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Clinicopathological Study of p21, p27 and p53 Expression in Primary Non Small Cell Lung Cancer

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Abstract : We studied the expression of p21 and p27 proteins as cancer-suppressing factors in primary lung cancer and also evaluated its clinical significance. With respect to p53, a suppressing factor genetically located upstream of p21 and p27 in the cancer suppressing system, we studied how its clinical significance changes in relation to two factors.

We used 139 cases with primary lung cancer (adenocarcinoma 83 cases, squamous cell carcinoma 56 cases ; 104 males, 35 females) as the subjects, immunohistochemistry was performed using antibodies against p21, p27 and p53 proteins. P21 protein expressed itself in 53 cases (38.1%), p27 protein in 64 cases (49.0%) and p53 protein in 67 cases (48.2%). Regarding clinicopathological factors, either p21 or p27 expressed itself more frequently in the cases of Stage I compared with cases of Stage II～IV. Regarding the grade of differentiation, the frequency of expression of p21 and p27 was significantly higher in the highly-differentiated type than in the moderately-differentiated type and the poorly-differentiated type. As for T factors, no significant difference in the expression of p21 and p27 was found between T1 and T2～4. Regarding the expression of these proteins in cases having distant metastasis or lymph node metastasis, both p21 and p27 showed significantly lower values in the positive metastasis cases than in the negative metastasis cases. As for the prognosis, the 5-year survival rate was significantly higher in the cases with expression of p21 and p27 than in the non-expression cases. The 5-year survival rate was significantly higher in p21-positive p53-negative cases than in the p21-negative p53-positive cases.

Likewise, the 5-year survival rate was significantly higher in the p27-positive p53 -negative cases than in the p27-negative p53-positive cases. The above results suggest that p21 and p27 proteins can serve as one of the factors to predict prognosis in primary lung cancer.

Key words : Lung cancer, p53, p21, p27, cyclin-dependent kinase, Cell cycle

Introduction

P53, a cancer suppressing gene has been reported to be concerned with the adjustment of the cell cycle via the Cip/Kip family (p21, p27) located downstream.[4)5)7)] The Cip/Kip family is supposed to obstruct the activity of the cyclin D/Cdk (cyclin-dependent kinase) 4, cyclin A/Cdk 2 and suppress the phosphory-

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lation of the Rb gene, thereby suppressing the cell cycle from the G1 stage.[1)-4)] Yokota et al.[16)] reported the missing of chromosome 17 single arm in which p53 gene is present in small cell carcinoma of the lung. Similar reports have since been published regarding other malignancies such as breast cancer,[5)] stomach cancer[6)21)] and malignant lymphoma.[15)19)] As a result p53 has been clearly shown to be a cancer suppressing gene and its mutation is concerned with carcinogensis.[22)24)] On the other hand, p21 not only obstructs the kinase activity of the cyclin/Cdk complex, but also combines directly with PCNA (proliferating cell nuclear antigen), one of the constituent proteins of DNA synthetase to obstruct DNA reproduction and, consequently, is concerned with the suppression of the cell cycle.[1)3)24)] P27 may be related to the suppression of the cell cycle as is p21, but it does not combine directly with PCNA, and it is supposed to play a role in the threshold value for the kinase activity of the cyclin/Cdk complex necessary for the progression of the cell cycle.[12)-14)22)] Many reports studies have reported a mutation of the p53 gene to cause a collapse of the proliferation-suppressing mechanism. The expression of p21 and p27 is thought to prevent the collapse of this proliferation-suppressing mechanism and therefore, such expression is expected to become a target of gene therapy in the future. At the time we examined the expression of p21 and p27 located in the downstream of p53, a cancer suppressing factor using resected cases of primary lung cancer as the subjects and studied its relation to both clinicopathological factors and prognosis.

Subjects and Methods

The subjects included 139 cases of primary lung cancer resected at our department from 1986 to 1995 (Histological type: adenocarcinoma-83 cases, squamous cell carcinoma-56 cases. Sex: 104 male cases, 35 female cases. Grade of differentiation: well-74 cases, moderate-31 cases, poor-34 cases. Stage: Stage Ⅰ-61 cases, Stage Ⅱ-9 cases, Stage Ⅲ-54 cases, Stage Ⅳ-15 cases. T factor: T1-34 cases, T2-70 cases, T3-26 cases, T4-9 cases. Lymph node metastasis: n (−) -74 cases, n (+) -68 cases. Distant metastasis: M (−) -124 cases, M (+) -15 cases (Table 1). After being fixed in formalin, the biopsied specimens were embedded in paraffin and sectioned at 3 μm. The sections were stained by the avidin-biotin complex (ABC) method for immunohistochemistry anti-p53 monoclonal antibody (Dako Co.), anti-p21 monoclonal antibody (Oncogene Science Co.) and anti-p27 monoclonal antibody (Noovocastra Co.) were used as primary antibodies. Anti-p53 monoclonal antibody was diluted 50-fold and made to react at room temperature for 1 hour. Anti-p21 monoclonal antibody and

Table 1. Clinicopathologilcal characteristics
n=139

Age (mean)	66.16
Gender	
Male	104
Female	35
Histology	
adenocarcinoma	83
squamous cell carcinoma	56
Differantiation	
well	74
moderate	31
poor	34
stage	
Ⅰ	61
Ⅱ	9
Ⅲ	54
Ⅳ	15
T factor	
T1	34
T2	70
T3	26
T4	9
N	
n (−)	71
n (+)	68
M	
M (−)	124
M (+)	15

anti-p27 monoclonal antibody were diluted 20-fold and made to react at 4℃ for 24 hours. Next, the number of cells in 3 visual fields of 1 by 1 cm at 200 magnifications was counted, and the results were considered to be positive when the mean value of the cells of which nuclei were stained positively in cancerous tissues exceeded 5%.[6)8)13)14)19)]

Clinicopathological factors were determined in accordance with the General Rule for Clinical and Pathological Record of Lung Cancer (4th revised edition). The χ^2 test was used to test significance. The 5-year survival rate was calculated by the Kaplan-Meier method and the data were compared by Wilcoxon's test. Statistical significance was defined as $p<0.05$.

Results

On immunohistochemistry for p21 and p27, the nuclei of the cancer cells were uniformly stained in the case of positive cells (Fig. 6 : 1a～1d, Fig. 7 : 2a～2d). The expression of these proteins was found in some normal bronchial cells as well. The expression of p21 protein was noted in 53 cases as 38.1% (29 of adenocarcinoma, 24 of squamous cell carcinoma), and that of p27 protein was found in 64 cases as 49.6% (39 of adenocarcinoma, 25 of squamous cell carinoma). The expression of p53 protein was found in 67 cases as 48.2% (32 of adenocarcinoma, 35 of squamous cell carcinoma). Regarding the relation between the expression of p21, p27 proteins and clinicopathological factors, both p21 and p27 proteins showed no significant difference in the expression of adenocarcinoma and squamous cell carcinoma ($p=0.346$). Regarding the expression of p21, Ⅰ) The type of differentiation ; a highly differentiated type showed a significant difference in the expression compared with the moderately differentiated type and poorly differentiated type

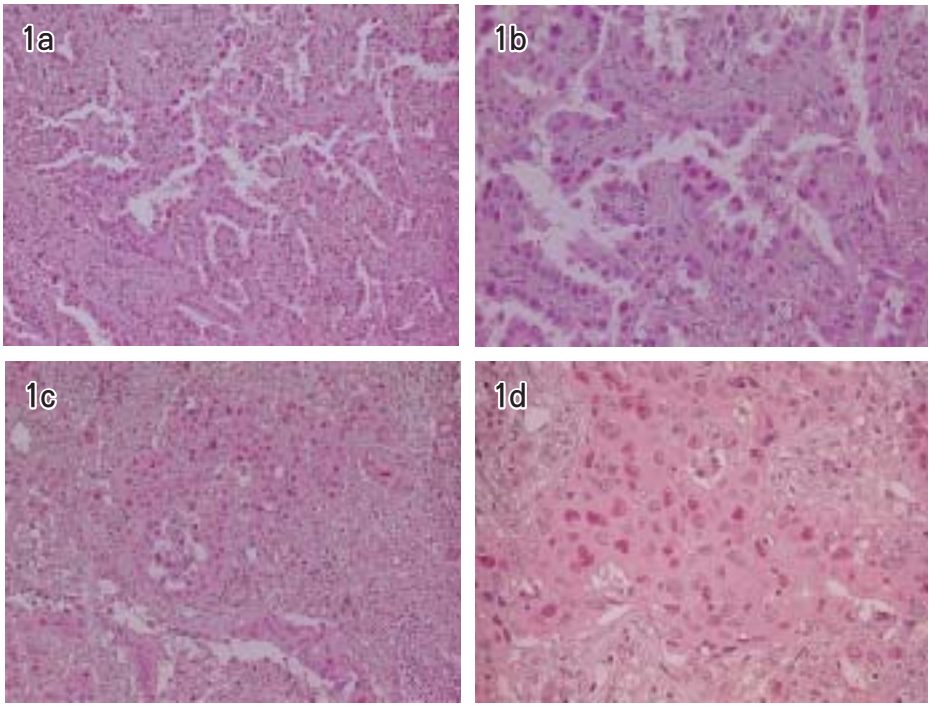

Fig. 6. 1a. 1b : p21 protein expression in adenocarcinoma. Magnification, ×100 and ×200
1c. 1d : p21 protein expression in squamous cell carcinoma. Magnification, ×100 and ×200

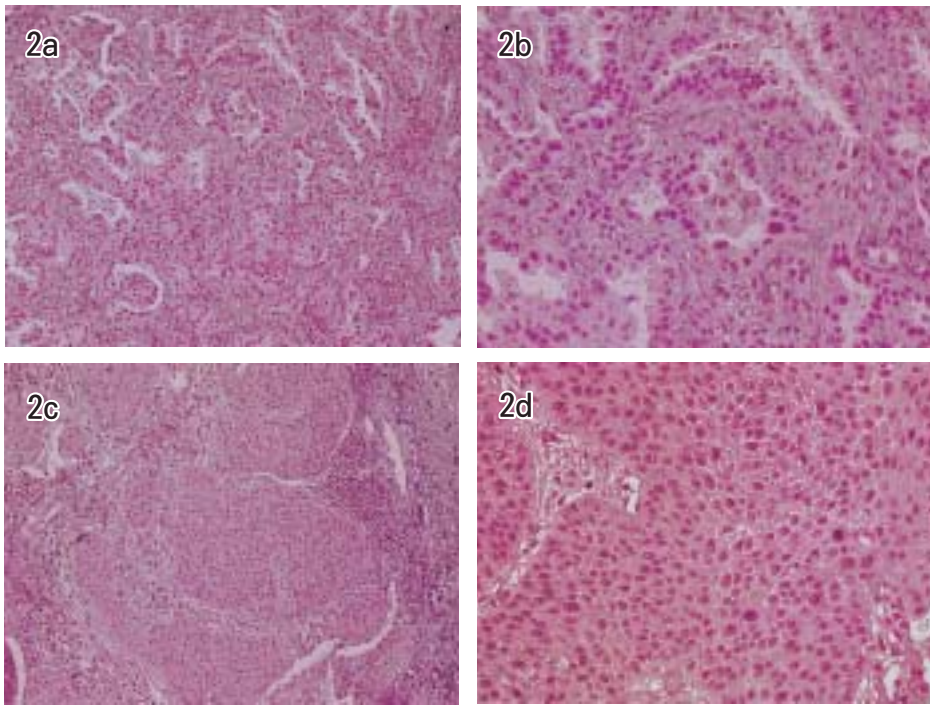

Fig. 7. 2a. 2b : p27 protein expression in adenocarcinoma. Magnification, ×100 and ×200
2c. 2d : p27 protein expression in squamous cell carcinoma. Magnification, ×100 and ×200

(p=0.043). Ⅱ) Stage ; the expression of p21 was significantly higher in the cases of stage Ⅰ than in stages Ⅱ～Ⅳ (p=0.001). Ⅲ) T factor ; T1 showed no significant difference from cases of T2～4 (p=0.408). Ⅳ) Lymph node metastasis and distant metastasis ; the expression of p21 was significantly lower in the positive group than in the negative group, with lymph node metastasis (p=0.0018) and distant metastasis (p<0.05) (Table 2). With p27 protein, as with p21 protein, Ⅰ) The type of differentiation ; the expression was significantly higher in the highly differentiated type than in the moderately differentiated type and poorly differentiated type (p=0.007). Ⅱ) Stage ; the expression was significantly higher in the cases of stage Ⅰ than in those of stages Ⅱ～Ⅳ (p=0.007). Ⅲ) T factor ; T1 showed no significant difference in expression of T2～4 (p=0.847). Ⅳ) Lymph node metastasis and distant metastasis ; the expression of p27 was lower in the positive group than in the negative group, with lymph node metastasis (p=0.005) and distant metastasis (p<0.033) (Table 3). Regarding the prognosis, the 5-year-survival rate was significantly higher in the cases with expression than in the non-expression cases for both p21 protein and p27 protein (p21(+)(61.3%) vs p21(−)(28.95%) p<0.05), (p27(+)(59.85%)vs p27(−)(33.40%) p<0.05) (Figs. 1, 2). Regarding the expression of p53 protein, no significant difference was found in any parameter, including sex, histological type, grade of differentiation, stage, T factor, lymph node metastasis, distant metastasis and survival rate (Table 4. Fig. 3). Regarding p53, the 5-year-survival rate was significantly higher in the p21 positive p53 negative, p27 positive p53 negative cases than in the p21 negative p53 positive, p27 negative p53 positive cases (p21 (+) p53 (−) (75.19%) vs p21 (−) p53 (+) (18.7%) =p<0.05) (p27 (+) p53 (−) (78.26%) vs p27 (−) p53 (+)

Table 2. p21 expression and clinicopathological characteristics

	p21expression		
n=139	p21 (+)	p21 (−)	P value
Age (mean)	68.87	64.06	
Gender			
Male	40	64	
Female	13	22	NS (P=0.890)
Histology			
adenocarcinoma	29	54	
squamous cell carcinoma	24	32	NS (P=0.346)
Differentiation			
Well	34	40	
moderate+poor	19	46	P=0.043
Stage			
Ⅰ	35	26	
Ⅱ+Ⅲ+Ⅳ	18	60	P=0.001
T factor			
T1	15	19	
T2+T3+T4	38	67	NS (P=0.408)
N			
n (−)	36	35	
n (+)	17	51	P=0.0018
M			
M (−)	51	73	
M (+)	2	13	P<0.05
p53 expression			
p53 (−)	21	51	
p53 (+)	32	35	P=0.025

Table 3. p27 expression and clinicopathological characteristics

	p27 expression		
	p27 (+)	p27 (−)	P value
Age (mean)	67.19	65.28	
Gender			
Male	53	51	
Female	11	24	P=0.045
Histology			
adenocarcinoma	39	44	
squamous cell carcinoma	25	31	NS (P=0.786)
Differentiation			
well	42	32	
mderate+poor	22	43	P=0.007
Stage			
Ⅰ	36	25	
Ⅱ+Ⅲ+Ⅳ	28	50	P=0.007
T factor			
T1	16	18	
T2+T3+T4	48	57	NS (p=0.847)
N			
n (−)	42	29	
n (+)	22	46	P=0.00153
M			
M (−)	61	63	
M (+)	3	12	P=0.033
p53 expression			
p53 (−)	23	49	
p53 (+)	41	26	P=0.00055

Table 4. p53 expression and clinicopathological characteristics

	p53 expression		
n=139	p53 (+)	p53 (−)	P value
Age (mean)	65.82	64.45	
Gender			
Male	52	52	
Female	15	20	NS (P=0.464)
Histology			
adenocarcinoma	37	46	
squamous cell carcinoma	30	26	NS (P=0.298)
Differentiation			
well	35	39	
moderate+poor	32	33	NS (P=0.820)
stage			
Ⅰ	32	29	
Ⅱ+Ⅲ+Ⅳ	35	43	NS (P=0.374)
T			
T1	16	18	
T2+T3+T4	51	54	NS (P=0.878)
N			
n (−)	39	32	
n (+)	28	40	NS (P=0.105)
M			
M (−)	63	61	
M (+)	4	11	NS (P=0.0772)

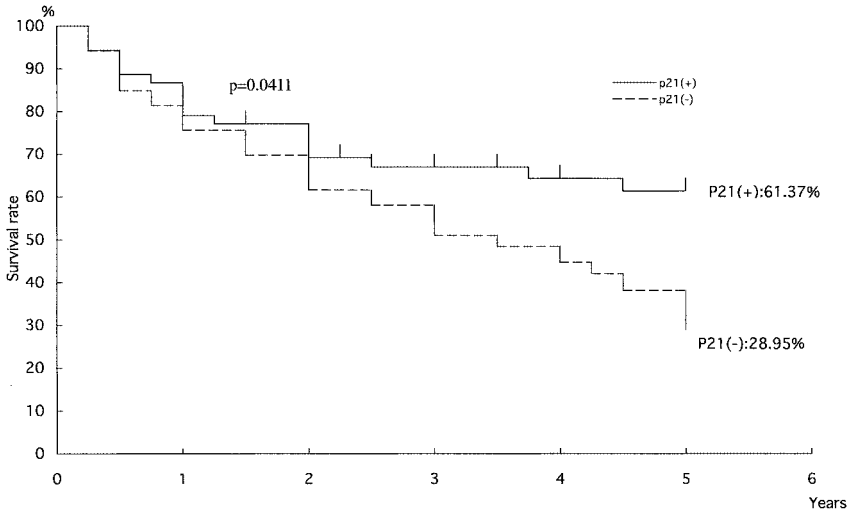

Fig. 1. Survival curve of patient in relation to p21 expression

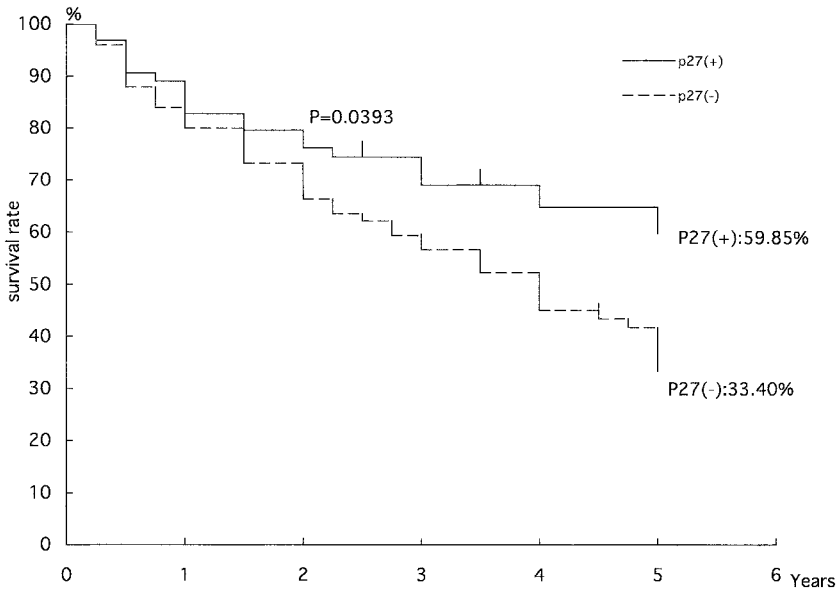

Fig. 2. Survival curve of patient in relation to p27 expression

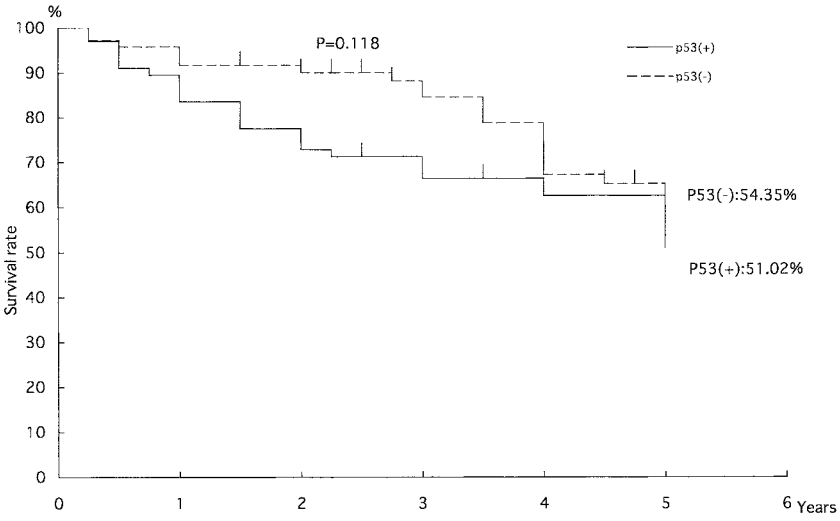

Fig. 3. Survival curve of patient in relation to p53 expression

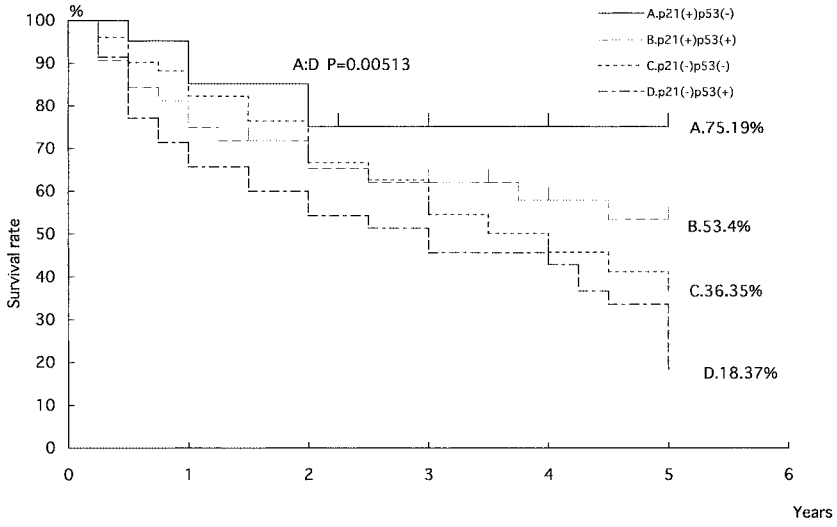

Fig. 4. Survival curve of patient in relation to p21 expression and p53 expression

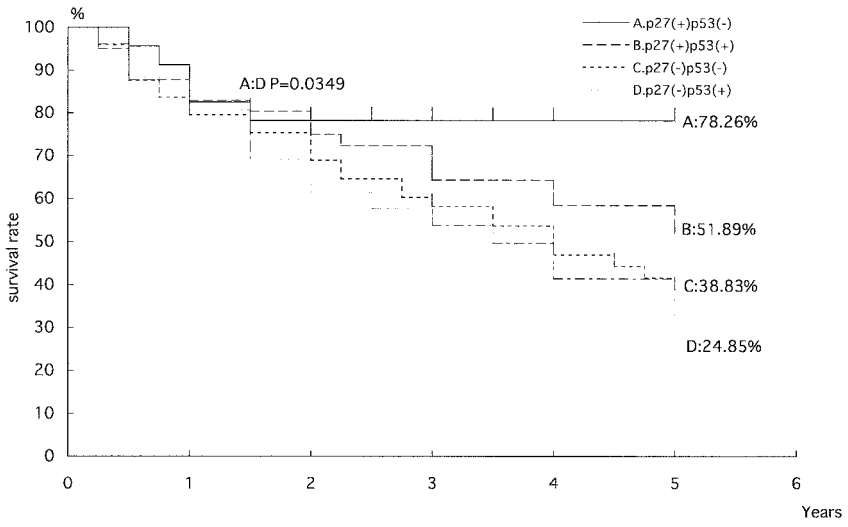

Fig. 5. Survival curve of patient in relation to p27 expression and p53 expression

(24.85%) $p<0.05$) (Figs. 4. 5).

Discussion

P21 and p27 are supposed to obstruct the activity of cyclin D/Cdk 4, cyclin A/Cdk 2 and suppress the phosphorylation of Rb gene, thereby suppressing the cell cycle from the G1 stage to S stage.[1)–4)] The abnormality of cyclin D at the DNA level has been shown in squamous cell carcinoma, and the abnormality of cyclin D is frequently observed in adenocarcinoma as well.[17)] P21 not only obstructs the kinase activity of a cyclin/Cdk complex, but also obstructs DNA reproduction and suppresses the cell cycle by combining directly with PCNA, one of the constituent proteins of DNA synthetase.[1)3)24)] At the chromosome level, the frequency of a missing chromosome 6 p21 is low, and a mutation of p21 gene itself has not yet been reported. On the other hand, p27 is supposedly concerned with the suppression of the cell cycle as is p21, but it is to said not directly combine with PCNA.[10)11)] P27 is present in chromosome 12 p13, and it is reported to be frequently missing in cancer cells with an abnormality of this chromosome.[1)–7)] However, no abnormality has been found in the remaining one half of the p27 gene pair and, therefore, the decrease in the expression of p27 is supposed to play a role as a threshold for the kinase activity of the cyclin/Cdk complex which is necessary for the progression of the cell cycle.[12)–14)22)]

In the present immunohistochemical study, p21–positive cases included many early stage cases and good prognosis cases in terms of the grade of differentiation, lymph node metastasis, distant metastasis and prognosis. On the other hand, the p21–negative cases consisted of many advanced cases and poor prognosis cases (Table 2). Regarding T factor, T1 showed no significant difference in the expression of p21 compared with the cases of T2～4. The expression of p27 protein was significantly high in the early stage cases and good prognosis cases, while p27–negative cases included many advanced cases and poor prognosis cases. The above results suggest that the cell cycle suppressing mechanism is maintained in the p21–positive cases and p27–positive cases, but is abnormal in the p21–negative cases and p27–nagative cases.In paticular, the prognosis of patients in whom the expression of p27 is small is reported to be poor in many cases. A low level of p27 present in the proliferating cells is absorbed by the cyclin D/Cdk4 complex and thus shows no Cdk obstructing activity. Presumably, the activity to decompose p27 specifically is high and not only a mutation of the p27 gene locus but also the proteolytic system working selectively on p27 is thus exists in cancer cells in which the expression of p27 is small. When viewed in relation to p53, no mutation of p53 gene is found and the cell cycle suppressing mechanism is maintained in the p53–negative p21–positive cases and p53–negative p27–positive cases, but this mechanism may be nullified by a mutation of the p53 gene in the p53–positive p21–negative cases and p53–positive p27–nagative cases. On the other hand, the prognosis was not necessarily good in the group in which p53, p21 and p27 were all positive (Figs. 4. 5). We also herein studied the association between p53 factor and various phathological factors, but found no particularly significant findings. No difference has been reported in the survival rate between the positive and negative cases regarding p53 protein. The survival rate for p53–positive cases reported to be significantly high in adenocarcinoma. Other studies have reported that p53 has nothing to do with the prognosis in squamous cell carcinoma, but that there are many p53–positive cases in advanced adenocarcinoma. However, these findings remain controversial.[25)26)] In immunohistochemical studies of p53, as is generally known, a bond mutation type p53 in addition to the wild type p53 is included in the cases with expression of p53, and where only one half of the of p53 gene pair has been found to cause this bond type mutation, the function of the wild type p53 is also lost. It is possible that p53–positive p21–positive cases and p53–positive p27–positive cases do not always show a good prognosis. The results of this study suggest that p21 protein and p27 protein can serve as one of the factors to predict the

prognosis of primary lung cancer and also as an indicator for the degree of malignancy of lung cancer. P21 and p27 proteins with TGF-β (transforming growth factor β) are thought to each act on the cell cycle suppressing mechanism via a different pathway.[4] There may be some gene control mechanism other than p53 for p21 and p27. In fact, many cases in which both p21 and p27 express themselves despite p53 being positive, have been observed, thus suggesting the presence of a factor that induces an expression without mediation of p53. The cell cycle suppressing mechanism is present in factor other than p53, p21 and p27. These factors are presumed to be related and to also influence one another in a complex manner regarding the suppression of cancer cell proliferation.

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