

**Short Communication****Frequency of Trisomy 20 in Nonmalignant Bronchial Epithelium from Lung Cancer Patients and Cancer-free Former Uranium Miners and Smokers<sup>1</sup>**

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**Abstract**

**Lung cancer is the leading cause of cancer-related deaths. The development of sensitive screening methods to identify at-risk individuals before emergence of clinical disease would permit early intervention that could decrease this mortality. Our previous studies have shown that cells with trisomy 7 can be detected in bronchial epithelium from cancer-free smokers and former uranium miners. However, the use of more than one molecular marker could increase the chance of identifying at-risk individuals. Trisomy 20, which is found in 43–57% of non-small cell lung cancers, is a candidate marker. The purpose of the current investigation was to determine the percentage of cells with trisomy 20 in persons with a high risk for lung cancer. Bronchial epithelial cells that had been assayed for trisomy 7 were assayed for trisomy 20 by fluorescence *in situ* hybridization. Trisomy 20 was detected in bronchial epithelial cells from lung cancer patients and from smokers and ex-uranium miners without lung cancer. In some cases, patients who were negative for trisomy 7 exhibited trisomy 20. Consequently, more people with field cancerization were identified using both markers. However, the two markers combined did not appear to stratify the risk for lung cancer.**

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**Introduction**

Lung cancer is the leading cause of cancer-related deaths in the United States (1). One reason for the high mortality rate is the lack of satisfactory early detection methods. If sensitive screening methods were developed, intervention strategies could be implemented to decrease the high mortality rate from this disease (2). Early detection of lung cancer is conceivable due to "field cancerization," in which the entire respiratory tract is exposed to inhaled carcinogens. Consequently, susceptible individuals would be expected to exhibit multiple, independently initiated sites throughout their lungs (3). This expectation is supported clinically by the high frequency of second primary lung tumors in persons who underwent resection for the first primary tumor (4). In addition, chromosome aberrations associated with lung cancer, such as LOH<sup>3</sup>, involving chromosomes 3 and 17, are seen in nonmalignant epithelium adjacent to lung tumors (5, 6). Furthermore, trisomy 7 is found in 43–64% of cytogenetically analyzed NSCLCs (7–9) and has been detected in the far margins of some resected lung tumors (10).

Recent studies by our laboratory (11) extended the above investigations on trisomy 7 to examine normal-appearing epithelium for this chromosome gain. BECs were harvested using routine clinical procedures, expanded *in vitro*, and assayed for trisomy 7 using FISH. Cells with trisomy 7 were detected in 33, 12, and 47% of sites from lung cancer patients, smokers, and ex-uranium miners, respectively, and there was no correlation with abnormal cytology. These results suggested that cells with specific chromosome aberrations that were present in normal-appearing epithelium could be detected in persons at risk for lung cancer.

Cytogenetic analysis has shown that trisomy 20 is a numerical chromosome aberration found in 43–57% of NSCLCs (7–9). The purpose of this investigation was to determine the frequency of trisomy 20 in nonmalignant BECs obtained from the populations previously examined for trisomy 7. The frequency for detecting trisomy 20 and 7 in the same site or independent sites was also determined to assess the potential for using these two markers together to stratify the risk for lung cancer.

**Materials and Methods**

**Subject Recruitment.** Thirty-two subjects were studied. Fifteen were cancer patients, who would be expected to exhibit an extensive cancerization field. Of these cancer patients, 12 had been evaluated for trisomy 7 (11). The remaining cancer patients were new volunteers. Seven smokers without lung cancer

<sup>3</sup> The abbreviations used are: LOH, loss of heterozygosity; NSCLC, non-small cell lung cancer; BEC, bronchial epithelial cell; FISH, fluorescence *in situ* hybridization; BEGM, bronchial epithelial growth medium; DAPI, 4,6-diamidino-2-phenylindole; SM, squamous metaplasia.

were evaluated in this study. Three were new volunteers, and four had been evaluated for trisomy 7. Cells from 10 uranium miners were evaluated. All of the miners were new volunteers and had not been evaluated for trisomy 7.

Of the 32 subjects, 21 were cigarette smokers (present and former), one was a pipe smoker undergoing a diagnostic evaluation for possible lung cancer, and 10 were former uranium miners, 8 of whom were former smokers. Seven individuals who had never smoked were used as controls.

**Bronchoscopic Collection and Processing of Bronchial Epithelium.** In each subject, a bronchoscope was directed into each upper and lower lobe, and the mucosal surface brushed with cytology brushes. Usually the second and third bifurcation within the upper and lower lobes were brushed, respectively. These sites were chosen for three reasons: (a) they are high-deposition areas for particles; (b) they are frequently associated with histological changes in smokers; and (c) they represent common sites of tumors (12, 13). Each site was brushed twice. One brush was used for cytology, and the other was grown in culture for cytogenetic analysis. The areas were 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 removed, placed in serum-free medium, and kept on ice until processed.

**Bronchial Epithelial Cell Culture.** Our previous investigation (11) examined the effect of cell culture on the percentage of trisomy 7 cells within the population as a function of cell doublings. These data showed that propagation in culture does not affect the incidence of trisomy 7. Thus, the cells recovered from the airways were cultured to expand the number of cells available for analysis. These cultures were established using serum-free BEGM (Clonetics, Inc.), which is selective for the growth of BECs (11, 14). Cells were removed from brushes by vigorous shaking in BEGM. Cells from one brush were prepared for cytological analysis, and cells from the other brush were washed, resuspended in BEGM, seeded onto 60-mm fibronectin-coated tissue culture dishes, and grown at 37°C in 3% CO<sub>2</sub> until 80% confluent. Before passage, aliquots of cells were cryopreserved. Cells were fixed in methanol/acetic acid (3:1) at passage 1 or 2 for FISH assays.

**FISH. Trisomy 20:** A SpectrumOrange<sup>™</sup>-labeled probe for 20q13.2 (LSI, Vysis, Downers Grove, IL) was denatured in hybridization buffer at 74°C for 5 min. The chromosome region 20q13.2 is a breast cancer amplicon; however, there is no evidence for amplification of this region in lung cancer (15). Therefore, this probe could be used as a chromosome enumerator. Chromosomal DNA in cells was denatured at 73°C in 70% formamide and 2× SSC for 5 min, then dehydrated successively in 70, 85, and 100% ethanol for 1 min each. The cells were hybridized overnight at 37°C. Slides were then washed successively in 50% formamide and 2× SSC, 2× SSC, and 2× SSC and 0.1% NP40 at 46°C. The cells were counterstained with DAPI and viewed using an Olympus BX60 fluorescence microscope with a DAPI/FITC/TRITC (tetramethylrhodamine isothiocyanate) triple bandpass filter.

Trisomy 2 and trisomy 7 were assayed as described previously (11). Trisomy 2 was assayed as a control for generalized polyploidy.

**Data Analysis.** The number of FISH signals was evaluated in 400 cells/site. Lung sites were considered positive for trisomy 2, 7, or 20 if the number of cells with three FISH signals was greater than the average control values in BECs for trisomy 2, 7, or 20, plus 3 SDs. For quality assurance, 20% of all of the

slides were scored by two trained technicians. Their results differed by <0.4%.

## Results

**Pathology and Exposure History.** Fifteen of the 22 smokers who underwent diagnostic bronchoscopy were diagnosed with NSCLC. Eight tumors were characterized histologically as squamous cell carcinomas, and seven tumors were adenocarcinomas. No tumors were found in the remaining seven smokers. Smoking histories ranged from 17–120 pack-years. The smokers' ages ranged from 34–81 years.

Former uranium miners worked underground for 18–35 years, with exposure levels to radon progeny on the order of 56–459 working-level-months. Eight of 10 uranium miners had smoking histories that ranged from 5–62 pack-years. The uranium miners ranged in age from 54–71 years; none of the miners had clinical evidence for lung cancer.

**Trisomy 20 in BECs.** Baseline values for trisomy 20 were determined by scoring 400 cells/site in BECs from never-smokers. Lung sites were considered positive for trisomy 20 if the mean number of cells with three FISH signals was greater than the average plus 3 SDs (mean = 2.1 ± 0.4 per 400 cells). The percentage of cells in lung sites considered positive for trisomy 20 was 2.3- to 4.4-fold greater than baseline values determined in never-smokers.

Forty-six sites from smokers with lung cancer were assayed for trisomy 20; 13 sites (28%) were positive (Fig. 1A). Twenty-five sites from smokers without lung cancer were examined for trisomy 20. Four sites (16%) exhibited trisomy 20 (Fig. 1A). Only 3 (8%) of 37 sites from uranium miners were positive for trisomy 20.

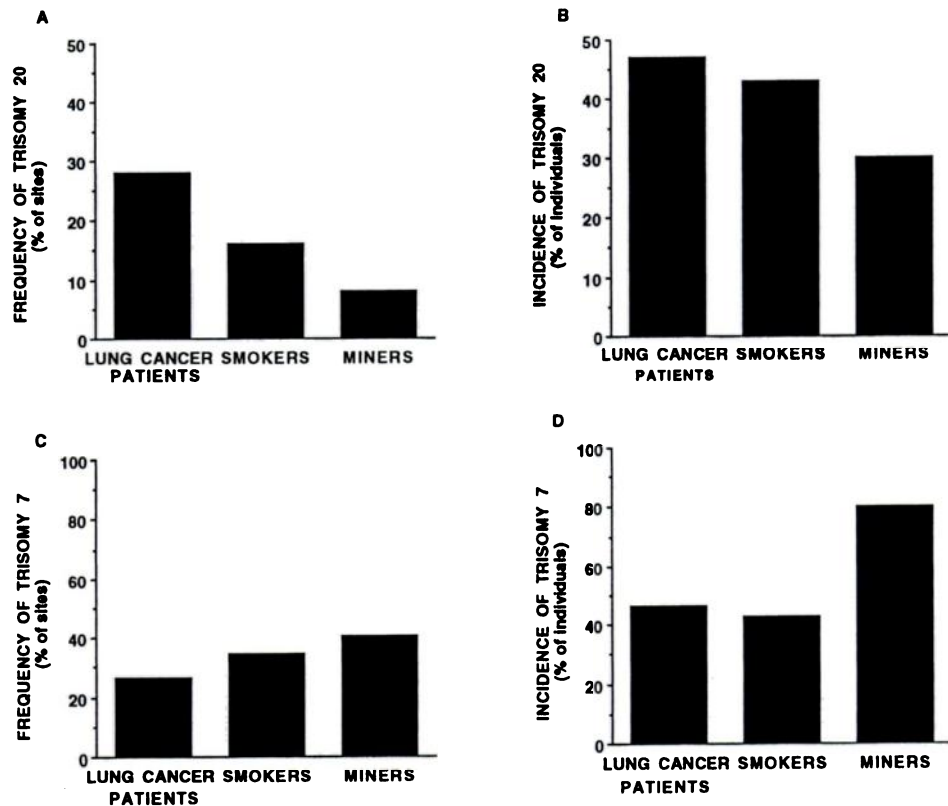
Presented in Fig. 1B is the frequency of trisomy 20 in patients from the three groups. Seven (47%) of 15 lung cancer patients exhibited cells with trisomy 20 in normal-appearing epithelium. Trisomy 20 was detected in 50% and 43% of lung cancer patients with squamous cell carcinoma and adenocarcinoma, respectively. Three (43%) of 7 smokers and 3(30%) of 10 former uranium miners had BECs that exhibited trisomy 20.

**Trisomy 7 in BECs.** Baseline values for trisomy 7 were determined previously (11) in BECs from never-smokers. Lung sites were considered positive for trisomy 7 if the mean number of cells with three FISH signals was greater than the average plus 3 SDs (mean = 1.4 ± 0.3 per 400 cells). The percentage of cells in lung sites considered positive for trisomy 7 was 2.3- to 4.8-fold greater than baseline values determined in never-smokers.

One of eight sites from the three new lung cancer patients exhibited trisomy 7. Five of the 10 sites sampled from the three new smokers were positive for trisomy 7. In former uranium miners, 15 (41%) of the 37 sites assayed were positive for trisomy 7 (Fig. 1C). Two of three new lung cancer patients were positive for trisomy 7. All of the three smokers who were added to the present study had trisomy 7 in their BECs, and 9 of the 10 miners were positive for trisomy 7 (Fig. 1D). Fig. 1, C and D, summarizes the results for the total study population.

**Trisomy 2 in BECs.** Trisomy 2 has been used in our laboratory (11) as a control for generalized aneuploidy because the abnormality is not commonly found in lung tumors (7–9). Therefore, to determine whether or not trisomy 20 in BECs is a sign of polyploidy or trisomy of a specific chromosome, a subgroup of samples was evaluated for trisomy 2. Trisomy 2 was assayed in eight individuals not examined in the original study; there was no evidence for trisomy 2 in any of them.

**Fig. 1.** Frequency and incidence of trisomy 20 and 7 in lung cancer patients, smokers without lung cancer, and former uranium miners without lung cancer. **A**, percent of lung sites positive for trisomy 20 (for lung cancer patients,  $n = 46$ ; for smokers,  $n = 25$ ; for miners,  $n = 37$ ). **B**, percent of individuals who exhibit trisomy 20 (for lung cancer patients,  $n = 15$ ; for smokers,  $n = 7$ ; for miners,  $n = 10$ ). **C**, percent of lung sites positive for trisomy 7 (for lung cancer patients,  $n = 49$ ; for smokers,  $n = 26$ ; for miners,  $n = 37$ ). **D**, percent of individuals who exhibit trisomy 7 in their BECs (for lung cancer patients,  $n = 15$ ; for smokers,  $n = 7$ ; for miners,  $n = 10$ ). Mean values for nonsmokers controls: for trisomy 20,  $2.1 \pm 0.4$ ; for trisomy 7,  $1.4 \pm 0.3$ .

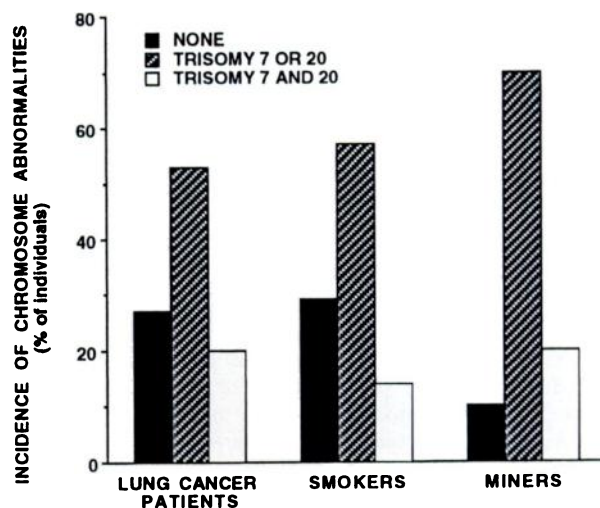


**Cytology.** Cytology has been published (11) for most of the study population. For the new lung cancer patients from whom eight sites were sampled, one showed SM, and one exhibited atypical glandular cells. Ten new sites were examined from the additional cancer-free smokers; one exhibited SM, and atypical glandular cell was detected in another. Thus, in the total study population of lung cancer patients and smokers, abnormal cytology was observed in 14 of 46 sites and 4 of 25 sites, respectively. The group of uranium miners in this study was entirely new; 37 sites were sampled within the group with 3 containing evidence of SM and 11 exhibiting hyperplasia.

The propensity to detect cytological and chromosome abnormalities in the sites sampled did not appear to be related. For example, of the 14 sites positive for cytology in the lung cancer cases, only 2 were positive for trisomy 20.

**Frequency and Incidence of Trisomy 7 and 20.** The two goals of our studies were to determine whether multiple chromosome markers can be used: (a) to better identify people with field cancerization; and (b) to stratify risk for lung cancer. Comparing frequencies (number of sites involved) or incidence (number of people) of both trisomy 7 and 20 is the first step in this process. Each population in this study is expected to exhibit field cancerization at a different frequency. Lung cancer patients, of course, have a 100% risk of lung cancer, and all of these patients should exhibit extensive field cancerization. Uranium miners who smoke can have as high as a 50% risk of lung cancer during their lifetime because of exposure to cigarette smoke and radon progeny (16). Smokers as a population have less than a 15% risk of lung cancer.

When incidences for trisomy 7 and 20 are compared (Fig. 2), 53% of the lung cancer patients had sites that were positive for trisomy 7 or trisomy 20; however, both markers were only



**Fig. 2.** Percentage of individuals with trisomy 7 and 20 and percentage of individuals with trisomy 7 or trisomy 20 from groups of lung cancer patients, smokers without lung cancer, and former uranium miners without lung cancer. For lung cancer patients,  $n = 15$ ; for smokers,  $n = 7$ ; for miners,  $n = 10$ . Mean values for nonsmoker controls: for trisomy 20,  $2.1 \pm 0.4$ ; for trisomy 7,  $1.4 \pm 0.3$ .

observed in 20% of these patients. Similarly, 57% of smokers without lung cancer had sites that were positive for trisomy 20 or trisomy 7, whereas both changes were observed in only 14% of these people. Seventy percent of former uranium miners had sites that were positive for trisomy 20 or 7. Strikingly, both changes were seen in only 20% of these persons. In addition,

the results stratified by frequency (total number of sites involved) did not differ significantly from the results for incidence (data not shown). Thus, the two markers combined did not help to stratify the populations by risk; however, more people with field cancerization were detected by using both markers together.

### Discussion

The role of trisomy is unknown in lung carcinogenesis, but the high association of this aberration with lung cancer suggests that trisomy plays an important role. These studies show that trisomy 20 is evident in cytologically normal BECs from smokers and former uranium miners without lung cancer. Although the frequency for this chromosome abnormality was relatively low (2- to 4-fold greater than baseline values) these values were consistent with the low percentage of cells (<10%) within the brush samples that exhibited abnormal cytology and the values seen previously for trisomy 7. However, the sampling procedure that collects cells over an area of approximately 1 cm, combined with expansion of the BECs in culture, would disrupt any small clones of cells containing this chromosome abnormality. This conclusion is supported by recent studies (17) that demonstrated clonal chromosome polysomy within biopsies of normal tissue adjacent to tumors.

The presence of trisomy 20 in nonmalignant bronchial epithelium from multiple sites further supports the role of field cancerization in lung carcinogenesis. According to this theory, exposure of the respiratory tract to inhaled carcinogens leads to the development of multiple initiated sites and, therefore, to tumor development. However, it is interesting to note that genetic aberrations were frequently found in cytologically normal tissue. In addition, 53% of lung cancer patients, 57% of smokers, and 70% of former uranium miners were identified as at-risk by the combination of two biomarkers, trisomy 7 and 20. This result and the data from other studies (18, 19) show that field cancerization may be more widespread than predicted. Epidemiological studies (1, 16) predict that smokers and uranium miners have different risks for lung cancer; however, their incidences of field cancerization using trisomy of chromosomes 7 and 20 as a marker of this phenomenon are similar. This leads to the conclusion that this type of damage does not always result in clinical disease.

Although the two combined markers did not help to stratify the population by risk, more people with field cancerization were detected by using both markers. The usefulness of multiple markers was also shown in a recent study (18) where LOH at 3p14, 9p21, and 17p13 was detected in bronchial biopsies from current and former smokers. In this study, 69% of the subjects had LOH at only one of the three loci; 31% had LOH in at least two loci; and LOH at all of the three loci was detected in only two biopsy specimens. Thus, screening for multiple chromosome aberrations could increase the chance of identifying individuals at higher risk, and trisomy 20 could be part of a battery of markers to identify these people.

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