Prognostic significance of dysadherin expression in patients with non-small cell lung cancer

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Objective: The aim of this study was to evaluate the expression of dysadherin and E-cadherin and to investigate their clinical significance as prognostic factors in non-small cell lung cancer.

Methods: Non-small cell lung cancer specimens were obtained from 131 patients undergoing clinically indicated operations at the Department of General and Cardiothoracic Surgery, Kanazawa University Hospital, between 1995 and 1997. All patients had undergone curative resection of the primary tumor, including systematic lymph node dissection. The avidin-biotin-peroxidase complex method was used for immunostaining of dysadherin and E-cadherin.

Results: Among the 131 lung cancer specimens, 46 (35.1%) tumors were positively stained with dysadherin. Preserved membranous E-cadherin staining was present in 45.8% (60/131) of cases. In this analysis dysadherin expression was not correlated with E-cadherin expression ($P = .1333$), but a significant association was observed between dysadherin expression and survival time. The overall survival of patients with dysadherin-positive tumors was significantly worse than that of those with dysadherin-negative tumors ($P = .0059$). Patients with reduced E-cadherin immunopositivity survived significantly shorter than those with preserved E-cadherin immunopositivity ($P = .0406$). The overall survival of patients with positive dysadherin and reduced E-cadherin expression was significantly worse than that of patients with negative dysadherin and preserved E-cadherin expression ($P = .0002$). Multivariate analysis revealed the independent prognostic value of dysadherin positivity, reduced E-cadherin expression, and lymph node metastasis on overall survival.

Conclusions: Dysadherin expression is an independent prognostic factor of survival in patients with non-small cell lung cancer, and combined immunohistochemical analysis of dysadherin and E-cadherin expression might provide further prognostic information.

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Cell-cell adhesiveness determines the polarity of cells and is generally reduced in human cancers. The cadherins are members of a large family of transmembrane glycoproteins that mediate calcium-dependent, homophilic cell-cell adhesion and play an important role in the maintenance of normal tissue architecture. A tumor-suppressor gene product, E-cadherin, and its undercoat proteins, catenins, which connect cadherins to actin filaments, are located at lateral borders concentrating on adherens junctions of epithelial cells and establish firm cell-cell adhesion. Hirata and associates¹ reported that decreased E-cadherin expression showed a correlation with lymph node metastasis in resected non-small cell lung cancer (NSCLC) and with the prognosis of patients with stage IIIA disease.

Recently, Ino and colleagues² identified a novel cancer-associated cell membrane glycoprotein, dysadherin, composed of 178 amino acids. The transfection of this

Figure 1. A and C, Immunohistochemical staining of dysadherin in the primary adenocarcinoma. (Original magnification: A, 100 $\times$; C, 400 $\times$.) Cell membranes are strongly stained. B, E-cadherin expression was reduced in tumor cells in which dysadherin was strongly expressed. (Original magnification 100 $\times$.) D, Immunohistochemical staining of E-cadherin in the primary squamous cell carcinoma. (Original magnification 200 $\times$.)

molecule causes E-cadherin downregulation and disturbs homotypic cell adhesion in the human hepatocellular carcinoma cell line PLC/PRE/5. Recent studies have demonstrated that the increased expression of dysadherin is correlated with decreased survival in patients with colorectal cancer,³ thyroid cancer,⁴ and pancreatic ductal carcinoma.⁵

In this study we examined for the first time the correlation of dysadherin expression and clinicopathologic findings and compared dysadherin expression and E-cadherin expression in patients with NSCLC.

Patients and Methods

Patients

NSCLC specimens were obtained from 131 patients (97 male and 34 female patients) undergoing clinically indicated operations at the Department of General and Cardiothoracic Surgery, Kanazawa University Hospital, between 1995 and 1997. All patients had undergone curative resection of the primary tumor, including systematic lymph node dissection. Their ages ranged from 47 to 89 years (mean $\pm$ standard deviation [SD], 66.1 $\pm$ 9.2 years). The sample selection was restricted to consecutive cases diagnosed as stage I, II, and IIIA. None of the patients received chemotherapy or radiation therapy preoperatively. Complete follow-up data were obtained for at least 60 months, and the mean observation period was 67.2 $\pm$ 29.1 months (range, 8-98 months).

Methods

Immunohistochemistry. The avidin-biotin-peroxidase complex method was used for immunostaining of dysadherin and E-cadherin, as described previously. In brief, a formalin-fixed and paraffin-embedded block was obtained for each case. Five-micrometer sections were mounted on poly-L-lysine-coated glass slides and dried. After deparaffinization with xylene and rehydration with a series of decreased alcohol concentrations, antigen retrieval was performed by placing microwave slides in citrate buffer for 15 minutes. Endogenous peroxidase activity was blocked in 0.3% hydrogen peroxide in phosphate-buffered saline (PBS; pH 7.2) for 15 minutes. After rehydration and washing in PBS, sections were incubated with 10% normal goat serum (DAKO) for 10 minutes at room temperature to block nonspecific binding of the second antibody. Then sections were incubated with anti-dysadherin antibody (1:500 dilution, Abcam) or anti-E-cadherin antibody (1:200 dilution, Takara) at 4°C. The sections were washed with PBS and incubated with biotinylated anti-goat immunoglobulin (Vectastain ABC Kit, Vector) for 20 minutes at room temperature and washed again in PBS and reacted with streptavidin-biotin system (DAKO) for 20 minutes at room temperature. Immune conjugate was visualized with PBS containing both 0.02% (wt/vol) 3,3-diaminobenzidine tetrahydrochloride and 0.03% (wt/vol) hydrogen peroxide. All sections were counterstained with Meyer hematoxylin. Negative controls were prepared by substituting PBS or Tris-buffered saline for the primary antibody.

TABLE 1. Correlation between expression of E-cadherin and dysadherin and clinicopathologic characteristics in patients with non-small cell lung cancer

Variables	n	E-cadherin expression (No. of patients [%])			Dysadherin expression (no. of patients [%])		
		Preserved	Reduced	P value	<50%	>50%	P value
Total	131	60 (46)	71 (54)		85 (65)	46 (35)	
Age (y)							
≤65	48	26 (54)	22 (46)	.2727	30 (62)	18 (38)	.6642
>65	83	34 (41)	49 (59)		55 (66)	28 (34)	
Sex							
Male	97	41 (42)	56 (58)	.1708	62 (64)	35 (36)	.6983
Female	34	19 (56)	15 (44)		23 (68)	11 (32)	
Pathologic stage							
I	74	38 (51)	36 (49)	.2124	54 (73)	20 (27)	.0872
II	20	6 (30)	14 (70)		11 (55)	9 (45)	
III	37	16 (43)	21 (57)		20 (54)	17 (46)	
Histology							
Adenocarcinoma	81	35 (43)	46 (57)	.8846	49 (60)	32 (40)	.0442
Squamous cell carcinoma	50	25 (50)	25 (50)		36 (72)	14 (28)	
Differentiation							
Well	46	21 (46)	25 (54)	.6098	33 (72)	13 (28)	.3041
Moderate	52	26 (50)	26 (50)		32 (62)	20 (38)	
Poor	33	13 (39)	20 (61)		20 (61)	13 (39)	

Evaluation of dysadherin and E-cadherin expression. All sections were analyzed in a blinded fashion without knowledge of the patient's clinical information. We assessed dysadherin expression as the percentage of positively stained tumor cells related to total tumor cells. Expression of E-cadherin was considered positive if tumor cells were stained as strongly as normal epithelial cells adjacent to the tumor, whereas those that stained weaker than normal epithelial cells were considered negative. We also assessed E-cadherin expression as the percentage of positively stained tumor cells related to total tumor cells.

Statistical Analysis

All results were analyzed with a statistical analysis software package (SPSS, Inc). The relation of clinicopathologic characteristics to the number of immunopositive tumor cells was analyzed by using the χ^2 test and analysis of variance where appropriate. Dysadherin, E-cadherin, tumor differentiation, histology, age, and sex were included in the assessment of prognostic factors. Cancer-specific survival was defined as the time between the operation and the last follow-up or cancer-related death. Overall survival curves were determined by using the Kaplan-Meier method and were analyzed with the log-rank test. The Cox proportional hazard model was applied for univariate

and multivariate analysis to confirm the prognostic effect of the factors on survival.

Results

Expression of Dysadherin

Dysadherin expression was present at the cell membrane of cancer cells (Figure 1, A and C). The possible association with dysadherin was examined by using a standardized scale of 0 to 4+ (0, none; 1+, 1% to 25%; 2+, 26% to 50%; 3+, 51% to 75%; 4+, 76% to 100% of cells). The percentage of tumor cells that stained positively for dysadherin was 46.4% $\pm$ 18.1% (mean $\pm$ SD). Therefore we set the cut-off value for dysadherin immunopositivity at 50%. Among the 131 lung cancer specimens, there were 46 (35.1%) tumors positively stained with dysadherin. Immunoreactivity for dysadherin was observed at the cell-cell boundaries of cancer cells and heterogeneously in tumor nests. Analyses of the associations between dysadherin and E-cadherin expression and clinicopathologic data are summarized in Table 1. Increased dysadherin expression was correlated with the tumor stage, although it was not statistically significant ($P = .0872$). There was no significant association between the dysadherin expression and age, sex, or tumor differentiation.

Expression of E-cadherin

Expression of E-cadherin was also observed at the cell-cell boundaries (Figure 1, C). The possible association with E-cadherin was examined by using a standardized scale of 0 to

TABLE 2. Correlation of E-cadherin and dysadherin expression

E-cadherin expression	Dysadherin expression	
	>50%	<50%
Preserved	16	44
Reduced	30	41

$P = .1333$.

Figure 2. Kaplan-Meier survival curves for dysadherin (A) and E-cadherin (B). Log-rank test for trend: $P = .0059$ and $.0406$, respectively.

4+ (0, none; 1+, 1% to 25%; 2+, 26% to 50%; 3+, 51% to 75%; 4+, 76% to 100% of cells). The percentage of tumor cells that stained positively for E-cadherin was $72.2\% \pm 16.8\%$ (mean $\pm$ SD). Therefore, we set the cut-off value for E-cadherin immunopositivity at 75%. Preserved membranous E-cadherin staining was present in 45.8% (60/131) of cases. There was no significant correlation between the reduced expression of E-cadherin and age, sex, staging, histology, or differentiation (Table 1). In this analysis dysadherin expression was not correlated with E-cadherin expression ($P = .1333$, Table 2).

Prognostic Value of Dysadherin and E-cadherin Expression

A significant association was observed between dysadherin expression and survival time. The overall survival of patients with dysadherin-positive tumors was significantly worse than that of those with dysadherin-negative tumors ($P = .0059$). The 5-year survival rates of the dysadherin-positive group and the dysadherin-negative group were 27.5% and 53.6%, respectively (Figure 2, A). Patients with reduced E-cadherin immunopositivity survived significantly shorter than those with preserved E-cadherin immunopositivity ($P = .0406$; Figure 2, B). Overall survival of patients with positive dysadherin and reduced E-cadherin expression was significantly worse than that of patients with negative dysadherin and preserved E-cadherin expression ($P = .0002$; Figure 3, A). The 5-year survivals of the former and the latter group were 56.0% and 14.3%, respectively. Similarly, in patients with pathologic stage I disease, worse prognosis could be found in patients with positive dysadherin and reduced E-cadherin expression than in patients with negative dysadherin and preserved E-cadherin expression (73.3% vs 23.1%, $P = .0088$; Figure 3, B).

Multivariate analysis revealed the independent prognostic value of dysadherin positivity, reduced E-cadherin ex-

pression, and lymph node metastasis on overall survival (Table 3).

Discussion

Cell-cell adhesion appears to be a vital process in tumor cell invasion and metastasis. In various cancerous tissues a reduced expression of E-cadherin has frequently been observed in cancer progression.⁶⁻¹² Ino and colleagues² reported previously that dysadherin, a cancer-associated cell membrane glycoprotein, has an anti-cell-cell adhesion function and inactivate E-cadherin function in a posttranscriptional manner and plays an important role in tumor progression and metastasis. In human subjects it was reported that dysadherin expression was associated with a high incidence of recurrence³ and decreased survival in certain types of cancers.³⁻⁵ To our knowledge, this is the first report to clarify the relationship among dysadherin expression, clinicopathologic features, and survival in patients with NSCLC.

In this immunohistochemical study patients with increased dysadherin expression had a tendency to show reduced E-cadherin expression in NSCLC, but a statistically significant difference could not be obtained. Sixteen (34.8%) of 46 patients with increased dysadherin expression had preserved E-cadherin expression. Sato and coworkers⁴ indicated a significant negative correlation between dysadherin expression and E-cadherin expression in 92 thyroid carcinoma specimens. In thyroid cancer it appears that transcriptional or posttranscriptional mechanisms, rather than structural abnormalities of the E-cadherin gene, are responsible for the downregulation of E-cadherin expression. Overexpression of dysadherin was shown to downregulate E-cadherin posttranscriptionally in liver cancer cells,² although the molecular mechanisms underlying this process are not clear. Shimamura and associates⁵ insisted that this discrepancy exists

Figure 3. Kaplan-Meier survival curves in relation to the combination of E-cadherin and dysadherin expression: **A**, overall; **B**, stage I.

because the differences of cell type and microenvironment in the human pancreas mask the dysadherin function in the downregulation of E-cadherin. We could find no correlation between the cell type, differentiation, and the result of immunohistochemical staining. This result suggests that a mechanism might exist for the reduction of E-cadherin expression other than that related to dysadherin expression.

When we analyzed the association between dysadherin expression and clinicopathologic features, we could demonstrate a significant association between the histologic type of tumor and dysadherin expression. In adenocarcinomas 39.5% showed positive dysadherin expression, which was statistically significant compared with that seen in squamous cell carcinomas, of which only 28.0% showed the same feature ($P = .0442$). In thyroid cancer dysadherin

expression was highest in undifferentiated carcinoma, next highest in papillary carcinoma, and lowest in follicular carcinoma.⁴ Concerning tumor differentiation, well-differentiated cancer has a greater tendency toward low positive dysadherin expression than poorly differentiated cancer, which showed no statistically significant difference ($P = .3041$).

It is interesting to note that the dysadherin expression levels in patients with lymph node metastasis were higher than in those without lymph node metastasis, although the difference was not statistically significant ($P = .057$). On the contrary, E-cadherin expression revealed no correlation with lymph node metastasis ($P = .3955$). In a multivariate analysis we found that the presence of lymph node metastasis, dysadherin overexpression, and reduced E-cadherin

TABLE 3. Prognostic factors in patients with non-small cell lung carcinoma according to univariate and multivariate analysis of Cox hazards model

Prognostic factors	Characteristics		Hazard ratio	95% CI	P value
	Unfavorable	Favorable			
Dysadherin	>50%	<50%	3.018	1.748-2.009	.0104
E-cadherin	Reduced	Preserved	2.76	1.676-4.577	.0142
Lymph node metastasis	Positive	Negative	3.414	1.907-4.877	.0081
Differentiation	Moderate/poorly	Well	1.738	1.034-3.887	.0544
Histology	Adenocarcinoma	Squamous cell carcinoma	1.232	0.803-1.978	.3093
Age	>65	≤65	1.121	0.8916-2.603	.4871
Sex	Male	Female	0.986	0.5398-2.016	.8367

CI, Confidence interval.

expression had significant independent prognostic ability. In this series there are 74 patients with stage I lung cancers. To eliminate the negative prognostic effects of lymph node metastasis, we evaluated the significance of dysadherin and E-cadherin expression restricted to patients with stage I disease. A statistically significant difference of prognosis could be found between the patients with positive dysadherin and reduced E-cadherin expression and the patients with negative dysadherin and preserved E-cadherin expression ($P = .0088$). Furthermore, we intended to verify that these factors are useful in stage IA lung cancers, but a statistically significant difference could not be observed ($P = .109$). Only 5 patients revealed positive dysadherin and reduced E-cadherin expression out of 44 cases.

In conclusion, dysadherin expression is an independent prognostic factor of survival in patients with NSCLC, and combined immunohistochemical analysis of dysadherin and E-cadherin expression might provide further prognostic information.

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