A Non-Invasive Gene-Expression Biomarker of Airway Response to Tobacco Exposure

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There are approximately 45 million current smokers and 46 million former smokers who are at increased risk for tobacco-related disease in the United States. The public health implications of this widespread environmental exposure are profound; tobacco smoke is the leading preventable cause of death in the United States and is projected to cause nearly 450 million deaths worldwide during the next 50 years. Despite the causal role of cigarette smoking in lung cancer and COPD, only 10-20% of smokers develop these diseases. There are few indicators of which smokers are at highest risk for disease, and it is unclear why individuals remain at high risk decades after they have stopped smoking.

Current standard methods for quantifying exposure to tobacco smoke are limited in their ability to accurately assess cumulative or past exposure, and they do not capture how an individual responds physiologically to that exposure. Given that only subsets of individuals exposed to tobacco smoke develop tobacco-related disease, biomarkers are needed that reflect the biological impact of exposure in a given individual. We have developed an approach to define how an individual has responded to cigarette smoke exposure that forms the basis of our U01 project. The approach is based on the concept that inhaled toxins create a "field of injury" in all exposed airway epithelial cells. The effects of this injury can be detected by identifying genes whose expression levels vary between exposed and unexposed individuals. These collective differences can be used to create a genomic gene expression profile (or signature) that measures host response to cigarette smoke exposure[1]. We have previously used this concept to develop a profile or biomarker that detects a lung-cancer specific gene-expression "injury" in histologically normal bronchial airway epithelial cells among smokers[2]. In this U01 project, we aim to extend the "field of injury" concept to epithelial cells collected from nasal or buccal (mouth) mucosa in a non-invasive fashion. By measuring global gene expression changes from these tobacco-smoke-exposed sites, we will develop a series of biomarkers that detect the host response to current tobacco exposure (active vs. passive vs. never smokers), intensity of current exposure, cumulative exposure among current smokers, time since last exposure (among smokers who recently quit), and lifetime exposure. These studies will begin by exploring the relation between nasal, buccal, and bronchial epithelial cell response to smoke exposure, and will then choose the site that is least invasive, most reproducible, and most affected by smoking for the remaining studies.

A challenge of tobacco-exposure-related studies is that the effects of smoking on gene-expression can be complex, potentially making it difficult to identify genes specifically deregulated by tobacco exposure and robust enough to predict the smoking status of an individual. To address this potential difficulty, we will also use an approach that makes use of experimentally generated gene expression signatures (using airway epithelial cells perturbed *in-vitro*) to specifically reflect the activation of various signaling pathways important for the

physiologic response to tobacco smoke. These pathway signatures reflect certain fundamental traits common to smoking related biological responses, including inflammation (NF- B), DNA damage (ATM), nitric oxide production (iNOS), glutathione metabolism (GPx) and epigenetic modifications (Sirt1). Importantly, these signatures provide tools that can assess the activation status of a pathway in a subject's airway epithelium by determining the extent to which the gene expression signature, identified in the *in vitro* training set, is represented in that airway sample. Therefore, we can predict the relative activation state of these pathways in response to tobacco exposure in samples from our study participants. For those pathways that are associated with tobacco exposure, the extent of pathway activation can be used to create potentially more robust (or complementary) biomarkers of tobacco exposure than possible from expression of individual genes alone.

Although some of the adverse health outcomes of tobacco smoke exposure, such as lung cancer, may be direct consequences of epithelial injury, others result from local inflammation and tissue structural alterations (e.g. COPD) or systemic inflammation (e.g. atherosclerotic heart disease) that are secondary consequences of smoking. We will therefore determine if the biomarkers of epithelial response to tobacco-smoke exposure are correlated with these downstream biologic consequences of tobacco smoke exposure. This portion of the project is motivated by the idea that since the biomarkers measure how an individual responds to tobacco-smoke exposure, they might also detect differences in the response to tobacco-smoke exposure that account for differences in the development of these long-term effects of tobacco-smoke. Specifically, we will correlate airway gene expression biomarkers with lung function and spirometric markers of small airways disease (FEF_{25-75}/FVC ratio) and blood markers of systemic inflammation (serum IL-6, IL-8 and TNF-alpha) and oxidative stress (glutathione and iNOS). Such correlations will link the gene-expression biomarkers to host responses relevant to diseases such as COPD and atherosclerosis that extend beyond the effects of tobacco smoke on respiratory epithelium. This work could potentially set the stage for a more detailed understanding of how heterogeneity in the response to smoke exposure contributes to variability in disease risk.

In summary, this project will yield robust and accurate biomarkers for tobacco exposure, including biomarkers for the intensity, dose, and recovery from exposure that can serve as noninvasive tools in large-scale population studies as part of the Genes and Environment Initiative.

References:

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