBROBLAST PLASTICITY AND CROSSTALK

Bidirectional interactions between epithelial cells and fibroblasts and interactions with matrix may strongly influence outcomes after lung injury. In addition, fibroblasts may exhibit phenotypic plasticity and exhibit heterogeneous phenotypes and fates after injury. The goals of this session of the workshop were to examine what is known regarding the effects of cell–cell and cell–matrix interactions on cell fate and to explore opportunities for influencing outcome after lung injury by modulating these interactions. Additional goals were to evaluate what is known regarding fibroblast fate and to explore opportunities for modulating cell fate to promote repair after injury.

Structural Considerations of Airway-Alveolar Fibroblasts

Ultrastructural studies of the adult mammalian lung indicate that interstitial mesenchymal cells (which we refer to as “fibroblasts”) form direct contacts with alveolar epithelial cell type 2 (AEC2) and capillary endothelial cells on both sides of the air–blood barrier (38). Numerous small apertures in the basement laminae that support AEC1 and AEC2 allow for cytoplasmic extensions and interdigitations of fibroblasts with adjacent epithelial cells (39, 40). Interstitial fibroblasts appear to form an interconnected reticular network that extends from the distal alveoli to the more proximal conducting airways (41).

Fibroblasts are structurally well positioned to “sense” and respond to structural and functional changes in airway and alveolar epithelial cells and in vascular endothelial cells. Additionally, fibroblasts participate directly in immune cell trafficking to the airways; neutrophils and eosinophils migrate in contact with the fibroblasts that “guide” them through apertures

in epithelial and endothelial basal laminae (39, 42, 43). In chronic obstructive pulmonary disease (COPD), a significant decline in the numbers of epithelial cell–fibroblast contacts with reduced numbers of basal lamina apertures are observed in emphysematous regions of lung parenchyma (40) and in conducting airways (44); this is associated with loss of airway caliber and thickening of the more proximal conducting airways.

Functional Integration and Crosstalk by Fibroblasts

In addition to direct cell–cell contact (“juxtacrine” signaling), communication between lung cells may occur through paracrine and autocrine signaling. The homeostatic mechanisms that maintain the differentiated state of resident lung cells are not well understood and are likely to require regulatory input from neighboring cells (45). In smokers and in patients with severe, chronic asthma, epithelial changes, such as squamous metaplasia and goblet cell hyperplasia, accompany the fibrotic remodeling response (46, 47). Such phenotypic changes may be perpetuated by bidirectional paracrine signaling between epithelium and mesenchyme (48–50). Fibroblasts may also regulate the phenotype and fate of cells by elaboration and remodeling of the extracellular matrix (ECM). The ECM provides mechanical support to the lung and critical contextual information for maintenance of epithelial cell phenotype in healthy lungs. Modulation of ECM facilitates tissue regeneration in response to injury, but sustained ECM alterations may lead to aberrant epithelial cell phenotype and function. Changes in ECM composition and structure affect cellular phenotype as a direct consequence of changes in signaling pathways controlled by integrins and other cell–ECM signaling molecules but also by virtue of the ability of the ECM to act as a reservoir and modulator of soluble signaling molecules.

There is a high degree of tissue-specific variability in epithelial–fibroblast interactions because epithelial differentiation is affected differently by mesenchyme derived from the trachea versus mesenchyme at distal structures, and mesenchyme derived from these different sites has differential sensitivity to soluble molecules produced by epithelial cells (51, 52). However, although much has been learned about the different types and functions of epithelial cells in the lung, the corresponding studies defining functional differences among lung fibroblasts are much less complete, limiting our ability to define functional interactions between epithelial cells and fibroblasts. Microarray studies have identified distinct gene expression profiles for lung fibroblasts as compared with other fibroblasts (53, 54) but not for distinct regions within the lung; much less is known about local changes in ECM composition and structure in the initiation and progression of lung diseases.

Fibroblast phenotype and fate. Fibroblasts are inherently versatile cells with remarkable plasticity. Although fibroblasts are of mesenchymal origin, no specific markers have been identified, and significant heterogeneity exists between different subsets within a population. Although reports of their ability to differentiate into epithelial and endothelial cells *in vivo* are limited, fibroblast differentiation into mesenchymal lineages (e.g., adipocytes, chondrocytes, and osteocytes) is well recognized. Fibroblast plasticity is well suited for their central role in tissue responses to injury; myofibroblast differentiation is a hallmark of wound healing responses in diverse organ systems, including the lung (55, 56). The origins of myofibroblasts and their relative contributions to lung repair and fibrosis remain unclear, although they may derive from local tissue mesenchymal progenitor cells (57), circulating fibrocyte-like cells (58–60), and epithelial–mesenchymal transition (16, 61, 62).

Recommendations

1. Develop technologies and methods to specifically mark and trace the lineage of resident fibroblasts.
2. Determine and characterize different subpopulations of fibroblasts in the airways and alveoli of the adult lung and their relationship to fibroblasts at other sites.
3. Develop tools and techniques to study cell–cell interactions *ex vivo* (e.g., fibroblast–epithelial interactions, 3D cell culture systems).
4. Elucidate mechanisms by which resident fibroblasts serve homeostatic regulatory functions in maintaining lung structure and function in health and how these regulatory mechanisms are subverted in disease (e.g., fibrosis, emphysema).
5. Define genetic, epigenetic, and age-related underpinnings of disease-associated fibroblasts and associated alterations in the ECM, including alterations of the basement membrane.
6. Correlate the phenotype and genotype of disease-associated fibroblasts with clinical phenotypes (e.g., creation of large cell repositories of well characterized patients with specific lung diseases and clinically relevant subphenotypes of disease); ideally, collection of longitudinal samples and data would allow for adequate clinical phenotyping.

GROUP 4: TRANSLATION TO HUMAN DISEASE

Pulmonary fibrosis complicates a heterogeneous group of disorders that are driven by chronic inflammation or repeated epithelial injury and involve regions of the lung varying from mainly airways to almost exclusively the lung parenchyma. More broadly, fibrosis is a final common pathway in many forms of chronic disease, affecting a range of tissues and leading to organ scarring and failure. Although fibrosis in various forms affects millions of patients in the United States, there is a paucity of adequate treatment options. Thus, there is a need to translate an understanding of basic disease mechanisms to the more pragmatic challenges associated with the clinical development of novel therapeutics. The most therapeutically challenging of these diseases in the lung is idiopathic pulmonary fibrosis (IPF). Although there have been many advances in clarifying the diagnosis and prognosis of IPF over the last decades, no medical therapy has significantly improved survival or halted the relentless progression of fibrogenesis (63, 64). The failure of antiinflammatory agents to favorably affect the course of IPF, coupled with the elucidation of defects that primarily involve AECs being associated with familial IPF, has turned attention to the pathobiology of epithelial–mesenchymal interactions in this disease. This has led to a number of clinical trials involving agents that, on the basis of preclinical studies in mouse models, were thought to focus on signaling pathways directly or indirectly involved in fibrogenesis. These include pirfenidone, etanercept, interferons, and, most recently, imatinib mesylate and bosentan. Unfortunately, none of these agents has demonstrated an unequivocal clinical benefit.

What lessons can one derive from the recent therapeutic failures? And what is needed in the field to inform and promote new clinical trials that better address critical elements of the pathobiology? Members of the workshop discussed key obstacles in translating basic studies to the clinical arena and attempted to identify several short- and more long-term advances that would increase the likelihood of developing promising therapies. Although much of the discussion was directed toward IPF, many of the principles elucidated are relevant to other

chronic lung diseases. The group identified four major areas for high-impact discovery.

Continued Basic Studies that Elucidate Key Drivers of Pulmonary Fibrosis in Humans

One lesson from recent clinical trials is that a molecular understanding of what drives the relentless fibrosis in IPF is unclear. A more complete understanding of the signaling pathways underlying fibrogenesis should result in new therapeutic targets more specifically directed toward fibrosis. In addition, because completely inhibiting a specific pathway may have adverse unintended consequences in the same or another cell type, more detailed knowledge of specific downstream targets and molecular interactions will be important.

New Animal Models that More Closely Resemble the Pathobiology of Progressive Pulmonary Fibrosis

A careful consideration of the model(s) in which preclinical data are obtained is important. Despite its limitations, the bleomycin-induced lung injury model remains the most widely used and best characterized model for studying potential antifibrotic agents. An important limitation of the bleomycin model is that fibrosis is highly dependent on inflammation induced by epithelial injury, whereas IPF has not proven responsive to antiinflammatory therapeutics. Inhibition of fibrosis in the bleomycin model by drugs given early after injury does not predict clinical responses. Although the bleomycin lung injury model has severe limitations, it has provided valuable insights into potential pathways involved in the pathogenesis of fibrosis, including molecular markers that reflect disease mechanisms impairing epithelial cell health and promoting fibrogenesis and that can reliably serve as interim indicators of a therapeutic response.

A major barrier to the development of therapeutics for fibrosis is the lack of interim indicators that reliably reflect drug effects on mechanisms of disease. Generating basic research tools and model systems to evaluate the relevance of target pathways to human disease and developing biomarkers to monitor the effectiveness of therapeutic intervention in clinical trials will greatly facilitate the development of novel therapies. Toward this end, understanding how molecular mechanisms regulating fibrotic disease in animal models overlap with human disease can lead to the identification of new biomarkers.

Personalized Medicine

Personalized medicine refers to therapy based on the elucidation of family history, environmental exposures, distinct clinical features and outcomes, biochemical phenotypes, and pathological characteristics that together identify dominant signaling pathways affecting the progression and outcome of disease in an individual patient. Chronic illness represents over 75% of medical care in the United States. These diseases are complex disorders that result from a combination of genetic factors and environmental exposures. We tend to label them as distinct diseases; however, the diseases are more appropriately labeled syndromes to indicate involvement of a variety of molecular pathways and clinical subphenotypes within each category. Moreover, the diseases themselves often have overlapping genetic and environmental components with common pathologic manifestations. The goal of personalized medicine is to match specific aberrant molecular pathways with clinical subphenotypes and develop specific therapy for each individual patient.

The overlap of COPD and IPF represents the blurring of disease categories based upon common environmental exposures and pathologic phenotypes. At first glance, COPD and IPF

appear to be diametrically opposed. Emphysema is a result of inflammation induced by cigarette smoke and proteolytic destruction of elastin and other ECM components, whereas IPF is not necessarily inflammation dependent and is characterized by relentless extracellular matrix deposition. However, around two thirds of patients with IPF are cigarette smokers. Moreover, epithelial cell apoptosis, matrix metalloproteinase activation, and net collagen accumulation are characteristic of COPD and IPF.

Even though the prognosis of IPF is generally poor, there is abundant evidence that there is substantial heterogeneity in the outcomes even within a biopsy-proven patient population. Cell plasticity may play a role with phenotypic switching, which can account for clinically distinct manifestations. Certain putative biomarkers of disease activity are elevated in some but not other patients with IPF. To effectively bring new therapeutic targets to the clinical arena, it will be crucial to identify at the outset the set of patients most likely to respond to a particular treatment. It may also be the case that the activity of one or only a few signaling pathways dominates disease progression in all patients, but there is little evidence to support this possibility.

Recommendations

1. Development of animal models of lung fibrosis that better reflect human disease. Elements of an animal model(s) that would potentially facilitate the identification of novel drug targets need to include:
 - a. Fibrosis must be persistent, ideally unrelenting, and lead to increased mortality due to fibrosis.
 - b. The model should include AEC injury.
 - c. Inducible AEC-specific transgenic models that manipulate specific genes that have been implicated in the pathobiology of usual interstitial pneumonia should be developed. Pathways to be considered should include telomerase, surfactant proteins A and C, components of vesicle trafficking, ER stress and unfolded protein response pathways, genes downstream of TGF- β 1 activation, and Wnt signaling pathways.
 - d. In addition to targeting AECs, models that genetically manipulate genes expressed by myofibroblasts also offer the potential to identify novel targets. These should also preferably be inducible systems.
2. Signaling pathways found to be critical in animal models should be tested directly in epithelial cells and fibroblasts isolated from patients or in lung tissues from patients to validate their role in human disease. Most of the transcriptional and biochemical profiling of fibrotic patients has been at the level of whole lungs. This will likely not provide sufficient resolution to identify critical epithelial or fibroblastic subphenotypes or signaling pathways within a patient population or applicable to a single patient.
3. Develop a biomarker of fibrogenic activity that is detectable by noninvasive imaging to facilitate monitoring of therapeutic response. In addition to development of better serum, urine, or bronchoalveolar lavage (BAL) markers of disease activity, there is much promise, and also challenge, in the development of functional imaging of fibrogenesis in lungs. Other than detection of the enhanced metabolic activity of lung tumors, no functional imaging is in clinical use in the lung. The development of such technology would greatly enhance the early quantification of functional impairment and the ability to monitor therapeutic candidates in pulmonary fibrosis.
4. Emphasize subgroup analysis of promising biomarkers (from blood, BAL, or urine) to elucidate connections between biochemical markers and lung disease progression in patient groups or individuals. There is no biomarker that tracks disease activity with enough variation to be useful in phenotyping subpopulations or predicting therapeutic response. Consider gender differences and reproductive history in the evaluation of response to lung injury and response to treatment.
5. Determine if there are both common and distinct patterns of epigenetic or genomic profiling of epithelial cells and fibroblasts isolated from, or assessed *in situ* in, fibrotic patients. If so, coordinate testing the correlation of such patterns with parameters of clinical and pathological disease progression.

CONSENSUS HIGH-PRIORITY RECOMMENDATIONS

1. Develop additional lineage-specific markers and drivers for epithelial cells and fibroblasts to enable generation of a cell type-specific gene expression database during the lifespan of a healthy mouse (a so-called LUNGMAP). Markers could be cell surface and transcription factors. This would serve as the reference database for all studies of adaptation, incident disease, and disease models and would allow lineage tracing and isolation of specific cell types for more detailed characterization. The approach should incorporate high throughput screens to generate reagents and the development of an epigenetic map. The information might best be collated on a central website, allowing immediate and ongoing access to all lung investigators. A transgenic core, a histology core, a high-level antibody-generating core, and strong bioinformatics and epigenetics core support are needed for this effort.
2. Generate an integrated gene expression database for tissue and primary cell isolates from healthy and diseased human lungs and provide the NIH-funded scientific community with highly curated, easily accessible tissue and disease-specific primary cell isolates. Cohorts of patients should be defined to allow phenotyping of specific disease subsets. Use resources such as the NHLBI Lung Tissue Research Consortium to get longitudinal information from patients and live cells from normal and diseased lungs. A tissue acquisition core (perhaps within a consortium) is needed for this purpose.
3. Explore opportunities for network and pathway analyses using the gene expression databases generated for human and animal model cells and tissues. Consider using a systems biology approach to investigate interactions between different cell types. Explore the opportunities provided by high-throughput DNA sequencing in specific pulmonary diseases.
4. Develop better animal models for progressive fibrosis. Focus on potential therapeutic targets that have been implicated in human pulmonary fibrosis and validated in an animal fibrosis model(s) evaluated after the inflammatory phase of injury.
5. Support investigator-initiated attempts to discover and test new biochemical markers that reflect disease mechanisms prominent in the pathobiology by elucidating underlying cell biology and molecular pathways important to fibrogenesis and mining information from studies of

disease progression of fibrosis in other organs that likely share common pathogenic mechanisms (e.g., the kidney).

6. Establish a collaborative infrastructure involving clinical and translational investigators to integrate large human tissue sample numbers with longitudinal collection to clinical annotation, including linkage to histopathology, functional measurements, and clinical outcome, to validate biomarkers. Where possible, determine if lung biopsies and BAL are superior to serum or urine samples for interim analysis of disease progression. Focus on defining subgroups of patients with common phenotypes tracked by biomarkers, especially noninvasive markers such as functional imaging.

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References

1. Adamson IY, Bowden DH. The type 2 cell as progenitor of alveolar epithelial regeneration: a cytodynamic study in mice after exposure to oxygen. *Lab Invest* 1974;30:35–42.
2. Adamson IY, Bowden DH. Origin of ciliated alveolar epithelial cells in bleomycin-induced lung injury. *Am J Pathol* 1977;87:569–580.
3. Adamson IY, Bowden DH. Bleomycin-induced injury and metaplasia of alveolar type 2 cells: relationship of cellular responses to drug presence in the lung. *Am J Pathol* 1979;96:531–544.
4. Bowden DH, Adamson IY, Wyatt JP. Reaction of the lung cells to a high concentration of oxygen. *Arch Pathol* 1968;86:671–675.
5. Cabral-Anderson LJ, Evans MJ, Freeman G. Effects of no2 on the lungs of rats: I. Morphology. *Exp Mol Pathol* 1977;27:353–365.
6. Evans MJ, Cabral LC, Stephens RJ, Freeman G. Acute kinetic response and renewal of the alveolar epithelium following injury by nitrogen dioxide. *Chest* 1974;65:628–655.
7. Evans MJ, Cabral LJ, Stephens RJ, Freeman G. Renewal of alveolar epithelium in the rat following exposure to no2. *Am J Pathol* 1973;70:175–198.
8. Evans MJ, Cabral LJ, Stephens RJ, Freeman G. Transformation of alveolar type 2 cells to type 1 cells following exposure to no2. *Exp Mol Pathol* 1975;22:142–150.
9. Evans MJ, Dekker NP, Cabral-Anderson LJ, Freeman G. Quantitation of damage to the alveolar epithelium by means of type 2 cell proliferation. *Am Rev Respir Dis* 1978;118:787–790.
10. Evans MJ, Johnson LV, Stephens RJ, Freeman G. Renewal of the terminal bronchiolar epithelium in the rat following exposure to no2 or no3. *Lab Invest* 1976;35:246–257.
11. Evans MJ, Cabral-Anderson LJ, Freeman G. Role of the clara cell in renewal of the bronchiolar epithelium. *Lab Invest* 1978;38:648–653.
12. Cardoso WV, Lu J. Regulation of early lung morphogenesis: questions, facts and controversies. *Development* 2006;133:1611–1624.
13. Stripp BR, Maxson K, Mera R, Singh G. Plasticity of airway cell proliferation and gene expression after acute naphthalene injury. *Am J Physiol* 1995;269:L791–L799.
14. Goodell MA, Jackson KA, Majka SM, Mi T, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, et al. Stem cell plasticity in muscle and bone marrow. *Ann N Y Acad Sci* 2001;938:208–218, discussion 218–220.
15. Wagers AJ, Weissman IL. Plasticity of adult stem cells. *Cell* 2004;116:639–648.
16. Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, Sheppard D, Chapman HA. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *Proc Natl Acad Sci USA* 2006;103:13180–13185.
17. Roper JM, Stavensky RJ, Finkelstein JN, Keng PC, O'Reilly MA. Identification and isolation of mouse type ii cells on the basis of intrinsic expression of enhanced green fluorescent protein. *Am J Physiol Lung Cell Mol Physiol* 2003;285:L691–L700.
18. Reddy R, Buckley S, Doerken M, Barsky L, Weinberg K, Anderson KD, Warburton D, Driscoll B. Isolation of a putative progenitor subpopulation of alveolar epithelial type 2 cells. *Am J Physiol Lung Cell Mol Physiol* 2004;286:L658–L667.
19. McQualter JL, Yuen K, Williams B, Bertoncello I. Evidence of an epithelial stem/progenitor cell hierarchy in the adult mouse lung. *Proc Natl Acad Sci USA* 2010;107:1414–1419.
20. Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, Randell SH, Hogan BL. Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc Natl Acad Sci USA* 2009;106:12771–12775.
21. Mucenski ML, Wert SE, Nation JM, Loudy DE, Huelsken J, Birchmeier W, Morrissey EE, Whitsett JA. Beta-catenin is required for specification of proximal/distal cell fate during lung morphogenesis. *J Biol Chem* 2003;278:40231–40238.
22. Goss AM, Tian Y, Tsukiyama T, Cohen ED, Zhou D, Lu MM, Yamaguchi TP, Morrissey EE. Wnt2/2b and beta-catenin signaling are necessary and sufficient to specify lung progenitors in the foregut. *Dev Cell* 2009;17:290–298.
23. Harris-Johnson KS, Domyan ET, Vezina CM, Sun X. Beta-catenin promotes respiratory progenitor identity in mouse foregut. *Proc Natl Acad Sci USA* 2009;106:16287–16292.
24. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008;133:704–715.
25. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 2007;449:557–563.
26. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nat Rev Cancer* 2009;9:265–273.
27. Ma L, Young J, Prabhala H, Pan E, Mestdagh P, Muth D, Teruya-Feldstein J, Reinhardt F, Onder TT, Valastyan S, et al. Mir-9, a myc/mycn-activated microRNA, regulates e-cadherin and cancer metastasis. *Nat Cell Biol* 2010;12:247–256.
28. Ho MM, Ng AV, Lam S, Hung JY. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. *Cancer Res* 2007;67:4827–4833.
29. Eramo A, Lotti F, Sette G, Pillozzi E, Biffoni M, Di Virgilio A, Conticello C, Ruco L, Peschle C, De Maria R. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ* 2008;15:504–514.
30. Bertolini G, Roz L, Perego P, Tortoreto M, Fontanella E, Gatti L, Pratesi G, Fabbri A, Andriani F, Tinelli S, et al. Highly tumorigenic lung cancer cd133+ cells display stem-like features and are spared by cisplatin treatment. *Proc Natl Acad Sci USA* 2009;106:16281–16286.
31. Pine SR, Ryan BM, Varticovski L, Robles AI, Harris CC. Microenvironmental modulation of asymmetric cell division in human lung cancer cells. *Proc Natl Acad Sci USA* 2010;107:2195–2200.
32. Park KS, Wells JM, Zorn AM, Wert SE, Laubach VE, Fernandez LG, Whitsett JA. Transdifferentiation of ciliated cells during repair of the respiratory epithelium. *Am J Respir Cell Mol Biol* 2006;34:151–157.
33. Willis BC, Borok Z. Tgf-beta-induced emt: mechanisms and implications for fibrotic lung disease. *Am J Physiol Lung Cell Mol Physiol* 2007;293:L525–L534.
34. Selman M, King TE, Pardo A. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med* 2001;134:136–151.
35. Chapman HA. Disorders of lung matrix remodeling. *J Clin Invest* 2004;113:148–157.
36. Myers JL, Katzenstein AL. Epithelial necrosis and alveolar collapse in the pathogenesis of usual interstitial pneumonia. *Chest* 1988;94:1309–1311.
37. Higgins DF, Kimura K, Iwano M, Haase VH. Hypoxia-inducible factor signaling in the development of tissue fibrosis. *Cell Cycle* 2008;7:1128–1132.

38. Sirianni FE, Chu FS, Walker DC. Human alveolar wall fibroblasts directly link epithelial type 2 cells to capillary endothelium. *Am J Respir Crit Care Med* 2003;168:1532–1537.
39. Burns AR, Smith CW, Walker DC. Unique structural features that influence neutrophil emigration into the lung. *Physiol Rev* 2003;83:309–336.
40. Sirianni FE, Milaninezhad A, Chu FS, Walker DC. Alteration of fibroblast architecture and loss of basal lamina apertures in human emphysematous lung. *Am J Respir Crit Care Med* 2006;173:632–638.
41. Evans MJ, Van Winkle LS, Fanucchi MV, Plopper CG. The attenuated fibroblast sheath of the respiratory tract epithelial-mesenchymal trophic unit. *Am J Respir Cell Mol Biol* 1999;21:655–657.
42. Walker DC, Behzad AR, Chu F. Neutrophil migration through preexisting holes in the basal laminae of alveolar capillaries and epithelium during streptococcal pneumonia. *Microvasc Res* 1995;50:397–416.
43. Behzad AR, Chu F, Walker DC. Fibroblasts are in a position to provide directional information to migrating neutrophils during pneumonia in rabbit lungs. *Microvasc Res* 1996;51:303–316.
44. Behzad AR, McDonough JE, Seyednejad N, Hogg JC, Walker DC. The disruption of the epithelial mesenchymal trophic unit in copd. *COPD* 2009;6:421–431.
45. Danto SI, Shannon JM, Borok Z, Zabski SM, Crandall ED. Reversible transdifferentiation of alveolar epithelial cells. *Am J Respir Cell Mol Biol* 1995;12:497–502.
46. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004;350:2645–2653.
47. Holgate ST, Holloway J, Wilson S, Bucchieri F, Puddicombe S, Davies DE. Epithelial-mesenchymal communication in the pathogenesis of chronic asthma. *Proc Am Thorac Soc* 2004;1:93–98.
48. Pan T, Mason RJ, Westcott JY, Shannon JM. Rat alveolar type ii cells inhibit lung fibroblast proliferation in vitro. *Am J Respir Cell Mol Biol* 2001;25:353–361.
49. El Ghalbzouri A, Ponc M. Diffusible factors released by fibroblasts support epidermal morphogenesis and deposition of basement membrane components. *Wound Repair Regen* 2004;12:359–367.
50. Waghay M, Cui Z, Horowitz JC, Subramanian IM, Martinez FJ, Toews GB, Thannickal VJ. Hydrogen peroxide is a diffusible paracrine signal for the induction of epithelial cell death by activated myofibroblasts. *FASEB J* 2005;19:854–856.
51. Spooner BS, Wessells NK. Mammalian lung development: interactions in primordium formation and bronchial morphogenesis. *J Exp Zool* 1970;175:445–454.
52. Shannon JM, Nielsen LD, Gebb SA, Randell SH. Mesenchyme specifies epithelial differentiation in reciprocal recombinants of embryonic lung and trachea. *Dev Dyn* 1998;212:482–494.
53. Chang HY, Chi JT, Dudoit S, Bondre C, van de Rijn M, Botstein D, Brown PO. Diversity, topographic differentiation, and positional memory in human fibroblasts. *Proc Natl Acad Sci USA* 2002;99:12877–12882.
54. Klapholz-Brown Z, Walmsley GG, Nusse YM, Nusse R, Brown PO. Transcriptional program induced by wnt protein in human fibroblasts suggests mechanisms for cell cooperativity in defining tissue micro-environments. *PLoS ONE* 2007;2:e945.
55. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: one function, multiple origins. *Am J Pathol* 2007;170:1807–1816.
56. Thannickal VJ, Lee DY, White ES, Cui Z, Larios JM, Chacon R, Horowitz JC, Day RM, Thomas PE. Myofibroblast differentiation by transforming growth factor-beta1 is dependent on cell adhesion and integrin signaling via focal adhesion kinase. *J Biol Chem* 2003;278:12384–12389.
57. Lama VN, Smith L, Badri L, Flint A, Andrei AC, Murray S, Wang Z, Liao H, Toews GB, Krebsbach PH, et al. Evidence for tissue-resident mesenchymal stem cells in human adult lung from studies of transplanted allografts. *J Clin Invest* 2007;117:989–996.
58. Hashimoto N, Jin H, Liu T, Chensue SW, Phan SH. Bone marrow-derived progenitor cells in pulmonary fibrosis. *J Clin Invest* 2004;113:243–252.
59. Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY, Belperio JA, Keane MP, Strieter RM. Circulating fibrocytes traffic to the lungs in response to cxcl12 and mediate fibrosis. *J Clin Invest* 2004;114:438–446.
60. Moore BB, Kolodick JE, Thannickal VJ, Cooke K, Moore TA, Hogaboam C, Wilke CA, Toews GB. Ccr2-mediated recruitment of fibrocytes to the alveolar space after fibrotic injury. *Am J Pathol* 2005;166:675–684.
61. Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, du Bois RM, Borok Z. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: potential role in idiopathic pulmonary fibrosis. *Am J Pathol* 2005;166:1321–1332.
62. Kim KK, Wei Y, Szekeres C, Kugler MC, Wolters PJ, Hill ML, Frank JA, Brumwell AN, Wheeler SE, Kreidberg JA, et al. Epithelial cell alpha3beta1 integrin links beta-catenin and smad signaling to promote myofibroblast formation and pulmonary fibrosis. *J Clin Invest* 2009;119:213–224.
63. Thannickal VJ, Roman J. Challenges in translating preclinical studies to effective drug therapies in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2010;181:532–533.
64. Collard HR, Ryu JH, Douglas WW, Schwarz MI, Curran-Everett D, King TE Jr, Brown KK. Combined corticosteroid and cyclophosphamide therapy does not alter survival in idiopathic pulmonary fibrosis. *Chest* 2004;125:2169–2174.