Detection of Trisomy 7 in Nonmalignant Bronchial Epithelium from Lung Cancer Patients and Individuals at Risk for Lung Cancer[1]

Richard E. Crowell, Frank D. Gilliland, R. Thomas Temes, Heidi J. Harms, Robin E. Neft, Evelyn Heaphy, Dennis H. Auckley, Lida A. Crooks, Scott W. Jordan, Jonathan M. Samet, John F. Lechner, and Steven A. Belinsky[2]

Departments of Medicine [R. E. C., E. H., D. H. A.], Surgery [R. T. T.], and Pathology [L. A. C., S. W. J.], Albuquerque Veterans Administration Medical Center and the University of New Mexico Health Sciences Center, Albuquerque, New Mexico 87131; Inhalation Toxicology Research Institute, Albuquerque, New Mexico 87115 [H. J. H., R. E. N., J. F. L., S. A. B.]; Department of Epidemiology and Cancer Control Program, University of New Mexico Cancer Research and Treatment Center, Albuquerque, New Mexico 87131 [F. D. G.]; and Department of Epidemiology, Johns Hopkins University, Baltimore, Maryland 21231 [J. M. S.]

Abstract

Early identification and subsequent intervention are needed to decrease the high mortality rate associated with lung cancer. The examination of bronchial epithelium for genetic changes could be a valuable approach to identify individuals at greatest risk. The purpose of this investigation was to assay cells recovered from nonmalignant bronchial epithelium by fluorescence *in situ* hybridization for trisomy of chromosome 7, an alteration common in non-small cell lung cancer. Bronchial epithelium was collected during bronchoscopy from 16 cigarette smokers undergoing clinical evaluation for possible lung cancer and from seven individuals with a prior history of underground uranium mining. Normal bronchial epithelium was obtained from individuals without a prior history of smoking (never smokers). Bronchial cells were collected from a segmental bronchus in up to four different lung lobes for cytology and tissue culture. Twelve of 16 smokers were diagnosed with lung cancer. Cytological changes found in bronchial epithelium included squamous metaplasia, hyperplasia, and atypical glandular cells. These changes were present in 33, 12, and 47% of sites from lung cancer patients, smokers, and former uranium miners, respectively. Less than 10% of cells recovered from the diagnotic brush had cytological changes, and in several cases, these changes were present within different lobes from the same patient. Background frequencies for trisomy 7 were 1.4 ± 0.3% in bronchial epithelial cells from never smokers. Eighteen of 42 bronchial sites from lung cancer patients showed significantly elevated frequencies of trisomy 7 compared to never smoker controls. Six of the sites positive for trisomy 7 also contained cytological abnormalities. Trisomy 7 was found in six of seven patients diagnosed with squamous cell carcinoma, one of one patient with adenosquamous cell carcinoma, but in only one of four patients with adenocarcinoma. A significant increase in trisomy 7 frequency was detected in cytologically normal bronchial epithelium collected from four sites in one cancer-free smoker, whereas epithelium from the other smokers did not contain this chromosome abnormality. Finally, trisomy 7 was observed in almost half of the former uranium miners; three of seven sites positive for trisomy 7 also exhibited hyperplasia. Two of the former uranium miners who were positive for trisomy 7 developed squamous cell carcinoma 2 years after collection of bronchial cells. To determine whether the increased frequency of trisomy 7 reflects generalized aneuploidy or specific chromosomal duplication, a subgroup of samples was evaluated for trisomy of chromosome 2; the frequency was not elevated in any of the cases as compared with controls. The studies described in this report are the first to detect and quantify the presence of trisomy 7 in subjects at risk for lung cancer. These results also demonstrate the ability to detect genetic changes in cytologically normal cells, suggesting that molecular analyses may enhance the power for detecting premalignant changes in bronchial epithelium in high-risk individuals.

Introduction

Although lung cancer is the leading cause of cancer death in the United States (1), early detection and intervention could decrease the high mortality rate associated with this disease if sensitive screening approaches could be developed (2–4). Early detection may be feasible because the entire respiratory tract is exposed to inhaled carcinogens; therefore, the whole lung is at risk for developing multiple, independently initiated sites. This "field cancerization" condition (5) is supported clinically by a high frequency of second primary tumors in lung cancer patients (6–9) and by the occurrence of progressive histological premalignant changes throughout the lower respiratory tract of cigarette smokers (10, 11). Moreover, recent studies using pathological tissues obtained after lung resection or autopsy have identified genetic aberrations associated with lung cancer in nonmalignant bronchial epithelium adjacent to tumors (12–16).

Although examination of pathological samples is useful for identifying genetic changes associated with carcinogenesis, this invasive approach for collection of clinical samples nec-

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[2] To whom requests for reprints should be addressed, at Inhalation Toxicology Research Institute, P. O. Box 5890, Albuquerque, NM 87185. Phone: (505) 845-1165; Fax: (505) 845-1198.

essary for early detection would not be appropriate for screening. However, bronchial epithelial cells harvested using routine clinical procedures could be examined for genetic changes as an initial approach for detecting individuals at high risk for lung cancer. This approach could also provide genetic markers for evaluating the effectiveness of chemoprevention regimens. Bronchoscopy provides direct access to viable cells within the airways and is a commonly used tool for obtaining samples from the lower respiratory tract, including bronchial epithelium (17). This procedure can be used to repeatedly sample the bronchial epithelium over time and to collect viable cells that can be expanded through tissue culture for functional assays.

Because of field cancerization, genetic abnormalities should be dispersed throughout the bronchial epithelium of persons at risk for lung cancer. The purpose of this investigation was to test this hypothesis by sampling nonmalignant bronchial epithelium from distinct locations within four different lobes of the lung from persons at risk for lung cancer and then assaying the bronchial cells for the presence of specific genetic abnormalities. Trisomy of chromosome 7 was examined in these cells, because this alteration is common in solid tumors, including lung cancer, of several different organ systems (18, 19). In addition, trisomy 7 has been detected in premalignant lesions such as villous adenoma of the colon (20), in the colonic mucosa of individuals with familial polyposis (21), and in the far margins of some resected lung tumors (22). Our results demonstrate that trisomy 7 can be detected in nonmalignant bronchial epithelium from patients with lung cancer distant to the site of the tumor and in individuals without tumors who are at high risk for lung cancer development. Together, these studies suggest that an extra copy of chromosome 7 may be an intermediate biomarker of ongoing field carcinogenesis.

Materials and Methods

Subject Recruitment. Bronchial epithelium was collected from 16 cigarette smokers undergoing a diagnostic workup for possible lung cancer and from 7 individuals with a prior history of underground uranium mining, 5 of whom were also smokers. Three individuals who had never smoked were also recruited to obtain bronchial epithelium not exposed directly to either tobacco smoke or radon progeny.

Pathology and Exposure History. Twelve of the 16 cigarette smokers who underwent diagnostic bronchoscopy were diagnosed with NSCLC.[3] Seven tumors were characterized histologically as SCCs, four tumors were ACs, and one tumor was an adenosquamous cell carcinoma. Lung cancer was not evident in the other four subjects. Smoking histories ranged from 15 to 120 pack-years (defined as the number of cigarettes smoked per day times the number of years smoked). All of the former uranium miners worked underground between 2 and 20 years, with a range of 27–527 working level months. Five of the seven miners had smoking histories that ranged from 20–60 pack-years.

Bronchoscopic Collection and Processing of Bronchial Epithelium. A protocol was developed for harvesting viable bronchial epithelium from the lower respiratory tract using a standard cytology brush during bronchoscopy. After introduction into the lower respiratory tract, the bronchoscope was directed into each upper and lower lobe, and the carinal margin of a segmental orifice, usually the second and third bifurcation within the upper and lower lobes, respectively, was brushed. These sites were chosen because (*a*) they are high-deposition areas for particles; (*b*) they are associated frequently with histological changes in smokers; and (*c*) they represent sites where tumors commonly occur (11, 23). The area was first washed with saline to remove any nonadherent cells. Sites were not brushed if a tumor was visualized within 5 cm of the site. After brushing, the brush was withdrawn, placed in serum-free medium, and kept on ice until processed. Each site was brushed twice. The procedure was well tolerated by all subjects, and no complications were noted related to the brushing procedure.

Bronchial cells were collected from only two of the sites in two of the subjects, from three sites in two subjects, and from all four sites in the remaining subjects. Although only two sites were brushed initially in case 1, cells were obtained from all four sites in this subject during a repeat bronchoscopy performed after the initial procedure did not yield a diagnosis. Samples were obtained from all four sites in the cancer-free current smokers and in the never smokers. In addition, bronchial epithelial cells derived at autopsy by Clonetics, Inc. (San Diego, CA) from four never smokers were also obtained to serve as additional controls. Only two sites sampled from most of the former uranium miners were available for analysis because cells recovered from the other sites had been used exclusively for cytology in another investigation.[4]

Bronchial Epithelial Cell Culture. Replicative cultures of the bronchial epithelial cells obtained by the procedure described above were established in our laboratory (24) using a serum-free medium (BEGM; Clonetics, Inc.) that is optimal for growth of these cells. Cells were removed from brushes by vigorous shaking in BEGM; cells from one brush were prepared for cytological analyses, and cells from the other brush were washed, resuspended in BEGM, seeded onto 60-mm fibronectin-coated plates, and grown at 37°C in 3% CO_2 and 21% O_2 until 80% confluence. Prior to passage, aliquots of cells were cryopreserved and stored at −145°C; other samples of cells were fixed in methanol-acetic acid (3:1). Next, the cells were washed four to six times in methanol:acetic acid and then dropped onto slides (about 2×10^5cells/slide). The effects of cell culture on the frequency of trisomy 7 in nonmalignant bronchial epithelium were examined by placing cells dispersed from brushes directly onto microscope slides followed by fixation.

Cytology. Cells from one brush from each bronchial collection site were prepared for cytological analysis by smearing the cells across a microscope slide. The cells were then fixed with 96% ethanol and stained according to the Papanicolaou procedure (25) to facilitate morphological evaluation by a cytopathologist.

Detection of Trisomy 2 and Trisomy 7. Trisomy 2 and trisomy 7 were determined by hybridization of cells with a biotinylated chromosome 2 or 7 centromere probe (Oncor; Gaithersburg, MD). The probes were denatured in hybridization buffer at 70°C for 5 min, and the slides were immersed in 70% formamide-2× SSPE at 70°C for 2 min. The probe was then applied to the slides, which were incubated in a humidified chamber at 37°C for 16 h. After incubation, the slides were washed in 0.25× SSPE (10 mM sodium phosphate monobasic monohydrate; 1 mM ethylenediamine tetraacetic acid disodium

[3] The abbreviations used are: NSCLC, non-small cell lung cancer; SCC, squamous cell cancer; AC, adenocarcinoma; EGFR, epidermal growth factor receptor; FISH, fluorescence *in situ* hybridization; LOH, loss of heterozygosity; BEGM, Bronchial Epithelium Growth Medium.

[4] Unpublished data.

salt, dihydrate; 150 mM sodium chloride, pH 7.4) for 5 min at 72°C, and the probe was detected with fluorescein-labeled avidin. Cell nuclei were visualized with propidium iodide.

Data Analysis. The number of centromeric hybridization signals in each cell were evaluated in 400 cells/slide, and the frequency of trisomy 7 on each slide was calculated by dividing the total number of cells expressing three hybridization signals by the total number of cells counted on each slide. Twenty % of the slides were scored by a second person, and frequencies for trisomy 7 differed by <0.4%. The total number of sites positive for trisomy 7 in subjects with SCC and AC were compared using Fisher's exact test.

Results

Cytology. Squamous metaplasia and atypical glandular cells, the only cytological abnormalities observed in lung cancer patients, were present in 32% of the samples (Table 1). These cytological changes were observed in <10% of the cells recovered from the diagnostic brush. Two subjects had three sites with cytological abnormalities, and five subjects had no cytological abnormalities. No samples contained tumor cells by cytology, although one of four sites in five subjects was collected from the same lobe where a tumor was later diagnosed.

Two of the 16 sites in smokers without lung cancer were cytologically abnormal (both in the same person; Table 2), whereas no atypical cells were present in the 12 sites from the three never smokers (Table 3). In former uranium miners, hyperplasia was present in bronchial cells collected from all four sites from one person, and in one site in two additional people (Table 2).

Culturing of Bronchial Epithelial Cells. The efficiency of establishing replicative cultures of the cells obtained by bronchial brushing was 100%. The serum-free medium used for these cultures is optimal for growing bronchial epithelial cells and does not support fibroblastic cell replication (25). Therefore, the cells were uniformly epitheloid in appearance. Growth potential was evaluated by passaging cells from all seven of the uranium miner cases and cases 1–6 from the lung cancer patients. Some of these cultures were maintained for up to nine passages (a minimum of 16 population doublings), and many underwent 30 divisions before senescence. However, none exhibited an indefinite population-doubling potential.

Detection of Trisomy 7 in Nonmalignant Bronchial Epithelium. Background rates of trisomy 7 were determined by examining normal human bronchial epithelial cell lines derived from autopsy cases of never smokers and bronchial epithelium collected from never smokers during bronchoscopy. In bronchial cell lines (passage 2) from four donors and bronchial epithelial cell samples obtained by bronchial brushing from the recruited never smokers (Table 3), only 1.4 ± 0.3% (SD) of the cells contained three hybridization signals for chromosome 7 with values ranging from 1 to 1.8%. These values agree with those reported by the manufacturer of the probe. Therefore, trisomy 7 frequencies of >2.0% (>2 SD above the mean for controls) were considered significantly different from controls.

Passage 1 or 2 bronchial cells from lung cancer patients were examined for trisomy 7. Eighteen of the 42 bronchial sites (43%) sampled from the 12 lung cancer patients contained trisomy 7 at frequencies ranging from 2.3 to 6.0% (Table 1; Fig. 1). Three subjects (cases 1, 2, and 11) displayed trisomy 7 in all sites collected during bronchoscopy, and in two subjects (cases 7 and 12), trisomy 7 was found in three of four sites (Table 1). Six of the 18 sites positive for trisomy 7 also contained cytologically abnormal cells. Trisomy 7 was found in six of seven patients diagnosed with SCC, whereas only one of four patients with AC displayed trisomy 7 in any site collected at bronchoscopy. Case 7, which had histological features of both SCC and AC, had one site positive for trisomy 7. The frequency of positive trisomy 7 sites in all patients with SCC within this small sample population was significantly greater than in AC patients ($P < 0.005$).

The reproducibility of detecting trisomy 7 at sites found to be positive for this abnormality was investigated in one patient (case 1) who required repeat bronchoscopy for clinical reasons. Trisomy 7 was increased similarly in the two sites brushed during both procedures, although cytological examination showed atypical cells in one site from the first bronchoscopy and cytologically normal cells from the same site collected

Table 1 Frequency of trisomy 7 in bronchial epithelial cells from lung cancer patients

Case	Age	Smoking (pack-yrs)	Tumor diagnosis	Brush location	Cytological diagnosis	Trisomy 7 (frequency, %)
1	64	104	SCC	RLL[a]	N	2.8[b]
				RUL	AGC	4.0[b]
				RLL[c]	N	3.0[b]
				RUL[c]	N	4.0[b]
				LLL[c]	N	6.0[b]
				LUL[c]	SM	4.3[b]
2	69	26	SCC	RUL	SM	2.8[b]
				LLL	SM	3.3[b]
				LUL	N	3.8[b]
3	65	120	SCC	RLL	AGC	2.0
				RUL	AGC	2.3[b]
				LLL	AGC	2.0
4	52	90	AC	RLL	SM	1.5
				RUL	N	1.8
				LLL	SM	1.5
				LUL	SM	1.8
5	70	50	SCC	RLL	N	1.5
				RUL	N	1.5
				LLL	N	1.5
				LUL	SM	1.3
6	61	93	AC	RLL	N	1.5
				RUL	N	1.3
				LLL	N	2.0
				LUL	N	1.5
7	58	40	SCC	RLL	N	1.8
				RUL	N	2.3[b]
				LLL	N	2.5[b]
				LUL	N	2.8[b]
8	59	120	AdSCC	RLL	N	1.5
				RUL	N	2.0
				LLL	N	2.5[b]
				LUL	AGC	2.0
9	65	71	SCC	RLL	SM	2.0
				RUL	SM	2.5[b]
10	63	45	AC	RLL	N	1.0
				RUL	N	1.8
				LLL	N	1.8
				LUL	N	1.3
11	61	95	AC	LLL	N	2.5[b]
				LUL	N	2.8[b]
12	76	17	SCC	RLL	N	2.0
				RUL	N	2.3[b]
				LLL	N	2.3[b]
				LUL	N	2.3[b]

[a] RLL, right lower lobe; RUL, right upper lobe; LLL, left lower lobe; LUL, left upper lobe; AGC, atypical glandular cells; SM, squamous metaplasia; N, normal cells; AdSCC, adenosquamous carcinoma.
[b] $P < 0.05$ as compared to never-smoker controls.
[c] Resampled 4 months later.

Table 2 Frequency of trisomy 7 in bronchial epithelial cells from cancer-free smokers and former uranium miners

Case	Age	Smoking (pack-yrs)	Radon exposure (WLMs)[a]	Brush location	Cytological diagnosis	Trisomy 7 (frequency, %)
13	81	15	0	RLL	N	1.8
				RUL	AGC	1.5
				LLL	N	1.8
				LUL	SM	2.0
14	34	24	0	RLL	N	1.3
				RUL	N	1.3
				LLL	N	1.0
				LUL	N	1.3
15	68	51	0	RLL	N	4.0[b]
				RUL	N	3.0[b]
				LLL	N	4.3[b]
				LUL	N	3.5[b]
16	45	30	0	RLL	N	1.3
				RUL	N	1.5
				LLL	N	2.0
				LUL	N	1.8
17	59	8	27	LLL	N	3.0[b]
				LUL	N	3.0[b]
18	65	9	516	LUL	N	1.3
				RUL	N	3.3[b]
19	64	30	235	LUL	N	1.5
				RLL	N	1.0
20	56	0	186	LUL	N	2.0
				RLL	N	2.3[b]
21	64	0	214	RLL	H	1.8
22	64	9	577	LUL	N	1.8
				RLL	H	0.8
23	67	31	124	LLL	H	1.3
				LUL	H	2.8[b]
				RLL	H	2.5[b]
				RUL	H	3.3[b]

[a] Abbreviations are as indicated in Table 1 footnote. WLM, working level month; H, hyperplasia.
[b] $P < 0.05$ as compared to never-smoker controls.

Table 3 Interphase analysis of chromosome 7 in normal human bronchial epithelial cells

Bronchial epithelial cell lines were established from never smokers (Clonetics) after autopsy and from volunteers. The normal distribution of chromosome 7 copy number as detected by FISH is shown by the percentage of cells exhibiting 1, 2, 3, or 4 hybridization signals. Four hundred cells containing hybridization signal were counted per donor.

Donor	Age	Brush location	Number of hybridization signals/cell (%)			
			1	2	3	4
1	6	NA[a]	3.5	92.0	1.5	3.0
2	17	NA	2.3	95.5	1.3	1.0
3	15	NA	1.5	94.7	1.8	2.0
4	41	NA	2.0	94.8	1.0	2.3
5	45	RLL	1.0	95.5	1.8	1.7
		RUL	0.5	98.3	1.0	0.2
		LLL	1.3	96.5	1.0	1.2
		LUL	1.0	96.3	1.2	1.5
6	35	RLL	1.0	96.8	1.0	1.2
		RUL	2.5	93.3	1.7	2.5
		LLL	2.0	94.8	1.5	1.7
		LUL	1.8	94.2	1.8	2.2
7	33	RLL	0.5	98.2	0.8	0.5
		RUL	0.5	97.2	1.3	1.0
		LLL	1.2	96.8	1.3	0.7
		LUL	1.0	96.0	1.5	1.5

[a] Abbreviations are as indicated in the legend to Table 1. NA, not applicable.

during the second procedure (Table 1). The other two sites collected during the second bronchoscopy also showed elevated frequencies of trisomy 7 in this patient.

Trisomy 7 was detected in cytologically normal bronchial epithelium collected from four sites in one (case 15) of the cancer-free smokers (Table 2). Bronchial cells from the other smokers did not contain this chromosome abnormality. In the former uranium miners (cases 17–23), seven of 15 sites collected during bronchoscopy were positive for trisomy 7. Three of the positive sites were found in one subject (case 23) and also contained basal cell hyperplasia. However, the other four samples positive for trisomy 7 showed no cytological abnormality.

Two of the former uranium miners (cases 18 and 23) developed lung cancer within 2 years of bronchial cell collection. SCC was diagnosed in the right upper lobe of both subjects. As noted in Table 2, both cases were positive for trisomy 7 in the right upper lobe brushing site obtained at the initial bronchoscopy.

Tissue Culture Effects on Trisomy 7 Expression in Bronchial Epithelium. The effect of tissue culture on trisomy 7 frequency was assessed by comparing the frequency of this chromosome abnormality in freshly isolated bronchial epithelium obtained directly from bronchial brushes ("preculture") to passage 1 cells. This comparison was conducted on cells collected from two different bronchial sites in three different subjects [(cases 11 and 16 and donor 7 (never smoker)]. Cultured samples positive for trisomy 7 in case 11 were also positive in preculture cells from the same bronchial collection site, whereas sites negative for trisomy 7 in cultured cells from case 16 and the never smoker were also negative in preculture cells (data not shown). Values for trisomy 7 differed by <0.3% between preculture and cultured cells. The effect of passaging cells on the frequency of trisomy 7 was also examined in bronchial cells from case 1. Trisomy 7 frequency was similar in cells from passages 1, 4, and 7.

Frequency of Trisomy 2 in Nonmalignant Bronchial Epithelium. Aneuploidy has been detected in bronchial squamous metaplasia, a likely precursor to SCC (26). To determine whether the increased frequency of trisomy 7 detected in the current study reflects generalized aneuploidy or a specific chromosomal duplication, a subgroup of samples was evaluated for trisomy of chromosome 2. The frequency of trisomy 2 in never smokers was 1.5 ± 0.4% (data not shown). Bronchial cells from eight subjects, six of whom had elevated frequencies for trisomy 7, were evaluated. The frequency for trisomy of chromosome 2 did not differ from never smokers (Table 4).

Discussion

The studies described in this report are the first to detect and quantify an increase in trisomy 7 in the airway cells of subjects at risk for lung cancer. The presence of trisomy 7 appeared to be a specific chromosome gain and not due to generalized aneuploidy in these cells. In addition, trisomy 7 in nonmalignant epithelium from lung cancer patients was associated with SCC tumor histology, suggesting that patients with this genetic change may be at greater risk for developing SCC than other histological forms of lung cancer. This supposition was supported by the fact that two cancer-free former uranium miners with bronchial cells positive for trisomy 7 ultimately developed SCC. Finally, these results demonstrate the ability to detect genetic changes in cytologically normal cells, suggesting that molecular analyses may enhance the power for detecting

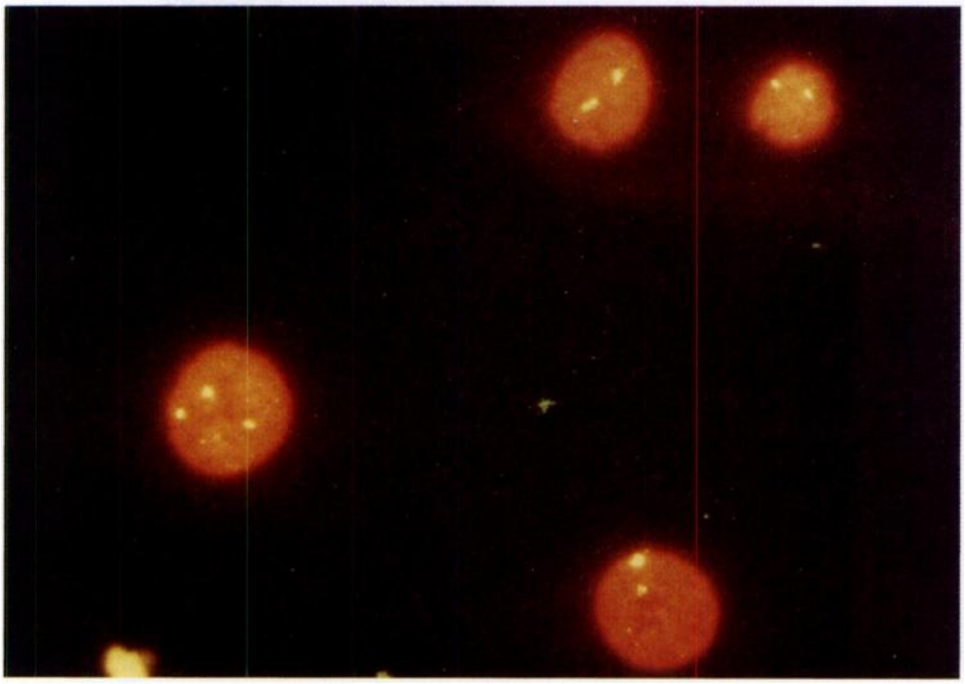

Fig. 1. FISH for chromosome 7 in bronchial epithelial cells. Trisomy 7 is apparent in one cell from this field. Magnification, ×530.

Table 4 Frequency of trisomy 2 in bronchial epithelial cells from lung cancer patients, cancer-free smokers, and former uranium miners

Case	Tumor diagnosis	Brush location	Trisomy 2 (frequency, %)
1	SCC	RLL[a]	1.5
		RUL	1.8
		LLL	1.8
		LUL	1.0
2	SCC	LLL	1.0
		LUL	1.0
7	SCC	RLL	1.5
		RUL	2.1
		LLL	1.8
		LUL	1.5
8	AC	RLL	0.3
		RUL	1.5
		LLL	0.8
13	None	RLL	1.0
		RUL	0.8
		LLL	1.0
		LUL	1.3
15	None	RLL	1.8
		RUL	2.0
		LLL	1.0
		LUL	1.3
19	None	LUL	1.9
		RLL	0.8
23	None	RLL	1.5

[a] Abbreviations are as indicated in legend to Table 1.

premalignant changes in bronchial epithelium in high-risk individuals.

Cigarette smoking and the exposure of underground miners to radon progeny are both well-established respiratory carcinogens (27, 28). Tobacco smoke contains numerous mutagens and carcinogens, and radon progeny that have been inhaled and deposited on the respiratory epithelium release α particles capable of damaging DNA (28). Although comparison between findings in the cigarette smokers and the former uranium miners is constrained by the number of participants in the two groups, trisomy 7 was found in both groups. These results are consistent with the synergism between smoking and radon progeny, which suggests commonality in the pathways by which the two carcinogens cause lung cancer (29).

The bronchial brushing method used for collecting cells from the lower respiratory tract is rapid (10–12 min total for two brushes at four different sites), well tolerated by the patient, and permits collection of viable bronchial cells that can be expanded through tissue culture at 100% efficiency. The stability of these cells in culture was evident by the fact that the frequency of trisomy 7 did not differ between primary brush cells and cells propagated for up to seven passages. Furthermore, this procedure is amenable to the production of sufficient cell numbers (1×10^8) at low passage (one or two) to accommodate multiple molecular analyses. Although the media used in culturing of bronchial epithelial cells did not appear to provide a selective growth advantage to cells harboring an additional chromosome 7, the modulation of medium supplements might lead to the establishment of clonal populations of premalignant cells. Such cell populations would greatly facilitate the identification of additional early gene changes in respiratory carcinogenesis.

The detection of trisomy 7 in multiple nonmalignant sites within the bronchial tree supports the theory of field cancerization (5), which states that diffuse exposure of the entire respiratory tract to inhaled carcinogens causes the development of multiple, independently initiated sites that can lead to tumor development. Although the frequency of this chromosome abnormality was relatively low (2.3–6.0%), these values were consistent with the low percentage of cells within each brush sample (10%) that exhibited abnormal cytology. These results are also similar to studies of chromosome gain in patients with head and neck cancer where trisomy 7 was detected at frequen-

cies of 2, 3, and 21% in histologically normal, hyperplastic, and dysplastic cells, respectively (30).

The detection of trisomy 7 in normal, hyperplastic, and metaplastic bronchial epithelium from cancer-free patients extends a recent report describing LOH at chromosomes 3p, 5q, and 9p in dysplastic premalignant bronchial lesions harvested from current and former smokers by bronchoscopy (31). The inability to detect LOH at these chromosome loci in normal or early premalignant epithelium may stem from a difference in sensitivity between the methodologies used. The low frequency of trisomy 7 and cytologically abnormal cells collected from bronchoscopy is consistent with a lack of clonality within the brush cells. FISH assays on interphase cells permit screening of individual cells, and sensitivity for detection is limited only by the number of cells examined. In contrast, microsatellite analyses for LOH cannot detect nonclonal changes but require that the chromosome alteration be present in approximately 40–50% of the sample (32, 33).

The role of trisomy 7 in lung cancer development has not been elucidated. Increased expression of EGFR, which is located on chromosome 7 (34), is observed in 50–80% of NSCLCs (16, 35, 36). EGFR expression appears greater in SCC than AC (35, 36) and is amplified in some cell lines derived from SCC (37). These findings corroborate our hypothesis that acquisition of trisomy 7 in bronchial epithelium could be prognostic for development of SCC. Moreover, expression of this gene is also increased in nonmalignant bronchial epithelium from NSCLC patients (16, 35) and in normal or premalignant epithelium adjacent to head and neck tumors (38). Thus, altered expression of EGFR could enable cells that have acquired additional genetic changes to proliferate continually and escape from terminal differentiation (39). In addition, the c-*met* oncogene is also located on chromosome 7 and is overexpressed in NSCLCs (40, 41). This oncogene encodes a transmembrane tyrosine kinase (42) that functions as a receptor for the hepatocyte growth factor (43) and is involved in sustaining the growth of NSCLC cells in culture (44).

Previous studies have detected mutations in p53 (12, 14, 35), chromosome losses at 9p21 (45) and 3p (46) in preinvasive bronchial lesions, and simple chromosome rearrangements in normal bronchial epithelium from proximal airways (47) of lung cancer patients. The prevalence of these genetic changes in normal epithelium from persons at risk for lung cancer should be quantified by FISH to define the temporal sequences of somatic genetic changes that precede the development of clonal lesions in the lung. This information will be invaluable in providing biological markers that can qualitatively estimate the extent of field cancerization in persons at risk for lung cancer and can be used to assess the efficacy of chemointervention trials. Ultimately, the efficiency for detecting these biological markers in bronchial epithelium *versus* exfoliated epithelial cells within sputum must be established to support the use of a "genetic-based" screening approach for individuals at high risk for lung cancer. The results of the current investigation have identified one potential biomarker, trisomy 7, that may be useful in early detection and intervention for lung carcinogenesis.

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R E Crowell, F D Gilliland, R T Temes, et al.

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