Association of the DNA repair gene XPD Asp312Asn polymorphism with p53 gene mutations in tobacco-related non-small cell lung cancer

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Lung cancer, a disease related mostly to tobacco smoke exposure and a leading cause of cancer-related death in industrialized countries, is frequently associated with mutations in the p53 tumor suppressor gene. Genetic differences resulting in inter-individual variation in DNA repair capacity may in part account for susceptibility of a cell to genotoxic agents leading to somatic mutations, including p53 mutations, and eventual transformation of a normal cell into a malignant phenotype. The objective of this study is to investigate the relationship between the polymorphisms of two DNA repair genes, the nucleotide excision repair xeroderma pigmentosum group D (XPD) gene (codons 312 and 751) and the base excision repair X-ray repair cross-complementing group 1 (XRCC1) gene (codon 399), and p53 mutations among lung cancer patients. Lung tumors from 204 smokers with non-small cell lung cancer (NSCLC) were analyzed for mutations in exons 5–8 of the p53 gene and genotypes of XPD and XRCC1. p53 mutations were found in 20% (40/204) of the patients. Patients with the XPD codon 312 Asn allele were less likely to have p53 mutations (13.8%) than XPD 312 Asp/Asp (27.3%) [odds ratio (OR) 0.43, 95% confidence interval (CI) 0.20–0.89, P = 0.023]. No association was found between p53 mutations and either XPD Lys751Gln or XRCC1 Arg399Gln. However, the p53 mutation frequency increased with the increased number of the combined genotypes among XPD 312WT (Asp/Asp), XPD 751VT (Lys/Gln or Gln/Gln) or XRCC1 399VT (Arg/Gln or Gln/Gln) (P = 0.01, trend test). These results suggest that individuals who smoke and have the XPD codon 312 Asp/Asp genotype may be at a greater risk of p53 mutations, especially if combined with other polymorphisms that may result in deficient DNA repair.

Introduction

Lung cancer is the leading cause of cancer-related deaths in the US. Cigarette smoking is the major cause of lung cancer (1–3). Cigarette smoke contains a myriad of chemical carcinogens and reactive oxygen species (ROS). Chemical carcinogens, including polycyclic aromatic hydrocarbons, aromatic amines and N-nitroso compounds, can form DNA bulky adducts by covalently binding with DNA and then irreversibly result in DNA mutations (4,5). For instance, benzo[a]pyrene can be bio-activated in vivo into benzo[a]pyrene-diol-epoxides (BPDE), which are well-known damaging metabolites and are related to a specific mutational spectrum in the p53 gene (6). Tobacco smoke and tobacco itself increase the production of ROS in cells, resulting in the production of oxidative lesions in DNA. The accumulation of ROS may inflict oxidative DNA damage indirectly, by inactivation of enzymes that are involved in DNA synthesis, or directly, by generating DNA strand breaks and base damage that can lead to mutations in tumor suppressor genes or oncogenes (7,8). Inactivation of the tumor suppressor gene p53 is common in a wide variety of human cancers, including non-small cell lung cancer (NSCLC) (9). Among NSCLC tumors, the frequency of p53 mutations are between 20 and 50%, of which >80% occur in exons 5–8 (10–16).

The removal or repair of DNA damage plays a key role in protecting the integrity of the genome from the insults of cancer-causing agents. DNA repair genetic polymorphisms may result in altered function and/or efficiency of DNA repair, and may contribute to inter-individual variation in DNA repair capacity (4,17,18). Bulky adduct lesions induced by smoking chemical carcinogens are repaired through the nucleotide excision repair (NER) pathway (19). Xeroderma pigmentosum group D (XPD) is involved in the NER pathway by functioning as an ATP-dependent DNA helicase with its 5′–3′ activity joint to the basal transcription factor IIH (TFIIH) (20). Three non-synonymous single nucleotide polymorphisms that induce amino acid changes have been found in the XPD gene at codon 199 (Ile → Met), codon 312 (Asp → Asn) and codon 751 (Lys → Gln) (21). Epidemiological studies have indicated that the XPD 312 and 751 polymorphisms might modify the risk of lung cancer (22,23). In addition to NER, damaged bases and DNA single strand breaks can be repaired through the base excision repair (BER) pathway (24). The X-ray repair cross-complementing group 1 (XRCC1) protein is implicated in the BER processes by serving as a molecular scaffold interacting with poly (ADP-ribose) polymerase (PARP), DNA polymerase-β and DNA ligase IIIα (25–30). Three polymorphisms in the XRCC1 gene that lead to amino acid substitutions have been described at codon 194 (Arg → Tryp), codon 280 (Arg → His) and codon 399 (Arg → Gln) (21). The XRCC1 Gln399 polymorphism resulting in single base substitution may affect binding with PARP, leading to a deficiency of DNA repair (31).

Abbreviations: BER, base excision repair; BDPE, benzo[a]pyrene-diol-epoxides; CI, confidence interval; DRC, DNA repair capacity; MGB, minor groove binder; NER, nucleotide excision repair; NSCLC, non-small cell lung cancer; OR, odds ratio; PARP, poly (ADP-ribose) polymerase; PBL, peripheral blood lymphocyte; PY, pack-year; ROS, reactive oxygen species; SCE, sister chromatid exchange; TFIIH, transcription factor IIH; XPD, xeroderma pigmentosum group D; XRCC, X-ray repair cross-complementing group 1.
There have been extensive studies investigating the prevalence of p53 mutations and their role in lung cancer risk (9–16). A few studies reported the relationship between polymorphisms of DNA repair genes, specifically XPD and XRCC1, and lung cancer risk (22,23,32,33). These studies have suggested that there is an association between XPD and XRCC1 polymorphisms and risk for lung cancer. Functional changes in repair capacity due to inheritance of certain polymorphisms could increase the chance that adducts produced from tobacco carcinogens result in p53 mutations. In this study, we investigated the relationship between polymorphisms in XPD and XRCC1 genes and presence of p53 mutations in specimens obtained from 204 smoking patients with NSCLC.

Materials and methods

Patients and tissue specimens

Patient recruitment and sample collection have been described elsewhere (10). Briefly, histologically confirmed incident adult lung cancer patients assessed at the University of Pittsburgh Medical Center between 1990 and 2002 were recruited. Lung tumor tissue samples were obtained from 204 Caucasian smokers with NSCLC (193 current smokers and 11 former smokers), including paraffin-embedded lung tissues from 37 patients and fresh-frozen lung tumor tissues from 167 patients. These patients consisted of 130 males and 74 females. Complete data for calculating total smoking exposure were available for 151 smokers. The patients’ mean pack-year (PY) ranged from 5 to 165 (median = 50), where one PY is defined as one pack of cigarettes per day for 1 year. Patient age at diagnosis ranged from 38 to 92 years (median = 66). The specimens’ histologies included in this investigation were as follows: 153 adenocarcinomas, 4 squamous cell carcinomas, seven adenosquamous carcinomas and four large cell carcinomas.

Analysis of mutations on the p53 gene

DNA was extracted from 167 fresh-frozen lung tissues as described previously (10). In addition, 37 paraffin-embedded tissue sections (25 μm) were individually deparaffinized in xylene, then ethanol and rinsed with water. The DNA was then extracted using the same DNA extraction method as was used for fresh-frozen tissues. p53 mutations occurring in exons 5–8 of the p53 gene were analyzed by PCR–SSCP as described previously (10).

Genotyping for DNA repair genes

All polymorphisms were analyzed in genomic DNA isolated from tumors using the ABI Prism 7700 sequence detector (TaqMan; Applied Biosystems, Foster City, CA). PCR primers and minor groove binder (MGB) probes were designed using the Primer Express 1.5 software (Applied Biosystems). Briefly, the 25 μl genotyping reaction mix contained 10–20 ng of genomic DNA, 1× TaqMan Universal PCR Master Mix (Applied Biosystems), 300 nM of each primer, 100–500 nM of one pair of specific oligonucleotide probes labeled with the reporter dye 6-carboxy-fluorescein (FAM), corresponding to wild-type homozygotes (XPD-312Asp/Asp, XPD-715Lys/Lys or XRCC1-399Arg/Arg) and with the reporter VIC corresponding to the variant homozygotes (XPD-312Asn/Asn, XPD-715Gln/Gln or XRCC1-399Gln/Gln), at the 5’ end and with a non-fluorescent quencher and MGB at the 3’ end. The PCR reaction was carried out as follows: 50°C, 2 min, for 1 cycle; 95°C, 10 min, for 1 cycle; 95°C, 15 s, 57–59°C, 1 min, for 45 cycles. Amplified DNA exhibiting each genotype was electrophoresed on an acrylamide gel to confirm amplimer size and sequenced to confirm each genotype. For quality control, genotype determinations were run as duplicates and we observed a 100% concordance rate. In every assay, oligonucleotide controls for the wild-type homozygotes, heterozygotes and variant homozygotes were included. After PCR, the genotype was analyzed with Sequence Detection System according to the fluorescence yield for the two different dyes. The genotyping of the DNA repair genes was performed with DNA from tumor tissue for all 204 patients. Furthermore, for 22 of these patients, we had both tumor tissue and matched peripheral blood lymphocyte (PBL) samples available that were also analyzed for the panel of genetic polymorphisms. Our results showed that the genotype identified in the tumor tissue was identical to the genotype found in the matched PBL for these 22 patients (data not shown). Therefore, although we cannot exclude the possibility that chromosomal loss at the repair gene locus might occur in some of the tumors, our results indicate such a fortuitous loss, if it occurs, may involve only a small fraction of the tumors and is highly unlikely to affect our overall results. For each polymorphism screened, individuals were grouped as WT (homozygous wild-type) and VT (homozygous and heterozygous variant). WT was defined as XPD 312Asp/Asp, XPD 715Lys/Lys and XRCC1-399Arg/Arg. VT includes XPD 312Asp/Asn or XPD 312Asn/Asn, XPD 715Lys/Gln or XPD 715Gln/Gln and XRCC1-399Arg/Gln or XRCC1-399Gln/Gln.

Statistical analysis

Fisher’s exact and χ² (Pearson’s correlation) tests were employed to test the association between genotypes and p53 mutation frequency dichotomized as mutation negative and mutation positive. The possible associations were evaluated by stepwise unconditional multivariate logistic regression models controlling for age and sex [odds ratio (OR) and 95% confidence interval (CI)] where appropriate. A trend test (a non-parametric test for trend across ordered groups) for the ordered groups generating from the genotype combination was also analyzed. All statistical tests were two-sided. A P of <0.05 was considered statistically significant. Analysis was performed using the STATATA 6.0 software for Windows.

Results

Genotype and allele frequencies for XPD and XRCC1 polymorphisms

The genotypes and frequency distribution of alleles among the 204 cases are presented in Table I. The XPD-312Asn allele, XPD-715Gln allele and XRCC1-399Gln allele represented 34, 37 and 36% of the 204 patients, respectively.

Characterization of the roles of XPD and XRCC1 polymorphisms in p53 mutations

In order to investigate whether a variation in DNA repair capacity associated with XPD and XRCC1 polymorphisms affect the occurrence of mutations in the p53 gene, we compared the XPD and XRCC1 genotypes with p53 mutation frequencies in lung tumors from NSCLC patients (Table II and Figure 1A). p53 mutations were detected in 40 of 204 (20%) tumors. Figure 1A shows the frequency of p53 mutations in relation to XPD 312 genotype. Individuals containing the XPD 312 WT had a 27.3% p53 mutation frequency. For comparison, individuals who were homozygous (Asn/Asn) or heterozygous (Asp/Asn) for the XPD 312 Asn allele had a lower p53 mutation frequency of 13.8 and 13.6%, respectively. Individuals with the XPD 312VT, which combined patients who were homozygous or heterozygous for the XPD 312 Asn allele, had a mutation frequency of 13.8%. Therefore, patients with XPD 312VT were less likely to have p53 mutations than those with an XPD 312WT (OR, 0.43; 95% CI, 0.20–0.89);

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No. of patients</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XPD 312Asn</td>
<td>88</td>
<td>43</td>
</tr>
<tr>
<td>Asp/Asp</td>
<td>94</td>
<td>46</td>
</tr>
<tr>
<td>Asp/Asn</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Asn allele frequency</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>XPD Lys715Gln</td>
<td>82</td>
<td>40</td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>94</td>
<td>46</td>
</tr>
<tr>
<td>Lys/Gln</td>
<td>28</td>
<td>14</td>
</tr>
<tr>
<td>Gln allele frequency</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>XRCC1 Arg399Gln</td>
<td>85</td>
<td>42</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>90</td>
<td>44</td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>Gln allele frequency</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>
P = 0.023; Table II). No evidence of association was found between the presence of p53 mutations and the XPD 751 or XRCC1 399 genotypes, even when heterozygous were combined with homozygous variant genotypes (P > 0.05) (Table II).

Further statistical analysis was performed among subgroups of patients with the combinations of XPD and XRCC1 polymorphisms. As it has been shown previously that XPD 312WT, XPD 751VT and XRCC1 399VT genotypes resulted in deficient DNA repair (22,23,32,33), they were defined as defective genotype subgroups in this analysis. The proportion of p53 mutations among the four groups, carrying 0, 1, 2 and 3 defective genotypes (XPD 312WT, XPD 751VT and XRCC1 399VT), were 0 (0/6), 14.7 (11/75), 21.7 (25/115) and 50% (39/78), respectively (Figure 1B). Individuals carrying defective genotypes had a higher risk of having p53 mutations than those with XPD 312 Asp/Asp genotype (OR = 0.43; P = 0.023). However, we did not find any significant association between the XPD 751 polymorphism or XRCC1 399 polymorphism and the presence of p53 mutations in the patients’ lung tumors. These results showed a significant association between DNA repair XPD gene codon 312 and p53 mutation frequency, suggesting this polymorphism may modulate the relationship between cigarette smoking and incidence of p53 mutations.

There have been a few studies evaluating the relationship between polymorphisms of DNA repair genes and lung cancer risk (22,23,32,33). The results of our study support some of the studies showing that XPD 312 polymorphism may affect the carcinogenesis process. For instance, XPD 312 Asp/Asp has a protective effect against lung cancer (22), and glioma (35). The molecular mechanism by which this polymorphism affects lung cancer risk is not understood. It has been shown that individuals with the 312Asn alleles had a lower level of DNA damage (chromatid aberrations) to UV (36), and a higher apoptotic response than the 312 Asp/Asp genotype (37). In addition, the latter genotype was associated with an increased BPDE–DNA adduct level (34). We have shown in this study that patients with XPD 312 Asp/Asp or Asn/Asn had a lower p53 mutation frequency than that of patients with XPD 312 Asp/Asp. This may be explained by the fact that a higher apoptotic response can eliminate mutation-prone cells and why individuals with an Asn allele are less susceptible to cancer (37). However, our results are in disagreement with those of Spitz et al. (23) who reported that this XPD 312 Asn/Asn genotype was associated with less optimal DNA repair enzymes.

### Discussion

We investigated the relationship between polymorphisms of DNA repair genes, XPD and XRCC1, and p53 mutations in lung tumors of patients with active smoking history with NSCLC. We hypothesized that if there were functional relevance for the polymorphic DNA repair enzymes in the removal of DNA damage caused by tobacco smoke carcinogens, we would detect differences in p53 mutation frequencies in lung tumors of smoking lung cancer patients.

Our results showed that the XPD-312Asn and XPD-751Gln variant alleles occurred at a frequency of 0.34 and 0.37, respectively. These frequencies were similar to those reported previously for NSCLC by Butkiewicz et al. (0.37 and 0.40, respectively) and lung cancer patients by Spitz et al. (0.29 and 0.36, respectively) (22,23). The XRCC1-399Gln allele frequency observed in our study (0.36) was also consistent with those of previous studies (22,32,34). In the present study, the p53 mutation frequency in NSCLC of 20% (40/204) was lower than that reported for these cancers in other studies (11–16). The difference in frequency may be due to several factors, including the level of sensitivity of the mutation detection methods used by the different groups, and/or the geographical differences of patients investigated in these studies (10).

We first evaluated the association between XPD 312 polymorphism and the presence of p53 mutations in lung tumors. Individuals with either an XPD 312 Asp/Asn or Asn/Asn genotype had a lower risk of having p53 mutations than those with XPD 312 Asp/Asp genotype (OR = 0.43; P = 0.023). However, we did not find any significant association between the XPD 751 polymorphism or XRCC1 399 polymorphism and the presence of p53 mutations in the patients’ lung tumors. These results showed a significant association between DNA repair XPD gene codon 312 and p53 mutation frequency, suggesting this polymorphism may modulate the relationship between cigarette smoking and incidence of p53 mutations.

### Table II. The association between XPD and XRCC1 polymorphisms and p53 mutation

<table>
<thead>
<tr>
<th>p53 mutation</th>
<th>Crude OR 95% CI: P</th>
<th>Adjusted ORa 95% CI: P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>XPD Asp312Asn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp/Asp</td>
<td>64</td>
<td>24</td>
</tr>
<tr>
<td>Asp/Asn</td>
<td>81</td>
<td>13</td>
</tr>
<tr>
<td>Aas/Asn</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>Aas/Asn+Asn/Asn</td>
<td>100</td>
<td>16</td>
</tr>
<tr>
<td>XPD Lys751Gln</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>67</td>
<td>15</td>
</tr>
<tr>
<td>Lys/Gln</td>
<td>76</td>
<td>18</td>
</tr>
<tr>
<td>Glu/Gln</td>
<td>21</td>
<td>7</td>
</tr>
<tr>
<td>Lys/Gln+Gln/Gln</td>
<td>97</td>
<td>25</td>
</tr>
<tr>
<td>XRCC1 Arg399Gln</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>69</td>
<td>16</td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>68</td>
<td>22</td>
</tr>
<tr>
<td>Glu/Gln</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>Arg/Gln+Gln/Gln</td>
<td>95</td>
<td>24</td>
</tr>
</tbody>
</table>

aOR adjusted for age, sex.

bReference group.

The separate logistic regression was run to combine the heterozygous and variant homozygous.

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**Note:** The table and discussion focus on the association between specific DNA repair gene polymorphisms and p53 mutation frequencies, highlighting the role of genetic variants in the carcinogenesis process of lung cancer. The findings suggest that certain polymorphisms may influence the risk of p53 mutations, which are critical in the apoptotic response and cancer development. Further research is needed to elucidate the molecular mechanisms underlying these associations.
The observed differential effects of XPD 312 and 751 polymorphisms on p53 mutation frequencies are not completely understood because of the currently limited information available on the functional significance of the XPD polymorphisms. Nevertheless, it has been shown that the 312 Asp amino acid of XPD is highly conserved through evolution (19,22), and may thus be functionally important for the XPD enzyme. Conversely, the 751 Lys amino acid in the C-terminus of the XPD protein is not evolutionarily conserved. Also, as a member of the multiprotein complex TFIIH, XPD has multiple cellular functions and may in this way interact with various proteins (43). Amino acid variation between codons 312 and 751 of XPD may also lead to different protein interactions, resulting in the expression of different functional phenotypes (36). Finally, it may be possible that the sequence variation at codon 312 could affect RNA stability or influence protein synthesis, leading to differences in apoptosis (23).

We also observed no association between XRCC1 Arg399Gln and p53 mutation frequencies. This result is in agreement with those of Butkiewicz et al. (22), who found no association between lung cancer risk and XRCC1 399 polymorphism in the Polish population. In disagreement with our results, other studies showed that XRCC1 399 polymorphism may play a role in cigarette smoking-induced lung cancer. Divine et al. (32) found a positive association of XRCC1 399Gln allele with an increased risk of lung adenocarcinoma. Furthermore, Park et al. (44) reported that XRCC1 399Gln was associated with an increased risk for squamous cell carcinoma of lung with lower degrees of cigarette use in the Korean population. XRCC1 399 Gln/Gln genotype was associated with higher levels of placental aflatoxin-B1 adducts and glycoporphin A mutations in erythrocytes (45), higher frequencies of DNA damage (sister chromatid exchange, SCE) in smokers (38), or a lower repair capacity of NNK-induced genetic damage (SCE) in cultured human lymphocytes from non-smoking volunteers, compared with those carrying the XRCC1 399 Arg/Arg genotype (46). The absence of an association of XRCC1 399 polymorphism with the p53 mutation status in our study may be due to several factors, including the small number of patients, or the histological subtypes of lung cancer analyzed in this study.

In addition to individual polymorphisms, we investigated the combination of the XPD and XRCC1 polymorphisms. We observed no p53 mutations among the six patients who had the combined genotypes with XPD 312VT, XPD 751WT and XRCC1 399 WT; XPD 751 WT or XRCC1 399 VT; 2, individuals carrying either two of the XPD 312 WT, XPD 751 VT or XRCC1 399 VT; 3, individuals carrying the combination of XPD 312 WT, XPD 751 VT and XRCC1 399 VT. Trend test for the ordered groups showed the statistically significant (P = 0.01).

repair capacity (DRC) in cultured cells. The occurrence of lung cancer in XPD 312VT subjects with a low frequency of p53 mutation also suggests that a different cancer pathway is activated in these individuals.

Our results showed there was no association between the XPD Lys751Gln polymorphism and p53 mutation status of the tumor. Indeed, a few studies reported an absence of correlation of XPD Lys751Gln polymorphism with lung cancer risk (22,38). There is also a lack of association between this polymorphism with the levels of polyphenol DNA adducts (39), or the risk of bladder cancer (40). However, a few studies suggested that XPD 751 polymorphism may be related to cancer risk. For instance, XPD 751 Gln allele might be associated with a high risk of lung cancer (23), and squamous cell carcinoma of the head and neck (41). On the other hand, other studies showed that individuals with the XPD 751 Lys/ Lys genotype were at a higher risk of basal cell carcinoma (42), or associated with a less suboptimal DRC (36).
Transitions had each at least one allele Gln for the XPD genotypes, or the combination of XPD and XRCC1 genotypes, or the combination of XPD and XRCC1 and p53 mutations, which could increase the chance for lung cancer patients.

Acknowledgement

This work was supported by a grant from the American Cancer Society (RPG-99-161-01-CNE) and the SPORE grant (P50 CA090440).

References


Received March 14, 2003; revised June 6, 2003; accepted June 28, 2003