Frequency of TP53 Mutations in Relation to Arg72Pro Genotypes in Non–Small Cell Lung Cancer

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Abstract

Mutations in the TP53 gene are important events during human lung carcinogenesis. The TP53 gene harbors several polymorphisms, and functional studies have shown that the Arg72Pro polymorphism alters both wild-type and mutant p53 protein activity. Thus, we hypothesized that certain Arg72Pro genotypes may influence the frequency and pattern of somatic mutations in TP53. We therefore examined the status of the Arg72Pro polymorphism and TP53 mutations in 260 non–small-cell lung cancer cases. Here we report a significant trend toward lower frequency of TP53 mutations with increasing number of Pro72 alleles (P = 0.02). Overall, Pro72 allele carriers had significantly lower frequency of TP53 mutations compared with Arg72 homozygotes (P = 0.02). In addition, carriage of the Pro72 variant was related to a lower frequency of mutations affecting the hotspot codon 273. Mutations at codon 273 accounted for 10.6% of the mutations in Arg72 homozygotes and 1.7% of the mutations in Pro72 allele carriers. Our results suggest that the genotype of the Arg72Pro polymorphism may modulate the frequency of TP53 mutations in non–small-cell lung cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(10):2077–81)

Introduction

Lung cancer is one of the leading causes of cancer mortality worldwide (1). Cigarette smoke is known to be the most important risk factor, but environmental and occupational exposures are also related to lung cancer. In addition, genetic variation results in gene-environment interactions and modulation of lung cancer risk (2).

p53 is a tumor suppressor and is activated to prevent carcinogenic effects of cellular stress such as DNA damage. On activation, p53 induces apoptosis, cell cycle arrest, or DNA repair (3, 4). The importance of this protein is underlined by the fact that the TP53 gene is mutated in more than half of non–small cell lung cancer (NSCLC) tumors and mutation of the gene is considered to be an early event in lung carcinogenesis (5, 6).

Several polymorphisms in the TP53 gene have been reported (7). The most studied single-nucleotide polymorphism, located in exon 4 of the gene, results in an amino acid change from arginine (Arg) to proline (Pro) at codon 72 (8). The Arg72Pro polymorphism is located in the proline-rich region, which has been shown to be important for the apoptotic functions of p53 protein (9). The Arg72 variant has been shown to better induce apoptosis and to repress cellular transformation (10, 11). The Pro72 variant, in turn, induces a higher level of G1 arrest than Arg72 (12). Recently, it was shown that the Pro72 variant induces transcription of DNA repair genes, and Pro72-expressing cells had higher DNA repair capacity than the corresponding Arg72 cells (13). A negative regulator of p53, iASSP preferentially inhibits the Pro72 variant. In contrast, the Arg72 variant escapes this negative regulation and thus has greater apoptotic potential leading to better response to chemotherapy and longer survival (14, 15). Given that the Pro72 is a weaker apoptotic inducer, an association between this variant and lung cancer risk would seem plausible. However, although the Arg72Pro polymorphism has been intensively studied, the results have not been conclusive (16, 17).

Unlike other known tumor suppressor genes, the somatic mutations found in TP53 are primarily missense mutations leading to single amino acid substitutions (18). Most mutations lead to loss of wild-type activity such as induction of apoptosis. However, the selection of missense mutations in tumors suggests that mutated p53 itself may have specific tumorigenic functions. Several studies have shown that expression of mutated p53 may interfere with wild-type p53 functions in a dominant negative fashion. Mutated p53 is able to form tetramers with wild-type protein thereby reducing transactivation capability of the complex (19). The effect has been suggested to be promoter dependent (20). Another function of mutated p53 is the ability to interfere with transactivation by p73 (21).

The question arises whether polymorphisms in the TP53 gene leading to altered wild-type functions could...
Table 1. Characteristics of NSCLC patients

<table>
<thead>
<tr>
<th>Cases (n = 260)</th>
<th>Male/female</th>
<th>Age* (y)</th>
<th>Smoking</th>
<th>Current/former</th>
<th>Cigarettes/d*</th>
<th>Pack-years*</th>
<th>Nonsmokers</th>
<th>Histology</th>
<th>Adenocarcinoma</th>
<th>Squamous cell carcinoma</th>
<th>Large cell carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>184/75</td>
<td>63.9 ± 10.3</td>
<td>164/73</td>
<td>15.1 ± 7.8</td>
<td>31.1 ± 18.1</td>
<td>8</td>
<td>107</td>
<td>101</td>
<td>46</td>
<td>46</td>
<td>46</td>
</tr>
</tbody>
</table>

* Mean ± SD.

Table 2. TP53 mutation frequency and genotypes in NSCLC patients

<table>
<thead>
<tr>
<th>Mut TP53</th>
<th>Total</th>
<th>% Mut</th>
<th>Odds ratio*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>85</td>
<td>63.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Arg/pro</td>
<td>50</td>
<td>50.5</td>
<td>0.56 (0.31-0.99)</td>
<td>0.02</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>10</td>
<td>41.7</td>
<td>0.36 (0.13-0.97)</td>
<td>0.02</td>
</tr>
<tr>
<td>Arg/Pro + Pro/Pro</td>
<td>60</td>
<td>48.8</td>
<td>0.52 (0.30-0.89)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Adjusted for sex, age, and smoking (pack-years).

1 Two cases failed genotyping.

2 Cochran-Armitage trend test.

3 Pearson χ² test.

The aim of this study was to investigate the relationship between the Arg72Pro variants and occurrence of somatic mutations in TP53 in NSCLC. Here we report that the Pro72 allele is preferentially retained and mutated whereas the Pro72 allele is lost in different tumors (25, 26).

Materials and Methods

Study Population. The study consisted of 260 NSCLC patients treated by surgery at university hospitals in Bergen and Oslo in the period between 1986 and 2001. Patients were selected consecutively whenever technically feasible. Tumor histology was confirmed by an experienced pathologist and only NSCLC cases were included in the study. Subjects gave written informed consent to participate in the study and to allow their biological samples to be genetically analyzed. All cases were Caucasians of Norwegian origin. The Regional Committee for Medical Research Ethics approved the study.

Genotyping. DNA was extracted from blood samples or histologically normal lung tissue according to standard protocols. The histologically normal lung tissue was cut from the resected lobe at a minimum distance of 2 to 3 cm from the border of the tumor. Absence of tumor tissue was confirmed by microscopic examination by a pathologist. Genotyping of the TP53 Arg72Pro single-nucleotide polymorphism (rs1042522) was carried out using a 5’TaqMan assay. Approximately 10 ng of genomic DNA was amplified in a 5-μL reaction mixture containing 4.5 pmol of each primer (5’-CCAGAT-

GAAGCTCCCAGAATGC and 5’-TGGGAGGGACA-

GAAGATGACA) and 1.0 pmol of each allele-specific MGB-probe (5’-VIC-TGCTCCCCCGTGCC and 5’-FAM-

CTCCCGCGGGC) in 1× TaqMan Universal PCR mastermix. After initial denaturation and enzyme activation at 95°C for 10 min, the reaction mixture was subjected to 40 cycles of 95°C for 15 s, 50°C for 15 s, and 60°C for 1 min. The reactions were done on an ABI 7900HT sequence detection system. Equal numbers of cases and controls were run simultaneously, and negative controls containing water instead of DNA were included in every run. Genotypes were determined in the SDS 2.2 software (Applied Biosystems). Approximately 10% of the samples were regenotyped by PCR amplification, restriction cutting by BstUI, and agarose gel analysis. There was a complete concordance between the two methods.

TP53 Mutation Analysis. Samples of tumor tissue were frozen at −80°C immediately after the operation. Tumor tissue of at least 80% purity was selected by examination of frozen sections of the samples. DNA was extracted from the tissue using standard proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation. Tumor DNA was screened either by single-strand conformational polymorphism or denaturing capillary electrophoresis as previously described, covering exons 4 to 9 of the TP53 gene (27-29). Exons showing aberrant band migration were sequenced either by direct sequencing using BigDye chemistry (Applied Biosystems) or by the use of an arrayed primer extension platform (APEX; ref. 30). Primers and conditions used in PCR reactions and sequencing are available on request.

Statistical Analysis. Calculations of associated odds ratios were done by unconditional logistic regression
adjusted for parameters such as sex, age, and smoking, where appropriate, with SPSS ver. 14 (SPSS, Inc.). Trend tests were done with the Cochrane-Armitage test in StatExact 4.0 (Cytel Software Corp.). Pearson $\chi^2$ test and Fisher’s exact test were used to compare proportions.

Results

Patients and TP53 Mutations. To study the relationship between germ-line polymorphisms and somatic mutations in the TP53 gene, we genotyped the Arg72Pro polymorphism in normal DNA from 260 NSCLC cases. Corresponding tumor DNA was screened and sequenced for TP53 mutations in exons 4 to 9. Characteristics of the patients are given in Table 1.

Mutations in TP53 were detected in 146 of the 260 (56.2%) NSCLC cases. Missense substitutions accounted for 92 (63.0%) of the mutations. In addition, we observed 19 (13.0%) nonsense, 21 (14.4%) insertions/deletions, 11 (7.5%) splice-site, and 3 (2.1%) silent mutations. The full set of observed mutations is available in Supplementary Table S1.

Relationship between TP53 Genotypes and Mutations. A statistically significant trend toward lower frequency of TP53 mutations with increasing number of Pro72 alleles was observed ($P_{trend} = 0.02$; Table 2). Combined, carriers of the Pro72 allele had significantly lower burden of TP53 mutations compared with Arg72 homozygotes (48.8% versus 63.0%, $P = 0.02$) resulting in a reduced odds ratio for harboring TP53 mutations of 0.52 (95% confidence interval, 0.30-0.89).

The distribution of hotspot mutations was different between the Pro72 allele carriers and the Arg72 homozygotes (Fig. 1). The most striking difference was seen in mutations affecting codon 273. Overall, this codon was the most frequently mutated codon among the NSCLC cases, accounting for 6.9% (10 of 146) of the mutations. However, nine of these mutations were found among Arg72 homozygous cases, whereas only one mutation in codon 273 was observed in Pro72 carriers (Fig. 1). This difference was statistically significant (10.6% versus 1.7%; $P = 0.05$, Fisher’s exact test). Similar results were obtained when restricting the analysis to missense mutations (15.7% versus 2.5%; $P = 0.07$, Fisher’s exact test).

Discussion

In the present study, TP53 mutations were significantly less frequent among patients carrying the Pro72 variant of the gene compared with the Arg72 homozygotes. Functional studies have pointed out that the Arg72 variant is a more efficient inducer of apoptosis and thereby a better repressor of cellular transformation (10, 11). Thus, a selective pressure toward inactivation of the Arg72 variant of p53 by mutation seems to be likely. This is consistent with the findings of Langerød et al. (31), which showed preferentially mutation of the Arg72 allele in Arg72/Pro72 heterozygote breast cancer patients. Similar results have been obtained in several cancers, and studies on Arg72/Pro72 heterozygotes have indicated that the Arg72 allele is preferentially mutated whereas the Pro72 allele is lost (25, 26, 32).

It is currently an ongoing discussion whether the Arg72Pro polymorphism also modulates the frequency of TP53 mutations in lung cancer. In a study consisting primarily of NSCLC cases, Mechanic et al. (33) reported a higher frequency of TP53 mutations associated with the Pro72 allele. However, their results may have been influenced by the low sensitivity of mutation detection due to the use of archival pathologic samples, resulting in an observed mutation frequency of only 25%. The study also included African American subjects, which are known to have a higher frequency of the Pro72 allele (34). Szymanowska et al. (35) recently reported that Pro72 allele carriers from Poland had higher frequency of TP53 mutations. However, their findings did not reach statistical significance and they actually reported that the Pro72 homozygotes had lower frequency of TP53 mutations than the Arg72 homozygotes. Recently, Hu et al. (36) reported a higher frequency of TP53 mutations associated with the Pro72 allele in a study consisting of Caucasian ($n = 142$) and African American ($n = 38$) NSCLC patients. The frequency of the Pro72 allele was significantly higher among the African American subjects, as also reported by others (34). Combined with a higher frequency of TP53 mutations among the African American subjects, this may have influenced the results. Nelson et al. (37) reported that the Arg72 allele was preferentially mutated in NSCLC but did not show any differences between constitutional genotypes of the Arg72Pro polymorphism.

Given the functional importance of the Arg72Pro polymorphism on both wild-type and mutant p53 activity, it could be hypothesized that certain genotypes could influence the selection of specific mutants. Mutation in one of the alleles in an Arg72/*Arg72/*Arg72 homozygote leaves the cell with one intact wild-type Arg72 allele. Thus, in these cells, mutations having dominant negative effects toward the remaining Arg72 allele would have an advantage. In contrast, mutation of the Arg72 allele in Arg72/Pro72 heterozygote or the Pro72 allele in Pro72 homozygotes leaves the cells with a weaker Pro72 allele. In these cells, other tumorigenic functions such as inactivation of the p73 could play a more important role.

![Figure 1. Frequency of mutations affecting TP53 hotspot codons among NSCLC cases.](image-url)
in the selection. This may explain why the Arg72 allele is preferentially mutated among Arg72/Pro72 heterozygotes and that the effect is more pronounced in recessive mutants (22, 38). Our study did not allow for determination of the dominant negative effects of the observed mutations. The IARC TP53 mutation database contains functional information on many mutations (39). However, these functional data are based on several publications obtained with different methods and with variable results.

When comparing the frequency of mutations affecting known hotspot codons in lung cancer, we observed differences between Arg72 homoyzygotes and Pro72 allele carriers. Whereas the hotspots in total accounted for 31.8% of the mutations in Arg72 homoyzygotes and 28.3% in Pro72 carriers, the distributions were different. Especially mutations in codon 273 were more often found among Arg72 homoyzygotes than among carriers of the Pro72 variant. Mutations in codon 273 are the most frequent TP53 alteration found in lung cancer. However, among Pro72 allele carriers this codon was only mutated in 1.7% of the cases. It is interesting to note that in the study of Langerod et al. (31), 10 of 63 (15.8%) mutations in Arg72 homoyzygotes affected codon 273, whereas only 2 of 31 (6.5%) Pro72 allele carriers had mutation in this codon. In addition, the two codon 273 mutations found among Pro72 allele carriers resided on Arg72 alleles. However, due to the small number of cases, the observed difference in frequency of codon 273 mutations may arise as a result of variation due to chance and needs to be confirmed in a larger study. It is tempting to speculate whether the Arg/Pro status of the codon 72 influences the functional properties of mutations at codon 273. Most of the functional studies have explored the mutations without knowledge of the polymorphic status at codon 72. Therefore, the effect of the different alleles on dominant negative functions remains unknown. Mutations of codon 273, such as Arg273His, do not change the conformation of the protein and thus do not bind to p73 (22, 40).

In addition to the Arg72Pro single-nucleotide polymorphism, the TP53 gene contains several polymorphisms. Therefore, assessing additional polymorphisms in linkage disequilibrium may give a more complete view of the variation in the TP53 gene. In a recent study, Mechanic et al. (41) showed that although the Pro72 allele was associated with lung cancer in African Americans, only one of the Pro72-containing haplotypes showed an association. Thus, the Arg72Pro single-nucleotide polymorphism alone may offer a limited insight into the relationship between genetic variation in the TP53 gene and disease phenotypes.

In summary, our results showed a relationship between the number of variant alleles of the Arg72Pro polymorphism and reduced frequency of somatic mutations in the TP53 gene in NSCLC. Carefully designed studies comparing the various characteristics of the mutations harboring either Arg72 or Pro72 variants, as well as other single-nucleotide polymorphisms, are needed to fully understand the relationship between germ-line polymorphisms and somatic mutations in the TP53 gene.

References


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