10.1053.paor.1999.0185 available online at http://www.idealibrary.com on IDEAL

ARTICLE

Clinical Importance of Correlations Between p53 Immunoreactivity and Clinicopathological Parameters in Lung Carcinoma

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Many studies have revealed the frequency of p53 abnormalities in lung cancer. However, clinicopathological studies of p53 abnormalities have yielded conflicting results. We examined the p53 immunoreactivity and studied the correlations of p53 status and clinicopathological parameters in 76 primary lung cancers. By using DO-7 antibody, different degrees of p53 immunoreactivity was detected in 8 of 30 small cell lung cancer (SCLC, 26.6%) and 22 of 46 non-small cell lung cancer (NSCLC, 47.8%), 6 of 19 adenocarcinoma, 16 of 27 epidermoid carcinoma cases. In the whole group, no correlation was detected between the p53 status and the histological types of tumor, local tumor invasion, nodal status, and distant metastasis and patient characteristics, such as age, gender or smoking habit. P53 status was also found to have no effect on survival. However, in the NSCLC group, there was a significantly higher p53 immunoreactivity in well- and moderately-differentiated tumors ($p<0.05$). Patients with p53 immunoreactivity had a poor therapeutic response in the whole group. We concluded that, although p53 immunreactivity may be found in NSCLC, this does not correlate with clinicopathological parameters except therapeutic response. In SCLC p53 immunreactivity can be negligible. (Pathology Oncology Research Vol 5, No 4, 285–290, 1999)

Keywords: p53, lung cancer, clinicopathology

Introduction

Primary lung cancer is one of the major causes of death in the world and is mainly related to environmental agents, especially smoking. Although diagnostic procedures, staging methods, and treatment possibilities develop, overall 5 year survival for lung carcinoma remains low at less than 15%. The 5-year's survival rates are 2–3% and 25–40% in operable and inoperable NSCLC patients, respectively. In SCLC, mean survival is only 11 months.

Factors such as clinicopathological stage of disease, histopathological type, performance status affect survival. But, it appeares that some cases, with the same histopathological type and stage, have a different prognosis. Therefore, it is thought that there are some molecular changes which affect prognosis. Several oncogenes and tumor-suppressor genes have been reported to be altered in lung tumors.[13] The p53 gene is thought to play an important role in the development of several other cancers, particularly colorectal carcinoma, breast cancer, urinary bladder cancer, brain tumors. The p53 gene encodes a nuclear phosphoprotein, which regulates normal cell growth. p53 acts as a tumor-suppressor gene, arresting cells in the G_1-S phase to give more time for repair, and if this is unsuccessful, leads cells to apoptotic death.[12] Conversion of p53 from the normal to mutant phenotype alters its histochemical characteristics, since the half-life of the protein is enhanced from 6–20 minutes to several hours. Mutant p53 has also been shown to bind to cellular proteins such as hsp70, which increase its stability. These two effects lead to a vast increase in the amount of p53 in affected cells, which reaches levels detectable immunohistochemistry.[8]

Although the importance of p53 mutations in the pathogenesis of lung cancer is clear, it is not clear whether the presence of p53 mutations or overexpression of p53 protein affects an individual patient's chances for survival. Some authors report poor prognosis associated with p53

Received: Febr 13, 1999; *accepted:* Oct 10, 1999

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 1219-4956/99/040285+06 $ 12.00/0

mutations, while others say that these changes have no effect or even improve prognosis.[3,6,9,18,19,22]

p53 protein also plays an important role in the therapeutic response. The effect of radiotherapy and chemotherapy on tumor cells is mainly associated with active apoptosis, which is induced by wild type p53, rather than a genotoxic effect.[28] The other serious problem in cancer therapy is chemoresistance. Positive correlation between chemoresistance and p53 status has been shown especially in gastrointestinal and urinary system malignancy, breast and ovarian carcinoma. Although there are limited studies, it is thought that this may be true in lung carcinoma.[7,26]

The aims of this study were to investigate the immunohistochemical detection of p53 protein in primary lung carcinoma; and to evaluate the correlation between p53 overexpression and clinicopathological parameters such as age, gender, smoking history, TNM staging, survival and overall treatment response.

Materials and Methods

We examined 46 NSCLC and 30 SCLC patients who received no previous treatment. The follow-up period ranged from 1month and 47 months; and survival data were available on all of them. Of the whole group, 18 (23.7%) patients were alive at the time of last control.

Staging included a routine detailed history and physical examination, biochemical survey, chest radiography, bronchoscopy, CT of thorax and brain, abdominal ultrasonography and whole body scintigraphy. The international staging system based on TNM parameters was used. To facilitate evaluation patients were split into early (stage 1-3A) and advanced (stage 3B-4) stage in NSCLC. The SCLC group was evaluated as limited and extensive disease.

Paraffin wax embedded tissue samples were obtained from bronchoscopic biopsies (66 samples), mediastinoscopic biopsies (3 samples) and surgical material (7 samples). Initially, samples which were not good enough for immunohistochemical study were excluded. Immunohistochemical detection was performed using the anti-p53 mouse monoclonal antibody DO-7 that recognises both normal and mutant p53 proteins from aminoacids 35–45. Four micrometer thick sections were deparaffinized and blocked with 3% H_2O_2 for 5–10 min. The sections were then rinsed with phosphate-buffered saline (PBS) for 5 min. The sections were treated with antigen retroviral solution (BioGenex HK 087-5K) in microwave oven for 15 min, left at room temperature for 30 min. and then washed in water three times. Protein blockage was done with non-immune blocking serum (BioGenex HK112-9K) for 20 min. After incubation with primary antibody p53 (DO7-BioGenex) by covering totally for 2 hours, sections were exposed to the secondary biotinylated antibody (supersensitive multilink-HK340-9K, BioGenex) for 20 min. After washing with PBS, sections were incubated with streptavidin-biotin complex (supersensitive label BioGenex, HK330-9K) for 30 min. To image, 3–3 diaminobenzidin (DAB) as a peroxidase labelled chromogen was used after rewashing with PBS. The sections were left for 10 min. and washed with water. Background was stained with haematoxylin-eosin for 5–10 seconds. Homogenous nuclear staining between 10% and 30% was accepted as grade 1 positivity, between 30% and 60% as grade 2 positivity, more than 60% as grade 3 positivity.

After surgical approach, radiotherapy (RT) and/or chemotherapy (CT) were applied to stage 3A disease. Any treatment modality was not applied to stage 1 and 2 disease after surgery. CT was preferred for the patients who had stage 3B with malignant effusion and stage 4 disease. RT was applied to the other stage 3B disease. Combined CT was used for all of the SCLC cases. If complete response obtained, prophylactic cranial radiotherapy and thoracic radiotherapy was applied. All CT protocols were based on cisplatin in either NSCLC or SCLC.

To evaluate therapeutic response, patients on CT were followed by chest radiography monthly for the first six months. Thoracic computed tomography and flexible bronchoscopy (FOB) were done after the third and sixth cycles of CT. After six cycles of CT, all systems were checked. In patients receiving RT, thoracic computed tomography, FOB and distant metastasis detection were done in the first month after RT completed. Patients were then followed at three months intervals. Four different categories were accepted for evaluating therapeutic response:

Complete response: Complete disappearance of all tumor lesions for at least 4 weeks after treatment.

Partial response: Reduction of 50% and more in the product of the longest perpendicular diameter of the lesion.

Stable disease: Neither more than 50% regression nor progression less than 25% in the size of measurable lesions.

Progressive disease: More than 25% in one or more existing lesions and/or the appearance of new malignant lesions.

Comparisons of the patients group were based on the use of time-to-event methods including Kaplan–Meier estimation, log-rank test, chi-square test and Fischer-exact test. Statistical significance was set at a significance level of 0.05.

Results

Forty-six NSCLC and thirty SCLC were studied. Of the NSCLC patients, 19 were adenocarcinoma and 27 were epidermoid carcinoma. 13 NSCLC patients (23.8%) were classified as early stage, 33 NSCLC patients (71.7%) as advanced stage. In the SCLC group, there were 9 (30%) with limited disease, and 21 (70%) with extensive disease. While the average age was 59 years (38–76) in the NSCLC group, it was 56 (42–70) in the SCLC group. In the whole group, there were 7 female cases, 4 and 3 in NSCLC and

Table 1. **Some characteristics of non-small cell lung cancer group**

Clinicopathological		*Adeno n=19*	*Epidermoid n=27*	*Total n=46*
Differentiation	Well	10	12	22
	Moderate	2	9	11
	Poor	7	6	13
Tumor	T_1	1	2	3
	T_2	6	8	14
	T_3	2	5	7
	T_4	10	12	22
Nodal Status	N_0	2	10	12
	N_1	1	3	4
	N_2	11	12	23
	N_3	5	2	7
Metastasis	M_0	7	20	27
	M_1	12	7	19

n: number of patients

SCLC, respectively. 40 cases in NSCLC and 25 cases in SCLC were smoker. There were 6 and 5 non-smoker cases in NSCLC and SCLC group, respectively. Other clinicopathological characteristics of NSCLC patients are shown in *Table 1*.

p53 immunoreactivity was detected 47.8% of the NSCLC group and 26.6% of the SCLC group. In the NSCLC group, p53 immunoreactivities were 31.6% and 59.3% in adenocarcinoma and epidermoid carcinoma, respectively. Detailed results are shown in *Table 2* and *Figure 1–4.*

No correlation was observed between p53 overexpression and age, gender, smoking history and histopathologic type. Negative correlation was detected between p53 overexpression and tumor differentiation in the NSCLC group ($p<0.05$) (*Table 3*). There was no correlation between p53 overexpression and local tumor invasion, distant metastasis, or stage in the whole group. Although there was a trend indicating an association between a lymph node involvement and p53 overexpression, this data was not statisitically significant in NSCLC group.

Median survivals were 9,5, 8 and 8 months in NSCLC, adenocarcinoma, epidermoid carcinoma and SCLC, respectively. Survival graphics of all cases and epidermoid or adenocarcinoma or small cell carcinoma separately showed parallel curves with no statistical difference between negative and positive cases. Patients with distant metastasis and negative p53 immunostaining had longer survival than positive cases; but statistically this data was not significant.

While RT was the most preferred treatment modality in epidermoid carcinoma (13 of 27), CT was applied to the vast majority of adenocarcinomas (14 of 19) and all of the SCLC. Details of treatment modalities are shown in *Table 4*. One of 30 SCLC patients underwent diagnostic surgery for solitary pulmonary nodule was treated combined with CT and RT. This patient was included to evaluate correlation between treatment response and p53 status as were the other two NSCLC patients to whom postoperative RT was applied. Only four NSCLC patients who underwent surgery were excluded for treatment response evaluation. Correlation between therapeutic response and p53 overexpression was not found ($p>0.05$). But the therapeutic response of patients who had positive and dense staining for p53 was worst in the whole group. Detailed results of therapeutic response and p53 overexpression are shown in *Table 5.*

Table 2. **p53 overexpression distribution in the whole group**

Histopathologic type	*Stage*	*p53 overexpression*					
		Positive (n=30)					*Negative*
		Grade-1	*Grade-2*	*Grade-3*	*Total*		*n=46*
SCLC n=30	Limited	2	–	–	2	8	7
	Extensive	1	3	2	6		15
NSCLC n=46	Early	2	2	2	6	22	7
	Advanced	5	7	4	16		17

NSCLC: non-small cell lung cancer,
SCLC: small cell lung cancer,
n: number of patients

Discussion

Cancer is thought to arise after accumulation of a threshold number of lesions in key regulatory molecules in previously normal cells, and these lesions are being increasingly well characterised. p53 protein, which has very important role in normal cells, has the most common genetic changes in lung carcinoma (25,27). p53 gene mutations were seen 75–90% and about 50% in SCLC and NSCLC, respectively (25). Although it is clear that p53 gene mutations play an important role in cell cycle and human cancers, there is conflicting data about how p53 mutations and p53 overexpression affect tumor characteristics and prognosis.

In NSCLC, using polyclonal antibody (PAb) 240, which recognises only mutant type of protein, Fontanini et al,[8] Iggo et al[10] and Marchetti et al[17] found p53 overexpression 70.9%, 35.9% and 58%, respectively. Dalquen et al[5] and Walker et al[31] detected p53 overexpression 47.8% and 68% with polyclonal antibody CM-1, respectively. Using PAb 1801, which recognises both normal and mutant type p53 proteins, p53

overexpression was found in range of 33–76% by several authors.[4,8,17,19,23,24,29] With DO-7 antibody, which has similar characteristics as PAb 1801, Ebina et al,[6] Nishio et al[20] and Lee et al[15] detected p53 overexpression in a range of 39–66%. In the literature, p53 overexpression has been reported in the range of 43-82% for epidermoid carcinoma, and 33–57% for adenocarcinoma.[5,10,14,18,20,21,30,32] Although authors who used an antibody which recognises only mutant p53 protein found numeric differences between subgroups of NSCLC, these results were not statistically significant.[8,10,17] Similar results were detected with an antibody that recognises both mutant and normal types of p53 protein.[5,21,31,32] Only Lee et al[15] found statistically difference in favour of epidermoid carcinoma. In SCLC, p53 overexpression rate has been reported in range of 20–80% by several authors.[11-14] This difference among rates has been explained as characteristic of SCLC's subgroups such as oat cell and non-oat cell small cell carcinoma. We found that p53 overexpression was 47.8%, 31,6%, 59,3% and 26,6% in NSCLC, adenocarcinoma, epidermoid carcinoma and SCLC, respectively. Although we detected a numerical difference in favour of epidermoid carcinoma; there was no statistically significant difference between the subgroups of NSCLC. The low rate in the SCLC group could be based on subgroup characteristics, but unfortunately we did not make this separation due to very small biopsy material. Despite this, our results are consistent with others', using the same antibody, though not consistent with others' done by antibody, which recognises only mutant type p53 protein.

When correlation between p53 overexpression and age, gender was evaluated, although Passlick et al[23] found more p53 overexpression in younger group, it is generally thought that there is no correlation, as our results suggest.[6,16,17,20,21] There are conflicting results about correlation between smoking and p53 overexpression. While Ebina et al[6] reported no correlation between p53 overexpression and smoking or number of cigars per day, Nishio et al[20] and Westra et al[32] detected positive correlation between p53 overexpression and smoking, but not number of cigars per day. Liloglou[16] also found more p53 overexpression in heavy smokers, but this result was not statistically significant. In our study, correlation between p53 overexpression and smoking or number of cigars per day was not found in the whole group, as reported by others.

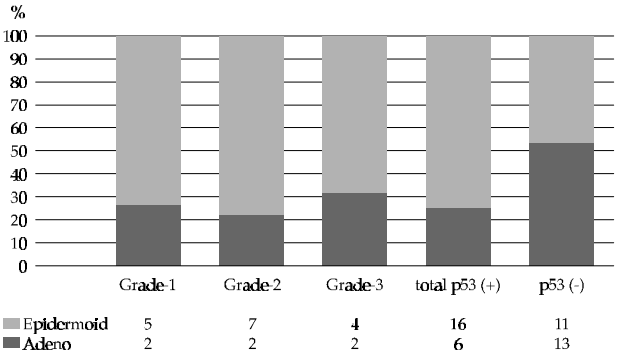

Figure 1. p53 overexpression distribution in non-small cell carcinoma

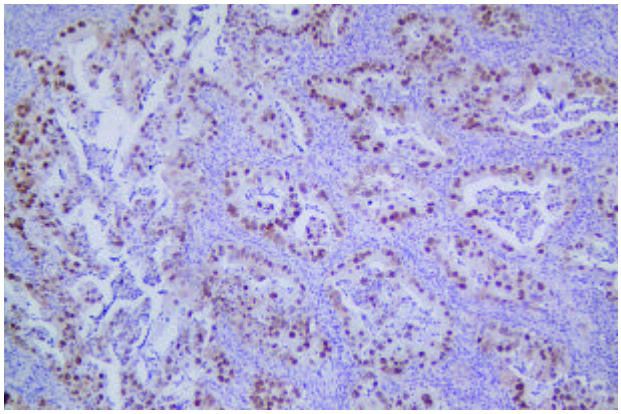

Figure 2. p53 immunreactivity in well differantiated epidermoid carcinoma (streptavidin-biotin x 200)

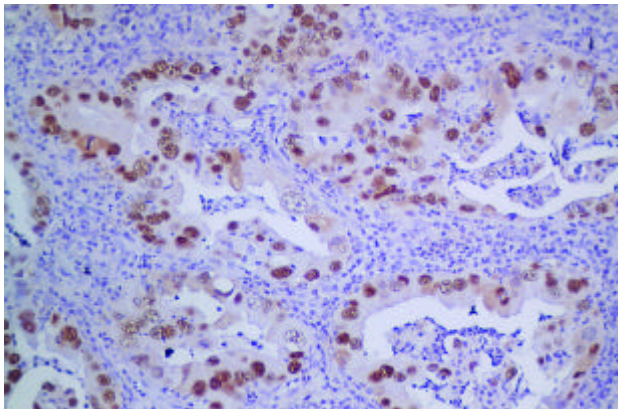

Figure 3. p53 immunreactivity in well differantiated adenocarcinoma (streptavidin-biotin x 400)

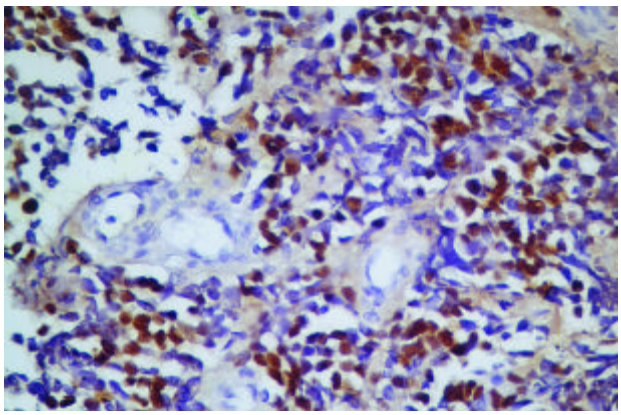

Figure 4. p53 immunreactivity in small cell carcinoma (streptavidin-biotin x 400)

While correlation between p53 overexpression and tumor differentiation was not found in some studies,[19,23,24] more p53 overexpression was detected in poorly differentiated tumors in two different studies.[29,32] To the contrary, we found more p53 overexpression in well and moderately differentiated tumors, and the result was statistically significant ($p<0.05$).

Table 3. **p53 overexpression distribution according to the tumor differentiation**

Differentiation	*p53 overexpression*				
	Positive (n=22)				*Negative*
	Grade-1	*Grade-2*	*Grade-3*	*Total*	*n=24*
Well	6	5	2	13	9
Moderate	–	4	2	6	5
Poor	1	–	2	3	10

n: number of patients

Table 4. **Treatment modalities**

Histopathologic type / *Treatment modalities*	*Adenocarcinoma N=19*	*Epidermoid carcinoma N=27*	*SCLC N=30*
CT	14	6	20
RT	2	13	–
Surgery	2	2	–
CT + RT	1	4	9
CT + Surgery	–	–	–
RT + Surgery	–	2	–
CT + RT + Surgery	–	–	1

CT: Chemotherapy
RT: Radiotherapy
SCLC: Small cell lung carcinoma
N: Number of patients

Table 5. **Therapeutic response and p53 overexpression**

Histopathologic type	*Therapeutic response*	*p53 overexpression*				*p*
		Positive (n=28)			*Negative*	
		Grade-1	*Grade-2*	*Grade-3*	*n=44*	
NSCLC n=42	Progressive disease	–	4	6	8	>0.05
	Stable disease	1	–	–	5	
	Partial response	3	2	–	8	
	Complete response	2	2	–	1	
SCLC n=30	Progressive disease	–	–	2	2	
	Stable disease	1	1	–	5	
	Partial response	1	1	–	8	
	Complete response	1	1	–	7	

NSCLC: non-small cell lung cancer,
SCLC: small cell lung cancer,
n: number of patients

Correlation between p53 overexpression and local tumor invasion has been reported different ways. Although there were some studies showed no correlation, Dalquen et al[5] found positive correlation.[14,21,24] In our study, significant positive or negative correlation was not found in the whole group. There are some studies which reported no correlation between p53 overexpression and nodal status.[21,24] On the other hand, other studies detected both numeric and statistically significant correlation.[8,14,17,31] We also found positive numeric correlation, but unfortunately the result was not significant. No correlation was detected between p53 overexpression and distant metastasis in all studies.[1,2,30] We also found no correlation. When correlation between p53 overexpression and stage was evaluated, no correlation was generally established.[4,5,19,23,29,32] However, in three other studies, statistically significant positive correlation was found.[1,2,8] In our study, no correlation was detected.

In the vast majority of studies, p53 overexpression did not affect survival.[4,18,20,24,29,30,32] But Korkoloupoulou et al[14] reported statistically significant negative correlation. Although Carbone et al[3] and Dalquen et al[6] found similar results, the results were not statistically significant. On the other hand, Morkve et al[19] and Lee et al[15] found that p53 overexpression was a positive prognostic factor and that there was longer survival in cases whom had p53 overexpression. In our study, neither positive nor negative effect of p53 overexpression on survival was found.

At the result of studies negative correlation was found between p53 overexpression and chemosensitivity in both lung carcinoma and other human malignancies, especially breast and ovarian carcinoma.[7,26] Rusch[26] reported that only 15% complete response in NSCLC cases had high p53 overexpression. In our study, although p53 overexpression showed a poorer response to both chemotherapy and radiotherapy in the whole group, the result was not statistically significant.

As a conclusion, regardless of p53 overexpression density, we consider that p53 overexpression can be detected in NSCLC rather than SCLC, but that it does not correlate with clinicopathological parameters. It appeares that p53 overexpression together with some clinical factors have an effect on survival. Finally, further studies including more patients, using more antibodies and genetic techniques are needed to evaluate the clinical effects of p53 overexpression.

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