Genetic Susceptibility to Lung Cancer as Determined by Lymphocytic Chromosome Analysis

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Abstract
Chromosomal anomalies were analyzed in the lymphocyte cultures among 96 untreated lung cancer patients and 74 clinically normal comparison subjects. The analysis revealed that >15% of the lung cancer patients showed structural or numerical rearrangements in chromosomes 1, 3, 5, 7, 9, 12, 14, and 21. A case control comparison showed that these aberrations were significantly higher in chromosome 7 [odds ratio (OR) = 2.32; 95% confidence interval (CI), 1.14 and 4.82], chromosome 9 (OR = 2.61; 95% CI, 1.27 and 5.48), chromosome 12 (OR = 4.10; 95% CI, 1.40 and 14.54), and chromosome 21 (OR = 7.57; 95% CI, 1.73 and 70.80) of the patients than in the controls. However, only chromosome 9 (OR = 3.57; 95% CI, 1.33 and 9.46) and chromosome 21 (OR = 6.94; 95% CI, 3.15 and 9.98) retained significance after stratifying on smoking status. Among the lung cancer patients, the breakpoints cluster in specific regions of some of these chromosomes. These regions are 1p13–q21, 3p21–q13, 7p12–q12, 7q22, 7q32, 9p13–q13, 12p13, 14q11, and 14q32. The distribution of lung cancer patients, according to histological types, showed that aberrations in chromosomes 1, 7, and 9 dominated the scenario of chromosomal changes in non-small cell lung carcinomas. Thus, the data on lymphocytic chromosome rearrangements in lung cancer patients not only indicate the importance of specific genetic changes in the etiology of lung cancer but also emphasizes the putative role of such analysis in determining primary genetic abnormalities in the large heterogeneous group of lung cancers.

Introduction
Although smoking is causally associated with the development of over 90% of all lung cancers, only 15% of smokers develop lung cancer (1), implying that the genetic profile of an individual plays a role in lung cancer predisposition. An understanding of the molecular genetic pathogenesis of lung cancer originated from classic cytogenetic studies (2–4). The theory of nonrandomness of certain chromosomal changes in human malignancies evolved progressively with the accumulation of cytogenetic data, largely on hematological malignancies rather than those from solid tumors (5, 6), because of the technical, analytical, and interpretative challenges presented by solid tumor analyses (7). There is increasing evidence that many of the primary chromosomal changes associated with solid neoplasms were first detected in PBLs (8–11). PBLs are ideal for the study of primary chromosomal defects in somatic cells because they actively proliferate in a short time, yielding an adequate supply of mitotic cells for chromosomal analysis, and offer the advantage of convenient collection. Our previous investigations in PBLs of patients with colorectal cancers, adenomatous polyps, and their asymptomatic family members showed lymphocytic cytogenetic abnormalities consistent with the chromosomes reported to be somatically involved in colorectal polyps and cancers (12–14). Similar investigations have also been carried out in renal cell carcinoma and breast cancer (8, 10). Pathak (15) and Pathak et al. (16) have hypothesized that predisposed individuals might be genetically mosaic, i.e., primary chromosomal defects associated with neoplastic transformation may not be necessarily inherited in every somatic cell but may be harbored only in a few somatic cells. Mosaicism has been recognized frequently in retinoblastoma (17, 18) and Wilms’ tumor patients (19).

To elucidate whether specific chromosomes are more frequently involved in structural and numerical abnormalities among lung cancer patients than in control subjects, we have evaluated chromosomal anomalies in the lymphocytes of 96 untreated lung cancer patients and 74 comparison subjects.

Materials and Methods

Study Population. This study used data from a case-control study of lung cancer in a minority population (i.e., African-American and Mexican-American). Detailed methods are presented elsewhere (Refs. 20 and 21), i.e., cases were newly diagnosed, histologically confirmed lung cancer patients who had not received prior treatment with radiotherapy or chemotherapy. The patients were recruited from The University of Texas M. D. Anderson Cancer Center; from county, community, and Veterans Administration hospitals in the Houston and San Antonio metropolitan areas; and from Galveston, Texas. There were no age, histological, or stage restrictions. Controls were identified from a convenience sample recruited from community centers, cancer-screening programs, churches, and employee groups. Only individuals without a history of cancer were eligible for participation as controls. The controls were frequency matched to the cases by sex, ethnicity, and age (±5 years).

After informed consent was obtained, a structured inter-
view of approximately 45 min in duration was conducted by trained interviewers/phlebotomists. Bilingual interviewers/phlebotomists conducted the interviews for the Mexican-American cases and controls using a questionnaire translated into Spanish and backtranslated into English by the Department of Mexican Studies at the University of Houston. Mexican-American study participants could choose either English or Spanish for the interview.

Data were collected on socio-demographic characteristics, recent and prior tobacco use, other life-style habits, and family history of cancer. Blood was drawn into heparinized tubes for cytogenetic and molecular genetic analyses.

**Lympocyte Cultures and Chromosome Preparations.** Peripheral blood samples of these 170 subjects were collected in heparinized vials. Lympocyte cultures were set up following the routine protocol of adding 1 ml whole blood to 9 ml of RPMI 1640 with folic acid (JRM Biosciences, Lenexa, KS) supplemented with 20% fetal bovine serum (Sigma Chemical Co., St. Louis, MO), 2 mM L-glutamine, 50 units/ml penicillin, 100 μg/ml streptomycin, and 1.3% phytohemagglutinin (Wellcome Diagnostic Laboratories, Research Triangle Park, NC). The cultures were incubated at 37°C for 72 h. All slides were coded before evaluation.

Chromosome preparations were made following routine Colcemid blocking (0.04 μg/ml for 45 min), hypotonic treatment (0.075 mM KCl for 20 min), and fixation (1:3 aceto-methanol by volume) procedures. Air-dried slides were prepared from the harvested cell cultures. Gietz (G-) banding was performed by trypsin treatment on 7- to 9-day-old slides. A minimum of 100 metaphases were analyzed using a Genetiscan (Perceptive System, Inc., Houston, TX), and all the abnormal metaphases together with three normal metaphases were karyotyped from each of the 170 individual cultures.

**Statistical Analysis.** Socio-demographic variables were compared between cases and controls using the χ² test for categorical data and Student’s t test for continuous variables. To test for significant associations between breakage on a specific chromosome and case-control status univariate, ORs were calculated as a measure of the relative risk. Ninety-five % CIs were computed using the method of Cornfield (22).

The modifying effect of smoking on specific chromosome breakage was tested in several ways. Stratified analysis was done for each chromosome based on categorized smoking status (yes or no). Pack-years were calculated in two ways: the first used the self-reported number of years smoked, while the second used the difference between beginning and ending smoking as the number of years smoked. Time since cessation of smoking behavior was also evaluated. Cigarette content information (e.g., tar and nicotine) was calculated using the reported brand and manufacturer’s estimates of content.

Logistic regression, calculated by the STATA program, was used to estimate risks adjusted for multiple factors (23). All models were fit with the matching criteria included as covariates. Confidence limits for the adjusted ORs were calculated using the associated logistic coefficient and the corresponding SE.

**Results**

Our report is based on data from 96 cases and 74 controls. Table 1 summarizes the distribution of select socio-demographic variables. Because our study was a subset of a larger case-control study of lung cancer in minority populations, we were not able to maintain exact frequency matching in our subset. As a result, there were more male cases (69.8%) than male controls (36.5%). The age distribution was also slightly skewed, with cases being significantly younger (59.5 years) than controls (64.5 years). Cases were more likely to be African-American than were controls (75.0% versus 59.5%). It is important to recall that these data are a subset from a case-control study that was frequency matched for age (± 5 years), sex, and ethnicity. Although the full study maintains the frequency matching, it is clear that this subsample is not matched. Therefore, we will include age, sex, and ethnicity in the multivariate analysis. There was no significant difference between cases and controls with respect to education or income.

As would be expected, there were more smokers in the cases (93.8%) than in the controls (41.2%). A summary of the smoking status by case-control status is presented in Table 2. The majority of cases were current smokers (68.7%), significantly more than controls (24.3%). Cases were also more likely to be former smokers. The OR for current smoking as a risk factor for lung cancer was 26.28 (95% CI, 8.98 and 84.99) and for former smokers 13.23 (4.02 and 46.86; data not shown).

Results were more likely to inhale deeply (75.6%) than were controls (38.7%). There was no significant difference in mean age of starting smoking between cases and controls (18.5 versus 17.29 years, respectively; P = 0.33). Both current and former smoking cases reported longer pack-years of exposure than did controls. We additionally evaluated the risk of tar and nicotine. Both of these factors showed an increased risk of lung cancer.

Exposure to chemicals from a defined list of potential occupational and avocational exposures had a nonsignificant OR of 1.55 (95% CI, 0.79 and 3.07; data not shown). Elevated risk was associated with exposure to dusts (OR = 3.38; 95% CI, 1.63 and 7.13) and fumes (OR = 2.53; 95% CI, 1.15 and 4.76).

The primary focus of our study was to associate specific chromosome breakage with development of lung cancer. Chromosomes 1, 3, 5, 7, 9, 12, 14, and 21 were rearranged in more than 15% of the lung cancer patients analyzed. Hence, for this...
evaluation, we chose breakage on chromosomes 1, 3, 5, 7, 9, 12, 14, and 21. Number and position of breaks were not included in this analysis. The distribution of breaks in univariate analyses is presented in Table 3. Chromosomes which showed significant association with case-control status included chromosome 7 (OR = 2.32; 95% CI, 1.14 and 4.82), chromosome 9 (OR = 2.61; 95% CI, 1.27 and 5.48), chromosome 12 (OR = 4.10; 95% CI, 1.40 and 14.54), and chromosome 21 (OR = 7.75; 95% CI, 1.73 and 70.80). Because smoking is such a significant determinant of lung cancer, we stratified based on smoking status. These results are shown in Table 4. Using the Mantel-Haenszel stratified OR, we found that chromosome 9 (OR = 3.57; 95% CI, 1.33 and 9.46) and chromosome 21 (OR = 6.94; 95% CI, 3.15 and 19.98) were associated with significantly elevated risk after stratifying on smoking status.

A variety of multivariate analyses were used to test the association of chromosomal breakage with case-control status. All analyses were adjusted for age, sex, and ethnicity. Each chromosome was evaluated with a variety of smoking variables to determine if the association was valid. Variables included in the analysis were smoking status, number of cigarettes, pack-years, tar, nicotine, age started smoking, age ceased smoking, length of time since cessation, and exposure to chemicals, fumes, or dusts. Results for chromosomes 7, 9, 12, and 21 are presented in Table 5. The other chromosomes were not significant in either the univariate or multivariate analyses.

![Fig. 1](image-url) shows representative structural abnormalities in chromosomes 1, 3, 5, 7, 9, 12, 14, and 21.

When the breakpoints of these structural rearrangements were plotted (Fig. 2), it was observed that certain regions were more recurrently involved among patients than among the controls. Regions 1p13–q21, 3p12–q13, 7p12–q12, 7q22, 7q32, 9p13–q13, 12p13, 14q11, and 14q32 exhibited higher frequencies of breakpoints among lung cancer patients than controls. There were no marked differences in chromosomes 5 and 21. The pattern of chromosomal involvement was associated with histological type (Table 6). Chromosome 1 was involved with uniform frequency in all histological types but was marginally higher in adenocarcinomas (48%). Chromosome 7 was most frequently involved in squamous cell carcinomas (63%) and chromosome 9 in large cell carcinomas (67%) and squamous cell carcinomas (52%).

### Discussion

The points at which changes in DNA organization are most likely to affect gene expression are chromosome breakpoints. Chromosome translocations, point mutations, or amplifications can activate oncogenes. Mutations may also inactivate a tumor suppressor gene. Functional loss of genes at specific chromosomal loci is a common genetic alteration in most human epithelial tumors and is thought to be critical for unmasking the recessive genetic changes for tumorigenesis. The breakpoints for specific chromosome rearrangements are often located at or near loci for proto-oncogenes (24, 25), consistent with the hypothesis that such lesions may alter the regulation of the oncogene products and promote unrestrained cell proliferation. Thus, cytogenetic aberrations like translocations, deletions, inversions, addition, or loss represent a gross change in the DNA and logically precede molecular genetic studies.
Genetic Susceptibility to Lung Cancer

Deletions of chromosomal material from chromosome 9 were also observed in other tumor types, including lung cancer. We observed structural aberrations in chromosome 9 in 43.8% of lung cancer patients, mainly at 9p13-q13 (Fig. 2). Lukeis et al. (37) reported loss of chromosome 9p in NSCLC and some 9p have been reported in melanoma, glioma, lung cancer, and certain leukemias (27–29). Merlo et al. (30, 31) have reported homozygous deletion on chromosome 9p in SCLC and NSCLC and loss of heterozygosity on 9q in human SCLC. Kamb et al. (26) performed studies on 290 cell lines derived from various tumor types and noted that, except for colon cancer and neuroblastoma, deletions in chromosome 9 were observed in all other tumor types, including lung. We observed alterations in chromosome 9 in 43.8% of lung cancer patients, mainly at 9p13–q13 (Fig. 2). Lukeis et al. (32) reported the loss of genetic material from 9p (9p22–9q13) in 90% of NSCLCs. Abnormalities involving translocation or loss of material from 9p have been reported frequently in NSCLC and cell lines (33, 34). Consistent numerical and structural abnormalities in chromosome 21 have been noted in lung cancers (32, 35). We found frequent loss of chromosome 21 in the lymphocytes of lung cancer patients, while structural aberrations were relatively fewer in this chromosome.

Studies of normal bronchial epithelium have demonstrated frequent involvement of chromosome 7 (36). Lee et al. (37) suggested a key role for chromosome 7 in the early stages of lung cancer. These reports have great relevance to our present findings. Involvement of chromosome 7 in numerical and structural rearrangements in PBLs was observed in 42.7% of the lung cancer patients. The breakpoints clustered in region 7q12–q13, 7q22, and 7q32 (Fig. 2). Among others who reported frequent involvement of chromosome 7 are Lukeis et al. (32), Whang-Peng et al. (38), and Nieuwint et al. (39) showing 7p11,

The chromosomes involved in structural or numerical aberrations in more than 15% of the patients were considered as important targets. These were chromosomes 1, 3, 5, 7, 9, 12, 14, and 21. The case-control analyses showed that the aberrations were significantly higher in chromosomes 7, 9, 12, and 21 (Table 3). However, in the multivariate analyses after adjusting for age, sex, ethnicity, and smoking, only chromosomes 9 and 21 retained significance (Table 5). Our data are in concordance with other cytogenetic and molecular findings of oncogene activation or tumor suppressor gene inactivation.

Recent reports suggest the presence of a putative tumor suppressor gene MTS-1 in proximity, almost within the IFN gene cluster, at chromosome 9p21 (26). Deletions of chromosome 9p have been reported in melanoma, glioma, lung cancer, and certain leukemias (27–29). Merlo et al. (30, 31) have reported homozygous deletion on chromosome 9p in SCLC and NSCLC and loss of heterozygosity on 9q in human SCLC. Kamb et al. (26) performed studies on 290 cell lines derived from various tumor types and noted that, except for colon cancer and neuroblastoma, deletions in chromosome 9 were observed in all other tumor types, including lung. We observed alterations in chromosome 9 in 43.8% of lung cancer patients, mainly at 9p13–q13 (Fig. 2). Lukeis et al. (32) reported the loss of genetic material from 9p (9p22–9q13) in 90% of NSCLCs. Abnormalities involving translocation or loss of material from 9p have been reported frequently in NSCLC and cell lines (33, 34). Consistent numerical and structural abnormalities in chromosome 21 have been noted in lung cancers (32, 35). We found frequent loss of chromosome 21 in the lymphocytes of lung cancer patients, while structural aberrations were relatively fewer in this chromosome.

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Fig. 1. Representative structural rearrangements involving chromosomes 1, 3, 5, 7, 9, 12, 14, and 21 from the lymphocytes of lung cancer patients. In each case, the altered chromosome is arranged on the left side and the normal homologue on the right side. Both chromatid- and chromosome-type aberrations, including some dicentrics and pericentric inversions in chromosome 9, have been recorded.

Table 6  Frequent chromosome abnormalities in lymphocytes of patients with different histological types of lung cancer.

<table>
<thead>
<tr>
<th>Histological types</th>
<th>Chromosomes</th>
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<tbody>
<tr>
<td></td>
<td>3 5 7 9 12 14 21</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>9 (33) 6 (22) 17 (63) 14 (52) 9 (33) 4 (15) 3 (11)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>10 (37) 9 (27) 12 (36) 12 (36) 7 (21) 8 (24) 9 (27)</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>11 (33) 12 (36) 7 (21) 8 (24) 9 (27)</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>12 (44) 2 (22) 6 (67) 1 (11) 1 (11) 2 (20)</td>
</tr>
</tbody>
</table>

* Figures in parentheses represent percentage (%).

There are relatively few reports highlighting rearrangements in chromosome 12 in lung cancers (35, 41, 43). However, some investigators have emphasized the importance of K-ras oncogene activation in lung carcinoma (44, 45). In our observation, not only have we found frequent involvement of chromosome 12 but more breakpoints are observed at 12p21, the location of the K-ras oncogene.

Many reports indicate that the pattern of chromosomes involved appears to vary between SCLC and NSCLC (40, 42, 7q1, and 7q22 as target regions, respectively. Polysomy of chromosome 7, either whole or in part, has also been observed in lung cancer (33, 40-42). Pastorino et al. (43) reported frequent involvement of chromosome 7 and an overexpression of epidermal growth factor receptor in the complex karyotypic rearrangements of lung tumor specimens. Stable overrepresentation of chromosome 7 has been noted by Erdel et al. (35) in squamous cell carcinoma and adenocarcinoma cell lines of human lung cancers.
Patients

Controls

Fig. 2. Distribution of breakpoints in selected lymphocytic chromosomes (1, 3, 5, 7, 9, 12, 14, and 21) of lung cancer patients and controls. Note the clustering of breaks in chromosomes 1p13 to q21; 3p21 to q13; 7p12 to q12; 7q22; and 7q32; 9p13 to q13; 12p13; and 14q11 and 14q32 of lung cancer patients. Each ▲ represents a single event. An additional 15 events in chromosome 9q13 in the patients are represented as (▲)5.

46–48). Some even point toward differences in chromosomal aberrations between the two major types of NSCLC, adenocarcinoma and squamous cell carcinoma (49). Although the number of SCLC patients studied in this investigation are fewer compared to those of NSCLC, it is clear (Table 6) that chromosomes 1, 7, and 9 are most likely involved in changes in NSCLC. Furthermore, while aberrations in chromosome 1 were fairly consistent in all histological types, abnormalities in chromosomes 7 and 9 were more frequent in squamous cell carcinoma than in adenocarcinomas. Interestingly, these are the chromosomes reported to be rearranged more frequently in NSCLC tumors and their cell lines (32, 33, 35, 38, 40–43, 50). Inexplicably, chromosome 3 was represented more in aberrations in NSCLC than in SCLC.

It could be argued that finding a specific abnormality in the PBLS of lung cancer patients may reflect the consequence of the disease status. The involvement of chromosome 7 in the normal bronchial epithelium or in normal nonmalignant lung tissue has been implicated as a primary chromosomal event by some investigators (36, 37, 43). We have also observed that chromosome 5, which is considered to be the primary event in the sequence of events occurring in colon cancer, was frequently observed to be altered, even in the lymphocytes of disease-free, first-degree relatives of adenomatous polyps or colon cancer patients (12–14). These results suggest that the presence of a chromosome aberration in lymphocytes (the non-target tissue) might represent the cause and not a consequence of the disease.

This relatively simple and inexpensive approach holds a promising future in determining which individuals are at a higher risk for developing lung cancer. However, it will be meaningful to conduct long-term surveillance of the comparison subjects with specific chromosome rearrangements to validate these aberrations as predictors of cancer risk.

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References

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