

A Case-Cohort Study of an Early Biomarker of Lung Cancer in a Screening Cohort of Yunnan Tin Miners in China¹

You-Lin Qiao, Melvyn S. Tockman,² Li Li, Yener S. Erozan, Shu-Xiang Yao, Michael J. Barrett, Wei-Hong Zhou, Carol A. Giffen, Xue-Chang Luo, and Philip R. Taylor

Cancer Prevention Studies Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892 [Y.-L. Q., P. R. T.]; Departments of Environmental Health Sciences [M. S. T., L. L., W.-H. Z.] and Pathology [Y. S. E.], The Johns Hopkins Medical Institutes, Baltimore, Maryland 21205; Labor Protection Institute and General Hospital, Yunnan Tin Corporation, Gejiu, Yunnan, People's Republic of China [S.-X. Y., X.-C. L.]; and Information Management Services, Inc., Silver Spring, Maryland 20910 [M. J. B., C. A. G.]

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 NCI³ 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 M_r 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 MAbs to the archived JHLP specimens, Tockman *et al.* (7) found that positively immunostaining specimens from those who would even-

Received 12/19/96; revised 8/25/97; accepted 8/25/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by National Cancer Institute Grant NO1-CN-25.

² To whom requests for reprints should be addressed, at Molecular Screening Program, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL 33612. Phone: (813) 632-1714.

³ 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.

tually develop lung cancer demonstrated a sensitivity of 91% among specimens collected (on average) 2 years before the clinical appearance of lung cancer. Specificity was 88% among specimens from individuals who remained free of lung cancer during the 7–8 years of follow-up (7). The JHLP cytologic specimens had been archived because they showed moderate or severe dysplasia. We have initiated the present study to confirm the accuracy and predictive advantage of detecting hnRNP overexpression in specimens from a recently established archive at the YTC in the People's Republic of China, which included all cytologic specimens (without morphologic preselection).⁴ We tested the hypothesis that detection of a tumor-associated antigen marker of carcinogenesis by sputum epithelial cells has greater accuracy (sensitivity and specificity) for early (localized) lung cancer than do routine morphologic screening methods. We compared the MAb 703D4 detection of hnRNP with current cytology and radiographic screening methods under field conditions to assess the accuracy and the timing of marker expression among miners who were highly exposed to tobacco smoke, radon, and arsenic in southwestern China.

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.⁴ The accuracy of hnRNP overexpression for detecting preclinical lung cancer was then compared with routine radiography and cytology collected at the initial screening of 6378 subjects in 1992.

⁴ Y. L. Qiao, P. R. Taylor, S. X. Yao, Y. S. Erozan, X. C. Luo, M. Barrett, Q. Y. Yan, C. A. Giffen, S. Q. Huang, M. M. Maher, M. R. Forman, and M. S. Tockman. Establishment of biologic specimen and data banks among Chinese tin miners to study early markers and etiology of lung cancer, submitted for publication.

Selection of Cases. Eighty-eight subjects had been clinically identified as "cases" by a preliminary radiologic or cytologic 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 conferences, 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 UICC tumor-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 cytology 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.

Anti-
agent
Muls
stituti
antihe
Vecte
Co.),
clude
yethy
ican
lines
ma),
labor
(John
Immu
pretre
includ
tion
biotin
ary at
subst
0.05%
PBS
Slides
cover
(HTB
contro
contro

Interp
Manu
immu
was b
immu
a scal
(4+),
Only
consid
that co
a 2+ i
the pre
presen
ing, o
unsati
Imagi
Image
on a Z
×2000
two lig
chrome
and 50
directe
C2400
faced
Imagin
on a Pa
Industr
remova
En
cytoted
come.
teristic

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 Saccomanno'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 Immunocytochemistry. Image acquisition was performed as described previously (15) on a Zeiss Axiomat optical microscope (final magnification, $\times 2000$), 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 Photonic 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

Table 1 Age distribution and selected exposure characteristics in the 1992 YTC case-cohort study

Indicator	Cases		Noncase subcohort		Total no.	χ^2 or Wilcoxon test, ^{a,b} P
	No.	%	No.	%		
Total	57	100.0	76	100.0	133	
Age (yr)						
40–59	21	36.8	25	32.9	46	0.5
60–69	26	45.6	32	42.1	58	
≥ 70	10	17.5	19	25.0	29	
Age at interview (yr)	62.7		63.3			0.53
Exposure						
Ever smoking	47	82.5	62	81.6	109	0.9
Never smoking	10	17.5	14	18.4	24	
Occupational exposure (yr)	31.7		28.4			0.08

^a χ^2 test for frequency distribution of categorical variables.

^b Wilcoxon rank sum test for mean of continuous variables.

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 in 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 χ^2 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 χ^2 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.

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 subcontrol members. An increased intensity of immunostaining, corresponding to a

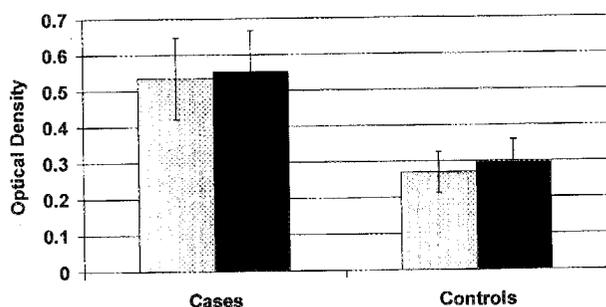


Fig. 1. hnRNP up-regulation, indicated by cytoplasmic absorbance among YTC cases and controls, □, A₅₁₀; ■, A₆₀₀.

greater mean optical density for both A₅₁₀ and A₆₀₀ wavelengths, 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 ≤TX, 53% of stage I, 50% of stage II, and 83% of stage ≥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 ≤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,⁴ 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 immunocytochemistry, 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 ≤I) or advanced stages (stage ≥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 cytomorphologic 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 overexpression 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 cytomorphological 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 preselection), 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 specimens 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-

Pearson

Chi

≤T

I

II

≥III

Tot

Me

Star

C

S

Imm

D

T

a Paired

b Positive

c Positive

T

a Defined

b Paired

3.56, P >

c Defined

d Positive

opment

marker b

per cell

Thi

itations.

of the ca

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

Clinical stage	Cell type					Total	Column %
	Squamous	Adenocarcinoma	Small cell	Other	Unknown		
≤TX	11	0	0	1	4	16	28.1
I	7	1	1	0	10	19	33.3
II	4	1	0	0	5	10	17.5
≥III	2	0	0	0	10	12	21.1
Total (row %)	24 (42.1)	2 (3.5)	1 (1.8)	1 (1.8)	29 (50.9)	57	100.0

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

Method of detection	Confirmed by Johns Hopkins University/ NCI review		Total	Sensitivity (%)	Specificity (%)	Accuracy ^a (%)
	Cases	Subcohort				
	Standard method					
Chest X-ray ^b						
Positive	24	7	31	42.1	90.8	69.9
Negative	33	69	102			
Sputum cytology ^b						
Positive	12	0	12	21.1	100.0	66.1
Negative	45	76	121			
Immunocytochemistry						
Densitometry ^c						
Positive	42	23	65	73.7	69.7	71.4
Negative	15	53	68			
Total	57	76	133			

^a Paired χ^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_{600} 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

Method of detection	Early stage (stage ≤I ^a)		Advanced (stage ≥II ^c)	
	No. of cases	Sensitivity ^b	No. of cases	Sensitivity
Chest X-ray				
Positive	9	25.7	15	68.1
Negative	26		7	
Sputum cytology				
Positive	11	31.4	1	4.5
Negative	24		21	
X-ray or cytology				
Positive	18	51.4	16	72.7
Negative	17		6	
Densitometry ^d				
Positive	26	74.3	16	72.7
Negative	9		6	
Total	35		22	

^a Defined as no evidence of cancer, stage TX, or stage I.^b Paired χ^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, $\chi^2 = 3.56$, $P > 0.05$.^c Defined as stage II-IV or unknown.^d Positive defined as in Table 3.

opment 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).

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

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 I) 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).

Acknowledgments

We thank the staff of the I.P.I and General Worker Hospital of YTC, People's Republic of China, for their cooperation with sputum specimen and data collection.

References

- Shaw, G. L., and Mulshine, J. L. Biomarkers and histology of premalignant and malignant lesions. In: P. Greenwald, B. S. Kramer, and D. L. Weed (eds.). *Cancer Prevention and Control*, pp. 111-133. New York: Marcel Dekker, Inc., 1995.
- Sacomanno, G., Archer, V. E., Auerbach, O., Saunders, R. P., and Brennan, L. M. Development of carcinoma of the lung as reflected in exfoliated cells. *Cancer (Phila.)*, 33: 256-270, 1974.
- Levin, M. L., Tockman, M. S., Frost, J. K., and Ball, W. C. Lung cancer mortality in males screened by chest X-ray and cytologic sputum examination: a preliminary report. *Recent Res. Cancer Res.*, 82: 138-146, 1982.
- Melamed, M. R., Flehinger, B. J., Zaman, M. B., Heelan, R. T., Perchick, W. B., and Martini, N. Screening for lung cancer. Results of the Memorial Sloan-Kettering study in New York. *Chest*, 86: 44-53, 1984.
- Frost, J. K., Ball, W. C., Jr., Levin, M. L., Tockman, M. S., Baker, R. R., Carter, D., Eggleston, J. C., Frozan, Y. S., Gupta, P. K., and Khouri, N. F. Early lung cancer detection: results of the initial (prevalence) radiologic and cytologic screening in the Johns Hopkins study. *Am. Rev. Respir. Dis.*, 130: 549-554, 1984.
- Tockman, M. S., Levin, M. L., Frost, J. K., Ball, W. C., Jr., Stitik, F. P., and Marsh, B. R. Screening and detection of lung cancer. In: J. Aisner (eds.). *Lung Cancer*, pp. 25-40. New York: Churchill-Livingstone, Inc., 1985.
- Tockman, M. S., Gupta, P. K., Myers, J. D., Frost, J. K., Baylin, S. B., Gold, E. B., Chase, A. M., Wilkinson, P. H., and Mulshine, J. Sensitive and specific monoclonal antibody recognition of human lung cancer antigen on preserved sputum cells: a new approach to early lung cancer detection. *J. Clin. Oncol.*, 6: 1685-1693, 1988.
- Mulshine, J. L., Tockman, M. S., et al. Lung cancer: rational strategies for early detection and intervention. *Oncology (Basel)*, 5: 25-32, 1991.
- Tockman, M. S., Gupta, P. K., Pressman, N., and Mulshine, J. L. Consideration in bringing a cancer biomarker to clinical application. *Cancer Res.*, 52 (Suppl.): 2715s-2718s, 1992.
- Mao, L., Hruban, R. H., Boyle, J. O., Tockman, M. S., and Sidransky, D. Detection of oncogene mutations in sputum precedes diagnosis of lung cancer. *Cancer Res.*, 54: 1634-1637, 1994.
- Zhou, J., Mulshine, J. L., Unsworth, E. J., Scott, F. M., Avis, I. M., Vos, M. D., and Treton, A. M. Identification of a heterogeneous nuclear ribonucleoprotein (hnRNP) as an early lung cancer detection marker. *J. Biol. Chem.*, 271: 10760-10766, 1996.
- Mulshine, J., Cuttitta, F., Bibro, M., et al. Murine monoclonal antibodies which distinguish non-small cell from small cell lung cancer. *J. Immunol.*, 131: 497, 1983.
- Rosen, S. T., Mulshine, J. L., Cuttitta, F., et al. Analysis of human small cell lung cancer differentiation antigens using a panel of rat monoclonal antibodies. *Cancer Res.*, 44: 2052-2061, 1984.
- Gupta, P. K., Myers, J. D., Baylin, S. B., Mulshine, J. L., Cuttitta, F., and Gazdar, A. F. Improved antigen detection in ethanol-fixed cytologic specimens. Modified avidin-biotin-peroxidase complex (ABC) method. *Diagn. Cytopathol.*, 1: 133-136, 1985.
- Tockman, M. S., Gupta, P. K., Pressman, N., and Mulshine, J. L. Cytometric validation of immunocytochemical observations in developing lung cancer. *Diagn. Cytopathol.*, 9: 615-622, 1993.
- Mountain, C. F. A new international staging system for lung cancer. *Chest*, 89 (Suppl.): 225s-233s, 1986.
- Kreyberg, L. Histological typing of lung tumors. In: *International Histological Classification of Tumors, Vol. 1*. Geneva: WHO, 1967.
- Tockman, M. S., Qiao, Y. L., Li, L., Zhao, G. Z., Sharma, R., Cavenough, L., and Erozan, Y. Safe separation of sputum cells from mucoid glycoprotein preserves surface antigen for immunocytochemistry and flow cytometry. *Acta Cytol.*, 39: 1128-1136, 1995.
- Sporn, M. B. Carcinogenesis and cancer. Different perspectives on the same disease. *Cancer Res.*, 51: 6215-6218, 1991.
- Stitik, F. P., Tockman, M. S., and Khoury, N. F. Chest radiology. In: A. B. Miller (ed.). *Screening for Cancer*, pp. 163-199. San Diego: Academic Press, 1985.
- Tockman, M. S., Mulshine, J. L., Piantadosi, S., Erozan, Y. S., Gupta, P. K., Ruckdeschel, J., Taylor, P. R., Zhukov, T., Zhou, W.-H., Qiao, Y.-L., and Yao, S.-X. Prospective detection of preclinical lung cancer: results from two studies of heterogeneous nuclear ribonucleoprotein A2/B1 overexpression. *Clin. Cancer Res.*, in press, 1997.

22. Ginsberg, R. J., Kris, M. G., and Armstrong, J. G. Cancer of the lung. In: V. T. DeVita, S. Hellman, and S. A. Rosenberg. (eds.). *Cancer, Principles and Practice of Oncology* pp. 673-679. Philadelphia: J. B. Lippincott Co., 1993.
23. Mulshine, J. L., Tockman, M. S., and Smart, C. R. Considerations in the development of lung cancer screening tools. *J. Natl. Cancer Inst. (Bethesda)*, 81: 900-906, 1989.
24. Hong, W. K., Lippman, S. M., Itri, L. M., *et al.* Prevention of second primary tumors with isotretinoin in squamous cell carcinoma of the head and neck. *N. Engl. J. Med.*, 323: 795-801, 1990.
25. Benner, S. E., Pajak, T. F., Lippman, S. M., Earley, C., and Hong, W. K. Prevention of second primary tumors with isotretinoin in patients with squamous cell carcinoma of the head and neck: long-term follow-up. *J. Natl. Cancer Inst. (Bethesda)*, 86: 140-141, 1994.
26. Pastorino, U., Infante, M., Maioli, M., *et al.* Adjuvant treatment of stage I lung cancer with high-dose vitamin A. *J. Clin. Oncol.*, 11: 1216-1222, 1993.
27. Avis, I., Mathias, A., Unsworth, E. J., *et al.* Analysis of small cell lung cancer cell growth inhibition by 13-*cis*-retinoic acid: importance of bioavailability. *Cell Growth Differ.*, 6: 485-492, 1995.

**Hsin-C
Herng-
Allan**

Graduate
Y-C. C.,
Pharmac
College o
Republic
National

Abstra

Both g
the dev
involve
polyme
focused
factors
(CYP)
GSTT
recruit
123 he
and ag
City at
alcohol
through
genoty
determ
and be
the ris
betel o
control
analys
GSTT
those t
GSTT
of 4.6
($P = 0$
associa
compa
not chi
among
associa
showin

Received
The cost
page chi
accordan
1 Suppo
ment of
2 To wh
Epidemi
Jen-Ai
cjchen@