Prognostic impact of p53 Pro72 homozygous genotype in non-small cell lung cancer patients

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Received June 27, 2007; Accepted September 3, 2007

Abstract. Mutations of the p53 gene represent the most common genetic alterations in human cancer. Several reports have focused on p53 polymorphisms as risk factors in lung cancer, in particular at codon 72 of exon 4, encoding either an arginine (Arg72R) or a proline (Pro72P) amino acid. Polymorphisms at codon 72 of the p53 gene were determined using a PCR-RFLP-based method. We analysed the relationship of this polymorphism to patient survival in 121 non-small cell lung cancer (NSCLC) cases. Interestingly, the 72P homozygous NSCLC patients often presented high-grade tumours and had significantly poorer survival rates than patients with R72 homozygotes or heterozygotes. Our results may help clarify discrepancies in the literature concerning the prognostic role of p53 codon 72 variants.

Introduction

Mutations of the p53 suppressor gene represent the most common genetic alterations in human cancer (1,2), leading to the loss of p53 functions such as apoptosis and cell-cycle arrest and repair (3-5). Besides these gene mutations, several reports have focused on p53 polymorphisms as risk factors for malignant disease. To date, at least 13 different polymorphisms have been identified in the human p53 gene (6,7). A polymorphic site at codon 72 of exon 4 encodes either an arginine amino acid (Arg72R) or a proline residue (Pro72P) (8) and is common in African-Americans and Caucasians. The two polymorphic variants of wild-type p53 differ biochemically and biologically (9,10) and their functional effect is unknown. Moreover, it is not as yet clear whether the expression of 72 variants contributes to the risk of cancer (11-14), chemotherapeutic resistance and poor prognosis in lung tumours, as well as in other cancer types. To further define the prognostic role of the p53 codon 72-genotype in lung cancer, we studied the relationship of this polymorphism to patient survival in 121 non-small cell lung cancer (NSCLC) cases.

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Abbreviations: NSCLC, non-small cell lung cancer; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism

Key words: TP53 Arg72Pro polymorphism, non-small cell lung cancer, prognosis

Materials and methods

Surgical specimens. Analysis was conducted of 121 NSCLC patients who had undergone curative surgical resection at the Service of Thoracic Surgery, University of Pisa, between 1992 and 1994. There were 111 male patients and 10 female patients (mean age, 64.01 years; median, 64; range, 45-88). The most common histologic type was squamous carcinoma (n=70), followed by adenocarcinoma (n=40), large-cell anaplastic carcinoma (n=6) and bronchiolo-alveolar carcinoma (n=5). According to tumour status, 30 cases were classified as T_1 (24.8%), 79 as T_2 (65.3%) and 12 as T_3 (9.9%). At the time of diagnosis, 41 patients showed metastatic nodal involvement and 80 did not. Of the patients, 74 were clinically staged as Stage I (S_1), while 17 and 30 were staged as Stage II (S_2) and Stage III (S_3), respectively. Data on clinical behaviour were available in all 121 cases (median follow-up, 123 months; mean, 119.85; range, 103-137). At the time of analysis, 57 patients were alive. After resection, tumour samples were in part frozen in liquid nitrogen and stored at -80˚C for molecular studies and, in the other, formalin-fixed and paraffin-embedded for histological classification following the World Health Organization Classification (1982) (15) and the guidelines of the American Joint Committee for Cancer Staging (1992) (16).

DNA extraction. Tissue samples were mechanically disrupted in liquid nitrogen and lysed with proteinase-K. DNA extraction was then performed using the spin column procedure (QIAamp Tissue Kit, Qiagen). The eluted DNA was used as a template in PCR-RFLP analysis.

p53 codon 72 genotyping. Polymorphisms at codon 72 of the p53 gene were determined using a PCR-RFLP-based method (17). Briefly, a 366-bp fragment of the p53 gene from lung DNA was amplified by PCR using forward primer 5'-GTCC

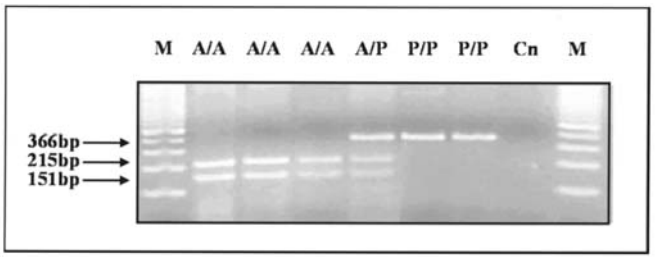

Figure 1. PCR/RFLP analysis of the p53 codon 72 polymorphism. A/A, homozygote Arg/Arg cleaved by BstUI yielding 215 and 151 bands; A/P, heterozygote Arg/Pro containing all three bands; P/P, homozygote Pro/Pro uncleaved by BstUI, yielding a single 366 band; Cn, negative control; M, molecular weight marker (100-bp ladder).

Table I. Clinical and pathological characteristics of p53 codon 72 genotypes in 121 NSCLC patients.

	Arg/Arg Arg/Pro	Pro/Pro	P-value
Gender			
Male	92	19	0.56
Female	9	1	
Age (years)			
≤64	48	13	0.15
>64	53	7	
Histotype			
Squamous	61	9	**0.03**
Adenocarcinoma	34	6	
Anaplastic	4	2	
Bronchiolo-alveolar	2	3	
T-status			
T_1	28	2	**0.02**
T_2	66	13	
T_3	7	5	
N-status			
N_0	70	10	0.15
N_1	17	4	
N_2	14	6	
Stage			
S_1	66	8	**0.001**
S_2	16	1	
S_3	19	11	

TCTGACTGCTCTTTTCACCCATCTAC-3' and reverse primer 5'-GGGATACGGCCAGGCATTGAAGTCTC-3'. The PCR product was digested with 40 units of BstUI (Fermentas, M-Medical, Milan, Italy) at 37˚C for 16 h and electrophoresed on 2% agarose gel, then stained with ethidium bromide. The Arg/Arg homozygote was cleaved by BstUI and yielded 215- and 151-bp bands. The Pro/Pro homozygote was not cleaved by BstUI and yielded a single 366-bp band. The Arg/Pro heterozygote contained all three bands (366, 215, and 151 bp) following restriction digestion (Fig. 1). PCR-digested products were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced, in order to confirm the genotyping results, with a cyclic sequencing kit (ALFexpressII, Amersham Biosciences) following the manufacturer's recommendations.

Statistical methods. Associations between the p53 codon 72 genotype and individual clinical and pathologic variables were assessed using the χ^2 test or Fisher's exact test. Using logistic regression, Arg/Arg and Arg/Pro genotypes were compared with the homozygous Pro allele. Survival curves were estimated using the Kaplan-Meier method. Associations between the codon 72 genotype and survival were assessed with the Cox proportional-hazard regression model.

Results

p53 codon 72 genotyping. The frequency of p53 codon 72 genotypes (Arg/Arg, Arg/Pro, Pro/Pro) in NSCLC patients were 46% (56/121), 38% (45/121) and 16% (20/121), respectively. The allelic frequencies of the Arg and Pro alleles were 0.6 and 0.4, respectively.

Relationship between p53 codon 72 polymorphisms and clinicopathological features. Table I shows the clinical characteristics of the 121 NSCLC patients in relation to polymorphic variants. The χ^2 test was performed, combining Arg/Arg and Arg/Pro, and the relative statistical significance was reported. Increased frequency of the Pro/Pro genotype was observed in cases of advanced tumour status (T_2 and T_3). No relationship was found between polymorphic variants and other clinical features such as age, gender and nodal status.

p53 codon 72 polymorphisms and prognosis. Kaplan-Meier survival analysis (Fig. 2) showed that NSCLC patients with the p53 Pro72 homozygous genotype had a poorer prognosis than patients with other genotypes. Disease-free interval was affected by codon 72 Pro/Pro (12 vs. 38 months in Arg allele carriers; Cox's F test, p=0.03), as was overall survival (21.5 vs. 56 months in Arg allele carriers; Cox's F test, p=0.03).

Discussion

Our distribution analysis of the three genotypes revealed allelic frequencies of 60% for arginine and 40% for proline, which is in agreement with previous studies (17).

The 72P homozygous NSCLC patients often presented high-grade tumours and different survival rates; patients with homozygous 72P were found to have significantly poorer survival than those with R72 homozygotes or heterozygotes (p=0.03).

This finding is consistent with some of the data in the literature, indicating that p53 Arg carriers induce apoptosis with faster kinetics and suppress transformation more efficiently than those carrying the p53 Pro/Pro variant. This could confer a more favourable response to radiation or chemotherapy (18) and be advantageous to survival (19-21). There is, therefore, a bias for the mutation and retention of the Arg allele in tumours arising in Arg/Pro germline heterozygotes (22).

On the other hand, one small study did not show any survival differences among codon 72 genotypes in lung cancer

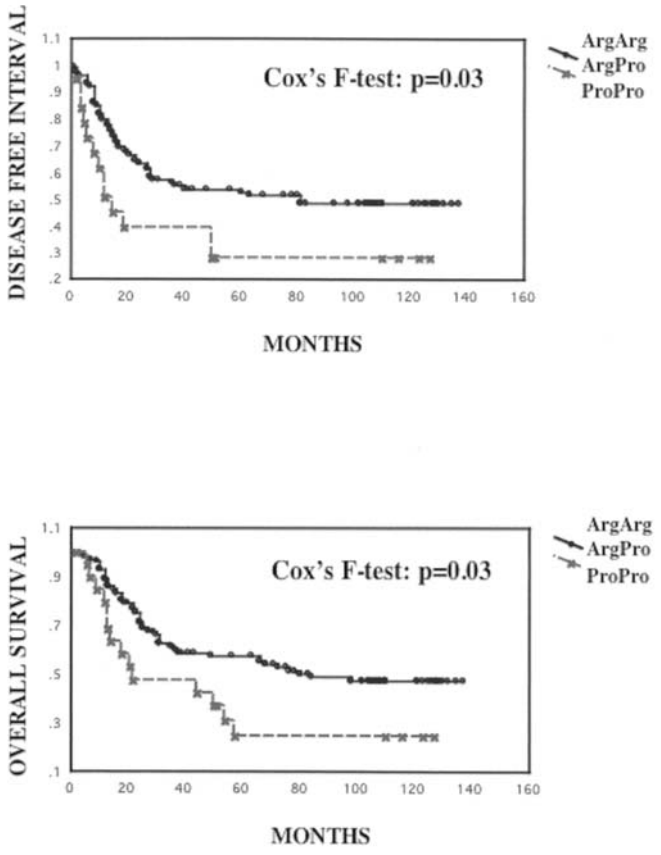

Figure 2. Kaplan-Meier disease-free interval and overall survival curves in relation to p53 codon 72 polymorphisms.

patients (23), and in a breast cancer study it was suggested that the 72P allele has a protective effect against death, with borderline significance. However, this effect was reduced by the inclusion of known prognostic variables in the analysis (24).

Recently, Matakidou *et al* (14) provided no evidence of a relationship between genotype and overall survival in 619 female lung patients; the authors confirmed these results, confining the analysis to 194 patients with NSCLC, without showing the data. Several factors may explain the conflicting results between studies, first of which is the heterogeneity of retrospective studies. Moreover, differences in the expression, polymorphisms and activity of several genes seem to play a role in gender differences in lung cancer, with implications for NSCLC prognosis (25).

In conclusion, our results suggest a prognostic significance for p53 codon 72 variant carriers, helping to clarify discrepancies within the data in the literature. Further analysis is required to investigate the possibility that the p53 codon 72 genotype may be a genetic marker for other genes that affect the prognosis of lung cancer patients.

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