Original Article

Prognostic Significance of p21 Protein Expression in Patients with Pulmonary Squamous Cell Carcinoma Following Induction Chemotherapy

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Background: The prognostic value of p21 protein expression in lung cancer patients has been assessed. However, its significance in those with pulmonary squamous cell carcinoma following induction chemotherapy (IC) remains unclear. We studied on patients who did or did not undergo IC (NIC) to elucidate the prognostic value of p21 protein expression.

Materials and Methods: p21 protein expression was assessed immunohistochemically and samples with greater than 10% positive tumor cells were considered positive. We then analyzed clinicalpathological features, including p53 protein expression and prognosis, in 43 patients who underwent IC group and 40 who did not IC (NIC) group.

Results: Positive nuclear p21 samples were obtained from 17 (41.5%) patients in the in IC group and 22 (55.0%) in the NIC group. In the IC group, there was no significant correlation between the histological effectiveness of chemotherapy and p53 protein expression, whereas a significant correlation was observed between that and p21 protein expression (p=0.048). Further, the prognosis for p21-positive patients tended to be better (p=0.0506) than for p21-negative patients, and was significant (p=0.048) in patients with pathological stage (p-stage) II or III disease.

Conclusion: Our findings suggest that p21 protein expression is a prognostic factor for primary patients with pulmonary squamous cell carcinoma following IC. (Ann Thorac Cardiovasc Surg 2007; 13: 9–14)

Key words: lung cancer, squamous cell carcinoma, induction chemotherapy, p53, p21

Introduction

The expression of p21 protein is encoded by the human WAF1/CIP1 gene (6p21.2) and directly induced by the wild-type p53 protein.[1] This protein binds to a variety of cyclin-dependent kinases and inhibits their activity,[2] regulates deoxyribonucleic acid (DNA) repair, and directly blocks DNA replication by inhibiting the proliferating-cell nuclear antigen.[3] It has been linked to terminal differentiation,[4] senescence,[5] and apoptosis inhibition.[6,7] In addition, p53-dependent induction of p21 protein causes cell cycle arrest after DNA damage.[1] In tumor cells, when p53 protein or an altered form of p53 is lost, p21 protein level becomes dramatically reduced or is totally absent.[8] Further, p21 protein is regulated independently of p53[9] in several situations, including cellular differentiation and normal tissue development.[4]

Some reports have focused on the correlation between p21 protein expression and survival.[10–15] However, there are few studies of patients with squamous cell lung cancer that have undergone induction chemotherapy (IC). We attempted to determine whether p21 protein expression provides additional prognostic information in squamous cell lung cancer patients following IC.

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Materials and Methods

Patients

Eighty-three consecutive patients with primary pulmonary squamous cell carcinoma, who underwent a complete resection between November 1989 and August 2000 were chosen and divided into 2 groups; those who did not undergo IC (NIC) group (n=40) and those who underwent IC group (n=43)(Table 1). Two of the patients with a tumor who showed a complete response to induction therapy were excluded from subsequent analysis.

Induction chemotherapy

For patients in the IC group, IC was performed using cisplatin (CDDP) at 17 mg/m^2/day from days 1 to 5 and vindesine (VDS) at 3 mg/m^2/day on days 1 and 8, with the regimen repeated on day 29. Following recovery from therapy, the operation was generally performed on day 58.

Assessment of chemotherapy effectiveness

The effectiveness of IC was assessed according to the recommendations for treatment of lung cancer by The Japan Lung Cancer Society.[16] This was classified according to diagnostic imaging and histological analyses. Four classifications were used based on the results of diagnostic imaging: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD); while 5 classifications of response were based on histological analyses; ineffective (Ef0), slightly effective (Ef1a), mildly effective (Ef1b), moderately effective (Ef2), and significantly effective (no viable cancer cells detected)(Ef3).

p53 and p21 staining, and definition of positivity

Since p53 protein directly induces the expression of p21 protein, p53 expression in cancer cells was analyzed. Following tumor identification by HE staining, serial 3-μm paraffin-embedded sections were prepared. The sections were then exposed to microwave radiation and the p53 antibody DO7 (diluted 200-fold)(DAKO Japan, Co., Ltd., Kyoto, Japan) was allowed to react with the sections for 60 minutes at 37˚C, after which they were stained immunohistologically using the avidin-biotinylated peroxidase complex (ABC) method.

The expression of p21 in cancer cells was analyzed as follows. Tissue sections were prepared as described above and then autoclaved for 15 minutes at 121˚C. The p21 antibody (diluted 50-fold)(DAKO Japan, Co., Ltd., Kyoto, Japan) was allowed to react with the sections overnight at 4˚C and then was stained immunohistologically using the ABC method.

Two thousand tumor cells per section were observed, and the numbers of p53- and p21-positive tumor cells determined. Sections with greater than 10% positive tumor cells were considered positive.

Table 1. Patient demographics and stages

	IC group	NIC group
Number	43	40
Age in years ± S.D.	64.3±6.8	66.3±8.1
Minimum	49	38
Maximum	79	82
Clinical stage		
O	0	0
IA	0	9
IB	4	10
IIA	0	0
IIB	9	7
IIIA	23	14
IIIB	0	7
Pathological stage		
O	2	0
IA	5	12
IB	17	12
IIA	2	1
IIB	6	7
IIIA	7	8
IIIB	4	0

S.D., standard deviation; IC, induction chemotherapy; NIC, no induction chemotherapy.

Statistical analysis

Fisher's exact test was used to compare the incidence of p53 and p21 protein expression between the NIC and IC groups. To investigate the effects of p53 and p21 protein expression, survival curves were calculated using the Kaplan-Meier method with cancer deaths considered as the end-point, i.e. we evaluated cancer specific survival. A log-rank test was used to analyze survival curves. P-values of less than 0.05 were considered to be significant.

Results

Immunohistological staining for p53 and p21

In the NIC group, 65.0% (26/40) of the patients were positive for p53 expression, while 61.0% (25/41) were positive in the IC group, which was not significantly dif-

Table 2. Clinical-pathological characteristics based on p21 protein expression in patients who underwent induction chemotherapy (IC) group

	p21		
	Positive	Negative	p-value
Number	17	24	
Age in years			
<65	8	14	.5
≥65	9	10	
Smoking (BI)			
<600	2	7	.3
≥600	15	17	
P-stage			
I	9	13	>.9
II, III	8	17	
Differentiation			
Well	5	4	.6
Moderate	7	10	
Poor	5	10	
Radiological response			
PR	6	12	.6
SD	10	11	
PD	1	1	
Pathological response*			
Ef0, Ef1a, Ef1b	8	19	.048
Ef2	9	5	
p53			
Positive	12	13	.3
Negative	5	11	

*Two cases were excluded because their pathological response was Ef3.
BI, Brinkman index; PR, partial response; SD, stable disease; PD, progressive disease.

Table 3. Clinical-pathological characteristics based on p21 protein expression in patients who did not undergo induction chemotherapy (NIC) group

	p21		
	Positive	Negative	p-value
Number	22	18	
Age in years			
<65	6	6	.7
≥65	16	12	
Smoking (BI)			
<600	5	1	.2
≥600	17	17	
P-stage			
I	13	11	>.9
II, III	9	7	
Differentiation			
Well	3	3	.09
Moderate	11	9	
Poor	8	6	
p53			
Positive	17	9	.1
Negative	5	9	

ferent. As for p21 protein expression, 55.0% (22/40) of the patients in the NIC group and 41.5% (17/43) in the IC group were positive, which was not significantly different.

Clinicopathological analyses based on p21 expression

In both the NIC and IC groups, no correlation was found between p53 or p21 expression and clinicopathological features such as age, Brinkman index (BI), p-stage, and tissue differentiation (Tables 2 and 3). However, no significant correlation was observed between p53 and p21 expression in either group (Tables 2 and 3). In addition, radiographic examinations revealed no significant difference in p21 protein expression between the 2 groups, whereas the prevalence of Ef2 was significantly higher (p=0.048) in p21-positive patients than in p21-negative patients (Table 2).

Relationship between prognosis and expression of p53 and p21

For the NIC group, no significant difference was seen in the survival curves between p53-positive and p53-negative patients (p=0.2731), or between p21-positive and p21-negative patients (p=0.8689). In the IC group, no significant difference was seen in the survival curves between p53-positive and p53-negative patients, however, the survival curve for p21-positive patients tended to be better than for p21-negative patients (Fig. 1). The IC patients were then subdivided into those with p-stage I carcinomas and those with p-stage II or greater. There was no significant difference seen between the survival curves for p21-positive and p21-negative p-stage I patients (Fig. 2). However, the survival rate of p21-positive patients with p-stage II or above was significantly better than that of p21-negative patients with p-stage II or above (p=0.048)(Fig. 3).

Comment

Although many studies of p21 status in lung cancer patients have been conducted,[10–14,17–24] the prognostic significance of p21 protein expression in cancer cells remains controversial. The significance of p21 protein expression may be important in cases following IC, as that induces

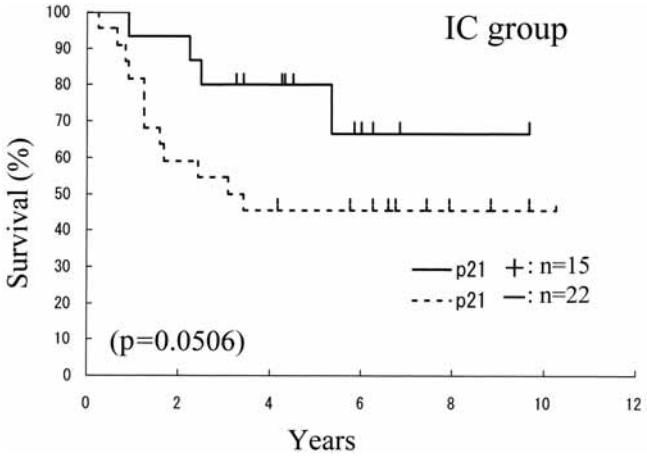

Fig. 1. Survival curves based on p21 status for all patients who underwent induction chemotherapy.
IC, induction chemotherapy.

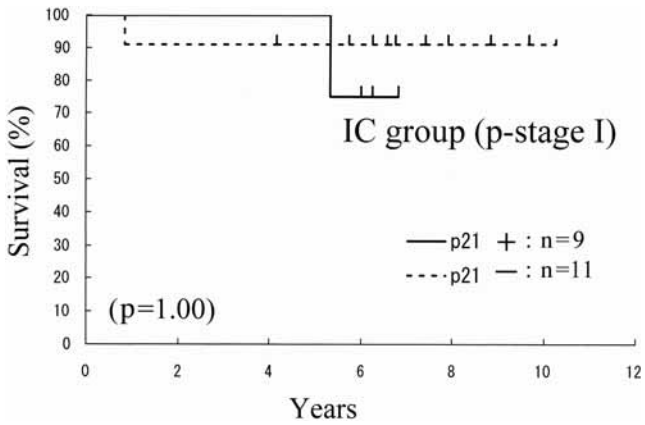

Fig. 2. Survival curves based on p21 status for patients with pathological stage I carcinoma who underwent induction chemotherapy.
IC, induction chemotherapy; p-stage, pathological stage.

apoptosis of cancer cells, which may lead to a pathological response. The present results demonstrated that pathological responses were significantly different between the p21-positive and -negative groups, as the ratio of Ef2 was 52% in the p21-positive group and 21% in the p21-negative group. That result may be attributed to the clearance of chemo-sensitive p21 positive cells, i.e. p21 negative cases might contain greater p21 positive cells before chemotherapy.

In addition to the pathological response following chemotherapy, cancer specific prognosis is a crucial issue. In our series, p21-negative was a prognostic indicator of completely resected squamous cell lung cancer following IC. However, that may have been dependent on the different characteristics of malignant cells between the p21-positive and -negative groups.

The expression of p21 protein has been associated with a poor prognosis in patients with breast cancer,[25] whereas it has been linked with a good prognosis in patients with bladder cancer[26] and advanced stomach cancer.[27] As for lung cancer, Komiya et al.[10] reported that the prognosis for p21-positive patients with squamous cell carcinoma was more favorable than that for p21-negative patients, and that the disease stage for p21-positive patients was less advanced than that for p21-negative patients. However, not all the patients in that study underwent postoperative chemotherapy and the effects of chemotherapy were not investigated. In the present study, no significant difference was observed between the survival curves of p21-positive and -negative patients in the NIC group.

Two contradictory hypotheses have been speculated

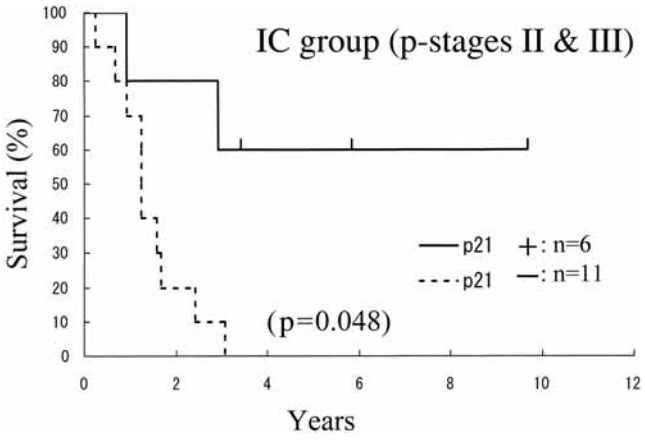

Fig. 3. Survival curves based on p21 status for patients with pathological stages II and IIIA carcinoma who underwent induction chemotherapy.
IC, induction chemotherapy; p-stage, pathological stage.

with regard to the relationship between the sensitivity to anticancer drugs and expression of p53 and p21.[27] When p53 and p21 expression is normal, cell growth is arrested following exposure to DNA-damaging anticancer drugs and apoptosis is induced, thus increasing drug sensitivity.[9,28] In contrast, it has also been speculated that DNA damage occurs in cells with normal p53 and p21 expression, and that cell death can be avoided due to the arrest of cell growth and the repair of damaged DNA, thus suppressing the effectiveness of anticancer agents.[29] Koga et al.[30] analyzed biopsy specimens from patients with advanced bladder cancer using immunohistological

staining and reported that p53-negative/p21-positive patients were significantly more responsive to chemotherapy as compared to p53-positive/p21-negative patients. Further, Nakashima et al.[31] studied patients with esophageal cancer and reported similar findings. The results of these 2 reports support the notion of increased drug sensitivity.

In the present study, biopsy specimens were not obtained preoperatively, thus p53 and p21 expression was analyzed immunohistologically using surgically excised specimens following chemotherapy. Forty patients with squamous cell carcinoma who did not undergo preoperative IC were used as the control (NIC group). There was no significant difference in p53 and p21 expression between the NIC and IC groups. However, the pathological effectiveness of chemotherapy in the IC group showed no correlation with p53 expression, whereas p21-positive patients in the IC group were shown to be more responsive to chemotherapy compared to p21-negative patients.

In conclusion, the expression of p21 protein in cancer cells was found to be a prognostic indicator in patients with completely resected squamous cell carcinoma following IC. Our results indicate that p21 expression is related to the sensitivity of pulmonary squamous cell carcinoma to chemotherapy.

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