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DNA Methylation biomarkers to assess therapy and chemoprevention for non-small cell lung cancer

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Gene promoter methylation in biological fluids is emerging as a biomarker that could be important for early detection of lung cancer and monitoring prevention and intervention. The prevalence of methylation of multiple gene promoters was evaluated in sputum and plasma from women at different risk for lung cancer. Lung cancer survivors participating in the selenium chemoprevention trial showed the highest prevalence for methylation in sputum and plasma compared to cancer-free smokers and never smokers. Sputum was superior for classifying methylation status of genes in tumor biopsies with a positive predictive value of 86% for the combined effect of four genes.

Lung cancer is the leading cause of cancer-related death in the United States and will soon reach epidemic levels worldwide. Mortality from this disease could be reduced through early detection and the identification of high-risk individuals who could benefit from chemopreventive strategies that can reverse or impede the progression of premalignant disease. Therefore, it is essential to develop biomarkers that can predict the efficacy of promising chemopreventive agents. In 1996, Dr. Larry Clark reported a study on skin cancer chemoprevention using L-selenomethionine.1 Although the primary endpoint of skin cancer prevention was negative, the rate of expected lung cancer in the group taking selenium decreased by approximately 50%. This finding led to the implementation of ECOG 5597, “A Phase III Chemoprevention Trial of Selenium Supplementation in Persons with Resected Stage I Non-Small Cell Lung Cancer.” This trial is testing the hypothesis that 200 μg of L-selenomethionine given as selenized yeast can decrease the rate of second primary tumors in patients who have undergone curative surgery for stages Ia (T1N0) or Ib (T2N0) non-small cell lung cancer.

Our studies have identified genes inactivated by aberrant cytosine-guanosine island methylation as candidate biomarkers for early detection of lung cancer.2–4 A nested, case-control study of high-risk smokers with chronic obstructive pulmonary disease revealed that a panel of genes could predict incident lung cancer 3–18 months prior to clinical diagnosis. Specifically, concomitant methylation of three or more of a six-gene panel was associated

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Declaration of interest. Steven A. Belinsky is a consultant to Oncomethylome Sciences. Under a licensing agreement between Lovelace Respiratory Research Institute and Oncomethylome Sciences, nested, methylation-specific PCR was licensed to Oncomethylome Sciences and the author is entitled to a share of the royalties received by the Institute from sales of licensed technology. The Institute, in accordance with its conflict-of-interest policies, is managing the terms of this agreement.

with a 6.5-fold increased risk and a sensitivity and specificity of 64%. These studies support the predictive power of gene promoter methylation. The goal for our correlative studies for the selenium trial is to determine the prevalence for methylation of an eight-gene panel in sputum and blood after tumor resection and follow people with positive methylation markers longitudinally to determine how selenium alters their methylation profile. In addition, we are determining whether aberrant promoter hypermethylation at baseline or subsequent time periods predicts development of second primary, recurrent lung cancer and mortality. A sample repository was established at the Lovelace Respiratory Research Institute in October 2001 to begin the collection of biological fluids. Sputum and blood are collected at time of entry into the study, and at 6, 12, 24, and 48 months during the study.

The first biomarker study with this ongoing clinical trial assessed whether methylation index (defined as the number of genes methylated in sputum or blood) would reflect the inherent difference in risk for participants in this trial compared to cancer-free smokers and never smokers.5 Resected lung cancer patients from ECOG5597 (*n*=86), cancer-free smokers (*n*=121), and never smokers (*n*=74) comprised the study population. Plasma was collected from all groups, whereas sputum was collected from lung cancer survivors and smokers. Nested, methylation-specific polymerase chain reaction was used to define the prevalence for methylation within the p16, O^6-methylguanine (MGMT) death associated protein kinase (DAPK), ras effector homolog 1 (RASSF1A), H-cadherin, PAX5 α, and PAX5 α gene promoters in sputum.2 Due to limited amounts of DNA, methylation of only p16, MGMT, and RASSF1A was defined in plasma. Methylation of these three genes was generally more common in sputum than plasma.5 However, importantly for both biological fluids, the prevalence at the individual gene level and methylation index increased as a function of increasing risk for lung cancer. With respect to methylation in plasma, with never smokers as the reference group, the odds for methylation of ≥1 gene in this fluid from smokers and lung cancer survivors was 1.8 (95%CI, 0.8–4.1) and 3.6 (95%CI, 1.4–9.1), respectively. The difference in odds for methylation of multiple genes in sputum was greater between smokers and lung cancer survivors. The odds (95%CI, 2.1–18.5) for methylation of three or more genes were 6.3-fold greater in sputum from lung cancer survivors compared to smokers after adjustment for age and smoking duration (Fig. 1). This study shows that concomitant methylation of multiple gene promoters in sputum is strongly associated with lung cancer risk and reinforces the use of gene methylation as a biomarker in the prevention trial. This finding has been replicated in a second study comparing prevalence for methylation in sputum from >250 trial participants to over 800 cancer-free smokers (Belinsky, unpublished data).

Another exciting area in which methylation may be an important biomarker is the monitoring of demethylation therapy for treatment and secondary prevention. Clinical trials with demethylating agents alone or in combination with histone deacetylation inhibitors have shown promising responses in the treatment of myeloid malignancies.6,7 An National Cancer Institute-supported phase I/II trial is now underway in lung cancer at Johns Hopkins and an adjuvant clinical trial is being planned. Monitoring the efficacy of demethylation therapy directly within the tumor may be difficult due to tumor location. To overcome this obstacle, we determined whether methylation detected in sputum and/or serum could serve as a surrogate for detecting gene methylation in primary lung cancer.8

A panel of eight genes (p16, MGMT, RASSF1A, DAPK, PAX5 α PAX5 β, H-cadherin) was evaluated by comparing methylation detected in the primary tumor biopsy to serum and sputum obtained from 72 patients with stage III lung cancer.8 The prevalence for individual gene methylation in sputum (21–43%) approximated that seen in the biopsy and was 0.7–4.3-fold greater than in serum. Importantly, sputum was superior to serum for classifying the methylation status of genes in the tumor biopsy. The positive predictive value of the top four

genes (p16, DAPK, PAX5 β, and GATA5) was 44–72% with a negative predictive value for these genes of ≥70%. Evaluating the combined effect of methylation of at least one of the four most significant genes in sputum increased the positive predictive value to 86%. These studies demonstrate that sputum can be used effectively as a surrogate for tumor tissue to predict the methylation status of advanced lung cancer in cases where biopsy is not feasible.

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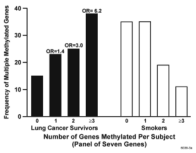

Figure 1.
Frequency of methylation of multiple genes in sputum obtained from lung cancer survivors and smokers.