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การตรวจหา p53-Abs ในผู้ที่อยู่ในกลุ่มเสี่ยง (ผู้ที่สูบบุหรี่จัด)

ในบรรดาผู้ที่สูบบุหรี่จัด (>20 pack-year) จำนวน 189 คน สามารถตรวจพบ p53-Abs ในซีรั่มได้จำนวน 8 คน (4.2%) ส่วนผู้ที่ไม่สูบบุหรี่ จำนวน 180 คน สามารถตรวจพบ p53-Abs ในซีรั่มได้จำนวน 2 คน (1.1%) คณะผู้วิจัยได้ติดตามผู้ที่มี p53-Abs ในซีรั่ม ทั้ง 10 คนนี้ เพื่อมาตรวจร่างกาย และตรวจหา p53-Abs เป็นระยะ ในกลุ่มผู้ที่สูบบุหรี่จัด มีเพียง 4 คนที่ตอบไปรณีย์บัตรว่าสุขภาพแข็งแรงดี และมีเพียงแค่ 1 คนเท่านั้นที่มาตรวจร่างกายอีกที่สถาบันมะเร็งแห่งชาติ และยังคงพบ p53-Abs ในซีรั่ม ในกลุ่มผู้ที่ไม่สูบบุหรี่มีเพียง 1คน ที่มาตรวจร่างกายอีกหลังจาก 1 ปี แต่กลับไม่พบ p53-Abs ในซีรั่มอีก จากการไม่ได้รับความร่วมมือจากอาสาสมัครโครงการทำให้การวิจัยในส่วนนี้ไม่สามารถสรุปผลการทดลองได้

คณะผู้วิจัยจึงได้ทำการศึกษาต่อเรื่องการนำการตรวจพบ p53-Abs มาใช้ประโยชน์ทางคลินิกในผู้ป่วยมะเร็งเต้านม อนึ่งโครงการวิจัยในเรื่องนี้เป็นโครงการที่ได้รับทุนสนับสนุนจากกรมการแพทย์ กระทรวงสาธารณสุข ซึ่งใช้เวลาในการวิจัย 2 ปี และใช้งบประมาณ 260,000 บาท แต่หลังจากดำเนินงานไปเพียง 1 ปี ได้รับเงินสนับสนุน 130,000 บาท ก็ได้รับแจ้งจากทางกรมการแพทย์ว่าไม่สามารถสนับสนุนทุนวิจัยในปีที่ 2 ได้เนื่องจากงบประมาณที่จำกัด คณะผู้วิจัยจึงได้นำเงินส่วนที่เหลือของ สกว. มาใช้ในการดำเนินการวิจัยต่อในโครงการมะเร็งเต้านมนี้ งานวิจัยในส่วนนี้ได้ดำเนินการจนเสร็จสิ้นโครงการ และได้เผยแพร่ในวารสาร Cancer Detection and Prevention

คณะผู้วิจัยได้ขอบคุณการสนับสนุนของ สกว.ใน acknowledgment (รายละเอียดดูได้จาก manuscript ภาคผนวก)

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ 2 ฉบับ

   1.1 Sangrajrang S., Sornprom A., Chernrungroj G', Soussi T. Serum p53 antibodies in patients with lung cancer: correlation with clinicopathological features and smoking. Lung Cancer 2003; 39:297-301.

   1.2 Sangrajrang S., Apornwirat W., Cheirsilpa A., Thisuphakorn P., Kalalak A., Sornprom A., Soussi T. Serum p53 antibodies in correlation to other biological parameters of breast cancer. Cancer Detection and Prevention 2003; 27(3):182-186.

2. การนำผลงานวิจัยไปใช้ประโยชน์

   จากผลการทดลองทำให้แพทย์ และบุคลากรทางการแพทย์ ทั้งในสถาบันมะเร็งแห่งชาติและที่อื่นๆสนใจที่จะนำการตรวจหา p53-Abs โดยวิธี ELISA ที่สะดวกสามารถทำได้ไม่ซับซ้อนมาประยุกต์ใช้ในผู้ป่วยมะเร็ง

3. การเสนอผลงานในที่ประชุมวิชาการ

   นำเสนอผลงานในการประชุม The 4th HUGO Pacific Meeting and 5th Asia-Pacific Conference on Human Genetics ที่โรงแรม Ambassador City Jomtien, พัทยา, วันที่ 27-30 ต.ค. 2545

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Serum p53 antibodies in correlation to other biological parameters of breast cancer

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Abstract

Breast cancer is the second most frequent cancer of Thai women. Mutation of *p53* is a common event in breast cancer. This alteration can result in cellular accumulation of p53 and may also found in serum p53 antibodies (p53-Abs). To clarify prognostic significance of these antibodies, we evaluated p53-Abs in 158 sera of patients with breast cancer. Thirty (19%) patients were found to have p53-Abs. The incidence of p53-Abs tended to be higher in patients with advanced disease group (stages III and IV) than patients with early disease group (stages I and II) ($P = 0.055$). Strong correlations were found between the presence of p53-Abs and p53 protein expression ($P < 0.001$) and lymph node status ($P = 0.021$). The presence of p53-Abs was associated with lack of estrogen (ER) receptor expression ($P = 0.035$) but was not related to progesterone receptor (PR) ($P = 0.567$). In addition, there was a statistically significant correlation between p53-Abs and proliferation associated antigen Ki-67 ($P = 0.006$), but no relation between c-erbB2 oncoprotein and p53-Abs was observed ($P = 0.112$). Additionally, no correlation was noted between the presence of p53-Abs and serum carcinoembryonic antigen (CEA) or carbohydrate antigen (CA15-3). Our findings indicate that p53-Abs appears to be a promising new parameter to evaluate the cellular biology and prognosis of breast cancer.

Keywords: p53 antibodies; Breast cancer; ELISA

1. Introduction

Activation of p53 in response to cellular or genotoxic stress induces several responses, including DNA repair, senescence, differentiation, cell cycle arrest and apoptosis [1,2]. These functions are achieved, in part, by the transactivational properties of p53, which activate a series of genes involved in cell cycle regulation. Mutation in the *p53* gene are found in 50% of all human malignancies. Most of the known *p53* gene alterations are missense mutations clustered in the evolutionarily highly conserved exons 4–8 [3,4]. These mutations result in a biologically inactive p53 protein that stably accumulates in the cell nucleus and can be detected by immunohistochemistry. In the absence of wild-type p53 protein, genetic aberrations are more likely to accumulate leading to genetic instability and cell transformation.

It has been demonstrated that *p53* mutations can lead to the production of p53-Ab which can be detected in the sera of patients with various types of cancers [5]. These antibodies recognize immunodominant epitopes localized in the amino-terminus and, to a lesser extent, in the carboxy-terminus of human p53 [6–8]. Antibodies specific for the central region is always very low or absent [6]. The mechanism by which p53 is presented in the immune system is unknown. Isotyping of p53-Abs has shown that they correspond mainly to IgG1 and IgG2 subclasses [9], corresponding to a secondary immune response. The usefulness of anti-p53 serology for detection of *p53* gene alteration has been studied in several malignancies including breast cancer [10–15]. The present study was performed to evaluate the prevalence of p53-Abs in correlation to p53 accumulation, ER, PR, c-erbB2, Ki-67 protein's expression, and circulating tumor markers CEA, CA15-3, and to the conventional clinicopathological parameters.

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2. Materials and methods

2.1. Serum collection

Patients had donated a blood sample for routine clinical examination prior to any treatment and excess sera were kept frozen at −80 °C and were used for the present analysis. For each patient, age, histopathological type and staging were recorded. Staging was defined according to the international TNM classification proposed by American Joint Committee on Cancer (AJCC) [16].

2.2. ELISA

p53 protein was prepared from recombinant baculoviruses by infecting Sf9 insect cell. The harvested cells were lysed, and protein was extracted. Ninety-six-well microtiter plates were coated with 100 μl of recombinant wild-type human p53 protein or control protein for 24 h at 37 °C. Immunoplates were blocked with PBS containing 2% casein and 0.2% Tween 20 to detect non-specific interactions. Duplicate immunoplates were then incubated with patient serum (100 μl per well) diluted 1:100 in PBS containing 5% non-fat milk at room temperature for 1 h and with anti-human IgG peroxidase conjugate human diluted 1:5000. The anti-human peroxidase activity was then visualized with 100 μl of tetramethylbenzidine solution. The reaction was stopped by adding 100 μl of 1 M sulfuric acid. Plates were then read at 450 nm using a MR5000 (Dynatech Laboratories). The result was then validated by comparison of the optical density plot of this series compared to the negative control, and the cut-off point was defined as 1.6 times the negative control [9].

2.3. Immunohistochemistry (IHC)

Immunohistochemical analysis was performed on tissues using conventional peroxidase method. After undergoing dewaxing, inactivation of endogeneous peroxidase, the sections were incubated with monoclonal antibodies in case of p53, Ki-67, ER, PR, (Dako, Denmark) and polyclonal antibody for c-erbB2 (Dako, Denmark) overnight at 4 °C. Subsequently, detected with biotinylated horse anti-mouse or anti-rabbit IgG antibody and streptavidine-biotin-conjugated horse radish peroxidase (Dako, Denmark). Peroxidase activity was detected using diaminobenzidine tetrachloride. For p53, ER, PR and Ki-67 nuclear staining of invasive tumor cells was scored as positive. For c-erbB2 membranous staining of invasive tumor cells was scored as positive. The threshold for p53 was 5%, for ER and PR was 10% and Ki-67 was 13%. ELISA and IHC were performed independently by two of the authors (SS and AK, respectively).

2.4. Assay for CEA and CA15-3

For the analysis of CEA and CA15-3, we used commercially available kits (Roche, Mannheim, Germany). The assay was performed according to the manufacturer's recommendations. The cut-off values were taken from the manufacturer's data, 5 ng/ml for CEA and 30.8 U/ml for CA15-3.

2.5. Statistical analysis

Data are presented as percentages or mean as appropriate. Statistical analysis of the data was performed using Microstat software. A χ^2-test was performed to determine the association between the presence of p53-Abs and clinicopathological features of the patients. Statistical significance was assessed at 5% level.

3. Results

3.1. Relationship between the presence of p53-Abs and clinicopathologic features

Of the 158 sera assayed from breast cancer patients (116 for preoperative evaluation and 42 for pre-chemotherapy investigation), 30 (19%) were positive for circulating p53-Abs including 3 early breast cancer (Table 1). Table 2 shows the relationship between the presence of p53-Abs and various clinical/pathologic characteristics. The presence of p53-Abs was independent of patient age. There was a difference in the incidence of p53-Abs between the early disease group (stages I and II) (14.3%) and the advanced disease group (stage IV) (26.7%), although this difference did not reach statistical significance ($P = 0.055$). A statistically significant relationship was noted between the presence of p53-Abs and local-regional lymph node involvement ($P = 0.021$). All tumors were invasive ductal carcinoma histological subtype. All patients were sero negative for HIV and Hepatitis B (HBs).

3.2. Relationship between the presence of p53-Abs and nuclear accumulation of p53 protein

A total of 28 (43.8%) of the 64 tumor assayed for nuclear accumulation of p53 were IHC positive (Table 3). Twenty tumors (71.4%) of the p53-Abs positive patients with available tissue had been immunohistochemically stained for cellular p53 accumulation (overexpression). Six tumors (16.7%) of p53-Abs positive patients with available tumor tissue had IHC negative. A highly significant association was found between p53 protein accumulation in tumors and the presence of p53-Abs ($P < 0.001$).

3.3. Relationship between the presence of p53-Abs and other biomarkers

Association analysis between hormone receptor status revealed a negative association between p53-Abs and estrogen receptor (ER) ($P = 0.035$) (Table 4), however, p53-Abs

Table 1
p53 antibodies in 30 breast cancer patients

No.	Age (years)	Stage	p53 protein	ER	RR	c-erbB2	Ki-67	CEA (ng/ml)	CA15-3 (U/ml)
1	40	IIA	+	−	−	−	ND	1.5	8
2	39	IIIA	+	−	−	+	ND	2	9
3	49	IIIA	+	−	−	−	+	4.3	18.3
4	38	I	−	+	+	+	−	1.8	15
5	62	IIA	+	−	+	+	+	6.3	20.3
6	57	IIA	ND	ND	ND	+	ND	2	10
7	43	IIA	−	−	−	−	−	2.7	18
8	29	I	−	+	+	+	−	2.4	18.6
9	71	IIIA	+	+	−	+	+	3.4	13
10	51	IIIA	+	−	−	+	+	2	17
11	45	IIIA	+	−	−	−	+	1	23
12	52	IIIB	+	−	+	+	+	1.8	21
13	51	IIA	+	−	+	ND	ND	2	10
14	39	IIA	−	+	+	+	+	2.9	15
15	55	I	+	−	−	−	−	3.1	18
16	50	IIIA	+	−	−	−	−	1.7	122
17	38	IIB	+	−	−	−	+	3.4	15
18	51	IIIA	ND	−	+	ND	ND	2.3	26
19	45	IV	+	−	−	−	−	1.5	10
20	43	IIIA	+	−	−	−	+	2	7.8
21	49	IIIA	ND	ND	ND	ND	ND	2.5	14
22	43	IIB	+	−	−	+	+	1.6	18
23	72	IIIB	−	+	+	−	+	3.7	10
24	32	IIIA	+	+	+	+	+	1.4	128
25	51	IIIA	+	−		+	+	3.9	27
26	37	IIB	+	−	−	−	+	1.8	19
27	41	IIA	−	−	−	+	−	1	23
28	46	IV	+	−	−	+	−	2	21
29	73	IIA	+	−	−	−	+	2.6	13
30	39	IIIA	ND	ND	ND	ND	ND	3.2	31

ND: not determined.

Table 2
Relationship between prevalence of p53-Abs with various clinicopathological features

Feature	No. of cases examined	p53-Abs (%)	*P*-value
Age (years)			
≤50	96	19 (19.8)	0.749
>50	62	11 (17.7)	
Stage			
I and II	98	14 (14.3)	0.055
III and IV	60	16 (26.7)	
Lymph node			
N+	86	22 (25.6)	0.021
N−	72	8 (11.1)	
Total	158	30 (19)	

Table 3
Relationship between the presence of p53-Abs and nuclear accumulation of p53 protein

Nuclear p53 staining	p53-Abs		*P*-value
	−	+	
p53+	8	20 (71.4)	<0.001
p53−	30	6 (16.7)	<0.001

Values in parentheses are percentages.

was not related to progesterone receptor (PR) expression ($P = 0.567$). The presence of p53-Abs was positively correlated with proliferation marker, Ki-67 ($P = 0.006$). No relationship was observed between p53-Abs and c-erbB2 oncoprotein expression ($P = 0.112$). Concerning the circulating tumor markers, there was no correlation between serum p53-Abs and CEA ($P = 0.668$) or CA15-3 ($P = 0.470$) statuses.

Table 4
Relationship between the presence of p53-Abs and other biomarkers

Various markers	p53-Abs		*P*-value
	−	+	
ER+	26	6 (18.8)	0.035
ER−	30	21 (41.2)	
PR+	11	9 (45)	0.567
PR−	30	18 (37.5)	
Ki-67+	12	15 (55.5)	0.006
Ki-67−	28	8 (22.2)	
c-erbB2+	27	14 (34.1)	0.0112
c-erbB2−	10	12 (54.5)	
CEA (mean ± S.D.; ng/ml)	2.3 ± 0.9 ($n = 45$)	2.5 ± 1.1 ($n = 30$)	0.668
CA15-3 (mean ± S.D.; U/ml)	21.4 ± 5.3 ($n = 30$)	20.6 ± 21.1 ($n = 30$)	0.470

Values in parentheses are percentages.

4. Discussion

p53-Abs were originally described in 1982, by Crawford et al. [10] in the serum of 9% of breast cancer patients using a Western blotting method. Using ELISA, more than 15 studies have been performed recently in breast cancer [5]. The frequency of p53-Abs in breast cancer range from 15 to 20% but the majority of these studies were performed either in Europe or in US. No study have been performed in Thailand where the frequency of breast cancer is lower than in other countries. In the present study, we detected p53-Abs in 30 (19%) of 158 sera patients with breast cancer. The presence of p53-Abs is strongly associated to the group of tumors with p53 protein accumulation ($P < 0.001$) indicating that this immune response is triggered by the accumulation of p53 in the tumor as previously described in lung cancer [17]. The relationship between *p53* mutation, p53 accumulation in the tumor and p53 antibodies in the sera have been analyzed in several multifactorial analysis. It is now clear that all *p53* mutations will not lead to p53 accumulation as about 15% of *p53* mutation are frameshift or nonsense mutations that will not lead to the synthesis of a stable protein [4]. On the other hand, the presence of p53 antibodies is almost invariably associated with *p53* mutation in the tumor [18,19]. Patients with more advanced disease (stages III and IV) (26.7%) had a higher incidence of p53-Abs than in early disease (stages I and II) (14.3%), although the difference was not statistically significant ($P = 0.055$). We found that p53-Abs was associated with a lack of ER expression but was not related to PR suggesting that tumors eliciting antibody responses defined a subgroup with poor prognosis as previously described [20].

Few studies have analyzed the prognostic significance of coexpression of biomarkers. The observed association may further contribute to understanding of the biology of breast tumors. In breast cancer, the overexpression of p53 protein have been shown to be associated with rapid tumor cell proliferation [21] and overexpression of c-erbB2 [22]. Our result shows a good correlation between the presence of p53-Abs and Ki-67 expression, however, we have not found the relationship between c-erbB2 expression and p53-Abs. It should be noted that there was a difference between the frequency of c-erbB2 expression observed in this study (65%) and in the reports of Western authors (14–34%) [23,24]. The high prevalence of c-erbB2 expression (66%) was also reported in breast cancer in young Kuwaiti women [25]. Several research groups including our own found that there was no relationship between p53-Abs and CEA or CA15-3 statuses [26]. In addition, Takeda et al. [27] reported that the presence of p53-Abs was more significantly associated with stages 0, I and II colorectal cancer than was CEA.

Numerous studies have attempted to evaluate the clinical value of p53-Abs. Lubin et al. [28] showed that p53-Abs have been detected in two heavy smokers several years before clinical diagnosis of lung cancer. Similarly, Triver et al. [29] reported the presence of p53-Abs prior to a diagnosis for breast, lung, and prostate cancer. This finding suggested that p53-Abs may facilitate the early diagnosis of cancer. In addition, p53-Abs can be used to monitor patients during treatment. Zalcman et al. [30] 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. This had raised the possibility that p53-Abs could be a good candidate biomarker for several cancers.

p53-Abs are found predominantly in human cancer patients with specificity of 96%, but the sensitivity of such detection is only 30%. Because p53 was not detected in sera from patients with non-malignant diseases (0.5–1%) [5], the authors nevertheless suggested that serological testing for p53-Abs, despite its low positive results, can be regarded as a specific method to identify subgroups of patients with cancer. The development of circulating serum antibodies against oncogene and tumor suppressor gene product represents an interesting model system for studying immune response in cancer patients. The simple and rapid ELISA procedure suggested the potential usefulness of p53-Abs in clinical implications. However, further investigations in larger prospective homogeneous series of patients are necessary before definitive conclusion.

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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.

Keywords: p53-antibodies; Lung cancer; Clinicopathologic features; Smoking

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 hydrocarbons (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 × 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 clinicopathologic 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).

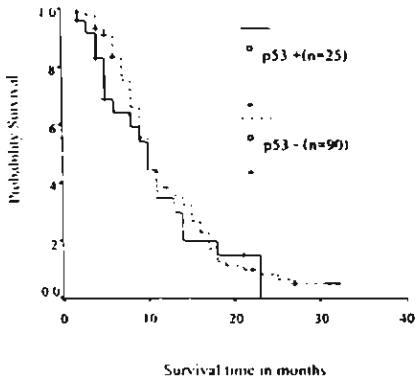

Fig. 1. Overall survival according to p53-Abs status.

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.

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 rep rted from Western countries [7]. Different frequencies of p53 gene mutation and p53 protein overexpression among the histologic types of lung cancer have been 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. Zaleman 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.

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