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Original Paper

Fas Expression in Non-small Cell Lung Cancer: its Prognostic Effect in Completely Resected Stage III Patients

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The aim of this study was to examine Fas expression in non-small cell lung cancer (NSCLC) and examine its correlation with clinicopathological features and prognosis. Fas expression was determined by an immunohistochemical analysis using the labelled streptavidin–biotin method from 220 paraffin specimens of completely resected primary stage I–III NSCLC. 80 (36%) of 220 cases were positive for Fas immunostaining. These 80 cases included 44 adenocarcinomas (33%) and 30 squamous cell carcinomas (40%). 33 stage I (33%) 13 (43%) stage II and 34 (37%) stage III tumours were Fas positive. No statistically significant differences were observed regarding the Fas status with respect to age, sex, histological type, or stage of disease. There was no significant difference in survival between early stage (stages I–II) disease pateints with positive Fas expression and those with a negative expression ($P = 0.719$). However, for patients with completely resected stage III tumours, the patients with positive Fas staining were found to survive for a longer period than those with negative staining ($P = 0.026$). © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: Fas protein, non-small cell lung cancer, immunohistochemical analysis, prognostic factor
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INTRODUCTION

FAS (ALSO known as APO-1 and CD95) and FasL play a key role in the regulation of apoptosis within the immune system [1]. Both proteins are highly expressed on activated T cells, with low levels of expression seen in resting T cells [2–5]. A ligation of Fas by either activating antibody or by FasL transmits a 'death signal' to the target cell, which potentially triggers apoptosis [6–8]. Several investigators have recently reported that colon carcinoma, melanoma, and hepatocellular carcinoma cell lines may express FasL and kill Jurkat cells in a Fas-mediated manner [9–11]. However, to our knowledge few reports have investigated its expression on solid tumours including lung cancer. Moreover, the relationship between Fas expression and clinical outcome in patients with malignant tumours is still unknown. In this study, we performed an immunohistochemical analysis of Fas in NSCLC to identify any correlation with the clinicopathological features and prognosis. In addition, we evaluated the relationship between Fas expression and bcl-2/p53 expression.

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PATIENTS AND METHODS

Patients

220 consecutive patients with completely resected stage I–III NSCLCs in the Department of Surgery II, University of Occupational and Environmental Health, Kitakyushu, Japan, from July 1991 to September 1996 were studied. The patients ranged in age from 38 to 84 years (mean 66.2 ± 9.1 years); 158 were male and 62 were female. All patients preoperatively underwent diagnostic procedures, including brain computed tomography (CT), body CT, and a bone scintigram without mediastinoscopy. The pathological types included 133 adenocarcinomas, 75 squamous cell carcinomas, 10 large cell carcinomas, and 2 adenosquamous cell carcinomas. According to the new international staging system for lung cancer [12], following a complete mediastinal lymph node dissection carried out in all patients, staging was as follows: 49 patients were stage IA, 50 patients were stage IB, 17 patients were stage IIA, 13 patients were stage IIB, 59 patients were stage IIIA, and 32 patients were stage IIIB. Of

the 32 stage IIIB patients, 24 had T_4N_{0-2} and 8 had $T_{1-3}N_3$ disease. A complete resection was defined as no gross or microscopic residual tumour remaining either at the surgical margins nor in the regional lymph nodes. Even if microscopic metastasis was present in the highest dissected mediastinal lymph node, it still was considered to be a complete resection. According to this definition, all 220 patients were considered to be complete resections.

Immunohistochemical examination of Fas

Immunohistochemical staining was performed on the 4 μm thick, 10% formalin-fixed, paraffin-embedded serial sections with a monoclonal antibody (MAb) to the Fas (APO-1; Dako, Japan) using the labelled streptavidin biotin (LSAB) method (Dako). APO-1 stains positively T Jurkat cells which express Fas [13, 14]. After deparaffinisation in xylene, the sections were incubated with 0.3% hydrogen peroxide for 15 min to quench the endogenous peroxidase activity. APO-1 was diluted 1:40 in phosphate-buffered saline solution (PBS) containing 2% bovine serum albumin. The sections were incubated with APO-1 at 37°C for 3 h, rinsed in PBS, and then developed with diaminobenzidine tetra-hydrochloride substance (DAB) for 8 min. A negative control was incubated without the primary antibody.

Positive or negative immunoreactivity for FAS was defined as follows: the sections for Fas were judged to be positive when the membrane and cytoplasm of the tumour cells were stained or when only the membrane of the tumour cells were stained. They were judged to be negative when neither the membrane nor cytoplasm of the tumour cells were stained or when only the cytoplasm of the tumour cells was stained. The results were evaluated independently by two observers without any knowledge of either the patients, molecular/biological data or clinical data.

Immunohistochemistry for bcl-2 and p53

bcl-2 And p53 immunohistochemical staining was similarly performed using MAbs to the bcl-2 protein (clone 124; Dako), and p53 protein (DO-1; Dako). The sections for bcl-2 were judged positive when 10% or more of the cytoplasm of tumour cells were stained, and the others were judged negative [15]. p53 immunoreactivity was judged positive when 10% or more of the nuclei of tumour cells were stained, and the others were judged negative [16]. Reactive lymphocytes and histiocytes were not included as positive cells.

Statistical analysis

The chi-square test and the unpaired Student's *t*-test were used to examine the association between Fas expression and clinicopathological features. The Kaplan–Meier method was used to estimate survival as a function of time, and survival differences were analysed by the log-rank test. The statistical difference was considered significant if the *P* value was less than 0.05. The patient follow-up time ranged from 10 to 2172 days (mean 650 days).

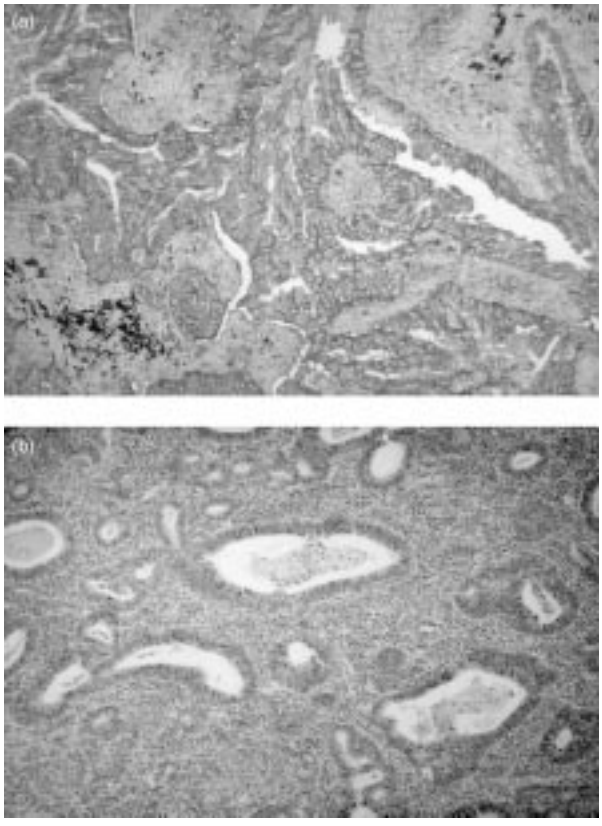

Figure 1. Immunohistochemical staining of Fas in NSCLC. (a) Positive Fas immunostaining of an adenocarcinoma; the section shows positive membrane staining of the tumour cells (original magnification ×200). (b) Negative Fas immunostaining of an adenocarcinoma; the section shows negative membrane and cytoplasm staining of the tumour cells (original magnification ×200).

RESULTS

Fas immunohistochemical staining and its relationship to clinicopathological features

Fas protein mainly localised to the membrane of tumour cells (Figure 1). Reactive lymphocytes and histiocytes were also stained. No differences were observed between adenocarcinoma and squamous cell carcinoma regarding the Fas immunostaining pattern. 80 (36%) of 220 patients were positively stained for Fas. The associations between Fas

Table 1. *Fas expression in* 220 *patients with resected stage I–III non-small cell lung cancer*

Clinicopathological factors	Fas positive *n* (%)	Fas negative *n* (%)	*P* value
All	80 (36)	140 (64)	
Sex			
Male	59 (37)	99 (63)	0.63
Female	21 (34)	41 (66)	
Mean age (± S.D.)	67.6 ± 7.8 yrs	65.3 ± 9.6 yrs	0.07
Histological type			
AD	44 (33)	89 (67)	0.32*
SQ	30 (40)	45 (64)	
LC	5 (50)	5 (50)	
ADSQ	1 (50)	1 (50)	
Pathology stage			
I	33 (33)	66 (67)	0.80†
II	13 (43)	17 (57)	
III	34 (37)	57 (63)	

AD, adenocarcinoma; SQ, squamous cell carcinoma; LC, large cell carcinoma; ADSQ, adenosquamous cell carcinoma; S.D., standard deviation. *P* value was calculated *between adenocarcinoma and squamous cell carcinoma; †between stage I–II and III.

Table 2. Relationship between Fas expression and bcl-2/p53 expression in 220 patients with resected stage I–III non-small cell lung cancer

	Fas positive ($n=80$) (%)	Fas negative ($n=140$) (%)	P value
bcl-2			
Positive ($n=44$)	12 (27)	32 (73)	0.161
Negative ($n=176$)	68 (39)	108 (61)	
p53			
Positive ($n=103$)	43 (42)	60 (58)	0.119
Negative ($n=117$)	37 (32)	80 (68)	

expression and the clinicopathological features are shown in Table 1. No significant correlation was observed between Fas expression and age at operation, sex, histological type or pathological stage.

Relationship between Fas and bcl-2/p53 expression

44 (20%) of the 220 tumours were positive for bcl-2 and 103 (47%) for p53 immunostaining. There was no relationship between Fas expression and bcl-2/p53 expression (Table 2).

Relationship between Fas expression and survival

Survival of all stage I–III NSCLC patients nor stage I–II only was not affected by Fas staining status (Figure 2a,b). However, in completely resected stage III NSCLC patients with positive Fas staining were found to survive for a longer period than those with negative staining ($P=0.026$) (Figure 2c). The differences in survival were not significant between patients with squamous cell carcinoma and adenocarcinoma ($P=0.184$ and 0.360, respectively, data not shown).

DISCUSSION

Fas antigen has been shown to be a cell surface molecule which can induce apoptosis [2, 7]. However, few reports have investigated Fas expression in surgically resected cancer specimens including NSCLC [13]. In this study, 80 (36%) of 220 NSCLC patients were positive for Fas. However, no relationship was found between Fas expression and any clinicopathological factors including pathological stages. Although we excluded stage IV disease from this study, 4 (33%) of 12 patients with distant metastasis (stage IV disease) were also positive for Fas expression and the percentage was almost equal to that of stages I–III. In previous studies, no data were available on the relationship between Fas expression and tumour stage in any cancers.

One of the most interesting findings in this study was the prognostic impact of the expression of Fas in patients with stage III NSCLC. The survival analysis revealed that the patients with negative Fas stage III NSCLC tumours had a worse prognosis. This fact may indicate that Fas-mediated apoptosis inhibits tumour progression especially in advanced lung cancer patients. In contrast, for patients with early-stage

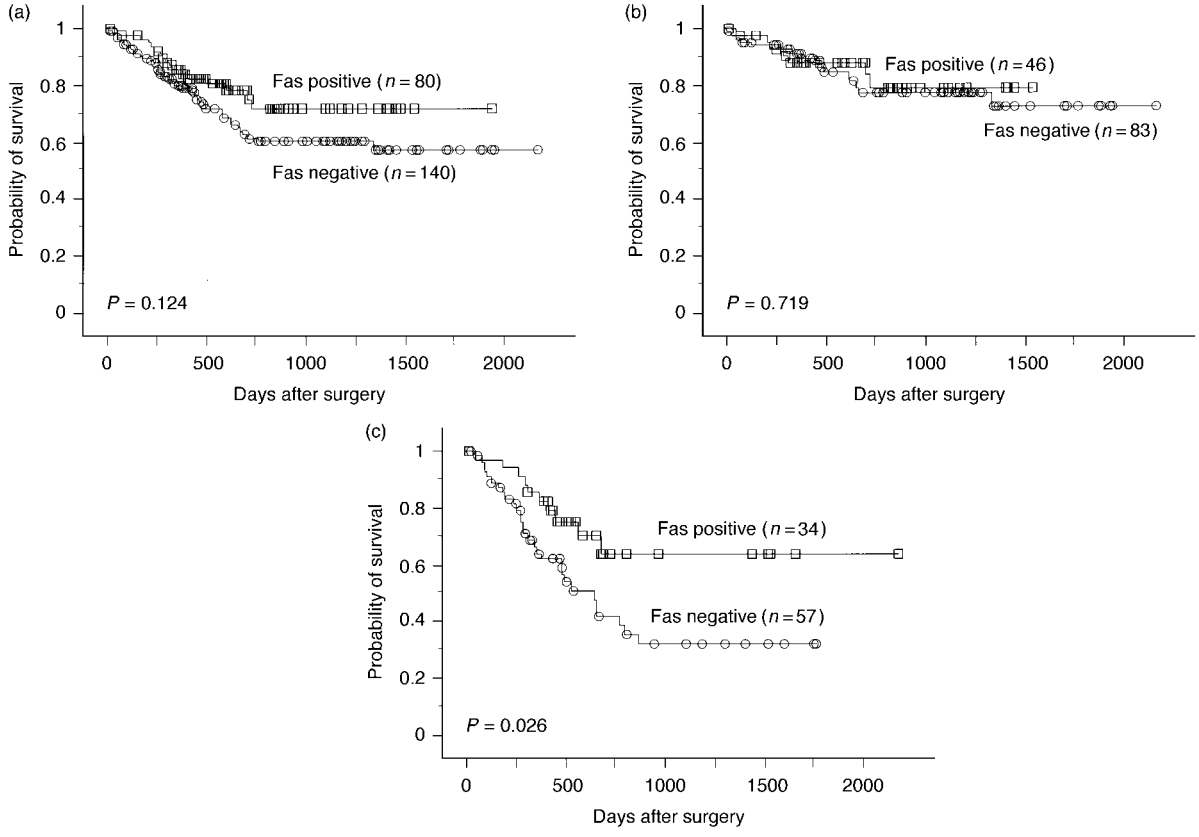

Figure 2. The probability of survival (death from any causes) according to Fas staining for (a) all 220 NSCLC patients. No difference was observed between the Fas positive and negative cases ($P=0.124$). (b) The 129 patients with stage I and II NSCLC. No difference was observed between the Fas positive and negative cases ($P=0.719$). (c) The 91 patients with stage III NSCLC. The difference in the survival curves was significant ($P=0.026$). Zero time on the abscissa represents the date of the pulmonary resection.

disease (stages I–II), Fas expression did not affect their prognosis. Tumour cells undergo multiple genetic alterations during tumour progression [17], and so a greater accumulation of genetic change exists in advanced human cancer (such as the group with stage III NSCLC in this study) than in early-stage disease. Therefore, in advanced human malignancies, apoptosis may play an important role in acquiring a better prognosis.

The *bcl-2* oncogene is a suppressor of apoptosis [18]. Itoh and associates [19] reported that overexpression of bcl-2 in a murine myeloid leukaemia cell line, FDC-P1, resulted in partial inhibition of Fas-induced cell death. This finding indicates that the balance between Fas and bcl-2 may play an important role in the induction of apoptosis. Ohsaki and colleagues reported that NSCLC patients with bcl-2 expression survived longer than those without bcl-2 expression [20]. In this study, 44 (20%) of the 220 tumours were positive for bcl-2 immunostaining, but no relationship was observed between the Fas and bcl-2 expression in our study. An alteration of the *TP53* tumour suppressor gene is the most frequently identified genetic change in human neoplasms including lung cancers [21, 22]. In this study, 103 (47%) of the 220 tumours were positive for p53 immunostaining, but no relationship was seen between Fas and p53 expression. The inhibition of apoptosis by bcl-2 or mutant p53 may thus play an important role in carcinogenesis, but their functions are not simple. Moreover, we consider that different and as yet unknown pathways for inducing apoptosis in NSCLC other than Fas, bcl-2, or p53 may also exist.

In conclusion, patients with completely resected stage III NSCLC with negative Fas expression survived for a significantly shorter period of time than those with positive Fas expression. An absence of Fas expression may thus indicate a subset of patients with stage III NSCLC who require appropriate postoperative adjuvant therapy, if a curative operation has already been performed.

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