Allelic Frequency of a p53 Polymorphism in Human Lung Cancer

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Abstract
p53 is a tumor suppressor gene that is mutated in diverse tumor types. Here we report the frequencies of common polymorphic variants at codon 72 of the p53 gene in germline DNA of lung cancer cases and controls as determined by a polymerase chain reaction strategy. The observed allelic distribution was found to be significantly different between African-Americans and Caucasians in this U.S. population. The frequency of polymorphic variants was similar in lung cancer cases and controls after adjustment for race. However, among lung cancer patients the proline variant at codon 72 was in excess in adenocarcinoma patients by comparison with other histologies.

Introduction
Mutations in the p53 gene have been identified in many forms of human cancer and appear to be important genetic events in cancer of the lung (1–7). Evidence is accumulating which suggests that specific mutations may be characteristic of tumor type or environmental exposure (7–10). Germline mutations in p53 have also been reported to be associated with the Li-Fraumeni cancer family syndrome and other inherited susceptibilities to cancer (11–13).

A common polymorphism at codon 72 in the p53 gene can be identified by restriction enzyme analysis (AccII). The frequency of the major, restriction site present (G CGC) allele compared to that of the minor, restriction site absent (G CCC) allele has been reported to be approximately 0.65:0.35 in two independent studies on healthy Caucasian subjects (14, 15). The gene products of the two possible polymorphic variants differ by the presence of either arginine (major allele), a large, polar amino acid residue, or proline (minor allele), a small, nonpolar amino acid residue (16). Taken together with information that implicates p53 mutations in human lung cancer, this suggested the possibility that the p53 geno-

type might be influential for an individual's risk of cancer. We hypothesized therefore that the presence of the minor allele would be overrepresented in lung cancer cases compared to controls.

In order to test this hypothesis, germline DNA samples (extracted from peripheral blood lymphocytes) of lung cancer cases and controls were analyzed using PCR/restriction analysis (AccII) methodology. With regard to the study design, since it is known that tobacco smoking is the major etiological factor in lung cancer development, that lung cancer occurs primarily in older people (age usually greater than 40 years), and that racial differences for lung cancer incidence have been noted (17), the study was carefully controlled for age, smoking, and race.

Materials and Methods
Lung Cancer Cases and Controls. Previously, we have reported the results of a lung cancer case-control study conducted on subjects accrued in two hospitals in the Baltimore-Washington metropolitan area between 1985 and 1989 (18–22). Briefly, the cases had a diagnosis of lung cancer confirmed by histological review, and these patients participated prior to radiation or chemotherapy. Two control groups were chosen; one comprised patients with chronic obstructive pulmonary disease, and the other consisted of patients with malignancies at anatomical sites other than the lung or urinary bladder. Cases and controls were closely matched with respect to age (mean for cases, 64 years; means for control groups, 62 years for individuals with chronic obstructive pulmonary disease and 61 years for individuals with cancer at anatomical sites other than the lung or bladder), race, and smoking history (average pack-years for cases, 57.4; controls, 57.1). Further details of study design, including exclusion criteria, have been described by Caporaso et al. (18). From the total population of 188 subjects originally studied, 150 were analyzed for the p53 polymorphism at codon 72 based on the availability of DNA.

Laboratory Assay. A facile PCR/restriction digest-genotyping test was developed based on previous reports (14, 15). The methodology used here, however, included an important modification (23): the primer flanking codon 72 in the 5' region contained a single base pair mismatch resulting in the formation of a new AccII restriction site between exons 2 and 3 (an A→C change at position 11863 of p53) as an internal control for completion of digestion. High-molecular-weight DNA was isolated from WBC of subjects accrued in a case-control study of lung

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² The abbreviation used is: PCR, polymerase chain reaction.
cancer. DNA samples were amplified for 30 cycles using a standard protocol (8) with either primer 1 and 2 (below) or by a heminested strategy using primers 1 and 2 followed by a second round of amplification with primers 1 and 3 (primer 1 = 5'-CCCATCCCAGCCCCCT-TGCTGAGTGGCGAGGCGG-3' [mismatch position underlined]; primer 2 = 5'-TCTGCTCCTCTCTGTCCCA-TGACCGACACCTGTGGCGAG-3'; primer 3 = 5'-ACACCCGCGCCCGCCCGCACCA3'). The resulting PCR products were digested to completion with Accll and analyzed by agarose gel electrophoresis (2% in Tris/borate buffer, pH 8.3).

Results
The results of PCR amplification of genomic DNA from lung cancer case-control study subjects using primers specific for exon 4 of p53 and subsequent restriction enzyme (Accll) digestion are shown in Fig. 1. In this figure, Lane U contains PCR-amplified but undigested control material, Lane SP contains a sample derived from an Accll restriction site present homozygous (arg/arg), Lane H contains a sample derived from a heterozygote (pro/arg), and Lane SA contains a sample derived from an Accll restriction site absent homozygote (pro/pro). The results demonstrated that the PCR products from a small subset of samples, confirmed their authenticity were consistent with the restriction analysis (data not shown).

The PCR analysis with restriction enzyme digestion was performed on 150 study subjects (78 cases and 72 controls), and the results are given in Table 1 and Fig. 2. There was no association of the p53 genotype with a diagnosis of lung cancer ($x^2$ = 0.73; $P = 0.69$). However, the observed allelic distribution was found to be significantly different between African-Americans and Caucasians ($x^2$ = 21.6; $P < 0.0001$). Thus, the most common allele in African-Americans is the restriction site absent allele (0.39:0.61), whereas the analysis of samples obtained from Caucasians was similar to the frequencies previously reported (0.65:0.35) (14, 15).

The data were further analyzed for differences among histological types of lung cancer and for different control groups (Fig. 2). A tendency toward increasing frequencies of the proline variant (Accll restriction site absent allele) was noted in lung cancer patients with adenocarcinoma compared to other histologies (squamous cell, small cell, and large cell carcinomas). This tendency was statistically significant in Caucasians ($x^2$ = 10.40; $df = 2; P = 0.006; n = 42$) but not African-Americans ($x^2$ = 1.44; $df = 2; P = 0.49; n = 36$). When the frequency of the proline variant in adenocarcinoma was compared to control subjects for Caucasians only a nonsignificant association was seen ($x^2$ = 4.15; $df = 2; P = 0.11$). The Mantel-Hanzel summary statistic also indicated a nonsignificant association ($x^2$ = 1.52; $df = 2; P = 0.22$). There were no associations between the p53 genotype and lung cancer risk factors (age, family history, gender, smoking, asbestos exposure, or debrisoquine metabolic phenotype) other than race. Thus, multivariate control for these factors did not result in any evidence of risk associated with the p53 polymorphism. Similarly, controlling for these variables did not alter the strongly significant ($P = 0.0001$) association between the p53 polymorphism and race.

Discussion
No association was found between the allelic frequencies of p53 and lung cancer, in either Caucasian or African-American study subjects. However, a tendency was found for the proline allele to be overrepresented in lung adenocarcinoma patients compared to other histological types of lung cancer, irrespective of race, although the association reached statistical significance only in Caucasians. These data need to be interpreted cautiously since this association was not significant when adenocarcinoma cases were compared with control subjects ($x^2$ = 4.15; $df = 2; P = 0.11$). Therefore, independent testing in a separate sample set is required.

Data did show that the proline variant of the Accll polymorphism in the coding region of p53 is more fre-

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Table 1: Distribution of p53 alleles by race

<table>
<thead>
<tr>
<th>Study group</th>
<th>pro/pro*</th>
<th>arg/pro</th>
<th>arg/arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blacks</td>
<td>26 (37b)</td>
<td>34 (49)</td>
<td>10 (14)</td>
</tr>
<tr>
<td>Whites</td>
<td>9 (11)</td>
<td>36 (45)</td>
<td>35 (44)</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>16 (21)</td>
<td>38 (49)</td>
<td>24 (31)</td>
</tr>
<tr>
<td>COPD</td>
<td>8 (17)</td>
<td>24 (51)</td>
<td>15 (32)</td>
</tr>
<tr>
<td>Other cancer</td>
<td>11 (14)</td>
<td>8 (32)</td>
<td>6 (24)</td>
</tr>
<tr>
<td>Pooled controls</td>
<td>19 (26)</td>
<td>32 (44)</td>
<td>21 (29)</td>
</tr>
<tr>
<td>Total</td>
<td>35 (123)</td>
<td>70 (47)</td>
<td>45 (30)</td>
</tr>
</tbody>
</table>

* pro/pro, proline homozygote; arg/pro, heterozygote; arg/arg, arginine homozygote, where the arginine allele is the Accll restriction site present variant and the proline allele is restriction site absent.

b Numbers in parenthesis, percentages.

* COPD, chronic obstructive pulmonary disease.

* Pooled controls are COPD patients combined with patients having malignancies at anatomical sites other than the lung or urinary bladder.
A third polymorphic variant in codon 72 was originally described by Matlashewski et al. (16) to be cysteine (TGC). By using a mismatch primer (C→A change introduced in codon 71) it should be possible to create a BspMI restriction site (ACCTGC) in the PCR product. With this approach, however, no cysteine variants were detected in this study. The most likely explanation for these results is the probability that the cysteine variant arose as an artifact of the original cloning procedure (16).

The current data are interesting because of the ethnic variation and possible association with adenocarcinoma. Therefore, this polymorphism is worthy of further study, particularly in view of the significant difference between various ethnic groups, especially since the medical and scientific data base on minorities is currently lacking.

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