Lung Cancer: Targets and Therapy

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ORIGINAL RESEARCH

Association of p21 Ser31Arg and p53 Arg72Pro polymorphisms with lung cancer risk in CAPUA study

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This article was published in the following Dove Press journal:
Lung Cancer: Targets and Therapy
21 November 2012
Number of times this article has been viewed

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Background: The aim of this study was to investigate how Ser31Arg polymorphisms in p21 may modify lung cancer susceptibility. Because p21 is the major downstream mediator of p53, we analyzed the combined effect of two polymorphisms, p21 Ser31Arg and TP53 Arg72Pro, to elucidate whether polymorphic variants determine the risk of lung cancer.

Methods: This was designed as a hospital-based case-control study, and included 675 cases and 675 control subjects matched by ethnicity, gender, and age. Genotypes were determined by polymerase chain reaction restriction fragment length polymorphism, and multivariate unconditional logistic regression was performed to analyze the results.

Results: Subjects who carried the p21 Ser31Arg allele had a higher risk of lung cancer (adjusted odds ratio [OR] 1.38; 95% confidence interval [CI] 0.99–2.03). This risk was increased in men aged younger than 55 years (adjusted OR 2.35; 95% CI 1.00–5.51). Smokers had an increased risk of lung cancer (adjusted OR 2.23; 95% CI 1.24–4.02). Men younger than 55 years carrying risk alleles for both genes (p21 Ser31Arg and TP53 Arg72Pro) had an increased risk (adjusted OR 5.78; 95% CI 1.38–24.19), as did smokers with both risk alleles (adjusted OR 4.52; 95% CI 1.52–13.50).

Conclusion: The presence of both variant alleles increased the risk of developing lung cancer in men, particularly in smokers younger than 55 years.

Keywords: molecular epidemiology, cell cycle, tobacco, susceptibility, oncology

Introduction

In Spain, 20,401 deaths were caused by lung cancer in 2009. In men, lung cancer is the second most common cause of death (17,279 deaths in 2009) and the death rate in women is currently rising (3122 deaths in 2009).[1] Factors such as genetic susceptibility,[2] occupation, air pollution, and diet are thought to be involved in individual variations in lung cancer risk. Several studies have demonstrated that genetic polymorphisms such as p53 Arg72Pro,[3] occupational exposure to carcinogens,[4] and air pollution are linked to a higher prevalence of lung cancer.[5] In addition, consumption of fruit and vegetables[6,7] and physical activity may be protective against lung cancer.[8] The importance of genetic susceptibility and its relationship with development of cancer has also been widely studied.[2,3,5,9,10]

p21 is a cyclin-dependent kinase inhibitor, and is important for regulating the cell cycle and controlling cells in the G1 phase. The tumor suppressor gene *TP53* expresses p53 protein that tightly controls p21 gene expression, and can block the cell cycle in response to a wide range of stressor stimuli. By interacting with proliferating cell nuclear antigen, p21 takes part in replication and DNA repair in the S phase of

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the cell cycle.[11,12] This promotes cell differentiation and inhibition of cellular senescence. As well as its role in cell cycle control, p21 is involved in DNA repair, such as nucleotide excision and base excision repair.[13–16]

At least 40 polymorphisms have been described for p21, of which only seven have a frequency higher than 10%. One of these polymorphisms, sited in codon 31, results in an amino acid exchange of serine to arginine, causing a cytosine to adenine conversion. The polymorphism is located in a highly conserved region, which includes a zinc finger. Because this region is involved in the molecular activity of p21, its cell cycle functions could be affected by the polymorphism.[17] Despite extensive investigation, the potential role of p21 polymorphisms in lung cancer remains unclear.[18–20]

Because p21 is regulated by the p53 tumor suppressor, and both have important roles in cell cycle regulation, we studied how polymorphisms of these genes may affect the risk of lung cancer, as a continuation of our previous study demonstrating a correlation between p53 Arg72Pro polymorphisms and risk of lung cancer.[3] The objective of this study was to examine the p21 Ser31Arg and p53 Pro72Arg polymorphisms in 675 lung cancer cases and 675 selected controls recruited for a hospital-based case-control study to elucidate whether the polymorphic variants determine the risk of lung cancer.

Materials and methods

Study population

CAPUA (Lung Cancer in Asturias [Cáncer de Pulmón en Asturias], Spain) is a hospital-based case-control study. Detailed methods of how participants were recruited for this study have been described elsewhere.[2,3,5,9,10] Briefly, histological analysis identified lung cancer cases that were recruited from two main hospitals in Asturias in Northern Spain (Cabueñes Hospital in Gijón and San Agustin Hospital in Avilés) from October 2000 to June 2010, following an identical protocol. Controls were selected from patients admitted to the participating hospitals for disorders or diseases believed to be unrelated to lung cancer. The controls were individually matched to the cases on the basis of ethnicity, gender, and age (±5 years). The final controls selected showed the following main specific pathologies: appendicitis (ICD-9 540; 8.8%), intestinal obstruction (ICD-9 560, 569, 574; 13.3%), injury (ICD-9 800–848, 860–869, 880–897; 32.5%), and inguinal or abdominal hernia (ICD-9 550–553; 41.1%). The ethical committees of the hospitals where recruitment took place approved the study, and written consent was obtained from each participant. In total, 879 cases and 803 controls agreed to participate in the study and were subsequently interviewed. Of these, 841 cases (95.7%) and 742 controls (92.4%) provided a blood or buccal cell sample. Finally, 675 cases and 675 controls were available for the study after DNA extraction, genotyping, and matching cases and controls.

Data collection

Trained interviewers used computer-assisted questionnaires to collect information on known or potential risk factors for lung cancer during the first hospital admission for diagnosis. Structured questionnaires included information on age, gender, sociodemographic characteristics, diet (including alcohol consumption), tobacco use (both recent and prior), and personal and family history of cancer (first-degree relatives). All eligible cases and controls included in our analysis were Caucasian.

For tobacco consumption, participants were grouped into three categories: never smokers (subjects who had not smoked at least one cigarette per day regularly for 6 months or longer in their lifetimes); former smokers (regular smokers who had stopped smoking at least one year before the interview); and current smokers (subjects who met none of the previous criteria). Smoking intensity (pack-years) was defined as the number of packs of cigarettes smoked per day multiplied by the number of years of smoking. Information about work status was classified according to list A and list B, and both lists were translated into codes of the International Standard Classification of Occupations and the International Standard Industrial Classification.[21,22]

Genotype analysis

Laboratory personnel were blinded to the case or control status of subjects. Peripheral blood samples (96.5% of total) or exfoliated buccal cells (3.5% of total) were used to extract genomic DNA, as previously described.[23] To determine p21 Ser31Arg polymorphism (rs 1801270), exon 3 of the p21 gene was amplified by polymerase chain reaction (PCR) using the following primers: forward primer 5′-CCCGGCCAGGTAACATAGTG-3′ and 5′-AACTCGAAGTTCCATCGCTC-3′ as the reverse primer. PCR was performed in a 10 µL mixture containing 20 ng of genomic DNA, 0.25 mM each dNTP, 0.5 units of Taq polymerase (Biotools, Madrid, Spain) and 10 pmol of each primer in 1 × PCR buffer. The PCR running conditions were 5 minutes at 94°C followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 62°C, and 30 seconds at 72°C, with a final step at 72°C for 7 minutes. After being digested overnight with BlpI at 37°C, DNA fragments were resolved on 1.5% agarose gel and stained with ethidium bromide.

After electrophoresis, homozygous Ser/Ser was represented by two DNA bands of 159 base pairs and 70 base pairs, the homozygous Arg/Arg genotype was represented by a 229 band of base pairs, and the heterozygous (Ser/Arg) genotype was represented by a combination of three bands (229, 159, and 70 base pairs). Genotype analysis of p53 Arg72Pro has been described eslewehere.[3]

Statistical analysis

Observed genotype frequencies and a χ^2 test with one degree of freedom were used to test for Hardy-Weinberg equilibrium among the controls. In order to compare the distribution of age and gender and the frequencies of alleles and genotypes, univariate analysis was performed. Differences in the distribution between cases and controls were tested using the χ^2, Fisher's Exact, and Mann–Whitney *U* tests where appropriate. Wolf's method was used to calculate the crude odd ratios (OR).[24] Multivariate unconditional logistic regression analysis with adjustment for age, gender, occupation (list A), and smoking status was performed to calculate the adjusted OR and 95% confidence intervals (CI). Gene-environment interactions were estimated using the logistic regression model, which included an interaction term as well as variables for exposure (smoking, occupation), genotypes (p53 and p21), and potential confounders (age and gender). All statistical analyses were performed with Stata version 8 software (Statacorp, College Station, TX).

Results

Subject characteristics

In this study, the analysis included 675 lung cancer cases and 675 controls. The distribution of gender, age, smoking status, pack-years, occupation (list A), and histological type for cases among the study subjects was assessed (Table 1A). The mean age of the subjects was 64.14 years in controls and 65.26 years in lung cancer cases. The main histological types were squamous cell carcinoma (40.72%), adenocarcinoma (30.09%), and small cell carcinoma (17.07%). Histological distribution varied according to gender: for men, the most common histological type was squamous cell carcinoma (43.29%), while adenocarcinoma was the main histological type for women (54.43%, Table 1B). In our population, the mean pack-year tobacco consumption was 61.95 in cases and 36.16 in controls. We determined the genotype distribution of the Ser31Arg polymorphism in p21 and analyzed the risk of lung cancer for the combined effect of the Ser31Arg polymorphism in p21 and the Arg72Pro polymorphism in p53.

Table 1A Characteristics of lung cancer cases and control patients

Variable	Cases (n = 675) n (%)	Controls (n = 675) n (%)	*P*[a]
Gender			
Male	595 (88.15)	595 (88.15)	
Female	80 (11.85)	80 (11.85)	1.000
Age (years), mean (SD)	65.26 (10.98)	64.14 (11.2)	0.064
Pack-years, mean (SD)	61.95 (36.16)	36.32 (31.13)	<0.001
<37	139 (20.62)	294 (43.68)	
≥37	490 (72.70)	191 (28.38)	<0.001
Family history of cancer			
No	366 (56.92)	400 (60.06)	
Yes (any cancer)	277 (43.08)	266 (39.94)	0.249
Lung cancer	76 (11.82)	45 (6.76)	0.007
Other cancer	201 (31.26)	221 (33.18)	
Missing	32	9	
Histological type			
Squamous cell carcinoma	272 (40.72)		
Adenocarcinoma	201 (30.09)		
Small cell carcinoma	114 (17.07)		
Nondifferentiated	43 (6.44)		
Large cell carcinoma	22 (3.29)		
Others	10 (1.50)		
Clinical diagnosis	6 (0.90)		
Missing	7		
List A, mean (SD)			
Yes	129 (19.79)	94 (14.48)	
No	523 (80.21)	555 (85.52)	0.011
Missing	23	26	
Years in list A, mean (SD)	17.45 (14.59)	15.88 (13.89)	0.418
Missing	23	26	

Note: [a]Two-sided χ^2 test and Mann–Whitney test when appropriate.
Abbreviation: SD, standard deviation.

Analysis of p21 Ser31Arg and p53 Arg72Pro polymorphisms

First, we investigated distribution of the Ser31Arg polymorphism. Because of the low risk of homozygous genotype frequency Arg/Arg, we placed all Arg carriers (homozygous and heterozygous) into one group. p21 Arg allele frequency was 0.08 in cases and 0.07 in controls. The frequency of Arg carriers was 16.08 in cases and 13.09 in controls (Table 2). Ser31Arg polymorphisms were in Hardy-Weinberg equilibrium in our control population (χ^2 HW = 0.29; $P = 0.589$).

Table 1B Histological distribution of population by gender

Histological type	Men	Women
Squamous cell carcinoma	255 (43.29)	17 (21.52)
Adenocarcinoma	158 (26.83)	43 (54.43)
Small cell carcinoma	101 (17.15)	13 (16.46)
Nondifferentiated	40 (6.79)	3 (3.80)
Large cell carcinoma	20 (3.40)	2 (2.53)
Others	10 (1.70)	0 (0.00)
Clinical diagnosis	5 (0.85)	1 (1.27)

Table 2 Genotype distribution of p21 Ser31Arg polymorphism and adjusted odds ratio for lung cancer

Genotype p21 Ser31Arg	Cases n (%)	Controls n (%)	Crude OR	Adjusted OR (95% CI)[a]
Ser/Ser	565 (83.70)	589 (87.26)	Reference	Reference
Ser/Arg	107 (15.85)	88 (12.26)	1.39	1.42 (0.99–2.03)
Arg/Arg	3 (0.44)	6 (0.89)	0.52	0.59 (0.09–4.07)
Arg carriers	110 (16.30)	86 (12.74)	1.33	1.38 (0.97–1.96)
Arg allele frequency	0.08	0.07		

Note: [a]Adjusted for age, gender, pack-years, familial history of lung cancer, and occupation (list A).
Abbreviations: CI, confidence interval; OR, odds ratio.

Analysis of the Ser31Arg genotype in p21 and susceptibility to lung cancer demonstrated that Arg carriers had a higher risk of lung cancer (adjusted OR 1.38; 95% CI 0.99–2.03, Table 2). Stratification for gender showed that men who were Arg carriers had an increased risk of lung cancer (adjusted OR 1.53; 95% CI 1.04–2.23). Because a statistically significant risk was found in men, and we had small numbers of women in our study populations, we focused on a stratified analysis only in men. Men who were Arg carriers and younger than 55 years had a two-fold increased risk of developing lung cancer (adjusted OR 2.35; 95% CI 1.00–5.51). According to smoking status, we found that carrying the Arg allele increased the risk of lung cancer in ever smokers (adjusted OR 1.75; 95% CI 1.21–2.54). This risk was particularly strong in current smokers (adjusted OR 2.23; 95% CI 1.24–4.02).

According to histological subtype, the p21 Arg allele polymorphism was associated with a higher risk of lung cancer in patients with small cell carcinoma (adjusted OR 2.11; 95% CI 1.15–3.86) and squamous cell carcinoma (adjusted OR 1.39; 95% CI 0.86–2.26). According to occupation (list A), there was no higher risk of lung cancer in people who had an occupation included in list A (adjusted OR 1.66; 95% CI 0.87–3.17, Table 4).

The frequency of the p53 Arg72Pro allele was 0.24 in cases and 0.27 in controls. This polymorphism was in Hardy-Weinberg equilibrium (χ^2 HW = 0.03; $P = 0.872$). There was no increase in risk of lung cancer for people harboring the p53 Arg72Pro allele (adjusted OR 1.19; 95% CI 0.91–1.54) (Table 3).

Stratified analysis of the Arg72Pro polymorphism did not show a higher risk in males (adjusted OR 1.17; 95% CI 0.88–1.53) for carriers of the Arg72Pro polymorphism. According to age, there was no increased risk in any of the three groups. Taking into account smoking status, the ever smoker group showed a higher risk of lung cancer (adjusted OR 1.43; 95% CI 1.09–1.86). No association between this polymorphism and increased risk of lung cancer stratified by histological type or occupation was observed (Table 5).

Combined analysis of p21 Ser31Arg and p53 Arg72Pro polymorphisms

Because p21 expression is regulated by p53, we investigated the combined effect of subjects harboring both risk alleles, ie, p21 Ser31Arg and p53 Arg72Pro, using Ser/Ser homozygotes in p21 and Arg/Arg homozygotes in p53 as references. We considered moderate-risk individuals to be those with at least one risk allele (ie, Arg carriers of p21 and Arg/Arg homozygotes of p53 or Pro carriers of p53 and Ser/Ser carriers of p21), and high-risk individuals to be those who carried an Arg allele of p21 and a Pro allele of p53, as shown in Table 6.

According to gender stratification, our results are consistent with those previously described. Men with a high-risk genotype showed an increased risk of developing lung cancer (adjusted OR 2.29; 95% CI 1.21–4.32). Because males comprised the statistically significant risk group, we performed the stratified analysis in men only.

Those with a high-risk genotype had an increased risk of developing lung cancer while young, with individuals

Table 3 Genotype distribution of p53 Arg72Pro polymorphism and adjusted odds ratio for lung cancer

Genotype p53 Arg72Pro	Cases, n (%)	Controls, n (%)	Crude OR	Adjusted OR (95% CI)[a]
Ser/Ser	341 (52.38)	318 (58.03)	Reference	Reference
Ser/Arg	267 (41.01)	198 (36.13)	1.26	1.16 (0.88–1.53)
Arg/Arg	43 (6.61)	32 (5.84)	1.25	0.59 (0.78–2.32)
Arg carriers	310 (47.62)	230 (41.97)	1.26	1.38 (0.91–1.54)
Pro allele frequency	0.27	0.24		

Note: [a]Adjusted for age, gender, pack-years, familial history of lung cancer, and occupation (list A).
Abbreviations: CI, confidence interval; OR, odds ratio.

Table 4 Stratified analysis of p21 Ser31Arg polymorphism

	Ser/Ser (ref)		Ser/Arg		Arg/Arg		Arg-carriers		Ser/Arg			Arg/Arg			Arg carriers		
	Case	Control	Case	Control	Case	Control	Case	Control	OR adjust[a]	CI	95%	OR adjust[a]	CI	95%	OR adjust[a]	CI	95%
Gender																	
Male	497	527	95	63	3	5	98	68	1.58	1.07	2.33	0.60	0.09	4.10	1.53	1.04	2.23
Female	68	62	12	17	0	1	12	18	0.68	0.27	1.69	–	–	–	0.65	0.26	1.61
Stratified analysis (male only)																	
Age (years)																	
<55	79	116	21	11	1	1	22	12	2.43	1.02	5.79	1.20	0.03	49.22	2.35	1.00	5.51
55–69	206	190	35	25	1	2	36	27	1.28	0.69	2.39	0.26	0.00	24.94	1.24	0.67	2.29
≥70	212	221	39	27	1	2	40	29	1.62	0.89	2.97	0.47	0.03	8.40	1.54	0.85	2.78
Smoking status																	
Never	7	116	0	13	0	4	0	17	–	–	–	–	–	–	–	–	–
Ever	490	411	95	50	3	1	98	51	1.73	1.19	2.52	2.54	0.25	25.46	1.75	1.21	2.54
Former	239	240	42	31	2	1	44	32	1.47	0.87	2.47	1.75	0.15	20.72	1.48	0.88	2.47
Current	249	162	53	17	1	0	54	17	2.18	1.21	3.93	–	–	–	2.23	1.24	4.02
Pack-years																	
<37	113	249	23	29	0	1	23	30	2.10	1.14	3.87	–	–	–	2.03	1.11	3.73
≥37	477	174	90	24	4	0	94	24	1.37	0.84	2.24	–	–	–	1.43	0.88	2.32
Histological type																	
Squamous cell carcinoma	215	527	38	63	2	5	40	68	1.39	0.85	2.29	1.40	0.18	10.70	1.39	0.86	2.26
Adenocarcinoma	136	527	22	63	0	5	22	68	1.09	0.61	1.96	–	–	–	1.05	0.59	1.87
Small cell carcinoma	80	527	20	63	1	5	21	68	2.15	1.16	3.98	1.24	0.06	24.15	2.11	1.15	3.86
List A																	
Yes	111	86	17	6	1	2	18	8	1.42	0.48	4.25	0.21	0.00	9.36	1.20	0.43	3.30
No	383	441	78	57	2	3	80	60	1.60	1.06	2.43	0.87	0.09	8.84	1.58	1.05	2.38

Note: [a]Adjusted for age, pack-years, familial history of lung cancer, and occupation (list A), when corresponding.
Abbreviations: CI, confidence interval; OR, odds ratio.

Table 5 Stratified analysis of p53 Arg72Pro polymorphism

	Arg/Arg (Ref)		Arg/Pro		Pro/Pro		Pro carriers		Arg/Pro			Pro/Pro			Pro carriers		
	Case	Control	Case	Control	Case	Control	Case	Control	OR adjust[a]	CI	95%	Adjusted OR[a]	CI	95%	Adjusted OR[a]	CI	95%
Gender																	
Male	303	280	236	177	41	29	277	206	1.12	0.84	1.49	1.47	0.83	2.58	1.17	0.88	1.53
Female	38	38	31	21	2	3	33	24	2.18	0.94	5.03	0.26	0.03	2.31	1.73	0.79	3.83
Stratified analysis (male only)																	
Age (years)																	
<55	49	58	38	42	9	7	47	49	0.95	0.48	1.87	1.50	0.45	5.03	1.03	0.54	1.96
55–69	129	107	91	67	16	11	107	78	0.94	0.59	1.50	1.32	0.53	3.29	0.99	0.63	1.54
≥70	125	115	107	68	16	11	123	79	1.44	0.92	2.25	1.74	0.69	4.38	1.48	0.96	2.27
Smoking status																	
Never	5	48	2	45	0	7	2	52	0.42	0.07	2.45	–	–	–	0.36	0.06	2.03
Ever	298	232	234	132	41	22	275	154	1.40	1.05	1.85	1.61	0.92	2.83	1.43	1.09	1.86
Former	139	138	116	71	22	13	138	84	1.69	1.14	2.50	1.92	0.90	4.12	1.72	1.18	2.51
Current	158	90	117	55	19	8	136	63	1.16	0.76	1.76	1.51	0.63	3.64	1.20	0.80	1.80
Pack-years																	
<37	63	135	35	79	12	11	47	90	0.98	0.58	1.64	2.69	1.08	6.67	1.17	0.72	1.89
≥37	234	95	199	53	29	11	228	64	1.55	1.05	2.30	1.10	0.52	2.31	1.47	1.01	2.14
Histological type																	
Squamous cell carcinoma	129	280	103	177	15	29	118	206	1.28	0.89	1.85	1.26	0.61	2.63	1.28	0.90	1.82
Adenocarcinoma	85	280	61	177	9	29	70	206	1.09	0.72	1.64	0.83	0.34	2.03	1.05	0.71	1.56
Small cell carcinoma	47	280	41	177	11	29	52	206	1.32	0.79	2.19	2.17	0.96	4.92	1.45	0.90	2.34
List A																	
Yes	59	48	62	24	5	6	67	30	1.77	0.90	3.49	1.03	0.24	4.45	1.66	0.87	3.17
No	242	232	173	153	36	23	209	176	1.00	0.73	1.38	1.51	0.82	2.81	1.07	0.79	1.45

Note: [a]Adjusted for age, pack-years, familiar history of lung cancer and occupation (list A), when corresponding.
Abbreviations: CI, confidence interval; OR, odds ratio.

Table 6 Stratified analysis of p21 Ser31Arg and p53 Arg72Pro polymorphisms

	Reference[b]		Moderate risk[c]		High risk[d]		Moderate risk			High risk		
	Case	Control	Case	Control	Case	Control	Adjusted OR[a]	CI	95%	Adjusted OR[a]	CI	95%
Gender												
Male	252	241	283	227	45	18	1.09	0.82	1.44	2.29	1.21	4.32
Female	33	30	31	28	7	4	1.20	0.53	2.72	2.48	0.55	11.14
Stratified analysis (male only)												
Age (years)												
<55	38	53	48	51	10	3	1.13	0.58	2.20	4.02	0.91	17.79
55–69	113	89	104	89	19	7	0.86	0.55	1.36	1.69	0.59	4.80
>70	101	99	131	87	16	8	1.41	0.91	2.18	2.37	0.89	6.30
Smoking status												
Never	5	44	2	49	0	7	0.38	0.07	2.15	–	–	–
Ever	247	197	281	178	45	11	1.29	0.98	1.70	3.61	1.80	7.22
Former	115	116	142	100	20	6	1.56	1.07	2.29	3.12	1.19	8.20
Current	131	78	138	71	25	4	1.12	0.74	1.68	4.25	1.41	12.82
Pack-years												
<37	53	117	48	101	9	7	1.10	0.67	1.79	3.52	1.21	10.21
≥37	193	78	233	77	36	4	1.25	0.85	1.82	3.85	1.32	11.25
Histological type												
Squamous cell carcinoma	108	241	121	227	18	18	1.12	0.78	1.61	2.59	1.18	5.69
Adenocarcinoma	72	241	74	227	9	18	0.95	0.63	1.43	1.44	0.54	3.80
Small cell carcinoma	38	241	50	227	11	18	1.30	0.78	2.15	4.33	1.70	11.06
List A												
Yes	54	42	60	35	12	1	1.21	0.63	2.32	5.69	0.64	50.60
No	196	199	222	192	33	17	1.05	0.77	1.44	1.99	1.01	3.94

Notes: [a]Adjusted for age, pack-years, familial history of lung cancer, and occupation (list A), when corresponding; [b]p21Ser/Ser and p53 Arg/Arg; [c]p21 Arg carriers and p53 Arg/Arg or p21 Ser/Ser and p53 Pro carriers; [d]p21 Arg carriers and p53 Pro carriers.

Abbreviations: CI, confidence interval; OR, odds ratio.

younger than 55 years having a four-fold increased risk (adjusted OR 4.02; 95% CI 0.91–17.79). Stratification for smoking status revealed a higher risk for ever smokers (adjusted OR 3.61; 95% CI 1.80–7.22) and especially for current smokers (adjusted OR 4.25; 95% CI 1.41–12.82). Regarding histological type, we found that those with the high-risk genotype showed an increased risk of developing squamous cell carcinoma (adjusted OR 2.59; 95% CI 1.18–5.69) and a four-fold increased risk of small cell carcinoma (adjusted OR 4.33; 95% CI 1.70–11.25).

Discussion

In the current study, we did not find a statistically significant association between the p21 Ser31Arg polymorphism and increased susceptibility to developing lung cancer in cases or controls. Nevertheless, it seems that the Arg allele may be related to an increased risk of lung cancer in men younger than 55 years and in those who smoke. The Arg allele may also increase the risk of small cell carcinoma. These results suggest that polymorphic variants may be less efficient and, thus, the capacity for cell cycle control by the p21 variant is limited compared with the wild-type protein.

With regard to the combined analysis of Ser31Arg and Arg72Pro polymorphisms, the results reported here are in agreement with those obtained for p21 Ser31Arg; in men who are carriers of risk alleles (ie, p21 Arg-carriers and p53 Pro carriers), there is an increased risk of lung cancer. This risk has been particularly noted in men younger than 55 years who smoke, and is more likely to result in squamous cell carcinoma or small cell carcinoma.

We focused on the male population because the histological distribution was different between men and women. The most common tumor observed in males was squamous cell carcinoma and in women was adenocarcinoma. Furthermore, in our study population, there were limited numbers of women.

In our study, the frequency of the Arg allele was similar to that obtained in other studies performed in Caucasian populations. Our frequency of Arg allele carriers was 0.07, which is similar to that reported by Koopmann et al (0.085),[25] Su et al (0.09),[20] and Popanda et al (0.07)[26] in European populations.

Thus, by analyzing the whole population, we show an increased risk of lung cancer in men who were Arg carriers. Presence of the Arg allele increased the risk in men younger than 55 years. The same was true for smokers but, interestingly, smokers who consumed less than 37 pack-years had a higher risk than heavy smokers. Thus, our data suggest that men who are Arg carriers develop cancer prematurely and with a smaller burden of external aggression, so this polymorphism may be a promising risk marker for this population group. The Arg allele frequency varies by ethnicity, so the genetic effects of p21 Ser31Arg polymorphisms might be different in distinct ethnic groups.

Because p21 plays a fundamental role in inhibiting cell cycle progression and apoptosis, and can inhibit tumor progression,[27] genetic polymorphisms may alter the normal function of the protein and lead to an increased risk of lung cancer. The association between Ser31Arg polymorphisms and risk of developing different kinds of tumors has been analyzed in several studies, with disparate results. Specifically, the presence of the Ser allele is associated with an increased risk of cervical and endometrial cancer,[28,29] and the presence of the Arg allele has been correlated with a higher risk of breast cancer and squamous cell carcinoma of the head and neck.[30,31]

Although a clear difference has not been demonstrated in p21 protein function (cyclin-dependent kinase inhibitor or cell cycle suppressor) due to the presence of the Arg allele, the possibility cannot be excluded that the Arg allele might affect other processes in which p21 is involved, either p53-dependent or related to other transcription factors.[18] Thus, there may exist post-transcriptional or translational modifications that also alter the protein function.

Lung cancer studies have yielded contradictory results. In a Swedish study involving 144 cases and 761 controls, the risk of lung cancer associated with the Arg allele was increased (OR 1.7).[18] Unlike our results, which point to an increased risk in Arg carriers, no association was found in a study of 155 cases and 189 controls in a Taiwanese population.[19] Su et al did not find an association between this polymorphism and lung cancer risk in a Caucasian population of 1220 cases and 1069 controls either, but there was a slight decrease in risk for former smokers (OR 0.69).[20] Choi et al demonstrated that Ser31Arg polymorphisms appeared to be in linkage disequilibrium with IVS2 + 16G > C (rs3176352) in a Korean population. Analysis of this haplotype for lung cancer susceptibility demonstrated a protective effect which was dependent on the number of variant alleles.[32]

A negative correlation between lymph node status and p21 expression was observed in a recent study, suggesting a possible role for p21 in the progression of nonsmall cell lung cancer. In the same study, a statistically significant correlation was found between expression and survival.[33] Our results differ from those obtained in other studies of lung, endometrial, and gastric cancers, for which the Ser allele

of p21 appears to confer increased risk.[26,29,34] This may be explained by the difference in allele frequencies between European and Asian populations.

Large sample sizes from a homogeneous population with similar ancestry (802 cases and 718 controls) and high participation of eligible cases (rate 91.4%) are notable strengths in our study. Furthermore, all of our cases were confirmed pathologically. A potential limitation could be the use of hospital-based controls. Because information on confounding variables was obtained retrospectively, recall bias could be present. However, estimators obtained for the most important confounding variable (tobacco) were in line with the literature. Residual confounding cannot be excluded, because these data could not be evaluated in the never smoked group, and the number of nonsmoker cases was limited. Moreover, given that the polymorphism frequency in our study was small, some strata had a small size, so the stratified analysis was limited.

Conclusion

The presence of both variant alleles (p21 Ser31Arg and p53 Arg72Pro) increased the risk of lung cancer in men, especially those younger than 55 years and in those who smoked. Therefore, these polymorphisms may have a potential role as markers of susceptibility for these groups of people.

Acknowledgments

We would like to thank all the patients who participated in the study. We are also indebted to the Instituto Universitario de Oncología, which is supported by Obra Social Cajastur-Asturias, Spain. This work was supported by the Fundación para el Fomento en Asturias de la Investigación Científica Aplicada y la Tecnología (BP09-031, IB09-133), Instituto de Salud Carlos III [FISS-PI060604], and Obra Social Cajastur.

Disclosure

The authors declare that they have no competing interests in this work.

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