Oral Epithelium as a Surrogate Tissue for Assessing Smoking-Induced Molecular Alterations in the Lungs

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Abstract The lungs and oral cavity of smokers are exposed to tobacco carcinogens. We hypothesized that tobacco-induced molecular alterations in the oral epithelium are similar to those in the lungs, and thus the oral epithelium may be used as a surrogate tissue for assessing alterations in the lungs. We used methylation-specific PCR to analyze promoter methylation of the *p16* and *FHIT* genes at baseline and 3 months after intervention in 1,774 oral and bronchial brush specimens from 127 smokers enrolled in a randomized placebo-controlled chemoprevention trial. The association between methylation patterns in oral tissues and bronchial methylation indices (methylated sites / total sites per subject) was analyzed in a blinded fashion. At baseline, promoter methylation in bronchial tissue was present in 23% of samples for *p16*, 17% for *FHIT*, and 35% for *p16* and *FHIT*; these percentages were comparable to methylation in oral tissue: 19% (*p16*), 15% (*FHIT*), and 31% (*p16* and *FHIT*). Data from both oral and bronchial tissues were available for 125 individuals, in whom the two sites correlated strongly with respect to alterations ($P < 0.0001$ for both *p16* and *FHIT*). At baseline, the mean bronchial methylation index was far higher in patients with oral tissue methylation (in either of the two genes; 39 patients) than in patients without oral tissue methylation (86 patients): 0.53 ± 0.29 versus 0.27 + 0.26 methylation index ($P < 0.0001$). Similar correlations occurred at 3 months after intervention. Our results support the potential of oral epithelium as a surrogate tissue for assessing tobacco-induced molecular damage in the lungs and thus have important implications for designing future lung cancer prevention trials and for research into the risk and early detection of lung cancer.

Lung cancer is the leading cause of cancer-related death in the United States (1), with a 5-year survival rate of only 15% (2). Intensive research into the therapy and prevention of lung cancer over the past 30 years has not substantially improved control of the disease. Prevention approaches of smoking cessation and chemoprevention focus on chronic tobacco smokers, who are the highest known risk group for lung cancer. Even chronic smokers who quit the habit remain at a substantially higher-than-average lifetime risk of lung cancer, particularly if they quit at age 50 years or older (3).

Chemoprevention has been limited by a lack of effective agents and by the logistics of clinical testing in this setting. The lifetime risk of chronic smokers for developing lung cancer is only 8% to 10%, strapping lung cancer chemoprevention trials with the need for thousands of trial subjects who must be treated and followed for up to 10 years to assess efficacy. The molecular revolution in cancer research contains potential solutions to both the efficacy and logistics problems of chemoprevention. Advances in the molecular understanding of multistep lung tumorigenesis are helping discover tolerable molecular-targeted preventive agents and are suggesting molecular markers with the potential to select the highest-risk chronic smokers for reducing the logistics of randomized controlled trials. We and others have identified early genetic and epigenetic alterations in tobacco-exposed lung epithelium, such as chromosomal deletions and promoter methylation of tumor suppressor genes (4–6). These alterations have been proposed as markers of lung cancer risk and as intermediate markers for assessing the effects of novel molecular-targeted chemopreventive agents in lung cancer prevention trials (7).

A major limitation on the use of these markers, however, is the anatomic nature of the lungs, which limits access to bronchial epithelium, particularly in relatively healthy smokers. Bronchoscopy to obtain bronchial tissue samples is invasive and expensive and can be done only in a limited number of patients. Sputum is another source of lung tissue for molecular analysis, but few of the many cell types in sputum samples come from airway epithelium, complicating data interpretation.

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Because the entire airway from the oral cavity to the lungs is exposed to tobacco carcinogens in smokers, we hypothesized that these carcinogens induce similar molecular alterations throughout the airway, making the oral epithelium a potential surrogate tissue for assessing tobacco-induced molecular alterations in the lungs. This surrogacy would greatly simplify screening for high lung cancer risk and early detection and would greatly facilitate repeated biomarker analyses of lung cancer chemoprevention in readily accessible oral tissue. To test our hypothesis, we compared the promoter methylation status of two important tumor-suppressor genes involved in early lung carcinogenesis, *p16* and *FHIT*, in bronchial cells with the methylation status of these genes in oral epithelial cells obtained from chronic smokers.

Materials and Methods

Trial design and subjects

Our study cohort came from a prospective placebo-controlled double-blind randomized chemoprevention trial conducted at The University of Texas M.D. Anderson Cancer Center among current and former smokers who had a minimum smoking history of 20 pack-years. Current smokers were defined as active smokers or those who had quit smoking less than 12 mo before their registration for the clinical trial; former smokers had quit smoking longer than 12 mo before their registration. Bronchoscopic and buccal brushing were donein participants at baseline and 3 mo after treatment with either celecoxib (200 or 400 mg twice daily) or placebo. Buccal brushing was done at one site, whereas bronchial brushing was done at six predetermined sites: the main carina, the bifurcation of the right upper lobe, the right middle and lower lobes, the left upper lobe, and the anterior bronchus of the left lower lobe, as shown in Fig. 1. The samples were collected after obtaining appropriate Institutional Review Board approval of the protocol and written informed consent from the subjects.

Sample processing and DNA extraction

Specimens obtained from bronchoscopic and buccal brushing were placed in DMEM (Life Technologies, Inc.) in sterile tubes and stored at 4°C for processing the same day. DNA was extracted by digestion of cells with 10× proteinase K-SDS solution [5 mg/mL proteinase K (Roche Molecular Biochemicals) and 10% SDS (Life Technologies)] at 42°C overnight followed by phenol and chloroform extraction.

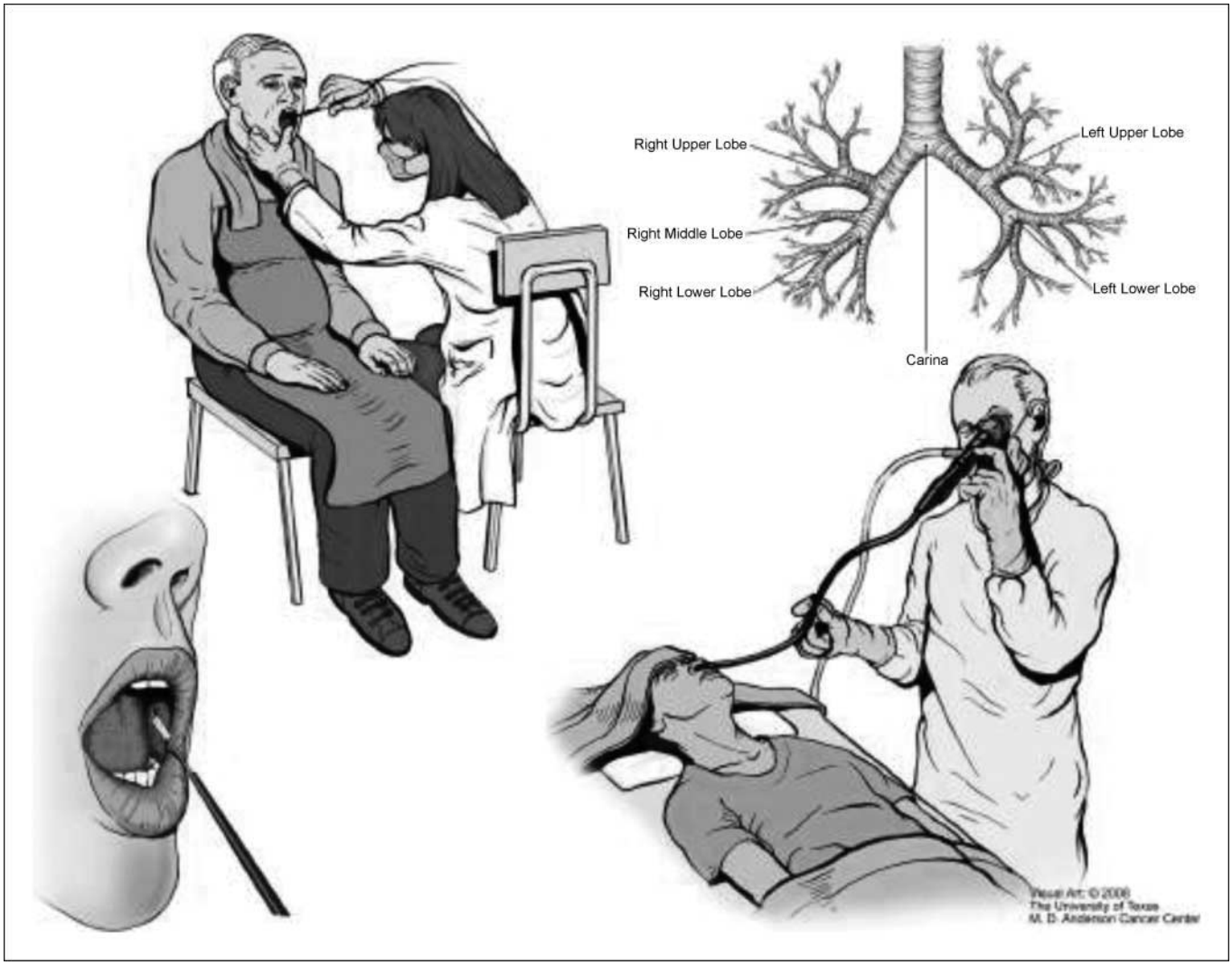

Fig. 1. A flexible bronchoscope is inserted through the nose or mouth to examine the airways after i.v. sedation (*bottom right*). Bronchial brushings are taken from six predetermined sites (*top right*). Oral brushing using a cytologic brush is done (*top left*) by rubbing the inner side of the left cheek (*bottom left*). From Visual Art © 2008, The University of Texas, M. D. Anderson Cancer Center; with permission.

Methylation-specific PCR

At least 100 ng of sample DNA, mixed with 1 μg of salmon sperm DNA (Life Technologies), were subjected to chemical modification following the protocol of Herman et al. (8). PCR was then conducted with primers specific for either the methylated or unmethylated versions of the *p16* and *FHIT* promoter regions (5, 9). The 12.5-μL total reaction volume contained 25 ng of modified DNA, 3% DMSO, all four deoxynucleoside triphosphates (each at 200 μmol/L), 1.5 mmol/L magnesium chloride, 0.4 μmol/L PCR primers, and 0.625 unit of HotStar Taq DNA polymerase (Qiagen). Negative controls included water to control for DNA contamination and normal tissue DNA was used as a negative control for methylation. DNA from the NCI-H460 lung cancer cell line treated with *Sss*I methylase (New England Biolabs Inc.) was used as a positive control. PCR products were separated on 2% agarose gels and visualized after staining with ethidium bromide.

Statistical analysis

Methylation status was determined at baseline and 3 mo after intervention with the brush site (both oral and bronchial) and participant (with multiple bronchial brushes) as the units of analysis. When the participant was used as the unit of analysis, that individual was considered to be methylation positive when any of the bronchial brush sites showed promoter methylation. The methylation index was determined for each gene by dividing the number of bronchial brush sites exhibiting promoter methylation by the total number of sites examined in each participant.

Statistical analysis was done using χ^2 test or Fisher's exact test for correlation among multiple genes and between methylated gene status and sex. Wilcoxon's rank-sum test was used for testing differences between methylated and unmethylated groups in median age, packs per day, years of smoking, pack-years, and years since having quit. Spearman's rank correlation was applied to estimate the association between smoking pack-years and methylation index. Kruskal-Wallis test was used to compare bronchial brush methylation index modulation among oral brush methylation modulation groups (10). All P values were determined by two-sided tests, and $P \leq 0.05$ was considered statistically significant.

Results

Two hundred four participants were randomized into this clinical trial; 127 completed 3 months of treatment, including 99 current smokers and 28 former smokers. These composed our study population. Of those 127, 58 were randomized to the placebo-treated group and 69 to the celecoxib-treated group, including 33 treated with 200 mg twice daily and 36 treated with 400 mg twice daily. The participants' average age was 53.6 years (range, 32.0-73.6 years), and they had smoked an average of 42 pack-years (range, 20-89 pack-years). Former smokers had quit an average of 7.8 years (range, 1-35 years) before study participation.

Baseline oral brush samples were not available for two subjects, and thus data from 125 subjects were used to analyze the relationship of promoter methylation between tissues from the two anatomic sites at baseline. At baseline, 762 bronchial brush samples from 127 subjects were analyzed. Because of DNA amplification failure, methylation status was not available in 8 (1%) of the samples for *p16* and 10 (1.3%) for *FHIT*. Promoter methylation was observed in 174 of the 754 (23%) bronchial brush samples for *p16* and in 124 of the 752 (17%) samples for *FHIT*. When methylation in either of the two promoters was considered positive, 267 of the 762 (35%) samples contained promoter methylation. Only 31 of the 744 (4%) samples with methylation status available for both promoters showed methylation in both promoters, suggesting that multiple mechanisms are involved in methylating the promoters during early carcinogenesis.

In total, 125 oral brush samples from 125 subjects were obtained for the study. Methylation data were not available from two samples (one for each promoter) owing to DNA amplification failure. Promoter methylation was detected in 24 of the 124 (19%) oral brushes for *p16* and in 19 (15%) for *FHIT*. When methylation of either promoter was considered positive, 39 of the 125 (31%) oral brushes were positive. To determine whether the subjects' characteristics may have affected their baseline methylation status, we evaluated possible associations between the characteristics listed in Table 1 and the baseline oral and bronchial methylation status. The number of pack-years of smoking was positively associated with *p16* methylation status ($P = 0.003$) or methylation in either of the two gene promoters (*p16/FHIT*; $P = 0.003$). When analyzed as a continuous variable, pack-years of smoking correlated with *p16* in bronchial samples ($P = 0.005$, Spearman's rank correlation) and in buccal samples ($P = 0.047$, Wilcoxon's rank-sum test). In contrast, pack-years of smoking did not correlate with FHIT methylation in bronchial or buccal samples.

The key issue is whether promoter methylation in the oral epithelium reflects molecular damage in the lungs. We first compared the methylation patterns in matched oral and bronchial samples. Of the 24 individuals with *p16* promoter methylation in oral samples, 22 (92%) had promoter methylation in at least one of the matched bronchial samples, whereas only 51 of the 100 (51%) individuals without promoter methylation in oral samples had promoter methylation in matched bronchial samples ($P = 0.0002$). Similarly, 18 of the 19 (95%) subjects with *FHIT* promoter methylation in oral samples had promoter methylation in at least one of the matched bronchial samples, whereas 29 of the 105 (28%) individuals without promoter methylation in oral samples had *FHIT* methylation in at least one matched bronchial sample ($P < 0.0001$). When methylation of either promoter was positive, 37 of the 39 (95%) individuals with *p16* and/or *FHIT* promoter methylation in oral samples had promoter methylation in at least one matched bronchial sample, compared with only 59 of the 86 (69%) individuals without promoter methylation in oral samples ($P = 0.001$; Table 2).

Despite these strong correlations, it is important to note that the sensitivity of oral methylation for detecting bronchial methylation was low. Twenty-two of the 73 (30%) individuals with *p16* promoter methylation in bronchial samples showed this methylation in oral samples; 18 of the 47 (38%) individuals with *FHIT* promoter methylation in bronchial samples showed this methylation in oral samples; 37 of the 96 (39%) individuals with methylation of either of the two promoters in bronchial samples showed methylation in oral samples (Table 2).

Because we analyzed up to six sites on the bronchial tree but only one oral cavity site for each subject, we developed a methylation index to quantify the extent of promoter methylation in an individual's bronchial tree. There was no significant difference in site-specific methylation in the lung (detailed later in this section); the percentage of overall methylation per lung site was similar to, but slightly higher than, that in the oral site, indicating greater molecular damage

Table 1. Gene promoter methylation status at baseline using patient (combining six bronchial brush sites within each patient) as an analysis unit

Characteristic		*p16*				*FHIT*				*p16/FHIT*			
		Bronchial		Oral		Bronchial		Oral		Bronchial		Oral	
		M	U	M	U	M	U	M	U	M	U	M	U
No. patients (%)		74 (58.3)	53 (41.7)	24 (19.4)	100 (80.4)	50 (39.4)	77 (60.6)	19 (15.3)	105 (84.7)	98 (77.2)	29 (22.8)	39 (31.2)	86 (68.8)
Age (y), *n* (%)													
<54	62 (48.8)	36 (58.1)	26 (41.9)	12 (20.3)	47 (79.7)	24 (38.7)	38 (61.3)	9 (15.3)	50 (84.7)	46 (74.2)	16 (25.8)	19 (31.7)	41 (68.3)
≥54	65 (51.2)	38 (58.5)	27 (41.5)	12 (18.5)	53 (81.5)	26 (40)	39 (60)	10 (15.4)	55 (84.6)	52 (80)	13 (20)	20 (30.8)	45 (69.2)
P		0.96		0.79		0.88		0.98		0.44		0.91	
Sex, *n* (%)													
F	58 (45.7)	39 (67.2)	19 (32.8)	12 (21.1)	45 (78.9)	25 (43.1)	33 (56.9)	10 (17.9)	46 (82.1)	49 (84.5)	9 (15.5)	20 (35.1)	37 (64.9)
M	69 (54.3)	35 (50.7)	34 (49.3)	12 (17.9)	55 (82.1)	25 (36.2)	44 (63.8)	9 (13.2)	59 (86.8)	49 (71)	20 (29)	19 (27.9)	49 (72.1)
P		0.06		0.66		0.43		0.48		0.07		0.39	
Smoker, *n* (%)													
Current	99 (78)	59 (59.6)	40 (40.4)	21 (21.9)	75 (78.1)	38 (38.4)	61 (61.6)	12 (12.5)	84 (87.5)	77 (77.8)	22 (22.2)	31 (32)	66 (68)
Former	28 (22)	15 (53.6)	13 (43.4)	3 (10.7)	25 (89.3)	12 (42.9)	16 (57.1)	7 (25)	21 (75)	21 (75)	7 (25)	8 (28.6)	20 (71.4)
P		0.56		0.28		0.67		0.11		0.76		0.73	
Pack-years, *n* (%)													
<30	36 (28.3)	12 (33.3)	24 (66.7)	3 (8.6)	32 (91.4)	14 (38.9)	22 (61.1)	6 (17.1)	29 (82.9)	20 (55.6)	16 (44.4)	9 (25)	27 (75)
30-40	32 (25.2)	23 (71.9)	9 (28.1)	6 (18.8)	26 (81.3)	15 (46.9)	17 (53.1)	4 (12.5)	28 (87.5)	29 (90.6)	3 (9.4)	9 (28.1)	23 (71.9)
41-50	24 (18.9)	15 (62.5)	9 (37.5)	5 (20.8)	19 (79.2)	8 (33.3)	16 (66.7)	6 (25)	18 (75)	19 (79.2)	5 (20.8)	10 (41.7)	14 (58.3)
≥50	35 (27.6)	24 (68.6)	11 (31.4)	10 (30.3)	23 (69.7)	13 (37.1)	22 (62.9)	3 (9.1)	30 (90.9)	30 (85.7)	5 (14.3)	11 (33.3)	22 (66.7)
*P**		0.0003		0.08		0.94		0.72		0.0003		0.34	
Quit-years, *n* (%)													
<1	99 (78)	59 (59.6)	40 (40.4)	21 (21.9)	75 (78.1)	38 (38.4)	61 (61.6)	12 (12.5)	84 (87.5)	77 (77.8)	22 (22.2)	31 (32)	66 (68)
1-10	18 (14.2)	9 (50)	9 (50)	3 (16.7)	15 (83.3)	9 (50)	9 (50)	6 (33.3)	12 (66.7)	14 (77.8)	4 (22.2	7 (38.9)	11 (61.1)
>10	10 (7.9)	6 (60)	4 (40)	0 (0)	10 (100)	3 (30)	7 (70)	1 (10)	9 (90)	7 (70)	3 (30)	1 (10)	9 (90)
P†		1		0.21		0.74		1		0.69		0.17	

Abbreviations: M, methylated; U, unmethylated.
*Comparing methylation between the groups <30 and ≥30 pack-years.
†Comparing methylation between the groups <10 and ≥10 quit-years.

in the lung. Subjects whose oral samples exhibited promoter methylation had significantly higher bronchial methylation indices than did those without methylation in the oral tissues [mean, 0.47 versus 0.18 for *p16* ($P < 0.0001$) and 0.50 versus 0.10 for *FHIT* ($P < 0.0001$); Table 3]. When methylation of either promoter was considered positive, the group with positive oral tissues had significantly higher methylation indices than did the group with negative oral tissues (0.53 versus 0.27; $P < 0.0001$).

Table 2. Association between oral methylation status and bronchial methylation status

Bronchial	Oral					
	p16		*FHIT*		*p16/FHIT*	
	U	M	U	M	U	M
U	49*	2	76	1	27	2
M	51	22	29	18	59	37
P†	0.0002		<0.0001		0.001	

*Data are expressed as number of patients.
†Fisher's exact test.

The availability of tissues at 3 months allowed us to test the robustness of the correlation between epigenetic alterations in oral and bronchial tissues. The 3-month correlations were consistent, as observed in the baseline samples. The group with oral tissue methylation had significantly higher bronchial methylation indices (0.39 with versus 0.14 without *p16* methylation, $P < 0.0001$; 0.57 with versus 0.08 without *FHIT* methylation, $P < 0.0001$; Table 3). Similarly, the group with oral tissue methylation of either promoter had a significantly higher methylation index than did the group without oral tissue methylation (0.50 with versus 0.21 without methylation; $P < 0.0001$).

We analyzed site-specific bronchial methylation. p16 promoter methylation ranged from 17.5% to 25.6% for each site. Similarly, *FHIT* methylation ranged between 13.3% and 21% per site. The methylation status of every bronchial site was significantly associated with the methylation status in oral samples for both *p16* and *FHIT* (data not shown).

The extent of promoter methylation in bronchial sites, or the number of sites with methylation per individual, was also compared with oral methylation. The following results pertain to p16 methylation: If none of the 6 bronchial sites showed methylation, only 2 of the 51 (3.9%) cases showed methylation in oral samples; if 1 to 3 of the 6 sites showed methylation, 11 of the 57 (19.3%) cases showed methylation in oral samples; if 4 to 6 sites showed methylation, 11 of the 16 (68.8%) cases showed methylation in oral samples. Similar relationships occurred for *FHIT* methylation: If none of the 6 bronchial sites showed methylation, only 1 of the 77 (1.3%) cases showed methylation in oral samples; if 1 to 3 of the 6 sites showed methylation, 10 of the 35 (28.6%) cases showed methylation in oral samples; if 4 to 6 sites showed methylation, 8 of the 13 (61.5%) cases showed methylation in oral samples. When the methylation status at specific brushing sites was evaluated after the 3-month intervention period, we observed consistent changes in methylation patterns between the oral cavity and the lungs (Table 4).

Discussion

Our results provide the first prospective data on the potential of oral tissue as a surrogate tissue for evaluating tobacco-induced molecular damage and cancer risk in the lungs. The two tumor-suppressor genes, *p16* and *FHIT*, are deeply implicated in lung tumorigenesis, and we found that chronic tobacco exposure induced similar methylation patterns in these two genes in oral and matched bronchial epithelial tissues, both at baseline and in a second sample set collected 3 months later from the same individuals. The consistent findings at two time points reflect the robustness of our findings. These results may have important implications for future lung cancer prevention trials.

Table 3. Correlation between oral methylation status and bronchial methylation index

		Bronchial methylation index		
	Oral	*n*	Mean ± SD, median (range)	*P**
Baseline				
p16	U	100	0.18 ± 0.22, 0.17 (0-0.83)	<0.0001
	M	24	0.47 ± 0.30, 0.50 (0-1.00)	
FHIT	U	105	0.10 ± 0.20, 0.00 (0-1.00)	<0.0001
	M	19	0.50 ± 0.29, 0.50 (0-1.00)	
p16/FHIT	U	86	0.27 ± 0.26, 0.17 (0-1.00)	<0.0001
	M	39	0.53 ± 0.29, 0.50 (0-1.00)	
3 mo				
p16	U	89	0.14 ± 0.21, 0.00 (0-1.00)	<0.0001
	M	36	0.39 ± 0.29, 0.33 (0-1.00)	
FHIT	U	113	0.08 ± 0.18, 0.00 (0-1.00)	<0.0001
	M	14	0.57 ± 0.28, 0.67 (0.17-1.00)	
p16/FHIT	U	83	0.21 ± 0.24, 0.17 (0-1.00)	<0.0001
	M	44	0.50 ± 0.31, 0.50 (0-1.00)	

*Wilcoxon's rank-sum test comparing bronchial brush methylation index between oral brush methylation groups.

Table 4. Comparison of modulation of promoter methylation status between oral and bronchial epithelia after 3-mo intervention

Oral baseline/ 3 mo	*n*	Bronchial methylation index (baseline/3 mo)	
		Mean ± SD, median (range)	*P**
p16			
M/M	7	−0.16 ± 0.19, −0.17 (−0.5-0)	0.003
M/U	16	−0.15 ± 0.40, −0.17 (−0.67-0.67)	
U/M	28	0.16 ± 0.34, 0.17 (−0.50-1.00)	
U/U	71	−0.05 ± 0.28, 0 (−0.83-0.67)	
FHIT			
M/M	3	−0.11 ± 0.25, −0.17 (−0.33-0.17)	<0.0001
M/U	16	−0.46 ± 0.30, −0.50 (−0.83-0)	
U/M	10	0.42 ± 0.33, 0.42 (−0.17-1.00)	
U/U	95	0.01 ± 0.22, 0 (−0.50-1.00)	
p16/FHIT			
M/M	16	−0.06 ± 0.37, −0.17 (−0.83-0.67)	0.007
M/U	23	−0.22 ± 0.38, −0.33 (−0.83-0.50)	
U/M	27	0.16 ± 0.37, 0.17 (−0.50-1.00)	
U/U	59	−0.05 ± 0.31, 0 (−0.83-0.50)	

*Kruskal-Wallis test comparing bronchial brush methylation index modulation among oral brush methylation modulation groups.

Intermediate end-point biomarkers provide a scientific tool for designing more efficient and cost-effective chemoprevention trials (11, 12). Intermediate end-point molecular analyses, however, depend on serially procured lung tissues, which hampers the feasibility of such trials, particularly in relatively healthy smokers. Therefore, potential surrogates for lung tissues are of intense interest. Sputum is a potential surrogate for bronchoscopy in assessing people at high risk for lung cancer (13) but has inherent limitations. For example, sputum is clinically difficult to obtain from a considerable number of smokers (14), particularly former smokers, who represent more than 50% of newly diagnosed lung cancer patients in the United States (15). Furthermore, serial sputum samples are highly variable in composition, varying with respect to epithelial versus inflammatory components and to the lobe of origin of cells. Serum protein and DNA analysis is another potential surrogate, but its efficacy in identifying individuals at risk is not established (16). Therefore, the demonstration of oral brushes as a surrogate for lung tissue in our study has significant implications for lung cancer screening and prevention studies.

We assessed promoter methylation of tumor-suppressor genes because it is considered a major mechanism underlying tumor initiation and progression (17). Promoter methylation, particularly in multiple tumor-suppressor genes, is associated with an increased risk of lung cancer in people (18). We previously reported that promoter methylation of tumor-suppressor genes can occur in the bronchial epithelium of chronic smokers without lung cancer (5). Our current results, including the correlation between *p16* methylation index and pack-years of smoking, indicate that methylation burden increases

with heavy smoking, notwithstanding our finding that, in contrast to *p16* methylation, *FHIT* methylation did not correlate with pack-years. *FHIT* inactivation occurs early in lung carcinogenesis, and thus *FHIT* methylation could have peaked in our study population of >20 pack-year smokers (mean of 42 pack-years; data not shown) before our analyses. Promoter methylation was present in current and former smokers, supporting our previous findings that smoking-induced genetic alterations persist in smokers who have quit for a long period (19). This finding suggests that methylation changes, which are transforming or permit the accumulation of additional changes, continue to confer an increased risk for lung cancer. Long-term follow-up study will be crucial for revealing the actual risk of lung cancer in patients with widespread aberrant methylation in the aerodigestive tract.

Promoter methylation patterns in the oral epithelium correlated strongly with those in the lungs, and the correlation was consistent over time. Based on these findings, we conclude that the oral epithelium can be used as a surrogate tissue in future lung cancer prevention studies. Although we analyzed only promoter methylation, the oral epithelium may be evaluated for other molecular markers, such as chromosomal deletions and amplifications, abnormal gene expression, and abnormal protein expression and/or modifications (20). A limitation of our study is the low sensitivity of oral methylation for detecting bronchial methylation. This low sensitivity is likely due, at least in part, to the larger number of bronchial than oral sampling sites. We found that each bronchial site had a similar frequency of promoter methylation at baseline to that in the oral site and that these strong associations remained at 3 months. We also showed that methylation at a single oral site associated strongly with the extent of methylation (methylation index) in the lungs. These results support the possibility that the numerical imbalance between bronchial and oral sites contributed to low sensitivity, suggesting that future studies should use specimens from multiple oral sites to increase detection sensitivity and allow quantification of damage in the field. This approach may provide a more accurate assessment of cancer risk and the effects of chemopreventive agents. It is also possible, however, that heavy tobacco smoking exposure is needed for promoter methylation to appear in the oral cavity. Future studies should also address whether oral methylation is an independent factor showing a high risk of lung cancer (and oral cancer) in current and former smokers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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