Expression of Heterogeneous Nuclear Ribonucleoprotein A2/B1 in Bronchial Epithelium of Chronic Smokers

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ABSTRACT

The monoclonal antibody 703D4, which binds heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1), has been reported to detect lung cancer more than a year earlier than routine chest X-ray or cytomorphology. To explore the biological basis of this detection, we studied the expression of this antigen in the central airways of smokers with evidence of bronchial metaplasia using specimens from a previously reported, randomized retinoid chemoprevention trial. By analyzing 1078 available biopsy specimens from 147 individuals at baseline and 68 individuals who completed the intervention, we frequently detected overexpression of hnRNP A2/B1 in normal and abnormal bronchial epithelium (i.e., in 41% of normal and 37% of squamous metaplasia samples). There was no correlation between hnRNP A2/B1 overexpression and the different histological changes. In cases with hnRNP A2/B1 overexpression, immunoreactivity was homogeneously expressed in all biopsied sites. For the 68 cases with serial biopsies, there was no significant modulation of hnRNP expression by retinoid intervention or smoking status. With lung cancer cell lines, 0.5–4 μM concentrations of 13-cis-retinoic acid reduced hnRNP A2/B1 overexpression by immunocytochemistry. We conclude that hnRNP A2/B1 overexpression is frequently found in central airways of chronic smokers, consistent with the pattern of expression that we reported previously in airways surrounding resected primary lung cancers. Oral 13-cis-retinoic acid at a dose of 1 mg/kg has no demonstrable effects on modulating hnRNP A2/B1 expression in proximal bronchial epithelium.

INTRODUCTION

Lung cancer is the most frequent cause of cancer death of both males and females in the United States, accounting for one in three cancer deaths (1). In the last 30 years, cancer-related survival for this disease has improved only minimally. Previous efforts to reduce lung cancer mortality through earlier cancer detection by using combined chest X-ray and sputum cytology have not shown an improvement in cancer-related mortality (2, 3). Tockman et al. (4) have reported a more sensitive detection technique using immunostaining of exfoliated cells from sputum samples with two lung cancer-associated monoclonal antibodies. Analyzing the contribution of the individual monoclonal antibodies to early detection of lung cancer, the monoclonal antibody 703D4 alone identified 90% of the detected, true-positive cases in the first published study (4, 5). This antibody has been shown recently to recognize hnRNP2 A2/B1 (6). This protein is expressed at low levels in all cells. As reported recently, the level of this protein and mRNA is frequently overexpressed in transformed bronchial epithelium and most NSCLC primary and metastatic cancer cells, allowing detection either by immunolocalization techniques or Northern blotting, respectively (6). When used for early cancer detection, this antibody was effective in recognizing all histological subtypes of early lung cancer (4, 7). Preliminary results from two, ongoing prospective early lung cancer detection trials also show that hnRNP A2/B1 overexpression as recognized by immunocytochemistry identifies 80% of the individuals progressing to lung cancer (7).

“Field cancerization” is a concept proposed by Slaughter et al. (8), which is fundamental to understanding the biology of upper aerodigestive tract carcinogenesis. This theory suggests that the whole upper aerodigestive tract is exposed to carcinogen such as tobacco combustion products, and that exposure results in multiple foci of malignant transformation. Lung cancer can progress through a series of morphological changes that are presumed to reflect progressive genetic injury. This process of progressive genetic injury takes decades to evolve from normal epithelium to invasive cancer. A number of morphological epithelial changes have been observed throughout the respiratory tract of both smokers and lung cancer patients (9). The morphological progression from hyperplasia through squamous metaplasia to invasive lung cancer was initially proposed by Saccmanno et al. (10) as an early detection tool. Despite individual cases being diagnosed in an early stage via conventional sputum
exam, this test has never been shown to be of benefit when evaluated in a prospective clinical trial. This may be related to the fact that conventional sputum cytology diagnoses early squamous cancer cases only, but most of the new lung cases are adenocarcinomas, and other nonsquamous lung cancers are not detected by conventional sputum cytology. For reasons that are not as yet fully understood, the overexpression of hnRNP A2/B1 is consistently informative as an early detection marker (4, 7).

The present study is a systematic effort to map the expression of this antigen in tobacco-injured airways.

The multistep process of carcinogenesis is often associated with an accumulation of many genotypic and phenotypic alterations. These changes could be the result of dysregulation of genes that control normal cell growth. To begin to study the critical changes involved in "field cancerization," investigators from M. D. Anderson Cancer Center mounted a prospective clinical trial. Using established criteria for a metaplasia index, they identified a cohort of chronic smokers who manifested morphological changes in their airway epithelium (11). All study participants underwent a baseline bronchoscopy in which six predefined airway sites, including all five lobes and the carina, were systematically biopsied. To determine whether administration of 13cRA would reverse the squamous metaplasia, study subjects were randomized to receive daily 13cRA (1 mg/kg) or placebo for 6 months. Upon completion of the drug course, all consenting subjects underwent a bronchoscopy with multiple biopsies as described for the baseline study. All of the bronchial biopsies from baseline and the repeat study after 6 months of intervention were stored. The metaplasia study involves the largest systematic, serial evaluation of the bronchial tree that has ever been conducted. The archive of the bronchial biopsies specimens from that study provided the tissue analyzed for hnRNP A2/B1 expression in this study (11).

Immunohistochemistry. Mouse monoclonal antibody 703D4 (5) was purified from mouse ascites using a protein A column and discontinuous glycine, NaCl/citrate gradient (Pierce, Rockford, IL). Purified antibody (4 μg/ml) was used to identify areas of hnRNP A2/B1 expression. Immunohistochemical staining was performed using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) following the vendor’s instructions with modifications reported previously (19).

Procedure for Slide Analysis. Tissue specimens from six bronchial sites were mapped for each case using light microscopy in corresponding hematoxylin-stained material. All bronchial epithelium were screened for the presence of the following histological abnormalities: BCH, GCH, SQM, and dysplasia. All morphological designations were determined by independent reviewers at National Cancer Institute and M. D. Anderson Cancer Center using published criteria (11, 20, 21). In most cases, a range of morphological changes along with normal tissue were evident. For each histologically abnormal tissue, we counted the number of atypical foci per total available number of high power fields (×40). No comparable effort was made to quantify the area of normal morphology. A staining distribution score (0, no positive cells; 1, 1–10%; 2, 11–50%; and 3, 51–100% of cells positive) and staining intensity score (0, no positive cells; 1, 1–10%; 2, 11–50%; and 3, + + +) was obtained for each specimen. Using the sum of these values, a staining index (SI, distribution score + intensity score: possible values, 0 and 2–6) was established for each tissue as published previously (22). The SI ≥2 was called positive as reported previously (23). Levels of hnRNP expression were scored blindly for both baseline and post 6 months treatment cases, with the normal and abnormal bronchial epithelium independently scored by different reviewers. Disagreements were resolved after joint review before any clinical correlation analysis.

hnRNP A2/B1 Modulation Studies in Vitro. Small cell lung cancer cell line H345 and NSCLCs H720 and H157 were established and maintained as published previously (24). The cells were seeded in six-well plates at 1–2 × 10^5 cells/ml in serum-free, hormonally defined medium (18). 13cRA (Sigma Chemical Co., St. Louis, MO) was added at a final concentration of 0–4 μM. After 24 h incubation, the cells were harvested, and cytosplins were prepared (5 × 10^6 cells/slide). The cells were formalin fixed for 10 min, and immunocytochemistry was performed using a Histo Stain kit (Zymed laboratories, Inc., San Francisco, CA), following the manufacturer’s protocol, with the
Modification that primary antibody was incubated overnight at 4°C in a humid chamber. An AEC chromagen/substrate was used as the detection of the antigen/antibody/enzyme complex.

The 13cRA treated and untreated control cells were harvested, and RNA was extracted with Trizol (Life Technologies, Inc., Gaithersburg, MD) following the manufacturer’s instructions. Ten μg of total cellular RNA from each cell line were used for Northern blot analysis as described previously (6).

Statistical Analysis. Descriptive statistics were used to summarize the hnRNP A2/B1 expression in each morphology at baseline and at 6 months. The Pearson’s χ² test was applied for contingency table analysis to reveal the association between biopsy site and between smoking quitters and nonquitters. Both site-specific and patient-specific hnRNP A2/B1 expressions were used as an analysis unit when appropriate.

RESULTS

Mapping of Normal and Abnormal Bronchial Epithelium in the Airway Biopsies. In each of the baseline 147 chronic smokers, all six biopsy specimens were examined. Careful mapping of morphological abnormalities revealed that 457 of 704 (65% of available sites) biopsies showed evidence of morphological abnormalities. For each individual, analyzing all available biopsies for morphological change, only 2 of 147 baseline cases showed completely normal morphology. Furthermore, 246 of 374 (66%) follow-up biopsy sites with 67 of 69 of the randomized subjects showed areas of morphological abnormality in their biopsies at 6 months treatment. As described in “Materials and Methods,” we quantitated each abnormal tissue in their biopsies at 6 months treatment.

hnRNP A2/B1 Expression in Bronchial Epithelium of Chronic Smokers. For both the baseline 147 cases of chronic smokers and the repeat biopsies, we evaluated the hnRNP A2/B1 expression in both histologically normal and abnormal bronchial epithelium (Fig. 2). A comparison of hnRNP A2/B1 expression over time. A two-sample test was applied for matched case analysis to reveal the change of mean SI between treatment groups and between smoking quitters and nonquitters. Both site-specific and patient-specific hnRNP A2/B1 expressions were used as an analysis unit when appropriate.
immunoreactivity in different histological subtypes is illustrated in Table 3. For the baseline cases, 146 individuals have focal areas of histologically normal tissue; 45% of them were hnRNP A2/B1 positive. For the 6-month treatment group, 41% (of 68) had hnRNP A2/B1 expression in areas of normal morphology. In individuals with BCH, GCH, and SQM of bronchial epithelium, hnRNP A2/B1 expression status from baseline to study completion ranged from 48 to 44%, 51 to 44%, and 41 to 34% for each histology, respectively. There was no statistically significant change in hnRNP A2/B1 expression from baseline to 6 months of treatment \((P > 0.01\) by \(\chi^2\) test) in BCH and SQM, but a statistically significant change in hnRNP A2/B1 expression was found in GCH from baseline to 6 months of treatment \((P = 0.04)\). However, when hnRNP A2/B1 expression was examined in matched cases, there was no statistically significant change in hnRNP A2/B1 expression from baseline to 6 months of treatment. When we analyzed hnRNP A2/B1 expression by biopsy site as well as each histological subtype alone, no statistically significant correlation could be found in either baseline or the 6 months of treatment group \((all P_s > 0.4)\). Analyzing hnRNP A2/B1 positive and negative cases from baseline, there was almost no change in hnRNP A2/B1 expression after 6 months because 27 of 28 positive baseline cases remained positive at the 6-month evaluation and 39 of 40 negative cases remained negative for a concordance rate of 97% (Table 4).

When the subset of biopsy samples that contained only histologically normal tissue was analyzed, positive hnRNP A2/B1 expression was found in 85 of 224 (38%) baseline cases and 43 of 107 (40%) of the final biopsy specimens, respectively (data not shown). The expression of hnRNP A2/B1 was detected in both ciliated and nonciliated bronchial epithelium as well as in underlying basal cells (Fig. 2). The pattern of hnRNP A2/B1 included both focal (detected in single or small groups of cells) and diffuse \((\pm 50\%\) of cells positive), as shown in Fig. 2. In positive cases, immunoreactivity was generally homogeneously expressed throughout all sampled sites.

**Clinical Correlation.** To evaluate for possible quantitative changes of hnRNP A2/B1 expression in bronchial epithelium of chronic smokers, SI was correlated with clinical features such as smoking history (pack years) and treatment. A comparison of hnRNP A2/B1 expression in matched 68 cases, stratified by treatment (13cRA and placebo) and smoking cessation, re-
Fig. 2  The hnRNP A2/B1 expression pattern in bronchial epithelium.  A, granular localization of hnRNP toward the apical portion of ciliated and nonciliated bronchial epithelium. Note faint staining of underlying basal cells (arrow; immunohistochemical staining, ×233).  B, hnRNP A2/B1 expression in basal cell hyperplasia at the bronchi, showing more than three basal cell layers in otherwise normal respiratory epithelium (immunohistochemical staining, ×233).  C, hnRNP A2/B1 expression in GCH of the bronchi demonstrating more than 50% of bronchial epithelium consisting of goblet cells (immunohistochemical staining, ×233).  D, hnRNP A2/B1 expression in squamous metaplasia of the bronchi. Note the flattening and stratification of the epithelial cells (immunohistochemical staining, ×116).  E, hnRNP A2/B1 expression in dysplastic bronchial epithelial cells. Note nuclear atypia (immunohistochemical staining, ×466).  F, hnRNP A2/B1 expression in histologically unremarkable and pretransitional epithelium. Note the right part of epithelial cells with several rows of elongated and slight atypia cells throughout the entire epithelial cell layers. The surface cells lack cilia and appear flattened (immunohistochemical staining, ×194).

vealed no statistically significant changes. In the placebo group, only one case showed decreased expression of hnRNP A2/B1 SI after 6 months. Conversely in the 13cRA arm, one subject showed an increase in hnRNP A2/B1 expression after 6 months. For hnRNP A2/B1-positive cases at baseline, no significant change in hnRNP A2/B1 SI was found by treatment (13cRA and placebo) or smoking cessation (Figs. 3 and 4). To evaluate for any change in hnRNP A2/B1 expression, due to 13cRA administration, each case was matched by site and analyzed for changes in antigen expression status from baseline to the study termination at 6 months, and again no significant changes in hnRNP A2/B1 status was observed.

**hnRNP A2/B1 Modulation Studies in Vitro.** Using immunocytochemistry to study the modulation of hnRNP A2/B1 expression after exposure to 13cRA in vitro, we observed a marked decrease in hnRNP A2/B1 expression as measured by a SI, which decreased from 4 to 2 for all of the cell lines tested with 2, 0.5, and 4 μM concentration of 13cRA, respectively. When compared with untreated cells, the decrease in immunoreactivity was due to reduction in both antigen distribution and intensity (Fig. 5). However, no significant decrease of mRNA of hnRNP A2/B1 was observed in lung cancer cell lines after the 13cRA treatment using Northern blot analysis (data not shown).

**DISCUSSION**

Lung cancer is a result of chronic exposure of bronchopulmonary epithelium to carcinogens. According to the field carcinogenesis hypothesis of Slaughter et al. (8), much of the respiratory tract epithelium has been mutagenized as the result
of exposure to tobacco combustion products or other carcinogens. The changes include a variety of molecular events throughout the airway; there are multiple foci of malignant genes. The changes include a variety of molecular events (2), and SQM are most frequently seen in the conducting airways adjacent to the primary cancer. Abnormalities like BCH, GCH, those cases, hnRNP A2IB1 was frequently expressed in both histologically normal- and abnormal-appearing peripheral airway epithelium (4). This study is the first evaluation of the expression of hnRNP A2/B1 in normal-appearing epithelium of chronic smokers. A tissue with SI ≥2 was called positive.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Summary of tissue and cases specific for hnRNP A2/B1 expression in bronchial epithelium of chronic smokers</th>
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<tbody>
<tr>
<td>Morphology</td>
<td>Tissue&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal</td>
<td>212/521 (41)</td>
</tr>
<tr>
<td>BCH</td>
<td>150/344 (44)</td>
</tr>
<tr>
<td>GCH</td>
<td>57/104 (55)</td>
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<tr>
<td>SQM</td>
<td>63/170 (37)</td>
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<sup>a</sup> A tissue with SI ≥2 was called positive.
<sup>b</sup> Each individual may have histologically normal and abnormal areas; some of them contained more than one abnormality.
<sup>c</sup> Difference significant at $P = 0.004$.

<table>
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<th>Table 4</th>
<th>Summary of hnRNP A2/B1 expression in matched cases (n = 68)</th>
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</thead>
<tbody>
<tr>
<td>hnRNP A2/B1 expression at baseline</td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
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The frequent finding of hnRNP A2/B1 in normal-appearing epithelium is uncertain. A growing body of data suggests that molecular changes occur frequently in “normal-appearing” lung tissue from patients with and without cancer. These changes include activation of dominant onco genes (myc family and K-ras), loss of recessive growth regulatory genes, or antioncogenes (p53; Refs. 26–32). A growing consensus in the lung cancer research community is that the most informative markers of early lung cancer will be molecular and not histological (33).

In the recent North American experience, using hnRNP A2/B1 overexpression as an early lung cancer detection tool, most hnRNP A2/B1-positive sputum specimens were from individuals who developed cancer without showing evidence of morphological change in their sputum (7, 34). These results are consistent with the finding that a variety of molecular or biochemical changes, including hnRNP A2/B1 overexpression, could precede morphological changes in cells evolving into invasive lung cancer (26–32). With a small experience such as with the bronchial metaplasia study, the complex patterns of expression related to the development of a lung cancer may not be evident. Larger studies with a longer term of follow-up are essential to approach these issues.

Although the pattern of hnRNP A2/B1 expression found in this analysis is complex, in positive cases, immunoreactivity was generally homogeneously expressed throughout all sampled sites. From a recent report from Crowell et al. (32), if trisomy of chromosome 7 that was found in one bronchial site, it was consistently found in other airway sites as well. Bronchoscopic evaluation can usually visualize up to the fourth or fifth order of bronchi. This central region that is accessible to the bronchoscope is also likely to be exposed to a uniform and concentrated dose of tobacco combustion products. Homogeneity of molecular injury as with hnRNP A2/B1 and trisomy of chromosome 7 may be common in this region. In general, bronchial biopsies will sample normal or metaplasia squamous epithelium. The majority of the surface area of the airway is distal to the reach of the bronchoscope, and the distal tissue is predominantly adenomatous in origin. Sampling of respiratory epithelium in the distal airways, including type I and type II pneumocytes as well as Clara cells, is not possible via a bronchial biopsy. The bronchial biopsy tissues evaluated in this study are actually complementary to our evaluation reported previously of nonmalignant tissues adjacent to resected primary NSCLC tumors (22). For surgical resection cases, lung tissues from the distal airway and alveolar tissue were routinely available. In those distal...
epithelial specimens, hnRNP A2/B1 expression in peripheral airway cells was reported as 14% for normal type II cells and 28% for type II cell hyperplasia. Additional studies are required to determine how reflective changes of hnRNP A2/B1 in proximal epithelium are relative to the status of distal respiratory epithelium.

In regard to the possible association between smoking and hnRNP A2/B1 expression, there was no difference in hnRNP A2/B1 expression level between current and former smokers in this report. In contrast, our previous study of hnRNP A2/B1 expression in the surrounding lung tissue adjacent to primary lung cancers, with stratification based on smoking history, showed that the "heavy" smoking group (>50 pack-years) had a significantly higher expression of hnRNP A2/B1 than did the group of "light" smokers (≤50 pack-years; Ref. 22). Also, hnRNP A2/B1 expression did increase with a longer smoking history. This contrasts with the current study, where smoking status did not significantly correlate with hnRNP A2/B1 expression status. With the size of the metaplasia study, stratification for subjects who recently stopped smoking (during the 6 months of the trial) compared with smokers who continued was not possible. The smoking groups for the current and previous reports are both small and different; therefore, no firm conclusion about the relationship of hnRNP A2/B1 expression and smoking is possible.

We had previously reported that the high correlation between hnRNP A2/B1 expression and the eventual development of lung cancer was consistent with hnRNP A2/B1 overexpression being a marker of early cancer (4, 7, 34). But another possibility is that hnRNP A2/B1 expression is induced by tobacco exposure in a similar fashion to CYP1A1 (35). This would be consistent with its homogeneous pattern of airway expression in positive cases. Alternatively, because restricted levels of hnRNP A2/B1 are universally expressed in mature lung tissue but high levels are expressed in fetal lung development (36), a case can be made for changes in that the level of expression of hnRNP A2/B1 represents an oncofetal antigen. From this perspective, this early field carcinogenesis marker could have application either as a risk assessment or early detection context.

In 1990, Hong et al. (37) demonstrated that the administration of 13cRA for 1 year at the dose of 50–100 mg/m2/day prevented the development of second primary cancers (especially for aerodigestive cancers) in a population of head and neck cancer patients who had been definitively treated for their first primary tumors. Recently, McLarty et al. (38) reported that a randomized, placebo-controlled clinical trial of β-carotene and retinol with 755 former asbestos workers revealed no significant reduction in cellular atypia in sputum samples. Arnold et al. (39) also reported no benefit in another chemoprevention trial using etretinate. In the 1994 study that provided the bronchial biopsies for this report, Lee et al. (11) reported that 13cRA has no effect...
hnRNP Expression in Chronic Smokers

In reducing squamous metaplasia. In that experience, only smoking cessation but not retinoid chemoprevention was significantly related to the reversal of abnormal bronchial morphology (11). For clinical application, the predictive significance of cellular atypia in sputum samples or squamous metaplasia is not clear. According to the Johns Hopkins University and M. D. Anderson experience, between 60 and 80% of cellular atypia revert back to normal without any intervention and do not progress to lung cancer (3, 4). Although extrapolations from this study of bronchial metaplasia must be made with care, bronchial metaplasia tissues obtained during the trial represent a unique window into the understudied phase of tobacco-induced lung injury.

In comparing the retinoid-treated group to the placebo group, no statistically significant difference was noted in hnRNP A2/B1 antigen expression between the two groups. For this analysis, we evaluated two measures of antigen density, including the staining intensity alone as well as the aggregate of antigen intensity and distribution, which is reflected in the SI, but the outcome was the same with both methods. However, we reported previously that the addition of 13cRA resulted in a significant dose-dependent, growth-inhibitory effect on the small cell lung cancer cell lines using an in vitro proliferation assay (18). From that study, we found that 13cRA concentrations of about 0.5–10 μM were required to modulate cancer cell growth in vitro. In the present study, although the modulation of hnRNP A2/B1 protein expression was observed by using 0.5–4 μM concentrations of 13cRA in vitro, no modulation was found in the in vivo setting with chronic smokers after 6 months of oral 13cRA (1 mg/kg) treatment (11). Retinol has been shown to modulate a closely related ribonucleoprotein, hnRNP A1, and this modulation has been reported as being linked to regulation of cell growth (17). A recent report from M. D. Anderson shows that changes in chromosomal loss at 9p21 found in preneoplastic lesions of the head and neck also persist despite retinoid intervention (40). The long-term follow-up on the successful M. D. Anderson head and neck cancer retinoid chemoprevention trial showed that the retinoid-treated patients ultimately resumed a rate of developing second cancers that was similar to the placebo arm (37, 41). This result is consistent with retinoids acting as antipromotional agents, but this effect would not correct the underlying tobacco-initiated molecular damage (15). A potential conclusion with the present study may be that retinoid chemoprevention does not affect hnRNP A2/B1 expression status, perhaps because hnRNP A2/B1 may reflect initiation events rather than an aspect of promotional biology. Alternatively, we have shown previously that albumin greatly reduces the tumor cell availability of retinoids, and this could be a significant factor with p.o.-administered 13cRA (18). The lack of in vivo effect of retinoid on hnRNP A2/B1 expression could reflect that p.o.-administered 13cRA at the 1 mg/kg dose does not result in sufficient epithelial delivery of active drug to effect hnRNP A2/B1 modulation.

Although the metaplasia trial that provided the specimens for this analysis is the largest trial of its kind to date, this effort represents an initial rather than a definitive study. In the metaplasia study, only one case in each arm changed hnRNP A2/B1 expression status after the intervention, and the clinical consequence of that change is unknown. With the size of the metaplasia study, only a few cases of lung cancer would be eventually expected to develop in this cohort. Without a more complete understanding of the molecular phenotype of smoking injury leading to an invasive lung cancer, a number of issues, such as variable rates of continued smoking, confound interpretation of these types of studies. To address these issues, a larger, randomized study would be required. This study should be conducted using a projected bronchial epithelial dose of delivered 13cRA that inhibits lung cancer growth and modulates hnRNP A2/B1 expression in vitro (18). We are presently studying whether such a high airway dose of retinoid can be tolerated when delivered directly as an aerosol (42), because we predicted previously that direct aerosol administration of chemoprevention agents represented a more logical way to deliver effective doses of aerodigestive chemoprevention agents.

We conclude that based on this comprehensive analysis of a small but important clinical archive of serial bronchial epithelial specimens from smokers, hnRNP overexpression occurs in about 40% of tissues, and different histological abnormalities are observed frequently. No differences exist between frequency of hnRNP A2/B1 expression and morphological changes or biopsy sites. The frequency of hnRNP A2/B1 overexpression in the airway of smokers is a surprising finding in light of its published utility as an early detection marker. The frequent and widespread distribution of hnRNP A2/B1 overexpression in the airways of chronic smokers is comparable to our previous description of hnRNP A2/B1 expression in the airways surrounding a primary lung cancer (22). The sputum-based, early detection test is based on antigen quantitation in single, intact bronchial epithelial cells that are recognizable cytologically. Additional studies, to ascertain whether exfoliated bronchial epithelial cells that can survive long enough to be recovered in
the sputum cells represent a particularly informative subset of bronchial epithelial cells, are needed to answer this most provocative question.

Whether hnRNP A2/B1 has a direct role in lung carcinogenesis must be determined because that information may refine the use of hnRNP A2/B1 expression, either for early detection or risk assessment applications. Oral 13cRA at 1 mg/kg has no significant effects on hnRNP A2/B1 expression in the bronchial epithelium of chronic smokers, but a question remains about whether optimal, epithelial concentrations of retinoids were achieved. Continued efforts to systematically map the molecular events leading to early lung cancer and to establish the changes modulated by defined chemoprevention agents may assist in the deconvolution of the critical steps in lung carcinogenesis.

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Expression of heterogeneous nuclear ribonucleoprotein A2/B1 in bronchial epithelium of chronic smokers.

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