Serum p53 antibodies in patients with lung cancer: correlation with clinicopathologic features and smoking

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Abstract

Abnormalities of p53 gene can lead to the production of p53 antibodies (p53-Abs) in the serum of cancer patients. This study was designed to investigate the prevalence of p53-Abs in 133 lung cancer patients and the distribution of these antibodies to clinicopathologic features and smoking status. Twenty five (18.8%) lung cancer patients were found to have p53-Abs. The presence of p53-Abs did not correlate with sex or age but showed frequent association with tumors of squamous cell carcinoma (31%) in comparison with adenocarcinoma (13.6%) ($P = 0.052$). There was a statistically significant difference in the incidence of p53-Abs between early disease group (stage I–II) and the advanced group (stage III–IV) ($P = 0.036$), however, there was no relationship between the presence of p53-Abs and overall survival. Interestingly, the frequent of p53-Abs was higher in smokers (27.1%) than in non-smokers (13.6%), though the difference was of borderline of statistical significance ($P = 0.061$). These findings suggested that p53-Abs could be a potential biomarker for the study of individual with lung cancer.
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1. Introduction

Lung cancer is one of the most common cancer among Thai men [1]. Because lung cancer does not show any symtoms in early stage of the disease, the majority of Thai patients with this cancer are diagnosed with metastasis. Searching for prognostic indicators of lung cancer is an important clinical issue. The p53, tumor suppressor gene, is a critical regulator of normal development involved in cell cycle control pathways, such as growth arrest, differentiation and apoptosis [2]. The mutant p53 proteins have a much longer half-life than that of the wild-type protein and thus accumulate in tumors cells. The accumulated proteins can be released from the tumor cells, recognized by the immune system in humans as a foreign protein and induce a humoral response with development of antibodies against the proteins. There is a generally a very good correlation between the presence of p53-Abs and p53 accumulation and/or mutation in the tumor [3]. Thus, the detection of p53-Abs can be used as a possible biomarkers for the occurrence of p53 gene mutation.

Cigarette smoking is the most important aetiology factor of lung cancer and account for more than 80% of lung cancer cases [4]. Cigarette smoke contains many known carcinogens, such as polyclyclic aromatic hydro-carbons (PAHs). Recent study has revealed that benzo[a]pyrene diol epoxide (BPDE), one of PAHs in cigarette smoking, preferentially forms DNA-adducts along exons of p53 gene in codons 157, 248 and 273, which are the major mutational hotspots in human lung cancer [5]. In this study, we reported the prevalence of p53-Abs in lung cancer patients, and the distribution of these antibodies to clinicopathologic features and smoking status.

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2. Materials and methods

2.1. Patients

The subjects in this study were recruited from National Cancer Institute (Bangkok) from May 1999 to January 2000. The primary lung cancer (133) were newly diagnosed and confirm by pathology and radiology report and had not received any therapy including radiotherapy, chemotherapy and surgical resection. Most patients presented in advanced stage (stage III–IV), only 17 patients underwent thoracotomy. Of the 116 inoperable patients, 76 had been treated with chemotherapy, the remaining had been treated with radiotherapy. Patients donated a blood sample for a routine clinical examination and excess sera were kept frozen at -80 °C and were used for the present analysis. For each patient, age, gender, histopathological type, staging, and smoking status were recorded. Staging was defined according to the international TNM classification proposed by the American Joint Committee on Cancer (AJCC) [6]. Control was obtained from healthy people who come to NCI for an annual physical check-up. For all samples a detailed history of smoking habits was recorded including daily consumption, age of commencement, duration of smoking, for ex-smoker, year since quitting smoking. Detailed information about smoking status, non-smokers were defined as never-smoker or those who had ever smoked < 0.1 pack-year (pack per day $\times$ smoking year), whereas, smokers meant current smokers or ex-smokers of ≥ 0.1 pack-year.

2.2. ELISA

p53 antibodies were identified using an ELISA with plates coated either with p53 or a negative control. The sensitivity and the specificity of this assay have been already described in previous works [7,8].

2.3. Statistical analysis

The chi-squared test was used to compare the association between the presence of p53-Abs and several clinicopathologic parameters. The Kaplan–Meier method was used to estimate survival possibility as a function of time, and survival differences were analyzed by the log rank test. P-values of less than 0.05 were considered statistically significant. All data analysis were performed using a standard statistical program.

3. Results

3.1. Correlation of p53-Abs and clinicopathologic features

p53-Abs were detected in 25 (18.8%) patients of 133 patients with lung cancer. Table 1 shows the relationship between the presence of p53-Abs and various clinical/pathologic characteristics. Neither age nor sex was correlated with the presence of p53-Abs. Most patients presented in advanced stage, comprising 28 patients (22.4%) in stage III and 67 patients (53.6%) in stage IV. The prevalence of p53-Abs were 0% (0/2), 7.1% (2/28), 21.4% (6/28) and 25.4% (17/67) in stage I, II, III, and IV, respectively. There was a statistically significant difference in the incidence of p53-Abs between the early disease group (stage I–II) (6.7%) and the advanced disease group (stage III–IV) (24.2%) ($P = 0.036$) when stage I–II were considered to be early disease; stage III–IV were advanced disease. By histological types, the p53-Abs rate was higher in squamous cell carcinoma cases (31%) than in adenocarcinomas cases (13.6%) with a P-value of 0.052. Small cell lung carcinoma (SCLC)

Table 1
The relationship between incidence of p53 autoantibodies with various pclinicopathologic features and smoking

Feature	No. cases examined	p53-Abs+ (%)	P
Sex			
Male	106	20 (18.9)	0.967
Female	27	5 (16.1)	
Age, y			
≤ 60	77	13 (16.9)	0.509
> 60	56	12 (21.4)	
Stage			
I–II	30	2 (6.7)	0.036*
III–IV	95	23 (24.2)	
I	2	0	
II	28	2 (7.1)	
III	28	6 (21.4)	
IV	67	17 (25.4)	
Histologic type			
Adenocarcinoma	59	8 (13.6)	0.052[†]
Squamous cell carcinoma	29	9 (31)	
Large cell carcinoma	4	–	
Small cell carcinoma	13	3 (23.1)	
Smoking status			
Nonsmokers	66	9 (13.6)	0.061[#]
Smokers	59	16 (27.1)	
≤ 20 pack-years	26	7 (26.9)	
> 20 pack-years	33	9 (27.3)	
Total	133	25 (18.8)	

* P-value for early disease (stage I–II) vs. advanced group (stage III–IV).
[†] P-value for squamous cell carcinoma vs. adenocarcinoma.
[#] P-value for smokers vs. nonsmokers.

had the prevalence of p53-Abs at 23.1% (3/13). We did not find any evidence of p53-Abs in large cell lung cancer cases (0/4) because of the small sample size.

Table 2 shows that 25 of 133 lung cancer patients (18.8%) were positive for p53-Abs and five of 200 controls (2.5%) were positive. A significant difference between cases and controls was found ($P < 0.001$).

3.2. Relationship between the presence of p53-Abs and smoking status

There was a trend of increase of p53-Abs with smoking, nine of 66 non-smokers (13.6%) and 16 of 59 smokers (27.1%) among lung cancer patients, but the difference was of borderline statistical significance ($P = 0.061$) (Table 1). Furthermore, no association was obtained between the presence of p53-Abs and various smoking group. The number of smoker:non-smokers were 24:4 in squamous cell carcinoma group and 27: 28 in adenocarcinoma group (data not shown).

3.3. Effect of p53-Abs on patient survival

We analyzed the association between p53-Abs and over all survival of 115 patients. At 2 years of follow up, 91 (79%) patients had died of the disease, 19 patients (16.5%) loss follow up, and only five patients (4%) still alive during this observation. As shown in Fig. 1, the Kaplan–Meier survival curve demonstrated that the presence of p53-Abs did not appear to be correlated with survival time ($P = 0.414$) by the log rank test). When a comparison was made within the group with early-stage (I–II), advanced stage (III–IV), squamous cell carcinoma, adenocarcinoma the effect of p53-Abs on survival was not statistically significant (Table 3).

4. Discussion

In the present study, we detected p53-Abs in 25 (18.8%) of 133 sera patients with lung cancer. This incidence is generally in accordance with previous reported from Western countries [7]. Different frequencies of p53 gene mutation and p53 protein overexpression among the histologic types of lung cancer have been
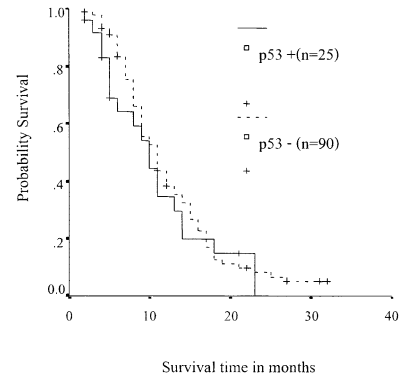

Fig. 1. Overall survival according to p53-Abs status.

Table 3
p53 autoantibodies and survival in lung cancer patients

Variable	P53-Abs($+$)	P53-Abs($-$)
Stage I–II		
No. of cases (n)	2	28
Mean survival time	All censored	20
Analysis of variance (P value)	NA	
Stage III–IV		
No. of cases (n)	23	72
Mean survival time (months)	9	10
Analysis of variance (p value)	0.917	
Squamous cell carcinoma		
No. of cases (n)	9	20
Mean survival time (months)	11	11
Analysis of variance (p value)	0.760	
Adenocarcinoma		
No. of cases (n)	8	51
Mean survival time (months)	13	13
Analysis of variance (p value)	0.853	

NA, not applicable.

reported in many studies [9,10]. Li et al. [11] showed that p53-Abs were more frequent in SCLC (42.9%) than those with squamous cell carcinoma (25%) or with adenocarcinoma (14.6%). A difference in prevalence of p53-Abs by histological type of lung cancer is also found in our study. In squamous cell carcinoma, nine of 29 (31%) patients had p53-Abs, whereas eight of 59 patients (13.6%) was found in adenocarcinoma ($P = 0.052$). Patients with SCLC might be expected to have higher incidence of p53-Abs, since the incidence of p53 mutation in SCLC is even higher than in squamous cell carcinoma [10]. However, they were only detected in 23.1% in our study, probably the small sample size of SCLC. The presence of p53-Abs is usually associated with poor prognosis and shorter survival for non small cell carcinoma (NSCLC) [12,13]. In other types of cancer, such as breast [14] colon [15] or head and neck [16], the presence of p53-Abs has been reported to be a marker of poor prognosis. Some groups have found no

Table 2
The incidence of p53 autoantibodies in study population

Population	No. cases examined	P53-Abs$+$ (%)	P
Healthy non smoker	100	1	0.002[†]
Healthy smokers	100	4	
Lung cancer patients	133	25	

[†] P-value for healthy smoker vs. lung cancer patients.

such correlation [17] and others have a favorable prognosis [18,19]. In the present study, the presence of p53-Abs was significantly associated with patients in advanced disease (stage III–IV) (24.2%) than in early disease (stage I–II) (6.7%) ($P = 0.036$). However, there was no difference in survival time between patients having lung cancer with p53-Abs and those without p53-Abs.

Cigarette smoke is closely associated with p53 mutation and overexpression. Husgafvel-Pursiainen et al. [20] reported that there were different frequencies of p53 mutation by smoking status with p53 mutations increasing from non-smokers (25%) to ex-smokers (38%) to current smokers (55%). Li et al. [11] observed that there was a mild trend with the frequencies of p53-Abs increasing from non-smokers (14.3%) to ex-smokers (16.7%) to current smokers (19.1%), and heavy smokers (41 pack-years and more) had the highest prevalence of the antibodies (28.6%). Similarly, our study showed that smokers (27.1%) had a higher frequency of p53-Abs than non-smokers (13.6%) with a P-value of 0.061. Lubin et al. [21] and Trivers et al. [22] found that the p53-Abs could be detected in ex-smokers or current smokers as early as 15 months prior to the diagnosis of cancers of the lung, breast and prostate. This finding suggested that p53-Abs may facilitate the early diagnosis of cancer.

To date, numerous studies have attempted to evaluate the clinical value of p53-Abs. Zalcman et al. [23] showed that there was a good correlation between the specific evolution of the p53-Abs titer and the response to chemotherapy in patients with lung cancer. A similar situation was described in colorectal [24] and ovarian cancer [25]. This raises the possibility that p53-Abs could be a good biomarker for lung cancer.

In summary, in this study we have demonstrated a higher prevalence of p53-Abs in lung cancer patients in a pattern by histological types consistent with prior studies and the suggestion that this could be related to smoking. These results suggest that p53-Abs could be a potential biomarker for the study of individuals with lung cancer or at-risk for the development of lung cancer. However, this application has to be explored in further studies.

Acknowledgements

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