Airway Gene Expression in Chronic Obstructive Pulmonary Disease

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Although cigarette smoking is the major cause of chronic obstructive pulmonary disease (COPD), only a subset of smokers develops this disease. There is significant clinical, radiographic, and pathologic heterogeneity within smokers who develop COPD that likely reflects multiple molecular mechanisms of disease. It is possible that variations in the individual response to cigarette smoking form the basis for the distinct clinical and molecular phenotypes and variable natural history associated with COPD. Using the biologic premise of a molecular field of airway injury created by cigarette smoking, this response to tobacco exposure can be measured by molecular profiling of the airway epithelium. Noninvasive study of this field effect by profiling airway gene expression in patients with COPD holds important implications for our understanding of disease heterogeneity, early disease detection, and identification of novel disease-modifying therapies.

Keywords: airway gene expression; chronic obstructive pulmonary disease; bioinformatics

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide. In the United States, COPD caused 127,000 deaths in 2005 (1), and the burden of disease is expected to increase in the coming decades (2). Although cigarette smoking is a well-established cause of COPD, it is unclear why only a subset of smokers develops this disease. Although cigarette smoking is a leading cause of COPD, smoking cessation is not uniformly effective at halting disease progression. Current therapies for COPD are effective for symptom reduction (3–5) and may slow the rate of decline of FEV_1 (3), but they do not arrest the longitudinal decline in lung function or decrease mortality (3–5).

Among smokers who develop COPD, the natural history is highly variable (6). This variability may be due to the diverse clinical, pathologic, and radiographic manifestations of this disease within and across individuals. Although spirometry detects the irreversible airflow obstruction characteristic of COPD (6), it does not quantify the alveolar septal destruction, airway wall thickening, or small airway inflammation associated with the disease (7–9). The variable natural history of COPD could be the result of diverse pathogenic mechanisms involving distinct patterns of molecular deregulation. The distinct clinical and molecular phenotypes associated with COPD could in turn be the result of heterogeneity in the physiological response to tobacco smoke exposure.

In this article, we review the smoking-induced field of injury in the airway, its applications to smoking-induced airway disease, and the implications this holds for understanding of distinct molecular phenotypes of COPD, molecular pathogenesis, and disease-modifying therapies.

THE SMOKING-INDUCED FIELD OF INJURY IN THE AIRWAY

Cigarette smoking creates a molecular field of injury throughout the airways and lung. Although this field effect was first observed in smokers with head and neck cancers (10), it has subsequently been described in smokers with and without lung disease. This field effect includes p53 mutations (11), EGFR mutations (12), loss of heterozygosity (13), microsatellite alterations (14), and mutations in mitochondrial DNA (15). Several groups have described epigenetic alterations in the smoking-induced airway field of injury, including increased telomerase activity (16) and alterations in methylation of p16 (17). These changes extend into the oral epithelium of the upper airway (18, 19).

Although several theories of the origin of this field effect have been proposed (20), a substantial portion likely reflects an individual's physiologic response to tobacco exposure. Because this response is likely to result in modulation of gene expression patterns in tobacco-smoke–exposed tissues, our group and others have hypothesized that profiling of the airway transcriptome may provide a detailed characterization of an individual's specific physiologic response to tobacco smoke. Because this response likely contributes to smoking-related disease pathogenesis, airway transcriptome profiling may provide an opportunity for the characterization of relatively easily accessed tissues in the airway to provide diagnostic and prognostic information about the presence of smoking-induced disease as well as disease risk.

SMOKING-INDUCED ALTERATIONS IN AIRWAY GENE EXPRESSION

Our group and others have shown that smoking induces large-scale gene expression changes in the airway. Smoking-induced alterations in gene expression occur in the intrathoracic large- and small airway epithelium (21, 22, 41) and in the extrathoracic airway epithelium of the nose and mouth (23) (Table 1). These alterations in airway gene expression result in changes in the corresponding proteins in the bronchial airway epithelium (24). The airway expression of regulatory miRNA is also altered by smoking, and these changes contribute to the regulation of specific smoking-modulated mRNAs (25) (Table 1).

Many of the genes that are altered by smoking code for proteins with functions involved in inflammation and oxidative stress, suggesting that a large component of the physiologic response to smoking is involved in antioxidant defense and detoxification of a number of the components of tobacco smoke. Consistent with this hypothesis, the majority of these alterations in gene expression are rapidly reversible upon smoking cessation, suggesting that their alteration by smoking represents an acute response to the toxic effects of inhaled cigarette smoke (26, 27). In addition to genes that are rapidly reversible upon smoking

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TABLE 1. AIRWAY GENE AND MICRO-RNA ALTERATIONS IN SMOKERS AND SMOKERS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Comparison	Airway Size*	Direction†	Selected Alterations‡
Healthy smokers vs. healthy nonsmokers	Large	Up-regulated	ADH7, ALDH3A1, AKR1B1, AKR1C1, AKR1C2, AKR1C3, ANXA3, CYP1B1, CYP4F11, GCLC, GPX2, KLF4, MAFG, NQO1, PGD, PLEKHB2, RAB11A, TALDO1, TRIM16, TXNRD1(21) AKR1B10, AKR1C2, CYP1A2, CYP1B1, CYP4F3, DUOX2, FLJ10786, GPX2, SRPUL(32) hsa-miR-337, hsa-miR-18a*, hsa-miR-189, hsa-miR-365, hsa-miR-181d(25)
		Down-regulated	CCND2, CXCL1, CYFIP2, MMP10, SLIT1, SLIT2, TNFSF13(21) AKR1A1, CYP2B6, CYP4Z1, DHRS6(32) hsa-miR-10b, hsa-miR-19b, hsa-miR-30a-3p, hsa-miR106b, hsa-miR-128a, hsa-miR-130a, hsa-miR-218, hsa-miR-362, hsa-miR-625(25)
	Small	Up-regulated	ADH7, ALDOA, AKR1B1, AKR1C3, G6PD, GCLC, GCLR, GPX2, GPX3, GSR, GSTA2, IDH2, PGD, TALDO1, TKT, TXNRD1(22) HES5, NOTCH3(33) ADH7, ALDH3A1, AKR1B10, AKR1C3, CYP1B1, NQO1(41)
		Down-regulated	CX3CL1, GADD45B(41)
	Upper airway	Up-regulated	CYP4F11, RAB11A, CEACAM5, AKR1B1, SLC7A11, ALDH3A1, NQO1, TKT, CYP4F3(23)
		Down-regulated	BCL11A, CX3CL1, CYFIP2, SLIT1, SLIT2, TNFSF13(23)
Smokers with COPD vs. healthy smokers	Large	Up-regulated	AKR1A1, AKR1B10, AKR1C2, CYP1B1, CYP2B6, CYP4F3, CYP4Z1, DHRS6, DUOX2, GPX2, P5, SRPUL(32)
		Down-regulated	CYP1A2, FLJ10786(32)
	Small	Up-regulated	
		Down-regulated	DLL1, HEY2(33)

Definition of abbreviation: COPD = chronic obstructive pulmonary disease.

* Large airway indicates samples obtained by brushing the main-stem bronchi or other large bronchi. Small airway indicates samples obtained by brushing tenth- to twelfth-generation bronchi. Upper airway indicates samples obtained from the nose or mouth.

† The direction of fold change of Group 1 compared with Group 2.

‡ Genes are represented by gene symbol, and micro-RNAs are represented by microRNA ID.

cessation, a subset of the genes that are altered by smoking are only slowly reversible or are altogether irreversible after smoking cessation. Such aspects of the physiologic response to smoking might form the basis of the continued risk for smoking-induced lung disease among former smokers (Table 2) (26).

AIRWAY GENE EXPRESSION AS A MOLECULAR DIAGNOSTIC FOR LUNG CANCER

Lung cancer, like COPD, is a smoking-related disease for which former smokers remain at elevated risk long after smoking cessation and for which heterogeneous responses to cigarette smoke presumably contribute to the development of disease. The airway field of injury hypothesis thus suggests that a detailed profile of the airway transcriptome may be able to distinguish individuals with lung cancer or individuals at risk for lung cancer from healthy smokers and former smokers, thereby providing a clinically useful tool for a disease that lacks effective early diagnostic strategies.

Our group has profiled gene expression in cytologically normal bronchial airway epithelium samples obtained from smokers undergoing bronchoscopy for suspicion of lung cancer. We have developed an 80-probeset signature that can serve as a sensitive and specific early diagnostic biomarker for lung cancer (28). The performance of this biomarker improves when used in combination with clinical features such as patient age, mass size, and lymphadenopathy (29), suggesting that the molecular information measured by the biomarker is independent of other recognized lung-cancer risk factors. In cases where clinical assessment by independent physicians was most uncertain about the likelihood of lung cancer, this combined clinicogenomic model improved the diagnostic accuracy, suggesting that this approach might facilitate the diagnostic evaluation of smokers suspected of having lung cancer when clinical uncertainty exists (29). A quantitative competitive reverse transcription PCR–based biomarker has also been developed by other groups, which similarly enhances diagnostic accuracy in cytologically nondiagnostic specimens (30).

TABLE 2. REVERSIBILITY OF SMOKING-INDUCED AIRWAY GENE EXPRESSION CHANGES

Enriched among Reversible Smoking-induced Gene Expression Changes by EASE Analysis*	Degree of Reversibility in Former Smokers	Selected Gene Symbols from Enrichment Category
Oxidoreductase activity	Rapidly reversible	SCD, PGD, TXNRD1, AKR1B1, G6PD, CYP1B1, DHRS3, ALDH1A3, ME1, GULP1, HGD, ALDH3A1, CYP1A1, CYP4F11, AKR1B10, PRDX1, TXN, AKR1C3, CBR1, AKR1C2, ADH7, VPS13D, CA12, AKR1C1
Electron transporter activity	Rapidly reversible	PGD, TXNRD1, AKR1B1, CYP1B1, DHRS3, ME1, ALDH3A1, TXN, NADK, AKR1C3, AGR2, AKR1C2, PHTF2, ADH7, AKR1C1, TXNDC5
Pentose phosphate pathway (*Homo sapiens*)	Rapidly reversible	PGD, TALDO1, G6PD, TKT
Oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	Rapidly reversible	PGD, AKR1B1, G6PD, AKR1C3, CBR1, ADH7, VPS13D
Oxidoreductase activity, acting on CH-OH group of donors	Rapidly reversible	PGD, AKR1B1, G6PD, ME1, AKR1C3, CBR1, ADH7, VPS13D
Carbohydrate metabolism (*Homo sapiens*)	Rapidly reversible	PGD, AKR1B1, TALDO1, G6PD, GALE, ALDH1A3, ME1, ALDH3A1, UGT1A6, TKT, ADH7, GMDS, AACS
Chromosomal location 16q13	Slowly reversible and irreversible	MT1G, MT1X, MT1F, CX3CL1

* Significantly enriched functional categories, pathways, and chromosomal cytobands among the genes altered with smoking as determined by EASE (26). These categories are ordered by their degree of reversibility in former smokers.

AIRWAY GENE EXPRESSION PROFILING IN COPD

Using airway biomarker development in lung cancer as a model, expression-based COPD biomarkers should be developed for disease screening, early disease detection, molecular classification, and prognostication. Although there are several studies of gene expression in the lung tissue of patients with COPD (31), there are few studies of gene expression in more readily accessible airway tissue and none that link COPD-specific changes in airway gene expression to the lung.

In the first study of the airway transcriptome in COPD, Pierrou and colleagues compared whole-genome expression profiling of bronchial brushings from 38 subjects with COPD, 18 healthy smokers, and 14 healthy nonsmokers (32). They compiled two lists of oxidative response genes, one generated from mining the literature and one generated based on protein sequence domains. They demonstrated enrichment of these oxidative response genes among genes that change in smokers and smokers with COPD (Table 1) and enrichment of binding sites for nuclear factor-κB, Nrf2, FOXO4, and several other transcription factors in the promoter regions of these oxidative response genes (32).

Small airway gene expression in COPD has also been examined using expression profiling of brushings obtained from tenth- to twelfth-generation bronchi of 13 smokers with COPD, 15 healthy smokers, and 20 nonsmokers. After defining a list of genes involved in Notch signaling using literature mining, Tilley and colleagues demonstrated down-regulation of genes in the Notch pathway among smokers with COPD (33) (Table 1). The relationship between COPD-associated gene expression changes in the small airways and those described in the large airway (32) remains unclear.

Additional studies that follow patients longitudinally are required to identify expression profiles associated with longitudinal lung function decline and disease progression. Examination of airway tissues that can be collected noninvasively is particularly well suited for such longitudinal studies, given the difficulty of obtaining serial lung tissue samples. Current studies have also been limited to subjects whose COPD has been characterized by spirometry only. Although this is the current clinical standard (6), complete histopathologic and radiographic classification of parenchymal destruction, airway wall thickening, and small airways inflammation, coupled with genome-wide gene expression studies to identify gene-expression correlates of these phenotypes, might facilitate a deeper understanding of the mechanisms that contribute to these known sources of clinical heterogeneity.

THE FUTURE OF THE AIRWAY GENE EXPRESSION IN COPD

Combining airway epithelial expression profiling of patients with COPD with detailed clinical, radiographic, and histologic phenotyping will elucidate the relationship between gene expression patterns and clinical subtypes. Similar to a previous study of lung adenocarcinoma (34), variability in gene expression among patients with COPD can be used to identify novel molecular subclasses of this disease.

COPD-associated patterns of gene expression changes in lower airway can be extended to the upper airway. This would facilitate disease screening and might allow detection of early disease not apparent by spirometry. Examination of upper airway gene expression might also allow the development of an expression-based, noninvasive biomarker for COPD risk.

Alterations in airway gene expression in COPD could be used to monitor response to therapy. As in a previous study of inhaled corticosteroids in patients with asthma (35), a biomarker for therapeutic response could be developed for patients with COPD. By understanding the specific profile of airway genes altered in COPD, novel therapies could be quickly screened by looking for reversal of the disease-associated gene expression pattern.

Finally, beyond characterizing airway gene expression changes that occur in COPD, it is important to explore the mechanisms by which these alterations occur. As with smoking and smoking-associated lung cancers, genetic susceptibility and epigenetic modifications might explain some of the alterations in airway gene expression in COPD. Several candidate genetic risk factors have been identified in genome-wide association studies of COPD (36–39). Bronchial biopsies from patients with COPD have decreased histone deacetylase activity and, unlike bronchial biopsies from patients with asthma, have no change in histone acetyltransferase activity (40). Integration of transcriptomic profiling with whole-genome microRNA, methylation, and genotyping studies in the airway will ultimately provide a comprehensive view of the molecular field of injury associated with COPD.

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