A Case-Cohort Study of an Early Biomarker of Lung Cancer in a Screening Cohort of Yunnan Tin Miners in China

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Abstract

We initiated the present study to evaluate the accuracy of a new epithelial biomarker of early lung cancer. We tested the hypothesis that expression of a tumor-associated antigen by exfoliated sputum epithelial cells has greater accuracy (sensitivity and specificity) for the detection of preclinical, localized lung cancer than do routine clinical detection methods. Monoclonal antibody (MAb) 703D4 recognizes heterogeneous nuclear ribonuclear protein (hnRNP) A2/B1. We compared the accuracy of hnRNP up-regulation with cytology and radiographic screening for lung cancer detection in miners who were highly exposed to tobacco smoke, radon, and arsenic in southwestern China. The results showed that MAb 703D4 detection of hnRNP expression by sputum epithelial cells had greater accuracy for the detection of lung cancer than did routine screening methods, particularly for early (localized) disease. Among 57 cases and 76 noncases at the first screening, overall MAb detection of hnRNP was more sensitive (74% versus 21% for cytology and 42% for chest x-ray) but had lower specificity (70 versus 100% for cytology and 90% for chest x-ray) than standard methods. Recognizing hnRNP up-regulation resulted in detection of approximately one-third more early cases than did the combination of X-ray and cytology. Detection of hnRNP A2/B1 expression appears to be a good initial screening test for lung carcinogenesis, as it identified 74% of those who developed subsequent clinical lung cancer. Future studies might separate individuals with high lung cancer risk by MAb detection, confirming the positives with markers having greater specificity (e.g., clinical studies that become positive later in the morphological progression).

Introduction

Primary lung cancer arises from the bronchial epithelium, and it is reasonable to expect the earliest changes of lung cancer will be detectable in epithelial cells that are shed from the bronchial mucosa (1). During the 1960s and 1970s, the only clinical marker that was available for detecting pulmonary neoplastic changes was the progression in altered morphology of exfoliated epithelial cells visualized by light microscopy (2). We now know that classical cytologic criteria of cancer are not sufficiently sensitive for lung cancer screening. Less than 10% of lung cancers in the NCI1 early lung cancer detection trials were detected by routine sputum cell morphology alone (3–6). Because no diagnostic cytomorphological features were recognized for the remaining 90% of lung cancer patients, no significant overall mortality reduction resulted from cytomorphological screening (7, 8).

The morphological changes seen in exfoliated cells, from normal to metaplasia, to slight, moderate, and marked dysplasia, and finally to neoplasia, are considered the benchmark steps of neoplastic progression in the lung (2). Although the NCI collaborative trials had shown that this progression is not recognized sufficiently often (i.e., sensitive enough) to be useful for lung cancer screening, archived epithelial cells showing this progression may be used to assess the timing of gene and peptide markers of carcinogenesis (9, 10).

During the late 1980s, seeking to detect premalignant changes in the airway epithelium, Tockman et al. (7) found that immunostained monoclonal antibodies directed against tumor-associated and differentiation antigens were markers of preclinical lung cancer. These investigators used the archived exfoliated sputum epithelial cells and paired tumors from participants in the NCI collaborative early lung cancer detection trial at Johns Hopkins University (the Johns Hopkins Lung Project or JHLP). From several possible antigen targets, they selected two antigen target markers that are similarly expressed both by resected tumor and by the paired premalignant sputum specimens. One of these antigens is a Mr 31,000 protein, recently characterized as a hnRNP, hnRNP A2/B1 (11). The second is a difucosylated ceramide, related to the Lewis-X family of antigens. Overexpression of hnRNP is detectable by a murine IgG2b MAb (MAb 703D4; Ref. 12), whereas the ceramide is detected by a rat IgM (13–15). Applying these MAb to the archived JHLP specimens, Tockman et al. (7) found that positively immunostaining specimens from those who would even-

1 The abbreviations used are: NCI, National Cancer Institute; JHLP, Johns Hopkins Lung Project; hnRNP, heterogeneous nuclear ribonuclear protein; MAb, monoclonal antibody; YTC, Yunnan Tin Corporation; LPI, Labor Protection Institute; DAB, diaminobenzidine.
Early Markers of Lung Cancer in Tin Miners

Materials and Methods

Overall Study Design

Approximately 6000 tin miners who are at high risk for lung cancer are screened annually with sputum cytology, chest x-ray, and personal interview as part of a lung cancer screening project that has been conducted by the Labor Protection Institute (LPI) of the YTC since 1973. In 1992, a biologic specimen bank was established for the primary purpose of examining biomarkers in sputum that may permit earlier diagnosis of lung cancer. The cohort entry criteria were defined as current or retired YTC workers who are at least 40 years old, with a confirmed history of at least 10 years underground and/or smelting experience. They could not have had proven active or verified history of previous malignancy (except nonmelanoma skin cancer) and they must have consented to the study. The complete study cohort included 8346 members who participated in the annual screening program at least once between 1992 and 1995 (7867 male and 479 female miners). Lung cancer risk factor information was collected by questionnaire at entry and periodically in follow-up years. Annual sputum specimens and chest x-ray films were stored, and single samples of other biologic specimens (blood, urine, toenails, buccal smear, and finger-stick blood, as well as tumor and adjacent normal tissue) were also collected and stored during the follow-up years of the study. Follow-up was conducted annually during the study to identify all newly diagnosed cancers.

Study Population

In a case-cohort design, tumor-associated antigen expression of sputum cells from lung cancer cases was compared with that of randomly selected, age-matched members of the cohort who remained cancer-free during the first year of the prospective study. The accuracy of hnRNP overexpression for detecting preclinical lung cancer was then compared with routine radiography and cytomorphology collected at the initial screening of 6378 subjects in 1992.

Selection of Cases. Eighty-eight subjects had been clinically identified as “cases” by a preliminary radiologic or cytomorphologic diagnosis of lung cancer as of March 1993. Upon further review, 5 controls subsequently became cases, and 16 cases remained unconfirmed after 2 years of follow-up; therefore, the number of total cases was reduced to 77. All cases were confirmed by a consensus diagnosis of “best information” diagnosis/cause of death by a panel of clinicians from YTC and Johns Hopkins Medical Institutions, who evaluated clinical material available through December 31, 1994. This panel examined all screening and hospital chest radiographs and sputum slides, histologic materials, abstracts of medical records, oncology clinical conference records, and mortality reports. Clinical stage assignment was based on the anatomic extent of the cancer determined from screening radiographs according to the categories of the UICCumor-node-metastasis staging system (16). For those cases with histologic confirmation, cell type was assigned according to WHO Diagnostic Criteria for Pulmonary Carcinoma (17). “Prevalence” lung cancer cases were defined as those with a suspicious or diagnostic finding on routine cytomorphology or radiography in the 1992 screening. “Incidence” lung cancer cases were defined as cases that had a normal 1992 screening examination but developed lung cancer between 1992 and 1994.

Selection of Subcohort Controls. A control subcohort (n = 639) was selected by taking a 10% random sample of the 1992 screening cohort (n = 6378), weighted by the age distribution of those (n = 88) with preliminary diagnosis of lung cancer in March 1993. For each of the 88 original cases, one control was randomly selected from the subcohort within the same 5-year age group (n = 88).

Selection of Sputum Specimens for Determination of Marker Expression. The immunostained sputum specimens of 16 of 77 (20.8%) lung cancer cases and 12 of 88 (13.6%) controls were considered unsatisfactory. In 7 of these 28 unsatisfactory specimens, the absence of alveolar macrophages left uncertain whether the sputum specimen sampled the airway below the glottis. The immunostaining of the remaining 21 specimens was unsatisfactory (either too dark or too light). Three additional cases (4%) produced no sputum specimen, and in one case, the sputum container was broken during shipment. Satisfactory, immunostained sputum slides were available for 57 cases and 76 randomly selected members of the control subcohort, providing approximately one control per case through the end of follow-up. Fifty-three of these 57 (92.9%) lung cancers were prevalence cases. Four additional incidence cases developed after initial screening during the observation interval (through December 31, 1994).

Experimental Procedures

Radiographic and Cytologic Methods. Details of clinical radiographic and cytologic procedures, including equipment, film, system of double reading (for radiography) and sputum induction, slide preparation, specimen preservation, slide staining, reading, and interpretation (for cytology) have been previously reported (21).

Screening Test Quality Control Procedures. All positive cytology slides were reread by a confirmatory reader. A 2% sample of all negative cytology slides were reread for both diagnosis and adequacy of preparation. All chest x-rays were read independently by two radiologists, and their interpretations were recorded separately. Differences were resolved by a referee (a third reader), whose interpretation was also recorded.

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**Antibody, Cell Lines and Reagents.** Immunostaining reagents included mouse MAb 703D4 (provided by Dr. J. Mulshine, Division of Clinical Sciences, National Cancer Institute, Bethesda, MD), biotinylated horse antimouse IgG, goat antihorse IgG, avidin-biotin-peroxidase complex (ABC kit; Vector Laboratories, Burlingame, CA), DAB (Sigma Chemical Co.), and hematoxylin. Specimen preservation reagents included DTT (Sigma) and Saccamanno’s preservative (2% polyethylene glycol 1450 plus 50% EtOH). Positive control American Type Culture Collection human bronchogenic cancer cell lines HTB-58 (squamous cell cancer), Calu-3 (adenocarcinoma), and OH-3 (small cell carcinoma) were maintained in our laboratory. OH-3 was kindly provided by Dr. Stephen Baylin (Johns Hopkins Oncology Center, Baltimore, MD).

**Immunostaining Protocol.** All sputum specimens were DTT pretreated (18). A modified immunostaining protocol (7, 14) included application of a specific primary MAb (703D4) solution for 60 min, followed successively by a biotinylated secondary antibody solution (horse anti-mouse) for 30 min, a biotinylated tertiary antibody solution (goat against the secondary antibody) for 30 min, the ABC reagent, and finally the substrate-chromogen solution (0.01% hydrogen peroxide and 0.05% DAB in PBS) for 30 min. All solutions were diluted with PBS to the protein concentration of the primary antibody. Slides were then counterstained with Gill’s hematoxylin and coverslipped. American Type Culture Collection cell lines (HTB-58 and Calu-3) were used for positive staining quality control, and nonimmune mouse IgG2b was applied to negative controls.

**Interpretation and Validation Procedures**

**Manual Interpretation Criteria for Immunostaining.** The immunostained slides were evaluated by a cytopathologist who was blinded to the case/control status of the specimen. The immunostaining frequency and intensity were graded by using a scale that ranged from negative (0+) to strongly positive (4+), compared with the negative and positive control slides. Only slides staining with an intensity of 2+ or greater were considered positive. Negative specimens were defined as those that contained pulmonary epithelial cells that did not stain with a 2+ intensity. The complete absence of alveolar macrophages, the presence of obscuring quantities of inflammatory cells, the presence of excessive levels of nonspecific background staining, or lack of staining resulted in a specimen scoring of unsatisfactory.

**Imaging Procedures for Quantitative Immunocytometry.** Image acquisition was performed as described previously (15) on a Zeiss Axioshot optical microscope (final magnification, ×2000), specially equipped for quantitative densitometry at the two light frequencies optimal for the DAB and hematoxylin chromogens with Omega narrow-band filters of 590–610 nm and 500–520 nm, respectively. The primary light path was directed to a Hamamatsu monochrome video camera (model C2400-77; Hamamatsu Photonc System Corp., Japan), interfaced to an Metamorph digital image processor (Universal Imaging Corp., West Chester, PA). Digital images were stored on a Panasonic LF-7010 optical disk drive (Matsushita Electric Industrial Co., Ltd., Disc System Division, Osaka, Japan) with removable read-write disks of 1-gigabyte capacity.

Each subject slide was scanned under low power by a cytotechnologist having no knowledge of the individual outcome. After two slides per subject were scanned, 5–10 characteristic fields that contained sputum epithelial cells with regular metaplasia were selected, and cellular optical densities were measured at each wavelength (15).

**Data Analysis**

The primary databases consisted of information collected from three sources: questionnaires, clinical workups, and laboratory results. The coded data were independently doubly entered into dBASE III-plus files by two operators at the Labor Protection Institute of the YTC. Range, validity, consistency checks, and corrections were performed between these two sets of entries to reduce transcription error and to assure the quality of the data. Each calendar quarter, copies of the computerized data files were electronically transmitted to NCI. Subsequent file management and testing used Excel, SPSS, and SAS programs on a personal computer or the mainframe.

Frequency distributions of selected characteristics were obtained for cases and subcohort controls. Differences among continuous variables were tested with a Wilcoxon rank sum test and, for categorical variables, with a χ² test. All statistical tests were performed based on a two-tailed probability.

The sensitivity and specificity of the screening tests were calculated to evaluate their ability to detect subsequent development of lung cancer. Sensitivity was defined as the proportion of miners with lung cancer whose screening test results were positive; specificity was defined as the proportion of miners without lung cancer whose screening test results were negative. Differences between these screening tests were evaluated using paired χ² tests. The accuracy [(true-positive + true-negative)/total number of subjects tested] of MAb detection of a hnRNP as a marker of subsequent lung cancer was compared with the accuracy of routine cytology and radiography screening to determine if the new screening method represented an improvement over existing technology.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Cases</th>
<th>Noncase subcohort</th>
<th>Total no.</th>
<th>χ² or Wilcoxon test *P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–59</td>
<td>21</td>
<td>25</td>
<td>46</td>
<td>0.5</td>
</tr>
<tr>
<td>60–69</td>
<td>26</td>
<td>32</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>≥70</td>
<td>10</td>
<td>19</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Age at interview (yr)</td>
<td>62.7</td>
<td>63.3</td>
<td>109</td>
<td>0.9</td>
</tr>
<tr>
<td>Exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever smoking</td>
<td>47</td>
<td>62</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Never smoking</td>
<td>10</td>
<td>14</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Occupational exposure (yr)</td>
<td>31.7</td>
<td>28.4</td>
<td></td>
<td>0.08</td>
</tr>
</tbody>
</table>

*χ² test for frequency distribution of categorical variables.

*Wilcoxon rank sum test for mean of continuous variables.

**Results**

Demographic Characteristics and Clinical Features. One hundred thirty-three subjects were included in the analysis, of which 57 were cases and 76 were subcohort controls. The age distribution and selected exposure characteristics of the study population are presented in Table 1. Due to the homogeneity of exposure resulting from cohort entry criteria and popularity of smoking among Yunnan tin miners, smoking and occupational exposures were similar among cases and subcohort members. An increased intensity of immunostaining, corresponding to a
greater mean optical density for both $A_{510}$ and $A_{600}$ wave-
lengths, is seen among the case specimens, compared to those of the noncase cohort (Fig. 1).

Table 2 presents the distribution of cell type by clinical stage at time of screening among confirmed lung cancer cases. With increasingly advanced stages, a larger proportion of cases did not undergo medical work-up, resulting in an unknown cell type. For example, 25% of stage $\leq$TX, 53% of stage I, 50% of stage II, and 83% of stage $\geq$III had an unknown cell type. (Stage TX tumors are defined as tumors proven by the presence of malignant cells in sputum or bronchial washing but not visualized by imaging or bronchoscopy.) More than 60% of the cases were detected at a stage $\leq$I, and the majority of these were squamous cell carcinomas (18 of 35; 51%).

Comparison of Clinical Lung Cancer Detection by Radiography or Cytomorphology with hnRNP Overexpression. Results of the initial clinical radiological and sputum cytologic screening obtained on the 57 cases and 76 sub-cohort controls are shown in Table 3. As previously described, a “positive” clinical screening test is defined as an initial chest x-ray or sputum cytology examination result interpreted as “cancer.” Of the 57 confirmed cases, the chest radiograph was positive in 24 (sensitivity, 42.1%). In contrast, only 12 of 57 (sensitivity, 21.1%) demonstrated a positive sputum cytomorphology.

Increased cytoplasmic staining indicating hnRNP overexpression can be quantified by computerized densitometry (Table 3). The sensitivity of MAb 703D4 detection of hnRNP overexpression was 74% for computer-assisted immunocyto-
metry, with an overall accuracy of 71%. Densitometry increased the sensitivity of lung cancer detection by more than 3-fold compared with cytomorphology screening of sputum (paired $\chi^2 = 40.2, P < 0.01$) and by nearly 2-fold compared with radiographic screening (paired $\chi^2 = 12.0, P < 0.01$).

hnRNP Overexpression and Tumor Stage. Table 4 presents the stage-specific comparison of sensitivities by mode of lung cancer screening. The 57 lung cancer cases detected at the 1992 screening are categorized by early stage (stage $\leq$I) or advanced stages (stage $\geq$II). Nearly 75% of early-stage lung cancer was identified by quantitative densitometry, compared with only 26% identified by chest x-ray and 31% by sputum cytology alone.

Table 5 shows the percentage of early-stage cases detected by routine radiography and cytomorphology screening compared with hnRNP overexpression among Yunnan miners. Quantitative densitometry detected lung cancer at an early stage almost half the time (46%), about 3 times the rate of chest x-ray and over twice as often as sputum cytology.

The hnRNP expression by level of cytomorphic pro-
gression among lung cancer cases is shown in Table 6. More than one-third of the cases (36.8%) had completely normal cytology but overexpressed hnRNP. If slight atypia was also included, cumulative MAb recognition of hnRNP overexpres-
sion included almost half the cancer cases (47%).

Discussion

No screening techniques that use standard clinical radiology, sputum cytology, or direct biopsies have been proven adequate for lung cancer detection at a curable stage (8). Attention to developments in tumor biology have now turned to detection of markers of the preneoplastic phase of carcinogenesis (19). A focus on carcinogenesis shifts emphasis away from detection of bulk malignancy, which, for many epithelial organs, are often metastatic at the time of diagnosis, and toward detection of individual cellular and genetic markers of potentially reversible progression. Validation of carcinogenesis markers requires marker detection in premalignant specimens from individuals who later develop cancer and the absence of the markers from those who remain cancer free (9). The hypothesis of this study was that hnRNP overexpression by airway epithelial cells occurs early in carcinogenesis and may be detected as a biomarker of lung cancer with greater accuracy and earlier in the course of carcinogenesis than standard clinical radiography and cytomorphic indices of lung cancer. This hypothesis was tested by the present design, which provided for the analysis of a sputum specimen from each miner at the beginning of the cohort observation period (without morphological preselec-
tion), in conjunction with an annual screening follow-up to determine the lung cancer status of each miner. A prospective test of this hypothesis requires that the putative markers spec-
imen be collected before disease onset. This requirement is met by the present design, which provides for collection of a sputum specimen from each miner at the beginning of the cohort observation period. Although prospective studies have the advantages of minimum recall bias, the ability to observe changes in exposure status over time, and direct estimation of risk, they are inefficient, particularly for less common diseases like lung cancer. Many normal specimens would have to be interpreted along with relatively few from individuals who develop cancer during the observation period. Consequently, a case-cohort design in this setting (as was used here) is cost effective.

Our study confirms that a marker of carcinogenesis, hnRNP overexpression by sputum epithelial cells, is a more sensitive indicator of lung cancer than are standard clinical tests. The carcinogenesis marker also can detect future lung cancer at an earlier stage than do standard clinical cytology and chest radiography. Results of standard cytologic screening in the present study are similar to those found 2 decades earlier by the JHLP, detecting similarly positive proportions among the YTC miners and JHLP smokers (21 versus 28%, respectively). The lower proportion of positive radiographs at the YTC compared with the JHLP (42 versus 77%) may reflect the lower threshold for suspicious abnormalities that the JHLP radiologists established for screening radiographs (20, 21). To detect markers of carcinogenesis, it was logical to examine the respiratory epithelium, which may be directly, noninvasively sampled by an induced sputum specimen.

hnRNP is not unique to tumor cells but is normally expressed at low levels by most eukaryotic cells. Qualitative (presence/absence) detection of marker expression is unlikely to be sufficiently specific to characterize antibody binding to this ubiquitous protein. These circumstances require the devel-

![Fig. 1. hnRNP up-regulation, indicated by cytoplasmic absorbance among YTC cases and controls. □, A$_{510}$; □, A$_{600}$](image-url)
Table 2  Distribution of cell type by clinical stage at time of screening among confirmed lung cancer cases in the 1992 YTC case-cohort study

Pearson nonparametric test: $\chi^2 = 2.00, P > 0.05$.

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>Squamous</th>
<th>Adenocarcinoma</th>
<th>Small cell</th>
<th>Other</th>
<th>Unknown</th>
<th>Total</th>
<th>Column %</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\leq$TX</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>16</td>
<td>28.1</td>
</tr>
<tr>
<td>I</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>19</td>
<td>33.3</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>17.5</td>
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<tr>
<td>$\geq$III</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>12</td>
<td>21.1</td>
</tr>
<tr>
<td>Total (row %)</td>
<td>24 (42.1)</td>
<td>2 (3.5)</td>
<td>1 (1.8)</td>
<td>1 (1.8)</td>
<td>29 (50.9)</td>
<td>57</td>
<td>100.0</td>
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</table>

Table 3  Sensitivity, specificity, and accuracy by method of detection in the 1992 YTC case-cohort study

<table>
<thead>
<tr>
<th>Method of detection</th>
<th>Confirmed by Johns Hopkins University/NCI review</th>
<th>Total</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Accuracy (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Subcohort</td>
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<td></td>
<td></td>
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<tr>
<td>Standard method</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Chest X-ray</td>
<td>Positive</td>
<td>24</td>
<td>7</td>
<td>31</td>
<td>42.1</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>33</td>
<td>69</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Sputum cytology</td>
<td>Positive</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td>21.1</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>45</td>
<td>76</td>
<td>121</td>
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</tr>
<tr>
<td>Immunocytochemistry</td>
<td>Densityometry$^a$</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>42</td>
<td>23</td>
<td>65</td>
<td>73.7</td>
<td>69.7</td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>53</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>76</td>
<td>133</td>
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</table>

$^a$ Paired $\chi^2$ test: densitometry vs. chest X-ray, $\chi^2 = 12.0$, $P < 0.01$; densitometry vs. sputum cytology: $\chi^2 = 40.2$, $P < 0.01$.

$^b$ Positive defined as chest X-ray or sputum cytology indicating cancer.

$^c$ Positive defined as linear combination of $A_{400}$ and $A_{510}$ greater than 0.

Table 4  A comparison of the sensitivities of different methods of detection as screening tests by stage at time of screening among lung cancer found at the 1992 screening

<table>
<thead>
<tr>
<th>Method of detection</th>
<th>Early stage (stage $\leq$III$^a$)</th>
<th>Advanced (stage $\geq$II$^b$)</th>
<th>No. of cases</th>
<th>Sensitivity$^c$</th>
<th>No. of cases</th>
<th>Sensitivity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Chest X-ray</td>
<td></td>
<td></td>
<td>9</td>
<td>25.7</td>
<td>15</td>
<td>68.1</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td></td>
<td>26</td>
<td></td>
<td>7</td>
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<td>Negative</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sputum cytology</td>
<td>Positive</td>
<td>11</td>
<td>31.4</td>
<td>1</td>
<td>16</td>
<td>72.7</td>
</tr>
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<td></td>
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<td>24</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>X-ray or cytology</td>
<td>Positive</td>
<td>18</td>
<td>51.4</td>
<td>16</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>17</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Densitometry$^d$</td>
<td>Positive</td>
<td>26</td>
<td>74.3</td>
<td>16</td>
<td></td>
<td>72.7</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>9</td>
<td>6</td>
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<tr>
<td>Total</td>
<td>35</td>
<td></td>
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<td>22</td>
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</tr>
</tbody>
</table>

$^a$ Defined as no evidence of cancer, stage TX, or stage I.

$^b$ Positive defined as in Table 3.

$^c$ Paired $\chi^2$ test: densitometry vs. chest X-ray, $\chi^2 = 12.5$, $P < 0.01$; densitometry vs. sputum cytology, $\chi^2 = 26.5$, $P < 0.01$; densitometry vs. X-ray or cytology, $\chi^2 = 3.56$, $P > 0.05$.

$^d$ Defined as stage II-IV or unknown.

This investigation was impaired by certain practical limitations. First, although not required for analysis, more than half of the cases lacked uniform histological confirmation. Cultural reluctance to proceed with a clinical evaluation for lung cancer limited the number of cases with histological confirmation. These cases were diagnosed by a positive chest X-ray and compatible clinical course. Squamous cell and adenocarcinoma occurred with lower frequency among the YTC miners than among the JHLP smokers (42 versus 62% and 3.5 versus 17.5% respectively). Development of rigorous densitometric quantitative criteria for marker binding based upon the number of probe adherence sites per cell and the frequency of labeled cells per specimen (15).
17.9%, respectively). The lower frequency of squamous and adenocarcinoma may be explained by the high frequency of lung cancers of unknown cell type among the YTC miners. Cell type was unknown in about half of the YTC lung cancer cases. As shown in Table 2, this may due to more aggressive occult tumor growth (particularly adenocarcinoma and small cell lung cancer) among occupationally exposed miners, which would effectively remove the patients from sampling before a medical evaluation and cell type determination could be completed.

Second, high-quality sputum specimens are essential for successful immunostaining. Here, the preserved specimens available for immunostaining were limited to the cells remaining after the more promising diagnostic material had already been removed for routine Papanicolaou staining by the pick-and-smear method. Nevertheless, we have demonstrated that immunostaining resulted in a sensitivity improvement of more than 3-fold over cytomorphology for the detection of preclinical lung cancer (74 versus 21%, respectively) and nearly doubled the sensitivity of the screening chest x-ray (74 versus 42%). If the immunostaining method were the initial screening method used, it is quite possible that its sensitivity rate might be still higher. The existence of satisfactory sputum samples is essential for analysis. A total of 20 cases were un evaluable here because a sputum sample either did not exist or was unsatisfactory. To assess the potential impact of missing data on these 20 cases, we recalculated screening parameters under two sets of extreme circumstances: with all the missing cases presumed to have tested negative and with all the missing cases presumed to have tested positive. These calculations indicate that sensitivity, specificity, overall accuracy, and positive predictive value were all largely insensitive to the missing data and that there is likely little impact on our results from missing these 20 case samples.

Third, a relatively high rate of positive immunostaining (30% false-positive) was observed among those miners who did not have cancer on their initial screening examinations. Although this reduced the overall accuracy to 71%, it must be realized from the high lung cancer incidence of this cohort, 5–10 new cases of lung cancer will be expected among this group over the next 5 years. To date, only the first screening specimen collection has been analyzed, and participants have been followed for only a short time. Year 1 cases of this cohort presented primarily as prevalence cases (53 of 57; 93%). The predictive value of hnRNP overexpression for detection of incidence lung cancer could not be tested here.

The inherent difficulty in screening for lung cancer today is that clinical sputum cytology and chest x-rays are simply not sensitive enough detection tools to permit this diagnosis at early, treatable stages (22, 23). Built upon an ongoing program of traditional screening at the YTC, the present study was designed to establish an archive of premalignant sputum that could be paired with subsequent histologic tumor specimens for the development and testing of new biomarkers. Fundamental progress in development and validation of cancer biomarkers depends on banking carefully obtained, serial premalignant sputum cells to monitor hnRNP expression (and other protein markers) as they progress in development and validation of cancer biomarkers.
and molecular markers) should be relevant to the development of lung cancer across different populations (with different exposures), so long as this marker is up-regulated by a common etiological/molecular pathway in the carcinogenesis of lung cancer. The present study suggests that this is the case for most lung cancer, regardless of histology, that occurs in a high-risk population. If confirmed by the completion of this study, monitoring hnRNP expression in sputum cells would be a validated technique for the detection of preclinical lung cancer in high-risk individuals. Its accuracy in a general population must still be determined by studies in populations at lower risk.

Clinical application of a sensitive lung cancer biomarker also depends on the availability of an effective, noninvasive treatment. The strategy which underlies introduction of a new diagnostic test is that the test’s rigor should correspond to the morbidity of the therapy (8). Existing cancer therapies are invasive (surgery) and tissue killing (chemotherapy and radiotherapy). Thus, it is appropriate that existing clinical tests for the presence of cancer are highly specific and successfully minimize the numbers of falsely positive individuals who might be harmed by treatment. Unfortunately, their high specificity restricts these tests to detection of more advanced lesions with low sensitivity to early, potentially reversible changes of carcinogenesis. Trials of screening with a highly specific but insensitive clinical tests has led to a high lung cancer fatality from the widespread metastases of advanced cancer. This high specificity but low sensitivity of routine lung cancer diagnostic tests is illustrated in Table 3.

In contrast, a noninvasive treatment that might reverse carcinogenesis carries substantially lower patient toxicity. Preliminary studies have shown that retinoids may have chemopreventive activity (24-26). The preinvasive stages of lung cancer would be favorable to evaluate whether aerosolized administration of retinoid compounds can increase the bioavailability of the retinoid to airway epithelial cells while reducing systemic toxicity (27). Clinical trials of such a low morbidity treatment are now needed to weigh the reduction morbidity and mortality that may result from a more sensitive diagnostic against the minimal possibility of harm associated with the low morbidity treatment of any falsely positive individuals. These trials should incorporate triage strategies whereby immunodiagnostic positive individuals would undergo successful tests of increasing specificity until a diagnosis is made.

Finally, the densitometric marker of carcinogenesis also may be repeatedly sampled as an intermediate endpoint of therapeutic effect. Prospective intervention trials could determine whether reduction in expression of a carcinogenesis marker may indicate therapeutic efficacy, well in advance of alteration of tumor bulk.

In conclusion, prediagnostic sputum samples have been analyzed for expression of potential early markers using a case-cohort approach. We have shown that detection of hnRNP overexpression by MAb 703D4 was more sensitive than standard chest x-ray and sputum cytology methods for lung cancer detection. The sensitivity of hnRNP overexpression exceeds standard methods by 2-3-fold but has limited specificity. This study also showed that hnRNP overexpression detects a greater prevalence of lung cancer at early stages (stage \( \leq 1 \)) than do standard methods. The hnRNP overexpression may be a good initial screening test for the early detection of lung cancer, particularly if it is combined with more specific markers (e.g., other protein or genetic markers expressed at the same time or later in the morphological progression).

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A case-cohort study of an early biomarker of lung cancer in a screening cohort of Yunnan tin miners in China.

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