Reduced DNA Repair Capacity in Lung Cancer Patients

Qingyi Wei, Lie Cheng, Waun Ki Hong, and Margaret R. Spitz

Abstract

Although lung cancer is the paradigm of a tobacco-induced malignancy, host-specific factors modulate susceptibility to tobacco carcinogens. Variations in DNA repair may influence the rate of removal of DNA damage and of fixation of mutations. To test the hypothesis that genetically determined DNA repair capacity (DRC) modulates lung cancer susceptibility, we conducted a pilot case-control study of 51 patients with newly diagnosed, previously untreated lung cancer and 56 controls identified from local community centers and frequency matched to the cases on age, sex, and ethnicity. The subjects were ascertained and interviewed for an ongoing molecular epidemiological investigation of lung cancer susceptibility. We measured DRC in the subjects’ peripheral blood lymphocytes by using the host-cell reactivation assay, which measures cellular reactivation of a reporter gene damaged by exposure to 75 μM benzo(a)pyrene diol epoxide. The mean level of DRC in cases (3.3%) was significantly lower than that in controls (5.1%) (P < 0.01). Only nine cases (18%) had DNA repair levels greater than the median value of repair in the controls. This median level of DRC in controls was used as the cutoff value for calculating the odds ratios. After adjustment for age, sex, ethnicity, and smoking status, the cases were five times more likely than the controls to have reduced DRC (odds ratio, 5.7; 95% confidence interval, 2.1—15.7). Younger cases (<65 years) and smokers were more likely than controls to have reduced DRC. These findings suggest that individuals with reduced DRC are at an increased risk of developing lung cancer.

Introduction

Lung cancer is a leading cause of death by malignancy in the United States (1), and it is the paradigm of a tobacco-induced cancer (2). However, genetic susceptibility, which includes epigenetic factors and gene-environment interaction, to carcinogenesis is also an important determinant of risk (3), because only 10—15% of cigarette smokers develop smoking-related lung cancers (4). This variation in susceptibility to tobacco carcinogens may be due to genetically determined variation in metabolism of carcinogens (5), including the polycyclic aromatic hydrocarbon B(a)P, a classic DNA-damaging carcinogen commonly found in tobacco smoke and in the ambient environment (6). B(a)P bioactivation in vivo by cytochrome P450 and peroxidases generates highly toxic electrophilic and free radical reactive intermediates, such as BPDE, that can irreversibly damage DNA by covalent binding or oxidation (7—9). Evidence from molecular epidemiological studies indicates that smokers often have higher polycyclic aromatic hydrocarbon-DNA adduct levels than do non-smokers (10—12). The levels of DNA adducts detected in nonneoplastic surgical lung parenchymal samples (13) and alveolar macrophages (14) are also higher in smokers than in former smokers, suggesting that tobacco exposure is a source of B(a)P.

Genetically determined DRC may influence the rate of removal of DNA damage and of fixation of mutations (15, 16) and thereby modulate lung cancer susceptibility (17). BPDE-induced DNA adducts are repaired by the nucleotide excision repair pathway (18). The premutagenic lesions induced by BPDE are repaired more efficiently in the transcribed strand than in the untranscribed strand of the hypoxanthine-guanine phosphoribosyltransferase gene, suggesting that BPDE-DNA adducts may block the transcription of essential genes and cause changes in chromatin structure (19). Furthermore, molecular sequencing analyses have consistently shown a high frequency of p53 G to T transversion mutations in tobacco-related human cancers, including lung cancer (20, 21), suggesting an association with exposure to specific carcinogens such as B(a)P in tobacco smoke and the involvement of inefficient DNA repair.

Therefore, we hypothesized that variations in DRC could modulate lung cancer susceptibility. To test this hypothesis, we conducted a pilot molecular epidemiological study in which we measured DRC using a HCR assay of BPDE-damaged plasmids harboring a reporter gene (22, 23). Cryopreserved lymphocytes were available from 51 patients with newly diagnosed, previously untreated lung cancer and 56 cancer-free controls and were tested for DRC. We report here an association between reduced DRC and increased risk of lung cancer.

Materials and Methods

Chemicals and Plasmid Treatment. BPDE (NCl L0137, 99% purity) was purchased from Midwest Research Institute (Kansas City, MO) as a white powder and was completely dissolved in tetrahydrofuran (Sigma Chemical Co., St. Louis, MO). The stock concentration (1 μM, or 0.3 mg/μl) was further diluted for the working solution, which was prepared once in a dark room, aliquoted into microcentrifuge tubes, and kept at —20°C without exposure to air or light. For plasmid treatment, purified plasmid (pCMVcat, a gift from Dr. Lawrence Grossman, The Johns Hopkins University, Baltimore, MD) was dissolved in Tris-EDTA buffer (pH 7.9) at a concentration of 500 μg/μl and 1-ml aliquots of the solution were placed in microcentrifuge tubes. BPDE working solution was added to the tubes to final concentrations of 0, 30, 45, 60, 75, and 90 μM, and the mixtures were incubated for 3 h in a dark room. These treatments were performed in one batch for all HCR assays. After treatment, the plasmids were precipitated three times with 70% ethanol, then dissolved in Tris-EDTA buffer at a final concentration of 50 μg/ml and assessed for conformational changes using 0.8% agarose gel electrophoresis.

Cell Lines. Four EBV-immortalized human lymphoblastoid cell lines from the Human Genetic Mutant Cell Repositories (Camden, NJ) were used: two apparently normal cell lines (GM00892B and GM00131A) and two XP cell lines (GM02345B [XP-A] and GM02346B [XP-C]), with deficient nucleotide excision repair. We used these two types of cell lines to test the sensitivity and specificity of cellular repair of BPDE-induced damage in the plasmids. All of the cells were cultured in T-25 flasks at 37°C in a 5% CO2 atmosphere in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 15% fetal bovine serum (Life Technologies, Inc.).

Study Subjects. The 51 cases included in this study were lung cancer patients who were recruited for an ongoing molecular epidemiological study of...
lung cancer (24, 25) from The University of Texas M. D. Anderson Cancer Center; from county, community, and Veterans Affairs hospitals in the Houston and San Antonio metropolitan areas; and from Galveston, TX between 1994 and 1995, and who provided sufficient additional blood (10–15 ml) required for the HCR assay. The histopathological types of tumors they had included 2 large cell carcinomas, 10 non-small cell carcinomas, 8 small cell carcinomas, 13 squamous cell carcinomas, 9 adenocarcinomas, and 9 unspecified tumors. The 56 apparently healthy control subjects were randomly recruited from community centers, cancer screening programs, and The University of Texas M. D. Anderson Cancer Center blood drives. Each subject completed a short questionnaire that elicited information about demographic variables and smoking history. The exclusion criteria were prior chemotherapy or radiotherapy for cancer, recent infections, and recent blood transfusion. These controls were frequency matched to the cases by age (±5 years), sex, and ethnicity.

HCR Assay. Approximately 10 ml of blood from each subject were drawn into a 15-ml heparinized Vacutainer (Becton Dickinson, Franklin Lakes, NJ). The isolated lymphocytes were frozen in 50% fetal bovine serum, 40% RPMI 1640, and 10% DMSO (Fisher Scientific Co., Pittsburgh, PA) and thawed in batches later for the HCR assays. The thawing medium consisted of 50% fetal bovine serum, 40% RPMI 1640, and 10% dextrose (Sigma Chemical Co.), which ensured a viability of 90% after thawing. The DRC of the cells was measured using the HCR assay as described elsewhere (22, 23). Briefly, the frozen cells in each vial (1.5 ml) were quickly thawed and mixed with 9 ml of thawing medium before the last trace of ice disappeared. After washing with the thawing medium, the cells were incubated in RPMI 1640 supplemented with 20% fetal bovine serum and 112.5 μg/ml phytohemagglutinin (Murex Diagnostics, Norcross, GA) at 37°C for 72 h. For transfection, 2 × 10⁶ cells were transfected using the diethylaminoethyl-dextran (Pharmacia Biotech Inc., Piscataway, NJ) method (26) with approximately 0.25 μg of untreated plasmid or plasmid treated with 75 μM BPDE. The transfections were performed in duplicate for each dose. The chloramphenicol acetyltransferase gene expression (and thus DRC) was measured 40 h after transfection as described previously (22, 23).

Statistical Analysis. DRC was first analyzed as a continuous variable. Student's t test was used to compare the differences between groups. Correlation analyses were performed to compare DRC and selected host factors. For calculation of crude ORs and CIs, the median DRC of controls was used as the cutoff value: values greater than this median were considered high repair and values below the median, low repair. To evaluate the dose-response relationship, the subjects were categorized by tertile of the DRC distribution in the controls. “Ever smokers” were defined as those who had smoked more than 100 cigarettes in their lifetimes, and they were divided into “former smokers,” who were defined as those who had quit smoking for more than 1 year previously, and “current smokers,” who were the others. “Nonsmokers” were those who did not fit the other categories. For logistic regression analysis, the variable smoking status was recoded as a dummy variable (0,0, nonsmoker; 0,1, former smoker; and 1,0, current smoker). A similar approach was applied to the variable ethnicity (0,0, non-Hispanic white; 0,1, Mexican American; and 1,0, African American) and the tertile of DRC (0,0, < 3.0% (lowest tertile); 0,1, 3.0–6.5% (middle tertile); and 1,0, >6.5% (highest tertile)). To assess the trend, the tertile variables were recoded as a continuous variable and fit in the logistical regression model. All of the statistical analyses were performed with Statistical Analysis System software (Version 6; SAS Institute Inc., Cary, NC).

Results

The repair-proficient and -deficient lymphoblastoid cell lines were first tested to determine the appropriate dose of BPDE for assays of PBLs. As shown in Table 1, there were 6–10-fold differences in the DRC of repair-proficient and -deficient cells treated with 30–90 μM BPDE. For example, a dose of 60 μM BPDE reduced the DRC to about 2.1% (±0.9%) in repair-deficient cells compared with about 24.6% (±5.7%) in repair-proficient cells. BPDE doses up to 90 μM did not induce any conformational changes in the plasmid (all were in the supercoiled form I; Fig. 1). To be consistent with previously published data, a dose of 75 μM BPDE was chosen for the assays of PBLs. This dose reduced the DRC to 10–30% in normal cells in a previous study (22), but less than 10% in normal lymphoblastoid cells (mean, 9.7%) in the study reported here (Table 1). It seemed, however, that normal PBLs from the controls (mean, 5.1%; Table 2) were more sensitive to BPDE than the normal lymphoblastoid cells in this study. The blastogenic rates of PBLs after phytohemagglutinin stimulation were mostly between 60 and 70%, and cell growth was similar for the cases and the controls, which is consistent with previous reports (22, 23). From this pilot study, we concluded that a 10-ml blood sample was adequate for a HCR assay using two doses, each performed in duplicate.

Stratified analysis by tumor histological type was performed first to compare the differences in the DRC means of each histological tumor type and the controls. However, 12 cases were diagnosed as non-small cell without further categorization, and the histological types of an additional 9 cases were not specified. We categorized all cases into three types: non-small cell (67%), small cell (16%), and unspecified (18%). As shown in Table 2, although cases in all of the histological categories had lower DRC than the controls, the differences between each category and the controls were statistically significant for non-small cell (P < 0.01) but only marginally significant for small cell and not significant for the unspecified type due to the relatively small sample size. Further analysis of adenocarcinoma, large cell, and squamous cell histological types within the non-small cell category did not show any significant differences in DRC (data not shown).

For the case-control analysis, the mean age was 62.6 years for cases and 61.3 years for controls. Sixty percent of the cases and 68% of the controls were men. The percentages of non-Hispanic whites, African Americans, and Mexican Americans were 27, 33, and 40%, respectively, for the cases and 23, 36, and 41%, respectively, for the controls. These differences in frequency distributions were not statistically significant (P > 0.05; Table 3), indicating that the frequency matching was adequate. However, significantly more cases (84%)

| Table 1 DRC* in normal and repair-deficient lymphoblastoid cell lines as measured using the HCR assay |
|------------------|----------|---------|---------|---------|---------|
| BPDE dose (µM)  | Cell lines | 0       | 30      | 60      | 75      | 90      |
| Repair-proficient | GM00131A | 46.8 ± 15 | 27.5 ± 6.9 | 10.8 ± 1.3 | 2.3 ± 0.1 |
|                   | GM00892B | 32.7 ± 4.5 | 21.6 ± 2.5 | 8.6 ± 0.8 | 1.1 ± 0.4 |
|                   | Averagea | 39.8 ± 13 | 24.6 ± 5.7 | 9.7 ± 1.5 | 1.7 ± 0.7 |
| Repair-deficient  | GM02345B | 2.7 ± 0.7 | 1.4 ± 0.3 | 0.4 ± 0.2 | 0.2 ± 0.1 |
|                   | GM02246B | 9.7 ± 1.5 | 2.8 ± 0.8 | 1.5 ± 0.9 | 0.3 ± 0.1 |
|                   | Averagea | 6.2 ± 4.0 | 2.1 ± 0.9 | 0.9 ± 0.8 | 0.3 ± 0.1 |

* Means of three experiments ± SD.

a The average of all six experiments used for comparison.
than controls (57%) were smokers \( (P < 0.01) \), with a crude OR of 4.03 (95% CI, 1.60—10.1).

The distribution of DRC in cases was skewed to lower values than the control distribution was (Fig. 2). The mean DRC values were lower in cases (3.3%) than in controls (5.1%), and the difference was statistically significant \( (P < 0.01) \); Table 3), with a crude OR of 4.67 (95% CI, 1.92—11.4) using the median of the DRCs of the controls as the cutoff value. The OR increased to 5.70 (95% CI, 2.1—15.7) after adjustment for age, sex, ethnicity, and smoking. The younger cases had a significantly lower mean DRC (2.70%) than controls of the same ages (4.62%; \( P < 0.05 \)), although for both cases and controls the older age groups tended to have higher means than the younger age groups (Table 3). There was an association between the tertile of DRC and age at diagnosis (mean ages were 59.8, 64.7, and 68.3 years, respectively), although the trend was not statistically significant \( (P > 0.05) \) due to the small number of subjects in each group (data not shown). In addition, the differences in DRC between cases and controls were greater in males, non-Hispanic whites, Mexican Americans, and smokers (Table 3).

Because frequency matching on age, sex, and ethnicity between cases and controls was used, these variables were further adjusted for any residual effect of the matching variables by logistic regression analysis. In a logistic regression model including age, sex, ethnicity, smoking status, and DRC (Table 4), smoking status was a strong, independent risk factor for lung cancer (trend test, \( P < 0.0001 \)): the ORs for both former (OR, 3.48; 95% CI, 1.09—10.1) and current smokers (OR, 10.5; 95% CI, 3.13—35.5) were significantly increased. DRC was also an independent risk factor (trend test, \( P < 0.01 \)) in this model: those in the middle and lowest tertiles of DRC had increased ORs (OR, 2.30; 95% CI, 0.67—7.92, and OR, 5.47; 95% CI, 1.56—19.2, respectively) for lung cancer. No further interaction between smoking status and DRC could be evaluated because of the relatively small number of subjects in each group.

**Discussion**

In this pilot study, we demonstrated that reduced repair of BPDE-induced DNA damage was associated with an increased risk of lung cancer, which suggests that reduced DNA repair capacity may play a role in the etiology of smoking-related lung cancer. We previously reported that age (in the age range from 20 to 60 years) had an effect on DRC in normal subjects (23). This pilot study included few individuals in this age range because of the relatively advanced age of lung cancer patients.

BPDE-induced adducts are repaired mainly by nucleotide excision repair (18). XP cells, which are deficient in nucleotide excision repair, cannot efficiently repair damage induced by BPDE. It has been demonstrated that mononuclear cells, including lymphocytes, are a
valid surrogate tissue for estimating the burden of DNA adducts in the lung (27); therefore, the DRC measured using the HCR assay in lymphocytes should be a valid estimate of repair in the target tissue. It has been shown that inefficient DNA repair of BPDE adducts has been associated with formation of mutation hotspots in essential genes (15).

The HCR assay was designed for molecular epidemiological studies (22, 23, 28). Because plasmids rather than the cells are treated, the assay measures intrinsic cellular repair capacity without the bias that comes from dose-dependent cytotoxicity (29) due to treatment of the cells. Although transfection efficiency was not monitored in this study because of the scarcity of available cells, the fact that cell growth after stimulation and baseline reporter treated, the assay measures intrinsic cellular repair capacity with efficiency for damaged and undamaged plasmids (30). The adaptation of this assay to cryopreserved cells makes it especially appealing because samples of cells can be stored and accumulated.

The advantages of this approach include that (a) many assays can be performed together in batches, thereby reducing experimental errors due to variation in daily operations; (b) prospective studies of susceptibility to smoking-related cancers. The significance of the assay results should be further verified in a larger, well-designed case-control study in which the interactions between smoking exposure, DNA repair, and other factors that may influence DNA repair are evaluated.

Acknowledgments

We thank Dr. Larry Grossman for providing pCMVcat and constructive advice, Dr. Reuben Lotan for critical review of the manuscript, Dr. Maureen Goode (Department of Scientific Publications) for editing the manuscript, Susan Hons for assistance in recruiting study subjects, Jiang Hong for data management, Yongli Guan for technical assistance, and Shirley Norris for assistance in preparing the manuscript.

References


Table 4 Logistic regression analysis of adjusted ORs and CIs in lung cancer cases and

<table>
<thead>
<tr>
<th>Factor</th>
<th>No. of</th>
<th>Crude OR (CI)</th>
<th>Adjusted OR (CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smothing status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>8</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Former</td>
<td>17</td>
<td>2.43 (0.87—7.67)</td>
<td>3.48 (1.09—10.1)</td>
</tr>
<tr>
<td>Current</td>
<td>26</td>
<td>7.09 (2.44—20.6)</td>
<td>10.50 (3.12—35.5)</td>
</tr>
<tr>
<td>Trend test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRC (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;6.5</td>
<td>6</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>3.0—6.5</td>
<td>18</td>
<td>2.55 (0.83—7.88)</td>
<td>2.30 (0.67—7.92)</td>
</tr>
<tr>
<td>&lt;3.0</td>
<td>27</td>
<td>4.03 (1.34—12.1)</td>
<td>5.47 (1.56—19.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| * Adjusted in a logistical regression model including age, sex, ethnicity, smoking status, and DRC.


Reduced DNA Repair Capacity in Lung Cancer Patients

Qingyi Wei, Lie Cheng, Waun Ki Hong, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/18/4103

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.