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The Oral Cavity as a Molecular Mirror of Lung Carcinogenesis

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The lung is a great challenge to oncologists and is where lethal human cancers develop more frequently than in any other organ site. Two large, expensive chemoprevention trials, the Alpha-Tocopherol and Beta-Carotene (ATBC) study and Beta-Carotene and Retinol Efficacy Trial (CARET), highlight the intractability of the lung thus far to effective cancer prevention (1, 2). Both of these prevention trials suggested neutral or harmful results (by primary analyses) in a combined population of more than 47,000 smokers who were followed up to 10 years. The large size and long duration of these trials reflect, in part, the limitation of smoking as the primary risk eligibility criterion; their inefficacy may reflect the limitations of the commonly used nontargeted agents (vitamin E, β -carotene, and retinol). These limitations may be overcome by molecular research designed (a) to identify the highest risk subgroups among smokers, which would in turn allow the initiation of smaller, shorter-term prevention trials, and (b) to identify targeted drugs with a strong molecular rationale that will increase their preventive efficacy in the lung.

Cigarette smoke creates a field of tissue injury throughout the lungs and head and neck. Clonal loss of heterozygosity (3, 4), p53 mutations (5), increased telomerase activity (6), and promoter methylation (7) can occur in large patches of histologically normal epithelial cells of the large airway in current and former smokers with or without lung cancer. The tumor suppressor genes *p16* and *FHIT* are commonly inactivated by promoter methylation in early tumorigenesis of the lung and head and neck, and this methylation is associated with smoking (8–10). Moreover, methylation of *p16* and other genes in sputum or lung samples (11) can persist after smoking cessation and is associated (strongest for *p16*) with an increased risk of lung cancer (12). Methylation markers including *p16* are also a prognostic factor for aggressive disease in patients with stage I non-small-cell lung cancer (13). These molecular markers offer promise for measuring cancer risk and for monitoring trials of potential cancer prevention agents in the lung and head and neck. In the lung, however, these and other molecular markers measured directly in tissues are difficult to monitor, a difficulty that will require creative approaches to overcome.

Monitoring molecular activity in the lung currently relies predominantly on bronchoscopy-directed biopsies and sputum specimens. Bronchoscopy requires a physician and is invasive and expensive, making it unfeasible for population-based studies. Sputum analysis is limited

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by the frequently unpleasant difficulty in producing sputum encountered by people who do not smoke or have no active inflammatory disorder (e.g., chronic bronchitis), by issues related to standardization of the timing of sample collection, and by the complex mixture of airway epithelial cells and other contaminating cells contained in sputum.

A relatively noninvasive way to potentially monitor molecular changes in the lung would be to assess surrogate tissue from the oral cavity. Patients with oral and lung cancers share common exposures to the same tobacco carcinogens, harbor tumors with many of the same genetic and epigenetic changes, are at similar risk for the development of second primary tumors, and exemplify the concept of extensive field carcinogenesis. This concept directly underlies the potential of the oral cavity for monitoring molecular activity in the lung.

Bhutani et al. (14) report elsewhere in this issue of the journal the first population-based study of oral tissue as a molecular mirror for gene promoter methylation in the lung. These investigators concluded that results in oral brushings were an effective surrogate for smoking-induced molecular changes (DNA methylation) in the proximal airways of the lung, where the brushings were taken. Promoter methylation of the tumor suppressor genes *p16* and *FHIT* was studied prospectively in more than 1,700 oral and bronchial brushings of epithelial tissues from 127 smokers. Oral brushings from one site in the oral cavity (the left buccal mucosa) were compared with bronchoscopic bronchial brushings from six standardized sites in the lung. The rates of p16 and/or FHIT methylation were similar at each of the six lung sites (i.e., no site-specific pattern). Other p16 results included single lung site methylation frequencies that generally were slightly higher than that in the oral cavity and a 59% rate of methylation in at least one lung site. The larger the field of methylation in the lung, the more likely that it extended to the oral cavity. No methylation of p16 or FHIT in any tissue (lung or buccal) occurred in 21% of patients. If the buccal mucosa had methylation, there was a strong chance of methylation in at least one of the six lung sites. However, methylation in the lung correlated less strongly with methylation in the buccal mucosa. Furthermore, if no methylation was detected in buccal cells, there still was about a 50% chance of methylation in at least one lung site. These results are most consistent with extensive field cancerization and clonal spread of cells harboring promoter methylation from active lung sites into the oral cavity. Extensive clonal loss-of-heterozygosity alterations have been described at a distance of more than 30 cm from the oral cavity to the esophagus (15). Alternatively, in some cases, independent promoter methylation events in the oral cavity may just represent markers of exposure throughout the aerodigestive tract epithelium.

A provocative dose-response relationship existed between the number of sites methylated in the lung and the presence of oral methylation, as reflected by the following p16 data: 0 of 6 lung sites methylated corresponded with 4% buccal methylation; 1 to 3 lung sites correlated with 19% buccal methylation; and 4 to 6 lung sites with 69% buccal methylation. A similar dose-response pattern occurred for FHIT methylation. Although pack-years of smoking correlated directly with bronchial p16 methylation index ($P = 0.005$) and with buccal p16 methylation ($P = 0.047$), surprisingly they did not correlate with FHIT methylation in bronchial or buccal samples (a nonsignificant inverse relationship occurred). These results point to more rapid and extensive clonal expansion of p16-devoid cells than of cells with

FHIT methylation, consistent with a key role for p16 in the cell cycle and cellular senescence.

The Bhutani et al. results suggest that the amount of genetic damage can be quantified by a methylation index, reflecting the percentage of lung sites showing methylation. The higher the lung methylation index, the more severe damage in the lung and the higher likelihood of finding methylation in the oral cavity. It is possible that sampling multiple oral sites would have increased the correlation between oral and lung methylation. It is also possible that including other commonly methylated genes such as *MGMT*, *DCC*, and others would have increased the methylation index in both the oral cavity and lung (16, 17). It is likely that genetic and epigenetic damage in the lung surpasses that in the oral cavity, due presumably to the extended exposure of the lung to inhaled tobacco smoke. This biological rationale is also supported by smokers' higher absolute risk of lung cancer compared with oral cancer. Methylation in the oral cavity correlated with the presence and amount of methylation in the lung, and although not as sensitive, identified smokers with the most methylation and thus, presumably, the highest risk of lung cancer. Smokers have very large clonal expansions and an extensive field effect, and it seems that heavier smoking induces more global methylation in an extensive diffuse field throughout the lung and oral cavity. In contrast, data indicate that nonsmokers with adenocarcinoma (18) usually have a limited field effect.

The findings of Bhutani et al. have important practical implications for screening and risk assessment and provide important molecular insights into field carcinogenesis. Molecular tests in surrogate oral tissue would be substantially less invasive, easier, and more feasible than are bronchoscopy and sputum assessments. It is likely, however, that a two-gene panel will not be sensitive enough for future screening studies. Future studies extending the Bhutani et al. results should incorporate panels of other epigenetic and genetic markers. For example, genome-wide expression profiling has found correlations between nonmalignant tissues of the lung and oral cavity in global transcriptome changes associated with tobacco smoke effects on pathways related to cell proliferation, inflammation, apoptosis, and tissue injury (19). New high-throughput assays for global and site-specific methylation (20) may increase the detection of these changes and provide more extensive information for comparing oral and lung exposure. Ultimately, panels integrating the status of the cancer genome and methylome with polymorphic markers for susceptibility and standard clinical, pathologic, and demographic risk factors could be used in individualizing risk assessment.

One next step in this emerging area of translational research is to conduct a case-control study of noncancerous oral and bronchial brushes in people with and without lung cancer to see if an expanded panel of methylated genes (and more quantitative measurements of the amount of methylation in each tissue site like quantitative methylation-specific PCR) in normal tissues correlates with the presence of lung cancer. An additional study of the status of this methylation panel in oral and bronchial brushings from nonsmoking noncancer controls matched in age to the Bhutani et al. study would also be helpful in establishing a baseline. Following this assessment, a large-scale population-based study with long-term follow-up would assess the risk association between the methylation panel and lung cancer development.

The study of Bhutani et al. represents a major advance in the use of surrogate tissue for assessing molecular alterations and potentially risk of cancer in the lung. For cancer prevention studies, assessing molecular markers in the oral cavity is a feasible, practical approach that, unlike bronchoscopy, can be applied on a population-wide basis. Furthermore, because promoter methylation is found in tumors and is associated with an increased risk of lung cancer in normal appearing tissues, it also represents a therapeutic target for various demethylating agents in cancer prevention and treatment.

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