Abstract  Purpose: KRAS mutations are found in ~25% of lung adenocarcinomas in Western countries and, as a group, have been strongly associated with cigarette smoking. These mutations are predictive of poor prognosis in resected disease as well as resistance to treatment with erlotinib or gefitinib.

Experimental Design: We determined the frequency and type of KRAS codon 12 and 13 mutations and characterized their association with cigarette smoking history in patients with lung adenocarcinomas.

Results: KRAS mutational analysis was done on 482 lung adenocarcinomas, 81 (17%) of which were obtained from patients who had never smoked cigarettes. KRAS mutations were found in 15% (12 of 81; 95% confidence intervals, 8-24%) of tumors from never smokers. Similarly, 22% (69 of 316; 95% confidence intervals, 17-27%) of tumors from former smokers, and 25% (21 of 85; 95% confidence intervals, 16-35%) of tumors from current smokers had KRAS mutations. The frequency of KRAS mutation was not associated with age, gender, or smoking history. The number of pack years of cigarette smoking did not predict an increased likelihood of KRAS mutations. Never smokers were significantly more likely than current or former smokers to have a transition mutation (G→A) rather than the transversion mutations known to be smoking-related (G→T or G→C; P < 0.0001).

Conclusions: Based on our data, KRAS mutations are not rare among never smokers with lung adenocarcinoma and such patients have a distinct KRAS mutation profile. The etiologic and biological heterogeneity of KRAS mutant lung adenocarcinomas is worthy of further study.

Since the identification of somatic epidermal growth factor receptor (EGFR) mutations, there has been heightened interest in the molecular basis of lung cancer in patients who never smoked cigarettes (1–3). Somatic mutations in EGFR have been identified in ~15% of all patients with lung adenocarcinoma, with the proportion increasing to 50% in patients who never smoked cigarettes. There is an inverse relationship between cigarette smoking history and frequency of EGFR mutations, with the frequency of EGFR mutations decreasing significantly among patients who smoked more than 15 pack years (4). Such refined understanding of the relationship between smoking history and presence of EGFR mutations has allowed the design of clinical trials which use smoking history to enrich the selection of patients for enrollment. In contrast to EGFR mutations, KRAS mutations were initially identified in patients with lung adenocarcinoma who had a history of heavy cigarette smoking and were thought to be uncommon in patients without a history of smoking cigarettes (5). These mutations are found in ~25% of lung adenocarcinomas in western countries but are less common in Asian populations (6, 7). KRAS mutations have been associated with poor prognosis in resected non–small cell lung cancer (NSCLC; refs. 11–13), lack of survival benefit from adjuvant chemotherapy (14), and resistance to erlotinib or gefitinib (15). More than 95% of KRAS mutations in lung cancer occur in codons 12 and 13. In both KRAS and TP53, transversions (substituting a pyrimidine for a purine or purine for a pyrimidine) are more common than transitions (substituting purine for purine or pyrimidine for pyrimidine) identifying a molecular signature for the carcinogenic effects of cigarette smoke (16, 17). A detailed analysis of KRAS mutations in relation to smoking history has not been done. Using a cohort of patients with lung adenocarcinoma, we sought to determine the frequency and type of KRAS mutations in a large series of patients with known smoking histories.

Materials and Methods

Tumor specimens were obtained from an institutional tumor bank of patients who had undergone NSCLC resections between 2002 and 2007, as well as from patients with metastatic NSCLC who had KRAS mutation testing done as part of clinical trials or during routine clinical practice. Because KRAS mutations are rare in squamous tumors, only...
Mutations in the KRAS oncogene are found in ~25% of lung adenocarcinomas in Western countries. Studies have linked KRAS mutations with poor prognosis in non–small cell lung cancer as well as resistance to treatment with erlotinib or gefitinib. These mutations have been reported to be strongly associated with cigarette smoking. However, previous studies which explored the association of smoking with KRAS mutation did not include large numbers of patients who never smoked cigarettes. We report that KRAS mutations are found in 15% of lung adenocarcinomas from patients who never smoked cigarettes compared with 22% in patients with a history of smoking cigarettes, a statistically insignificant difference in this study. Furthermore, the frequency of KRAS mutation was not associated with age, gender, or smoking history, making it difficult to predict which tumors have KRAS mutations by any clinical characteristics. Based on these data, we believe that molecular testing for KRAS mutations is necessary to identify this subgroup of patients with a different response to some treatments.

In 482 lung adenocarcinomas, KRAS mutations in codons 12 or 13 were found in 21% (102 of 482; 95% confidence intervals, 18-25%). Patients whose tumors harbored KRAS mutations were not significantly different from patients with KRAS wild-type tumors with regard to gender, age, or prior smoking history (Table 1). KRAS mutations were identified in 15% (12 of 81; 95% confidence intervals, 8-24%) of never smokers, 22% (69 of 316; 95% confidence intervals, 17-27%) of former smokers, and 25% (21 of 85; 95% confidence intervals, 16-35%) of current smokers (Fig. 1). No tumor with a KRAS mutation had a mutation in EGFR. There were no significant differences in frequency of KRAS mutations by category of smoking history (Mantel-Haenszel \( \chi^2 P = 0.12 \)).

Next, we examined the frequency of KRAS mutation by pack years of cigarette smoking (Table 2), and found no trend (Mantel-Haenszel \( \chi^2 P = 0.19 \)). To determine whether there was a cutoff for pack years of cigarette smoking above which KRAS mutations were more frequent, a receiver operating characteristic curve was generated. The area under the receiver operating characteristic curve was 0.56 (data not shown) suggesting no value in using pack years of cigarette smoking to predict KRAS mutational status.

To determine whether the type of KRAS mutation identified in never smokers correlated with the previously described dominance of transversions in smoking-associated cancers, we compared the type of KRAS mutation found in never smokers to those found in former or current smokers (Table 3). Never smokers were significantly more likely (Fisher’s exact test \( P < 0.0001 \)) to have a transition mutation. The ratio of transition/transversion for never smokers was 17:73 for former or current smokers.

In these patients with lung adenocarcinoma, we find that KRAS mutations are not rare in never smokers. This is a striking finding given the widespread perception that cigarette smoking and KRAS mutations are invariably linked (reviewed in ref. 16). The association between cigarette smoking and KRAS mutations has been inferred from a number of series that included a relatively small numbers of patients who never smoked cigarettes. For example, Nelson et al. examined tumors from 365 patients with NSCLC, of which only 22 were never smokers (18). Among the patients in that series in which KRAS mutational analysis was done, there were only 16 never smokers, none of whom had KRAS mutations. However, another series which included some never smokers did identify KRAS mutation in 14% (3 of 21) of never smokers (19). A difference between our series and previous series is the method
of collection of smoking history. We determined smoking history using prospectively collected smoking questionnaires completed by patients with a diagnosis of lung cancer. These patients completed a detailed questionnaire which included the age of onset of smoking, the average number of cigarettes per day, the number of years in which they smoked cigarettes, and the time that the patient quit smoking cigarettes. The characteristics of patients included in this analysis are similar to the patient population seen at our institution with regard to age, gender, and smoking history.

The KRAS mutations observed in these never smokers, in addition to being more frequent than previously reported, are more likely to be transitions, unlike the transversions more common in patients with a history of cigarette smoking. In both KRAS and TP53, transversions (substituting a pyrimidine for a purine, or purine for a pyrimidine) are more common than transitions (substituting purine for purine, or pyrimidine for pyrimidine; refs. 16, 17). The etiology of G→T transversions in tumors from patients with lung cancer is thought to be related to exposure to polycyclic aromatic hydrocarbons found in cigarette smoke (20). In the case of TP53, investigators have recently noted that TP53 G→T transversions were distinctly uncommon in lung adenocarcinomas with EGFR mutations, a mutation more commonly seen in never smokers (21).

Because patients without smoking history represent ~15% of patients with lung cancer, it is critical that any analysis seeking to examine the biology of these tumors examine a relatively large number of patients with NSCLC (22, 23). Relatively little is understood about the biology and epidemiology of lung cancer in never smokers. A number of possible causative factors have been suggested including exposure to environmental tobacco smoke or radon, as well as genetic and hormonal abnormalities (reviewed in refs. 24, 25). The distinct profile of KRAS mutations observed here in never smokers further suggests that such cancers are only rarely caused by environmental tobacco exposure. Whether this etiologic heterogeneity within KRAS mutant lung adenocarcinomas is associated with differences in cooperating genetic lesions and overall biological behavior warrants further investigation.

Finally, because tumors from never smokers may have KRAS mutations, and such mutations have been associated with resistance to erlotinib and gefitinib (15), molecular analysis of NSCLC specimens for KRAS mutations may improve a clinician’s ability to predict response and resistance to therapy with erlotinib or gefitinib.

Table 2. Frequency of KRAS mutation by smoking history in pack years

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Wild-type</th>
<th>Total</th>
<th>Frequency (%)</th>
<th>95% Confidence intervals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never smokers</td>
<td>12</td>
<td>69</td>
<td>81</td>
<td>15</td>
</tr>
<tr>
<td>&lt;5 py</td>
<td>3</td>
<td>25</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>6-10 py</td>
<td>3</td>
<td>25</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>11-15 py</td>
<td>6</td>
<td>13</td>
<td>19</td>
<td>32</td>
</tr>
<tr>
<td>16-25 py</td>
<td>10</td>
<td>45</td>
<td>55</td>
<td>18</td>
</tr>
<tr>
<td>26-50 py</td>
<td>40</td>
<td>106</td>
<td>146</td>
<td>27</td>
</tr>
<tr>
<td>51-75 py</td>
<td>16</td>
<td>51</td>
<td>67</td>
<td>24</td>
</tr>
<tr>
<td>&gt;75 py</td>
<td>12</td>
<td>46</td>
<td>58</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>380</td>
<td>482</td>
<td>21</td>
</tr>
</tbody>
</table>

Abbreviation: py, pack years.

Table 3. KRAS mutation type as a function of smoking history

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Nucleotide</th>
<th>Former/current</th>
<th>Never</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>G12A</td>
<td>GGT→GCT</td>
<td>13</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>G12C</td>
<td>GGT→TGT</td>
<td>38</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>G12V</td>
<td>GGT→GTT</td>
<td>20</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>G13C</td>
<td>GGC→TGC</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>G13D</td>
<td>GGC→GAC</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>G12D</td>
<td>GGT→GAT</td>
<td>15</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>G12S</td>
<td>GGT→AGT</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Frequency of KRAS mutation by smoking history. Mantel-Haenszel χ² test for trend was used to calculate P value.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
References
Frequency and Distinctive Spectrum of \textit{KRAS} Mutations in Never Smokers with Lung Adenocarcinoma

Gregory J. Riely, Mark G. Kris, Daniel Rosenbaum, et al.


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