Benzo[a]pyrene Diol Epoxide-induced 3p21.3 Aberrations and Genetic Predisposition to Lung Cancer

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Abstract

3p deletion, a common chromosome defect in lung cancer, occurs more frequently in the lung tumor tissues of smoking patients than it does in those of nonsmoking patients. This pilot study evaluated whether 3p aberrations induced by benzo[a]pyrene diol epoxide (BPDE), the metabolic product of benzo[a]pyrene, a constituent of tobacco smoke, were more common in the peripheral blood lymphocytes of 40 lung cancer patients than they were in those of 54 matched controls. Our hypothesis was that 3p sensitivity to BPDE reflects the susceptibility of a specific locus to damage from carcinogens in tobacco smoke. BPDE-induced chromosome 3p21.3 aberrations were significantly more frequent in cases (34.1 per 1000) than they were in controls (22.1 per 1000; P < 0.0001). However, no such difference was observed for 6q27, a control locus. Using the median value in the controls (20 per 1000) as a cutoff point to classify BPDE-induced sensitivity at 3p21.3 and after adjustment by age, sex, ethnicity, and smoking status, 3p BPDE sensitivity was associated with an elevated risk of 14.1 (95% confidence interval: 3.5, 56.2) for lung cancer.

Subjects and Methods

Study Subjects. Cases and controls were identified from two ongoing molecular epidemiological studies of lung cancer that have been described previously (11). The cases were patients with newly diagnosed, histologically confirmed lung cancer who were registered at the Departments of Thoracic Surgery and Thoracic Medical Oncology at the University of Texas M. D. Anderson Cancer Center. There were no age, sex, ethnic, or stage restrictions. We chose to study previously untreated patients to minimize the potential confounding influence of radiotherapy and chemotherapy on the chromosomal analyses. Patients were referred to our center for definitive treatment. The study coordinator conducted a daily review of computerized appointment schedules for the hospital outpatient clinics that serve lung cancer patients to enroll patients before initiation of therapy. Once a patient was determined to be eligible for the study, an appointment would be made for an in-person interview, to be conducted at the time of the next follow-up appointment.

Controls without prior history of cancer (except nonmelanoma skin cancer) were identified from a control pool database that has been described previously (11). Briefly, this potential control database was derived from subscribers to a large private multispecialty provider (Kelsey-Seybold Clinic), which includes a health-maintenance organization, capitated patients, and fee-for-service components in the Houston metropolitan area. Thus far, we have 39,264 respondents in our potential control database. After each case was identified, a list of five controls was generated from the potential control database by computer. The randomly selected control subject would be contacted by telephone to confirm his/her willingness to participate. If the person refused to participate or was deemed ineligible, another potential control would be selected from the potential control pool. Controls were frequency matched to the cases by sex, age (±5 years), ethnicity, and cigarette smoking status. An appointment would be scheduled at a Kelsey-Seybold clinic site that was convenient to the participant. Each participating control subject was given a $30.00 gift certificate for use at a local grocery store and parking validation.

Each participant was asked to sign an informed consent form for the blood drawing and for completion of a personal interview (conducted by M. D. Anderson Cancer Center staff) that lasted about 1 h. Epidemiological data, including demographic information, smoking status, and family history of cancer information, were collected by personal interview. The blood samples were delivered to the epidemiology laboratory by the interviewer.

BPDE Sensitivity Assay. (1/-)-anti-BPDE was purchased from Midwest Research Institute (Kansas City, MO). We used tetrahydrofuran (Sigma Chemical Co., St. Louis, MO) as the solvent. The 1 mM stock was aliquoted into microcentrifuge tubes (500 µl each) and stored at -20°C in the dark. Lymphocyte cultures were established as follows: whole blood (1 ml) was cultured in 9 ml of RPMI 1640 tissue culture medium (JRM Biosciences, Lenexa, KS) with 10% FCS and 1% phytohemagglutinin (Wellcome Research Laboratories, Research Triangle Park, NC) at 37°C for 72 h. BPDE was then added to each culture to a final concentration of 2 µM for 24 h. After routine blocking with colcemid, hypotonic treatment, and fixation, cell suspensions were stored at -20°C until they were processed for FISH experiments.

3p aberrations were detected by FISH with a 3p21.3-specific DNA probe for the human semaphorin IV gene (approximately 75 kb; Oncor Inc., Gaithers-
controls and also to study it as a continuous variable. Crude and adjusted ORs were calculated and compared in terms of smoking status or other smoking variables. In Results and Discussion, sex, and age. There were no significant differences between cases and controls concurrently. The posthybridization wash was in 2X SSC (pH 7.0) for 5 min at 72°C without agitation. Signals were detected by incubating the slides for 10 min at 37°C in FITC-conjugated avidin (Oncor Inc.) and then washing the slides in 1X BST buffer (0.5 M NaHCO3, 1.5 M NaCl, and 0.25% Tween 20 (pH 8.0)) three times for 3 min each time. If a signal was weak, it was amplified by incubation with antiavidin antibody for 10 min at 37°C. Then the slides were washed in 1X BST again, incubated with FITC-antiavidin antibody for 10 min at 37°C, and then washed in 1X BST buffer again. Finally, the slides were counterstained with propidium iodide/antifade, the cells were viewed through a fluorescent microscope (Leeds Inc., Irving, TX) with individual FITC filters, and the images were captured with an Image System (PSI, Houston, TX).

The following scoring criteria were applied to the FISH signals: (a) nuclei did not overlap; (b) signal intensity was about the same; (c) minor hybridization spots, which are smaller and less intense than real signals, were excluded; (d) signals were only counted when they were completely separate from each other; and (e) paired or close signals were counted as one signal. The laboratory personnel who scored the 3p aberrations did not know the samples’ case-control status. The most common abnormal signals were one signal and three signals, which may reflect deletion and translocation, respectively.

Statistical Analysis. Laboratory and questionnaire data for this project were merged and edited, and clean system files were created by using SAS statistical software packages. We examined the association between 3p BPDE sensitivity, assessed as the number of chromosome 3p21.3 aberrations in 1000 interphases, and risk of lung cancer in the case-control study. Our analytic approach was to dichotomize 3p BPDE sensitivity at the median value for controls and from 5 per 1000 to 57 per 1000 (mean: 22.1 ±10.7 per 1000) for controls (P < 0.0001). In contrast, no difference in BPDE-induced chromosome 3p21.3 aberrations was observed at 6q27, a control locus: the number of BPDE-induced chromosome 6q27 aberrations ranged from 6 per 1000 to 18 per 1000 (mean: 10.9 ± 3.8 per 1000).

Results

This study comprised 40 lung cancer cases and 54 controls (Table 1). The cases and controls were well matched with respect to ethnic¬ity, sex, and age. There were no significant differences between cases and controls in terms of smoking status or other smoking variables. In terms of histological distribution, there were 3 patients (7.5%) with small cell lung cancer, 6 (15.0%) with squamous cell lung cancer, 21 (52.5%) with adenocarcinoma, 1 (2.5%) with large cell lung cancer carcinoma, and 9 (22.5%) with nondifferentiated small cell lung cancer. Stage information was only available for 30 cases. Among these cases, about 27% were stage I and II.

The number of BPDE-induced chromosome 3p21.3 aberrations ranged from 15 per 1000 to 54 per 1000 (mean: 34.1 ± 9.7 per 1000) for cases and from 5 per 1000 to 57 per 1000 (mean: 22.1 ± 10.7 per 1000) for controls (P < 0.0001). In contrast, no difference in BPDE-induced chromosome aberrations was observed at 6q27, a control locus: the number of BPDE-induced chromosome 6q27 aberrations ranged from 6 per 1000 to 18 per 1000 (mean: 10.9 ± 3.8 per 1000).

Table 1 Distribution of selected characteristics of lung cancer cases and controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Casesa</th>
<th>Controlsa</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Non-Hispanic white</td>
<td>33 (82.50)</td>
<td>43 (79.63)</td>
<td></td>
</tr>
<tr>
<td>Mexican-American</td>
<td>2 (5.00)</td>
<td>6 (11.11)</td>
<td>0.53</td>
</tr>
<tr>
<td>African-American</td>
<td>5 (12.50)</td>
<td>5 (9.26)</td>
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</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (42.50)</td>
<td>28 (51.85)</td>
<td>0.37</td>
</tr>
<tr>
<td>Female</td>
<td>23 (57.50)</td>
<td>26 (48.15)</td>
<td></td>
</tr>
<tr>
<td>Mean age, yr (SD)</td>
<td>62.70 (9.44)</td>
<td>60.48 (9.64)</td>
<td>0.27</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1 (2.50)</td>
<td>3 (5.88)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>20 (50.00)</td>
<td>22 (43.14)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>19 (47.50)</td>
<td>26 (50.98)</td>
<td>0.65</td>
</tr>
<tr>
<td>Number of cigarettes/day (SD)</td>
<td>26.79 (16.83)</td>
<td>23.44 (13.97)</td>
<td>0.37</td>
</tr>
<tr>
<td>Years smoked (SD)</td>
<td>33.82 (15.33)</td>
<td>33.03 (15.33)</td>
<td>0.83</td>
</tr>
<tr>
<td>Pack-years smoked (SD)</td>
<td>50.33 (37.15)</td>
<td>43.38 (34.47)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

a Values are no. (%), unless otherwise noted.
BPDE-INDUCED 3p21.3 CHANGES AND LUNG CANCER

<table>
<thead>
<tr>
<th>3p21.3 BPDE sensitivity</th>
<th>No. (%)</th>
<th>OR (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>Univariate</td>
<td>Adjusted*</td>
<td></td>
</tr>
<tr>
<td>Non-sensitive</td>
<td>3 (7.50)</td>
<td>26 (48.15)</td>
</tr>
<tr>
<td>Sensitive</td>
<td>37 (92.50)</td>
<td>28 (51.85)</td>
</tr>
</tbody>
</table>
| Dose responsea           | 11.5 (95% CI: 3.3, 38.9) for lung cancer. After adjustment for age, smoking status, and pack-years smoked. There were no significant associations (data not shown).

Discussion

Benzo[a]pyrene has been reported to be one of the most potent in vivo and in vitro carcinogenic compounds in tobacco smoke. Previously, we have demonstrated that bleomycin sensitivity is an excellent cancer risk predictor (12–14). Our data further suggest that sensitivity to BPDE may be a more relevant and more important lung cancer susceptibility marker than is bleomycin sensitivity (4). BPDE sensitivity was associated with a significantly elevated risk for lung cancer, with an OR (95% CI) of 11.5 (95% CI: 3.3, 38.9) for lung cancer. After adjustment for age, sex, ethnicity, and smoking status, the OR for BPDE sensitivity at 3p21.3 was 14.1 (95% CI: 3.5, 56.2).

When we categorized the subjects by the median value and 75th percentile distribution of 3p21.3 aberrations in the controls, there was a significant gradient of increasing risk of lung cancer with increasing number of 3p21.3 aberrations (Table 2). We also assessed the relationship between the 3p21.3 aberration profile and age, sex, ethnicity, smoking status, and pack-years smoked. There were no significant associations (data not shown).

Acknowledgments

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References


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