Advancing Respiratory Research

James P. Kiley, PhD

Respiratory diseases remain a major public health problem in the United States and worldwide, with increasing morbidity and mortality. Substantial progress has been made to advance understanding of the basic mechanisms of lung disease and to optimize clinical management of patients with a range of respiratory diseases. Despite this progress, our knowledge of how to predict disease prior to symptoms, improve disease definition and subclassification, and target novel and new treatments in a more personalized manner still remains inadequate. This article highlights several future opportunities and challenges related to genomics and molecular characterization of lung disease, lung injury and repair, translational lung research, the microbiome, and sleep and circadian biology as potential frontiers to advance progress in respiratory biology in health and disease.

CHEST 2011; 140(2):497–501**Abbreviations:** Th = T helper

Despite several decades of excellent progress in advancing understanding of the basic mechanisms of lung disease and optimizing the clinical management of patients, respiratory diseases are an increasing public health problem in the United States. They currently account for one-fifth of all deaths, will likely soon move from the fourth to third leading cause of death and disability, and are associated with an economic burden of > \$100 billion per year. To reverse this trend, we need to better predict disease prior to symptoms, improve disease definition and subclassification, and target novel and new treatments in a more personalized manner.

These areas of research reflect input and feedback from the broad respiratory community and offer unprecedented opportunities for making rapid and significant progress in pulmonary science. As such, an important objective is to help investigators antic-

ipate a few potential directions for lung research in ways that will best benefit from and contribute to progress in pulmonary science.

GENOMICS AND MOLECULAR CHARACTERIZATION OF LUNG DISEASES

Most lung diseases are heterogeneous, long-term, and progressive by the time they come to medical attention. Current diagnostic tools are only oriented

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to established disease and are suboptimal for capturing the breadth of abnormalities present. Advances in molecular biology now provide new platforms for redefining pulmonary diseases. Specific molecular markers have proven useful in the diagnosis of cystic fibrosis, α_1 -antitrypsin deficiency, and respiratory infections.^{1–3} Extending molecular profiling using high-throughput technologies across the spectrum of lung diseases may be valuable in diagnosing and stratifying diseases.

Complex molecular phenotypes or “fingerprints” involving hundreds of molecular changes can be identified using informatics analyses of high-throughput genomic measures. Differences between diseased and normal states have been seen in DNA, including both sequence and methylation; in RNA; in proteins, including concentrations, distribution, structure, and

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Affiliations: From the Division of Lung Diseases, National Heart, Lung, and Blood Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD.

Correspondence to: James P. Kiley, PhD, Division of Lung Diseases, National Heart, Lung, and Blood Institute, National Institutes of Health, Two Rockledge Centre, 6701 Rockledge Dr, Ste 10042, MSC 7952, Bethesda, MD 20892-7952; email: kileyj@nhlbi.nih.gov

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posttranslational modifications; and in metabolites. One fingerprint can be obtained through genome-wide association studies using array-based genotyping technologies for the detection of common genetic variants. Next-generation sequencing may soon enable investigators to explore the roles of rare genetic variants.

Molecular phenotypes that reflect both genetic and environmental factors, such as RNA, methylation, and protein, are of special interest for lung diseases. These molecular phenotyping measurements are often quite sensitive to disease status and environmental perturbations and sometimes require smaller sample sizes to reveal distinctive signatures. Many lung diseases have already been associated with unique expression signatures at mRNA levels⁴⁻⁸ and microRNA levels,⁹⁻¹¹ and some findings have potential clinical applications.^{12,13} Recent technological advances provide reliable and cost-effective methods for measuring RNA expression and sequence, DNA-methylation profiling, chromatin modification, protein expression profiling, and so forth. Further research in this area may yield practical biomarkers that can be used as diagnostic and/or prognostic tools, clues to pathogenetic mechanisms that will lead to novel therapies, better understanding of how environmental factors drive the development of lung diseases, and a fuller understanding of disease processes based on systems analyses of massively parallel genomics data. Combined analyses of multiple genomics measurements are especially desirable and may significantly enhance our ability to understand complex biologic processes and their alterations in disease.

Many current genomics studies measure cross-sectional differences among individuals, but such cross-sectional data do not capture the dynamic behavior of responses to environmental insults or of disease onset or progression. Repeated genomic measures over time and fingerprinting before and after environmental challenge or development of disease will provide an additional dimension for system analyses and may ultimately allow a more comprehensive understanding of lung health and disease.

LUNG REPAIR AND REGENERATION

Currently, there are no interventions to halt the damage once it has begun or to restore pulmonary function. Lung tissue regeneration offers a new approach to inadequate solutions for end-stage lung diseases and will require innovative approaches to “rebuild” the lung. Recent studies have demonstrated that a reimplanted tissue-engineered trachea can function in a human and that gas exchange is possible using decellularized lung bioscaffolds reseeded with

pluripotent cells reimplanted into a rodent model.¹⁴⁻¹⁶ Regenerative strategies are underway for other organ system disorders, such as neurodegenerative disease, trauma to skin and bones, and blood and heart diseases, which may suggest strategies to understand the potential for lung regeneration. The lung is unique in that exposures can either result in maintenance of lung function or lead to irreversible remodeling and dysfunction.

The molecular events that lead to effective lung repair vs maladaptive remodeling and fibrosis remain poorly understood. Unlike the skin and gastrointestinal tract, the lung is not a highly proliferative tissue. Recent evidence suggests that there may not be true lung stem cells but rather pluripotent cell populations that respond after significant injury in order to repopulate the epithelium for functional recovery. Subpopulations of basal cells in the trachea, secretory Clara cells in the small airways, and type 2 cells in the alveoli are believed to be pluripotent and capable of repopulating other functional cell phenotypes after lung injury in animal models.^{17,18} Controversy exists, however, in determining if cells within the human lung are truly pluripotent progenitor cells or cells capable of transdifferentiation, in part because the tools and models needed to explore the biology of lung repair in specific cell subsets are lacking. In addition, mesenchymal stromal cells seem to function as paracrine mediators of lung repair,¹⁹ but the mechanisms are not well understood. Whether lung cell plasticity exists is unknown. Epithelial-mesenchymal transition and/or mesenchymal-epithelial transition are considered potential components of lung fibrogenesis and potentially repair in response to inflammatory insults. However, evidence for these mechanisms is not well established in human lung models, except in cancer, which may involve different processes than those in acute and chronic lung diseases. More sophisticated studies using newer models and tools are needed to elucidate the biology of lung repair and regeneration. The ultimate goal is to address an important gap in knowledge in the lung field that would lead to development of new regenerative medicine strategies for reversing debilitating and life-limiting disorders, such as acute lung and/or tracheal injury, bronchopulmonary dysplasia, cystic fibrosis, pulmonary fibrosis, and COPD.

TRANSLATIONAL LUNG MEDICINE

As successful and enlightening as past basic and clinical research programs have been, the new challenge is to probe pathobiology across systems and levels—from one organ to another and from molecular processes and signatures to expression of clinical

symptoms in patients and populations. Such information will need a new research paradigm for successful translation into better respiratory health outcomes. Chronic lung diseases are complex with multiple gene-gene and gene-environment interactions resulting in multiple pathobiologic processes and disease expression, chronicity, and progression. This complexity and heterogeneity lead to differing, but sometimes coexisting, phenotypes and subphenotypes. Traditional disease definitions rely on grouping patients with similar presentations, often ignoring differing environmental exposures, clinical course, and underlying pathobiologies. This reductionist approach leads to an oversimplification of disease definition and treatment as demonstrated in a recent study on severe asthma.²⁰ Five independent clusters were identified that do not track with traditional disease definitions used to define severity and disease-management strategies, underscoring the need to redefine disease definition and severity classifications. A new paradigm is needed that will relate phenotypic traits to fundamental biologic processes instead of relying on the end point expression of clinical symptoms. One such study analyzed heterogeneity of disease at the molecular level and demonstrated two distinct molecular phenotypes, “high” and “low,” with respect to T helper (Th) 2 cell inflammation in a population of patients with asthma and healthy control subjects.¹² Although Th2 cell-driven inflammation is considered to be one of the central mediators of asthma pathobiology, these results indicate that therapies targeting Th2 cell inflammation may only be helpful in a subset of patients with asthma.

These examples indicate progress toward an integrated and systems view of disease pathobiology that may be applied to disease definition and predict treatment outcome. However, challenges remain around computational techniques and tools to obtain high-quality data, sample size, and a thorough understanding of disease mechanism such that data can be used to construct new disease paradigms. Nonetheless, definition of pathobiologic traits and mechanisms used to identify subphenotypes in patient populations will enable prediction of disease development, severity, and progression; aid the development of surrogate outcome measures that can correlate therapy to clinical outcome; and facilitate the identification, validation, and development of targets for mechanism-based interventions for prevention, diagnosis and treatment of respiratory diseases.

LUNG MICROBIOME

Attempts to investigate the lung microbiome are relatively new compared with other organs, even

though infectious agents are known to be or are suspected of having key roles in a number of chronic lung conditions. The late start-up of lung microbiome research may be due, in part, to the widely held belief that the normal lung is “sterile,” despite being constantly in contact with the external environment and downstream to the nasopharynx and oral cavities, which are known to be heavily populated with microbes. Another obstacle is getting truly noncontaminated representative samples of the lower respiratory tract. Nonetheless, exploration of the lower respiratory tract microbiome has now begun and evidence is mounting that normal lungs are not sterile. For instance, a recent study using samples obtained by bronchial brushing from patients with asthma and healthy controls has shown that the bronchial tree contains a characteristic microbiota that appears to be disturbed in asthmatic airways.²¹ Another study obtained samples from patients with COPD by mini-BAL and used the 16S ribosomal RNA PhyloChip (Affymetrix, Inc; Santa Clara, California) to identify bacteria. They showed that patients with COPD have a distinctive community of bacterial taxa that includes both known and previously unknown pathogens.²²

Determining the lung microbiome will enable us to learn which microbes are present and where they are located. Starting from this basic knowledge, we may be able to study how these microbes grow, interact among themselves, and relate to other microbial species coexisting in the same niche and with the cells in the lung, and how they are altered by factors such as the physical environment and cigarette smoke. This knowledge will enhance understanding of the role of the lung microbiome in preserving health or causing disease, and in the divergent effects observed in HIV-infected vs uninfected individuals. Understanding the role of the lung microbiome along with other components of the respiratory tract in health and disease may lead to new ways of thinking about respiratory disease and new therapeutic targets for translation into better preventive and treatment strategies. In the future, this may provide an opportunity to study gene-gene and gene-environment interactions under specific disease conditions and interactions of the lung microbiome with microbial populations located elsewhere in the body.

SLEEP AND CIRCADIAN BIOLOGY

Sleep and circadian rhythms are fundamental to biologic organization and tightly coupled to behavioral, physiologic, and genomic functions. Future research is needed to elucidate novel mechanisms by which circadian and sleep-regulating pathways are linked to systems and cellular function in the heart,

lung, and blood and to translate these discoveries to improved understanding of cardiopulmonary and hematologic disease pathogenesis and therapeutics.

Exciting opportunities for circadian research include elucidating clock-coupled genomic pathways in sustaining heart, lung, and blood functions through regulation of cell metabolism, oxidative stress, cellular repair, and posttranslational modification. Interrogating clock genes and clock-coupled pathways in cardiopulmonary and hematologic conditions will enhance understanding of disease pathophysiology and potentially identify new therapeutic targets. Clock gene expression reacts with many environmental exposures associated with heart, lung, and blood diseases, including nutrient content and meal timing, smoke exposure, and drug administration.²³⁻²⁵ Research is needed to examine the clock-coupled mechanisms as an interface for environmental exposure and epigenetic modification in cardiopulmonary and hematologic systems. Future studies may integrate circadian dynamics into genomic, proteomic, and microbiome analyses to enhance genomic discovery and systems biology modeling of cardiopulmonary and hematologic diseases. Recent, unexpected data show non-transcriptional circadian oscillations in human red blood cells, opening the door to develop new biologic and computational models of circadian regulation and system integration.²⁶

Clinical research to identify circadian patterns of disease symptoms and biomarker expression may be applied to improve diagnostics and inform therapeutic strategies. New research is indicated to investigate drug delivery “time of day” effects in therapeutic outcomes and to target circadian-based pathways in pharmacologic and genetic therapeutics for heart, lung, and blood disease.

Untreated sleep apnea is linked to cardiometabolic disease risk, including hypertension, stroke, cardiac arrhythmia, heart failure, and diabetes, as well as all-cause mortality.^{27,28} Important research directions include elucidating mechanisms by which sleep apnea is coupled to cardiopulmonary and metabolic pathophysiology, including autonomic, oxidative stress, inflammatory, and glucose-regulating pathways. Research is needed to differentiate sleep apnea from obesity mechanisms in cardiometabolic disease. An advanced understanding of sleep apnea pathogenesis, including mechanisms of upper airway and ventilatory control, will catalyze the development of new mechanical, pharmacologic, and genetic treatment strategies for patients with sleep apnea. Finally, research is needed at the cell biology level to elucidate mechanisms by which sleep apnea intersects with immune responses, stem cell regulation, and posttranslational processes (eg, uncoupled protein response) central to heart, lung, and blood functions.

SUMMARY

Chronic respiratory diseases are an increasing burden to the health-care system in the United States and globally. Early prediction of risk, prevention measures, preemptive approaches, and participation by individuals and communities will be essential to reversing this trend. Sustained progress will require developing sophisticated tools and approaches to stimulate innovation and new discoveries to transform how we prevent and diagnose respiratory disease and how we choose, monitor, and guide treatment. Examples include computational tools; databases for sharing (eg, the database of Genotypes and Phenotypes [dbGaP]); RNA interference, tissue engineering, and cell-based approaches; high-throughput proteomic, genomic, metabolomic, and glycomic technologies; epigenomic and microbiomic strategies; systems biology; imaging; nanotechnology; synthetic biology; and microfluidics. Clearly, these are only a few of the many frontiers in respiratory medicine. However, advancing these and related exiting research opportunities will take an enthusiastic, talented, and appropriately trained investigative community building ties between basic and clinical researchers, awareness of the need for multidisciplinary approaches, and a commitment to push new approaches and paradigms to advance treatment and eventual elimination of respiratory diseases.

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