Estrogen Receptor α and β are Prognostic Factors in Non–Small Cell Lung Cancer

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Abstract

Purpose: Estrogen receptor-α (ER-α) and -β (ER-β) play important roles in the carcinogenesis of breast tumors. Similarly, there have been several reports of ER expression in lung cancers, but the results have not been consistent, and the receptors' prognostic value remains unclear. Our goal was to investigate ER expression in non–small cell lung cancer (NSCLC) and to assess whether their expression correlates with prognosis.

Experimental Design: ER expression was examined using immunohistochemical methods with sections from 132 resected NSCLC specimens. Kaplan-Meier survival curves were analyzed to determine the significance of ER expression in the prognosis of NSCLC patients.

Results: ER-α was detected in the cytoplasm of 73% of the specimens analyzed, whereas ER-β was detected in the nucleus of 51%. ER-α expression correlated with poorer overall survival ($P < 0.001$), as did the absence of ER-β expression ($P = 0.048$). Likewise, at histopathologic stage I, ER-α expression ($P = 0.028$) or the absence of ER-β ($P = 0.037$) correlated with a poorer prognosis, and ER-α(+)ER-β(−) patients had a significantly worse prognosis than ER-α(−)ER-β(+) patients ($P = 0.00007$). Multivariate Cox regression analysis revealed the absence of ER-β to be an independent factor predictive of poor disease outcome (hazard ratio, 1.9; 95% confidence interval, 1.1-3.4; $P = 0.0264$).

Conclusions: ER-α expression and the absence of ER-β expression are associated with a poorer prognosis among NSCLC patients. In particular, the absence of ER-β could serve as a marker identifying patients at high risk even at an early clinical stage.

Lung cancer is now the leading cause of cancer death throughout the world. Despite recent improvements in its treatment, the prognosis for lung cancer patients remains poor. The pathologic staging of non–small cell lung cancer (NSCLC) is a key determinant of the patient's prognosis and the treatment options. For instance, in Japan, the 5-year survival rate after surgery at stage IA is ~80%, whereas the 5-year survival rate following surgery at stage IIIA is only ~30%. Nevertheless, we have often encountered patients in whom the course of their disease differed substantially from what would be predicted based on their clinical staging, which highlights the need to consider additional predictive factors.

A focus on estrogen receptors (ER) as targets of hormonal therapy has played a significant role in the treatment of breast cancer; in general, the prognosis of patients expressing ERs is better than that of patients not expressing them (1, 2). There have been several studies examining ER expression in lung cancers, but their results remain inconclusive, and the relationship between ER expression and prognosis remains unclear (3–12). Some clinical analyses have shown the incidence of ER expression to be as high as 90% among lung cancer patients (3, 4), whereas others detected little or no ER expression (5–12). At the cellular level, Stabile et al. observed that lung cancer cells express both ER-α and ER-β; the former is localized in the cytoplasm, the latter in the nucleus (13). The purpose of the present study was to determine whether ER expression influences the prognosis of NSCLC patients and to evaluate the role of the interaction between ER-α and ER-β in these patients.

Patients and Methods

Patients. This study was approved by the Human Ethics Committee of Nakadori General Hospital, Akita, Japan. The study participants included 76 males and 56 females, ranging in age from 38 to 81 years (median, 66 years), that were diagnosed with NSCLC in Nakadori General Hospital between 1995 and 1997. The clinical features of these patients are summarized in Table 1.

Immunohistologic staining. Specimens of resected lung cancer tissue obtained from surgical treatment were fixed in formalin, embedded in paraffin and cut into 5-μm sections. The sections were then deparaffinized in xylene and ethanol, placed in 0.1 mol/L

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Received 1/27/05; revised 4/5/05; accepted 4/22/05.

Grant support: Nakadori General Hospital.

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Table 1. Clinical and histologic features in 132 patients with NSCLC

Variables	All patients	ER-α expression		P*	ER-β expression		P
		Negative	Positive		Negative	Positive	
Total	132						
Age (y)				0.68			0.71
Median		66	68		68	68	
Range		38-81	42-80		38-81	42-81	
Gender				0.14			0.48
Male	76	17	59		35	41	
Female	56	19	37		30	26	
Smoking	71	14	57	0.78	36	35	0.98
Male	67	13	54		34	33	
Female	4	1	3		2	2	
Stage				0.20†			0.17†
Ia	46	13	33		19	27	
Ib	20	7	13		13	7	
IIa	9	3	6		6	3	
IIb	15	4	11		10	5	
IIIa	27	6	21		11	16	
IIIb	8	1	7		2	6	
IV	7	2	5		4	3	
Histology				0.65‡			0.033‡
Adenocarcinoma	102	28	74		55	47	
Squamous cell Carcinoma	28	7	21		9	19	
Large cell Carcinoma	2	1	1		1	1	
Grade				0.017§			0.72§
Well	50	20	30		24	26	
Moderate	55	9	46		26	29	
Poor	27	7	20		15	12	

*P values were obtained from the χ^2 test (two-sided).
† Stages I and II versus stages III and IV.
‡ Adenocarcinoma versus squamous cell carcinoma.
§ Well versus moderate, poor.

citrate buffer (pH 6.0) and irradiated with microwaves (750 W) for 15 minutes. The primary antibodies used were rabbit polyclonal anti-ER-α (HC-20, Santa Cruz Biotechnology, Santa Cruz, CA; 1:50 dilution in PBS) or anti-ER-β (H-150, Santa Cruz Biotechnology; 1:10 dilution in PBS) antibody. The immunostaining was carried out using the EnVision method (DAKO, Kyoto, Japan) according to the manufacturer's instructions. Brown staining was considered positive. The positive control for both ER-α and ER-β was breast cancer tissue; the negative control consisted of using PBS instead of primary antibody. All controls gave satisfactory results. Staining was categorized into eight grades according to previously described immunohistochemical scores (14–16). Briefly, each slide was examined in its entirety under a light microscope, and initially a proportion score was assigned, which represented the estimated proportion of positive tumor cells (0, none; 1, <1/100; 2, 1/100 to 1/10; 3, 1/10 to 1/3; 4, 1/3 to 2/3; and 5, >2/3). Next, an intensity score was assigned, which represented the average intensity of the positive tumor cells (0, none; 1, weak; 2, intermediate; and 3, strong). The proportion and intensity scores were then added to obtain a total score, which ranged from 0 to 8. Slides were scored by pathologists with no knowledge of the ligand-binding results or patient outcomes.

Statistical analysis. Two groups were compared using the χ^2 test. The probabilities of overall survival were calculated using the Kaplan-Meier method and compared using the log-rank test. For determination of factors related to overall survival, a Cox proportional hazard model was used. These analyses yielded hazard ratios, their 95% confidence intervals and P values. Values of $P < 0.05$ were considered significant.

Results

Expression of ER-α and ER-β. Figure 1 shows representative immunohistochemical staining of ER-α in the cytoplasm of NSCLC cells (Fig. 1A and B) and of ER-β in the nucleus (Fig. 1C and D). Ninety-six (73%) of the tumors tested were ER-α-positive, whereas 67 (51%) were ER-β-positive. ER-α expression occurred significantly more frequently among moderate or poorly differentiated carcinomas than among well differentiated ones ($P = 0.017$). On the other hand, the incidence of ER-β expression was significantly greater among squamous cell carcinomas than adenocarcinomas ($P = 0.033$). There was no

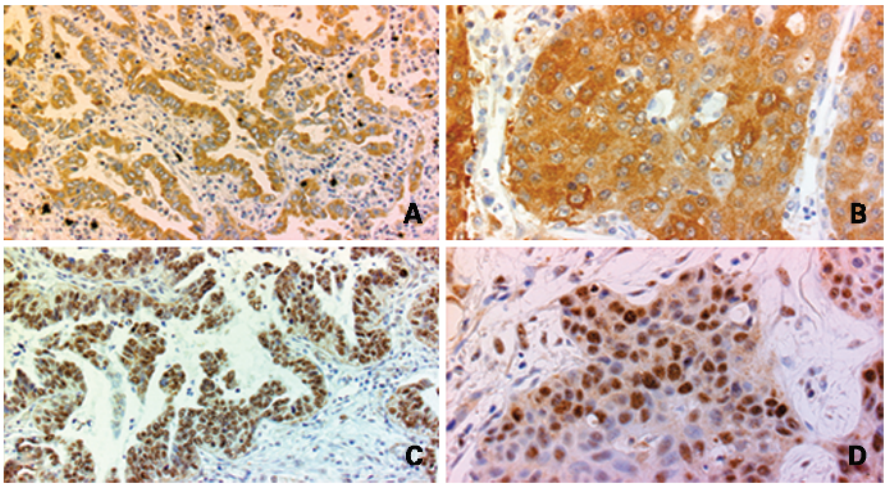

Fig. 1. Immunohistochemical staining of lung cancer specimens expressing ERs. *A* and *C*, adenocarcinoma cells showing ER-α immunoreactivity localized in their cytoplasm (*A*) and ER-β immunoreactivity localized in their nucleus (*C*). *B* and *D*, squamous cell carcinoma cells showing ER-α immunoreactivity in their cytoplasm (*B*) and ER-β immunoreactivity in their nucleus (*D*).

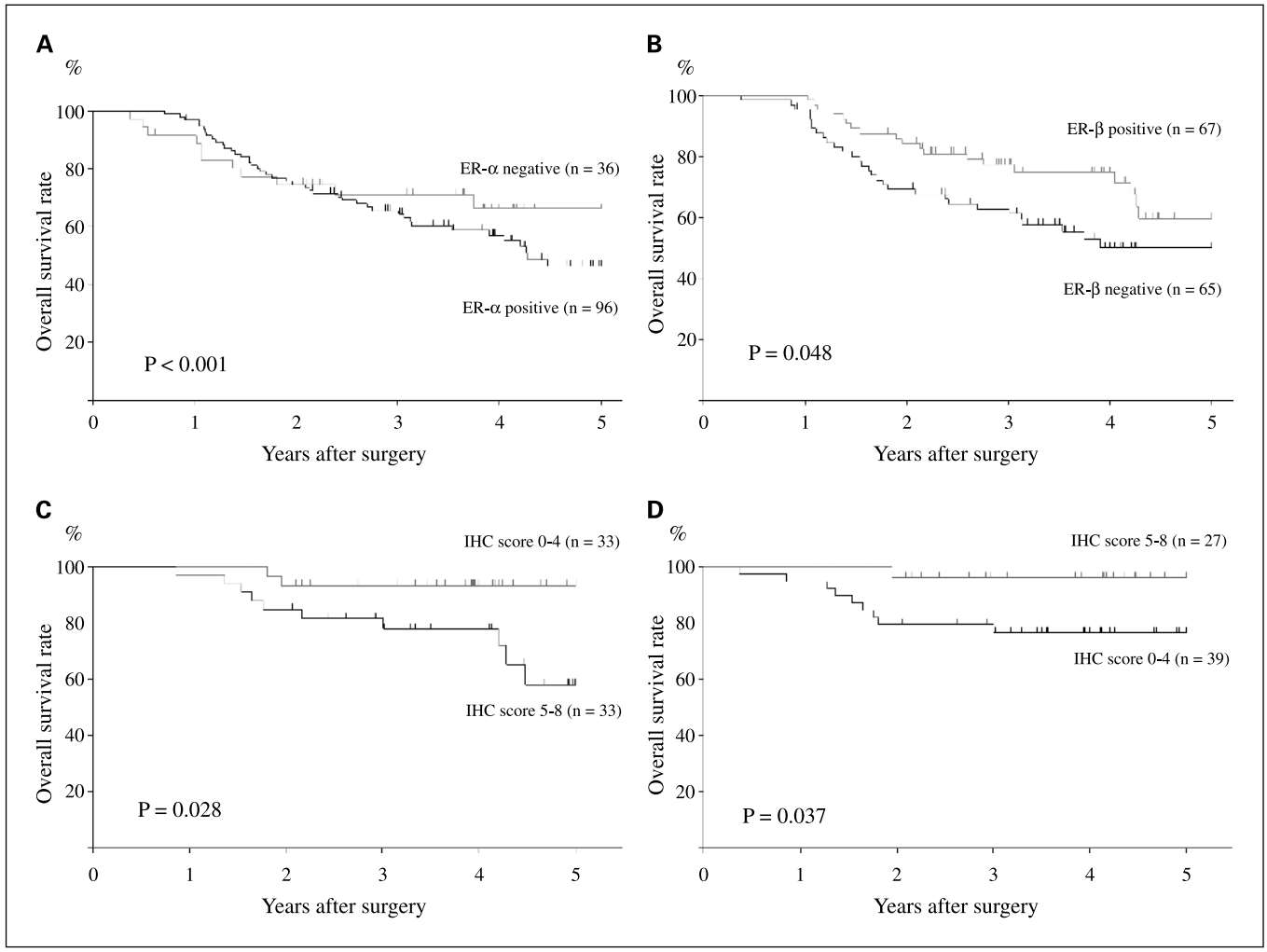

Fig. 2. *A* and *B*, Kaplan-Meier analysis showing the overall survival among NSCLC patients categorized according to their ER-α (*A*) or ER-β (*B*) expression status. *C* and *D*, overall survival curve for 66 patients with stage I NSCLC categorized according to their ER-α (*C*) or ER-β (*D*) expression status. *P* values were calculated using the log-rank test.

Table 2. Comparison of ER status results as determined by immunohistochemical staining

Immunohistochemical score	ER-α	ER-β
	No. (%)	No. (%)
0	36 (27)	65 (49)
2	7 (5)	3 (2)
3	10 (8)	8 (6)
4	12 (9)	5 (4)
5	7 (5)	8 (6)
6	24 (18)	13 (10)
7	19 (15)	13 (10)
8	17 (13)	17 (13)

correlation between the expression of ER-α or ER-β and any of the clinical variables listed in Table 1.

Clinical outcome. The overall Kaplan-Meier survival curves for ER-α and ER-β expression are shown in Fig. 2. Univariate analysis revealed expression of ER-α to be linked to poor overall survival ($P < 0.001$; Fig. 2A), whereas expression of ER-β was associated with better overall survival ($P = 0.048$; Fig. 2B). The immunohistochemical scores and the correlation between the expression of ER-α and ER-β in NSCLC tumors are shown in Tables 2 and 3, respectively. Univariate analysis showed that ER-α(+)ER-β(−) patients had a significantly poorer prognosis than ER-α(−)ER-β(+) patients ($P = 0.00007$; Fig. 3). Moreover, at histopathologic stage I, patients whose cancer received an ER-α immunohistochemical score of 5 to 8 (Ia, $n = 23$; Ib, $n = 10$) had a significantly poorer prognosis than those whose cancers received a score of 0 to 4 (Ia, $n = 23$; Ib, $n = 10$; $P = 0.028$; Fig. 2C). Conversely, patients whose cancer received an ER-β immunohistochemical score of 5 to 8 (Ia, $n = 21$; Ib, $n = 7$) had a significantly better prognosis than those whose cancer received a score of 0 to 4 (Ia, $n = 26$; Ib, $n = 13$; $P = 0.037$; Fig. 2D). There was no significant difference between patients with ER-α or ER-β immunohistochemical scores of 0 to 4 and 5 to 8 at histopathologic stages II or III (data not shown).

Table 3. Correlation between ER-α and ER-β in NSCLC

Antigen	ER-β positive	ER-β negative
ER-α-positive	45 (34%)	51 (38%)
ER-α-negative	22 (17%)	14 (11%)

Multivariate analysis of overall survival. We then carried out a multivariate analysis of overall survival using the Cox proportional hazards model to determine whether the prognostic value of ER-α or ER-β expression persisted even when other prognostic factors were considered. Of the factors listed in Table 4, histologic grade, stage, and ER-β were found to be significant independent variables that correlated with overall survival ($P = 0.0107$, $P < 0.001$, and $P = 0.0264$, respectively). The ER-β-negative value for overall survival yielded a hazard ratio of 1.9, with a 95% confidence interval ranging from 1.1 to 3.4.

Discussion

Although there have been a number of studies investigating the expression of ERs in lung cancer, the results remain inconclusive, and the relationship between ER expression and prognosis remains unclear (3–13). Using monoclonal and polyclonal anti-ER antibodies, Stabile et al. confirmed ER expression in lung cancer cells, identifying ER-α and ER-β in the cytoplasm and nucleus, respectively (13). Our findings obtained using polyclonal antibodies against the COOH terminus of the ERs are consistent with those earlier findings, but we observed no positive staining when we used a monoclonal antibody against the NH_2 terminus of ER-α (1D5, DAKO; data not shown). This suggests that the ER-α isoform localized in the cytoplasm of lung cancer cells is a variant lacking part of the NH_2 terminus (17–19), which is noteworthy, as the expression of such an ER-α variant in hepatocellular carcinomas is indicative of extremely aggressive tumors that are unresponsive to the estrogen antagonist tamoxifen (20).

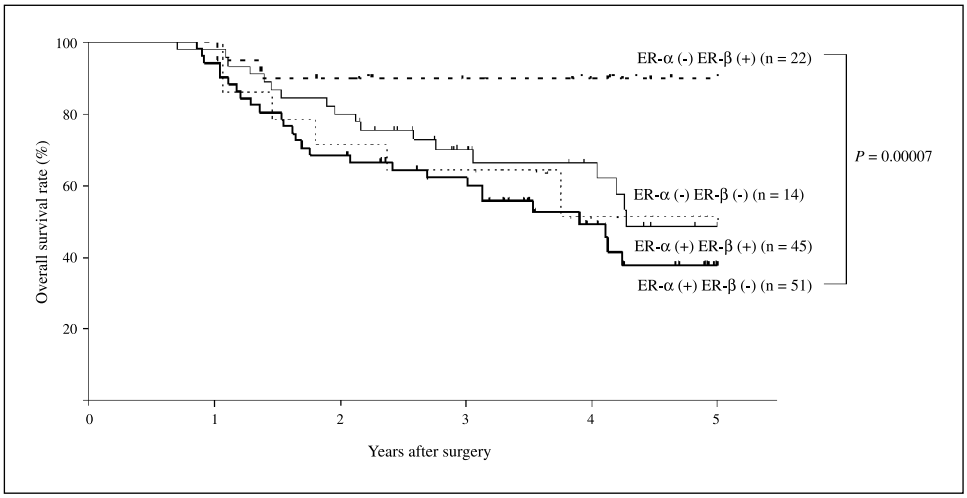

Fig. 3. Kaplan-Meier analysis showing overall survival among NLCLC patient subtypes defined by ER immunohistochemistry. *P* values were calculated using the log-rank test.

Table 4. Univariate and multivariate Cox proportional hazard analysis for overall survival of 132 patients with NSCLC

Factors	Univariate	Multivariate	
	P	Hazard ratio (95% confidence interval)	*P*
Age (y)			
<66 (median; $n = 60$) versus ≧66 ($n = 70$)	0.7745	1.4 (0.8-2.4)	0.2749
Histologic subtype			
Squamous cell carcinoma ($n = 28$) versus adenocarcinoma ($n = 102$)	0.4889	1.3 (0.6-2.7)	0.4913
Histologic grade			
Well differentiated ($n = 50$) versus others ($n = 82$)	0.0162	2.6 (1.2-5.3)	0.0107
Histologic stage			
I, II ($n = 90$) versus III, IV ($n = 42$)	<0.001	3.4 (1.9- 6.1)	<0.001
ER-α expression			
Negative ($n = 38$) versus positive ($n = 94$)	<0.001	1.3 (0.6-2.8)	0.4458
ER-β expression			
Positive ($n = 66$) versus negative ($n = 66$)	0.0481	1.9 (1.1- 3.4)	0.0264

NOTE: Hazard ratios, 95% confidence intervals, and two-sided *P* values were obtained from the Cox proportional hazards models.

The other main question addressed in this study was whether expression of ER-α or ER-β is a prognostic factor in lung cancer, and whether that prognosis is affected by an interaction between the two receptors. In breast cancer, expression of ER-α is generally indicative of a better prognosis (1, 2), which we anticipated would be the case in lung cancer as well. Our univariate analysis showed the exact opposite, however, which may mean that the ER-α variant expressed in the cytoplasm of lung cancer cells contributes to the aggressiveness of the tumor, as it does in hepatocellular carcinomas. Because the number of patients negative for ER-α was relatively small in the present study, ER-α could not be shown to be an independent prognostic factor of overall survival in lung cancer in our multivariate analysis. ER-β, by contrast, was found to be an independent prognostic factor of overall survival. Similarly, our results suggest that the expression of ERs is an important prognostic factor for NSCLC at histopathologic stage I.

Evidence suggests that in breast cancer cells, ER-β may bind to ER-α, thereby inhibiting its activity (21–24). Although our data reflect the opposing actions of ER-α and ER-β, their respective localizations within cells differ, suggesting that if ER-β acts to inhibit ER-α function, it works in some way other than through direct binding.

Recently, Fasco et al. reported that the expression of ERs in lung cancer is gender-dependent, and that ER-α expression occurs more often in the lungs of women than men (25). We found no such distinction, although the number of women smokers in our sample was small.

At present, there are few therapeutic options available for lung cancer patients. Our findings suggest that hormone therapy may be a useful new strategy for the treatment of lung cancer, although further study is needed to determine the sensitivity of the cytoplasmic ER-α variant to such therapy, and to determine the mechanism by which ER-β inhibits the ER-α variant in these cells.

In summary, expression of ER-α and the absence of ER-β expression were each associated with poor prognosis. In particular, the absence of ER-β was found to be an important indicator that could serve as a marker identifying patients at high risk even at an early clinical stage.

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