**Advances in Brief**

**Genetic Analysis of Second Primary Lung Cancers in Patients Surviving Small Cell Lung Cancer**

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**Abstract**

We performed genetic analysis on 12 second primary non-small cell lung cancers in patients surviving small cell lung cancer to assess the potential contribution of smoking to the development of these tumors. Mutations of TP53 were found in three (25%) tumors, KRAS2 in three (25%) tumors, and CDKN2 in two (18%) tumors. Four (50%) mutations (one each in TP53 and CDKN2 and two in KRAS2) were G:C to T:A transversions on the coding strand, a mutation accounting for approximately one-third of mutations in smoking-related tumors but uncommonly found in lung cancers not associated with smoking. The genetic changes in these second lung cancers are more representative of smoking-associated malignancies than lung cancers arising in patients occupationally exposed to irradiation and atomic bomb survivors.

**Introduction**

Combined chemotherapy and chest radiotherapy has been increasingly used over the past 2 decades for patients with SCLC because it prolongs survival compared to chemotherapy alone (1). Patients treated with chest radiotherapy have been found to have a 4-fold greater risk of developing a second cancer compared to patients treated with chemotherapy alone (2). More recently, combined chemotherapy and chest radiotherapy for patients with locally advanced non-SCLC and adjuvant systemic chemotherapy and breast irradiation for patients with localized breast cancer have been more commonly used. Second primary tumors are likely to be seen with increasing frequency in these patients who have multiple risk factors for the development of second cancers.

SCLC is the histological subtype of lung cancer most strongly associated with smoking. Patients surviving 2 years after SCLC are at risk for death from recurrent SCLC, second primary cancers, and other nonmalignant, smoking-related diseases. Lung cancer is the most common second primary cancer in these patients (2). Two-year cancer-free survivors of SCLC have a 10-fold increased risk of lung cancer, and this risk increases to 24-fold after 10 years from diagnosis (2). In addition to smoking, survivors of SCLC have additional potential risk factors for lung cancer, including treatment-related factors (chemotherapy and frequently chest radiotherapy), as well as possible genetic predisposition.

Approximately one-third of mutations of the TP53 gene and the RAS gene family in lung cancer are G:C to T:A transversions on the coding strand (3). This change is thought to be due to the mutagenic action of components of tobacco smoke (3). Cancers not associated with smoking, including lung cancer in nonsmokers, uncommonly have these changes (3). Additional genetic loci have been found to be altered in lung cancer, including the CDKN2 gene (4), and have a similar pattern of point mutations as found in TP53 and the RAS gene family. Analysis of multiple genetic loci provides more data than analysis of a single locus from the same number of tumor samples. We have performed genetic analysis of five loci in second primary lung cancers of patients surviving SCLC to determine whether mutations in these cancers are characteristic of those attributed to smoking.

**Patients and Methods**

Among 611 2-year cancer-free survivors of SCLC included in the North American cohort study, 50 patients developed non-SCLC (2). An additional patient developed non-SCLC since the time of the original report. Nineteen tumors were available, and of these, 12 samples contained amplifiable DNA sufficient for genetic analysis. Clinical information was available for these 12 patients. Tumor samples of the initial SCLCs were available from two patients. DNA extracted from formalin-fixed, paraffin-embedded tissues was screened for mutations of TP53, CDKN2, KRAS2, HRAS1, and NRAS using PCR-RFLP (5). Samples containing mutations were subjected to DNA sequence analysis (5). Statistical significance of comparisons was analyzed using the two-sided Fisher's exact test.

**Results and Discussion**

There were 10 men and 2 women; median age at diagnosis of SCLC was 59 years (range, 46–60 years). Ten patients had limited stage. All patients were initially treated with multimodality chemotherapy and 10 had received chest radiotherapy. The median can...
**Table 1** Characteristics of 12 patients developing second primary lung cancers after SCLC

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age at diagnosis</th>
<th>Stage</th>
<th>Cancer-free interval (yr)</th>
<th>Chest radiotherapy</th>
<th>Tobacco (pack-yr)</th>
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<td>46</td>
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<td>15</td>
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<td>30</td>
</tr>
<tr>
<td>2</td>
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<td>L</td>
<td>13</td>
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<td>40</td>
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<td>L</td>
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<td>70</td>
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<td>57</td>
<td>L</td>
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<td>yes</td>
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<tr>
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<td>L</td>
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<td>8</td>
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<td>7</td>
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<td>L</td>
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<td>64</td>
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<td>4</td>
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<td>50</td>
</tr>
</tbody>
</table>

Median: 59
Range: 46-69

a E, extensive; L, limited.

b Patient 6 smoked an unknown amount after diagnosis of SCLC.

cancer-free interval was 7.5 years (Table 1). All patients smoked cigarettes prior to diagnosis of SCLC with a median of 58 pack-years (range, 27-90). Five patients continued to smoke cigarettes after diagnosis of SCLC. The tumors were predominantly squamous histology with four adenocarcinomas (Table 2).

Five of the 12 tumors contained a total of 8 mutations: 3 (25%) in TP53, 3 (25%) in KRAS2, and 2 (18%) in CDKN2 (Table 2). No mutations were found in HRAS1 or NRAS. The frequency of mutations was not different from that of all lung cancers, or for TP53, from that found in lung cancers of nonsmokers. Four of eight (50%) mutations were G:C to T:A transversions on the coding strand, one each in TP53 and CDKN2, and two in KRAS2. No mutations were found at codon 249 of TP53. Seven patients had no mutations detected. Patients 1 and 10 had two mutations each, including three of the four G:C to T:A transversions. The remaining G:C to T:A transversion was found in patient 4. Patient 1 had the longest cancer-free interval (15 years) but continued to smoke cigarettes during this time. Patients 4 and 10 had cancer-free intervals of 8 and 4 years, respectively, and both patients stopped smoking at initial diagnosis after 90 pack-years. No mutations were detected in the two patients (patients 5 and 9) not treated with chest radiotherapy.

TP53 and the three RAS loci was also performed on the initial SCLC tumor from patients 4 and 12. A G:C to T:A transversion of TP53 (codon 298, GAG → TAG) was detected in the initial SCLC tumor from patient 4, whereas no mutation was found in TP53 of patient 12 or in any RAS gene from either patient. These results indicate that second lung cancers after treatment of SCLC arise independently of the initial SCLC. Similar findings have been described for second primary lung cancers occurring after cancers of the head and neck [7] and after non-SCLC [5, 8].

The frequency of G:C to T:A transversions on the coding strand in the second lung tumors of these 12 patients (50%) is significantly greater than the frequency of this mutation found in TP53 of lung cancers not associated with smoking (0 of 16 G:C to T:A transversions on the coding strand; Ref. 3; P < 0.01). This finding is consistent with tobacco smoking contributing to the development of these cancers and agrees with the approximately 4-fold increased risk for the development of a second
cancer among patients surviving SCLC who continue to smoke relative to patients who stop smoking (2). However, the presence of G:C to T:A transversions on the coding strand in the tumors of two patients who stopped smoking at the time of initial diagnosis of SCLC and had cancer-free intervals of 8 and 4 years suggests that the genetic changes resulting in second primary lung cancers in these patients has already occurred prior to treatment for SCLC.

Genetic alterations associated with tumors developing after use of therapeutic radiotherapy have not been reported. However, lung cancers associated with occupational exposure to radon gas in one study of uranium miners (9), most of whom smoked, and in nonsmoking survivors of atomic bomb blasts (10) had no G:C to T:A transversions on the coding strand. A second study of uranium miners exposed to about 5-fold higher level of radon gas in a different geographic region of the United States showed that about two-thirds of TP53 mutations were G:C to T:A transversions on the coding strand (11). However, 16 of 19 of these mutations were at codon 249, a site rarely mutated in any cancer except those hepatocellular carcinomas associated with the fungal toxin, aflatoxin B1. One proposed explanation for these unusual mutations is the presence of a mycotoxin in the mines of the second study of uranium miners (12). Thus, it appears that lung cancers associated with low-level exposure to irradiation have a different pattern of mutation than that found in the current study.

Patients with SCLC are also exposed to potentially carcinogenic chemotherapeutic agents. Workers occupationally exposed to mustard gas, an alkylating agent, have an increased risk for lung cancer (13). The frequency of TP53 mutations in lung cancers of these patients is similar to that found in lung cancers not associated with mustard gas exposure. However, four of eight (50%) of the mutations in lung cancers of patients exposed to mustard gas were G:C to A:T transitions, including two tumors that contained two of these transitions (13). None of the transitions were at CpG dinucleotides, a characteristic finding for spontaneous mutation. Fifteen % of TP53 mutations in lung cancers are G:C to A:T transitions at non-CpG sites (3). None of the eight mutations in our study were of this type (Table 2), although the difference between the frequency of G:C to A:T transitions in our study and that found in lung cancers of mustard gas workers did not quite reach statistical significance ($P = 0.08$) due to the small number of samples in both studies.

Analysis of additional samples and/or genetic loci may find a different pattern of mutations in subgroups of patients surviving SCLC and implicate other potential risk factors, such as chemotherapy, radiotherapy, and genetic factors. Studies of genetic alterations in second primary tumors may have implications in several settings. In addition to patients with SCLC, many patients with Hodgkin’s disease, breast cancer, and advanced non-SCLC have multiple risk factors for development of metachronous lung cancers, including cigarette smoking, treatment with chemotherapy, and exposure to therapeutic radiotherapy. A clearer understanding of the factors contributing to the development of these tumors may lead to strategies to modulate these risks and decrease the rate of development of these tumors.

References


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