"PIK"ing Out New Epigenetic Markers in Lung Disease

Over the past 10 years, genome-wide association studies have successfully identified genetic variation at a number of specific loci associated with chronic obstructive pulmonary disease (COPD) risk. The COPDGene (Genetic Epidemiology of COPD) study alone has led to the discovery of 20 risk loci (1). In addition to these genetic contributions to COPD, a variety of external factors, including exposures during the perinatal period, recurrent infections, and chronic environmental irritants (e.g., smoking, pollution, and biomass fuel), clearly contribute to the pathogenesis of this disease. The mechanisms by which these exposures predispose to disease remain an area of active interest. Epigenetic mechanisms, broadly defined as regulation of gene transcription that does not result from changes in DNA sequence, have been hypothesized to be key mediators of gene–environment interactions in disease pathogenesis (2).

Methylation patterns of DNA are widely recognized to be impacted by environmental stimuli, forming a cellular memory of past injuries that condition future responses. DNA methylation patterns may form an important biological bridge between genetic risk factors and environmental exposures that are characteristic of many complex medical conditions (3, 4). To date, epigenetic studies of large cohorts have demonstrated important associations between the risk of developing COPD and specific DNA methylation patterns in former smokers (5). DNA methylation changes have also been associated with increased overall mortality (6–8). However, the association between DNA methylation and mortality in current and former smokers with or without COPD has not previously been examined.

In a study presented in this issue of the *Journal*, Morrow and colleagues (pp. 1099–1109) analyzed peripheral blood for genome-wide DNA methylation in a subset of patients from two large multicenter studies of individuals with or at risk for COPD (9). They report DNA methylation at seven CpG sites that were associated with changes in mortality. These sites were identified in samples from the COPDGene study and were replicated in the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints Study) cohort. The two replicated sites with the largest effect sizes and arguably with the most supporting biological plausibility—cg03971555 and cg12033075—are located in the "shore region" of a CpG island adjacent to *PIK3CD* (phosphatidylinositol-3-kinase catalytic subunit δ). Hypermethylation at both sites was associated with decreased survival, lower lung function at enrollment, and increased risk of COPD. These results are impactful in that they implicate the well-studied PI3K signaling pathway as a potential mediator of disease phenotypes and mortality risk in smokers.

The *PIK3CD* gene encodes a class I PI3K protein that is found in many cells and enriched in leukocytes. By phosphorylating inositol lipids, PI3Ks trigger signaling cascades in many cell types that regulate diverse biological processes, including cell growth, survival, proliferation, motility, and differentiation. For example, in the immune system, PI3K is involved in B-cell development, proliferation, migration, and antigen presentation; T-cell receptor signaling; T-cell expansion, differentiation, and migration; natural killer cell development and migration; and neutrophil and mast cell responses (10). Within the lung, constitutive activation of the pathway is seen in non–small cell lung carcinoma, and abnormalities of PI3K signaling within the lung epithelium have been implicated by single-cell RNA-sequencing studies of interstitial pulmonary fibrosis (11, 12) The precise roles that PI3K signaling plays in lung development, homeostasis, injury, repair, and regeneration remain unclear.

It is commendable that the authors included analysis of DNA methylation and gene expression in both lung tissue samples and peripheral blood. Their data suggest a potential direct regulatory role for DNA methylation at these particular CpG sites in PI3K signaling. These findings are both novel and exciting, not only because they highlight methylation of *PIK3CD* sites as a potential biomarker to identify smokers who may be at the highest risk of developing complications such as COPD, but also because they suggest an underlying mechanism by which *PIK3CD* may directly impact COPD risk.

Together, these findings raise many interesting questions for researchers in the burgeoning field of pulmonary genomics and epigenetics. Additional studies are needed to determine whether the reported DNA methylation changes directly regulate the expression of *PIK3CD* or are merely markers for another yet-to-be-identified regulatory element. A better understanding of the signaling mechanisms that control *PIK3CD* activity in target cells is necessary to better elucidate the potential biological significance of these findings and their relationship with mortality risk. The dynamics of PI3K signaling in each cellular compartment (e.g., immune, vascular, mesenchyme, and epithelium), and the precise biological roles the PI3K pathway plays in each cell type merit further exploration. For example, the pattern of PIK3CD expression and the observation of *PIK3CD* methylation changes in leukocytes suggest that immune cell function may play a central role in the association between these DNA methylation changes and mortality, and encourage future studies to identify mechanisms by which PI3K signaling may modulate immune responses to alter smoking-related mortality risk.

The present study highlights the role of epigenetic alterations in the pathogenesis of human lung diseases. It will be important to gain a more complete understanding of how adverse environmental exposures lead to changes in the epigenome, the biological significance of these changes, and how epigenetic pathways can be manipulated for therapeutic benefit. DNA methylation is just one tool in a long list of epigenomic tools that the cell uses to regulate gene expression. Changes in local chromatin accessibility, large-scale chromatin structure/conformation, and histone post-translational modifications are other examples of epigenetic modifications that

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occur across the genome and need to be further explored in the context of pulmonary function and disease. Extensive research into the epigenomic changes observed in cancer cells has led to the discovery of a wide array of specific pharmacologic inhibitors and CRISPR-Cas9–based epigenome technologies as therapeutics. The current study by Morrow and colleagues provides a template for discovering new epigenetic biomarkers and points the way for targeted basic studies, with the eventual goal of discovering new classes of treatments for pulmonary disease. ■

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Treatment of Acute Exacerbation of Idiopathic Pulmonary Fibrosis
A Call to Arms

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, fibrosing interstitial lung disease with an unfavorable prognosis (1). Different from many other chronic lung diseases, deaths of individuals with IPF are primarily related to progression of lung fibrosis rather than occurring due to comorbidities (2). Acute exacerbations (AEs) of IPF (AE-IPF), characterized by the development of widespread acute lung injury, are an important cause of IPF-related disease progression and mortality, which may even occur in individuals with limited fibrosis and well-preserved lung function (3). When it comes to AE-IPF, there are important lacunae in knowledge, including understanding of pathogenesis and triggers, optimal strategies for prevention, and best approaches to (early) diagnosis. Although considerable progress has been made in the management of IPF, optimal treatment of AEs has yet to be defined, and varies considerably across the globe (4). Despite current international guidelines giving a (weak) positive recommendation for the use of glucocorticoids to treat AE-IPF, there are no proven, effective therapies for this devastating complication of IPF (1, 3). Currently used therapeutic approaches, usually glucocorticoids or immunosuppressants, are based on expert consensus and small, uncontrolled, or retrospective studies (3, 4). Before now, with the exception of a trial examining a procalcitonin-guided antibiotic treatment approach, prospective, clinical randomized placebo-controlled trials (RCTs) have been nonexistent in AE-IPF (3, 5).

Given the uncertainty around best management of AE-IPF, in this issue of the *Journal*, the article by Kondoh and colleagues (pp. 1110–1119) is very timely (6). Kondoh and colleagues report the outcomes of a multicenter RCT of recombinant thrombomodulin alfa in AE-IPF. This drug has been

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