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The Role of E-cadherin Expression in Non-Small Cell Lung Cancer

The purpose of this study is to evaluate the clinical significance of E-cadherin expression in lung cancer. E-cadherin expression was detected by immunohistochemistry using a monoclonal antibody (HECD-1). Strongly positive (++) E-cadherin tumors were classified as a type of preserved E-cadherin expression (Pr type), while the others (+, − tumors) were classified as a type of reduced E-cadherin expression (Rd type). The frequency of Pr type in squamous cell carcinomas (59.0%) was higher than Rd type. However, in adenocarcinomas, the frequency of Rd type was higher than Pr type. E-cadherin expression pattern was significantly correlated with differentiated state (Pearson correlation coefficient 0.394, $p<0.001$). E-cadherin expression of well-differentiated tumors was more frequently preserved than that of poorly differentiated tumors (60.0% vs. 25.9%). With regard to the correlation between E-cadherin expression and stages of lymph node metastasis in non-small cell lung cancers, the percentage of tumors with Pr type E-cadherin expression declined from 66.3% (≤N1) to 38.6% (≥N2), indicating that loss of E-cadherin expression is responsible for acquisition of invasive potential of lung cancer as well as the possible role of E-cadherin in the histological differentiation of lung cancer.

Key Words: *Cadherins; Lung Neoplasms; Lymph Nodes*

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INTRODUCTION

Cancer has the malignant potential to manifest as tumor cells to spread from the primary tumor and form metastasis in distant organs. A number of different steps in the metastatic process is associated with alterations in the adhesive properties of tumor cells (1). Apparently, a prerequisite for this process is that the carcinoma cells detach from their primary site and become motile, meaning these cells have reduced adhesion to neighboring tumor cells (2, 3). Considering cell adhesion, various molecules, including integrins (4), selectins (5), CD44 (6) and cadherins (7-10), have been examined for their possible association with tumor spread and invasion.

E-cadherin is a mediator of epithelial cell-to-cell adhesion and is considered to be an important member of the family of calcium-dependent cell adhesion molecules. The cell adhesion molecule E-cadherin plays a key role in the establishment and maintenance of epithelial tissue structure, and its down-regulation is potentially important in the formation of metastasis from carcinomas (7).

Multiple studies have suggested that loss of E-cadherin expression is responsible for acquisition of invasive potential (8-10). In human squamous cell carcinoma of the head and neck, loss of E-cadherin expression is correlated with dedifferentiation and metastasis (8). Mayer et al. (9) demonstrated that E-cadherin represents a differentiation marker whose down-regulation might play an important role in early gastric cancer metastasis. Inhibition of cadherin with antibodies promotes the invasiveness of the tumor cells (10). Therefore, the suppression of cadherin activity might trigger the release of tumor cells. This could occur either by suppressing of cadherin gene expression or by expressed cadherin molecules losing their function (10-12).

We investigated the relationship between the expression of E-cadherin and the clinicopathological features in non-small cell lung cancers by immunohistochemical staining using monoclonal antibodies to E-cadherin.

MATERIALS AND METHODS

Study population

This study population consisted of 175 patients diagnosed with lung cancer between January 1992 and September 1998 at Chonnam National University Hospital, Kwangju, Korea. Tissue samples were obtained at the time of surgical resection or bronchoscopic biopsy. The

survival times were calculated from the date of histologic diagnosis. Stage was recorded according to the TNM staging system for bronchogenic cancer (13). Surgical stage was assigned by the results of mediastinal lymph node sampling and primary tumor characteristics at the time of resection. For inoperable disease, stage was assigned according to clinical information available, chest and upper abdomen CT, or the results of biopsy from a metastatic site. All clinical information was taken by chart review retrospectively.

Immunohistochemistry

Three μm tissue sections were cut from paraffin blocks and mounted on Probe-On slides (Fisher Scientific, Pittsburgh, PA, U.S.A.). Paraffin was removed by heating and exposure to xylene, and the tissue was rehydrated in decreasing concentrations of ethanol. The sections were preincubated with 3% hydrogen peroxide in distilled water for 10 min to block endogenous peroxidase activity after the slides were rinsed in phosphate-buffered saline (PBS; pH=7.2). The slides were then placed in a 0.1 M citrate buffer (pH 6.0-6.5) and autoclaved for 5 min. The sections were incubated with primary antibody E-cadherin (HECD-1) at room temperature for 2 hr. After incubation with the primary antibodies, the samples were rinsed with PBS and re-incubated with a biotinylated secondary antibody (anti-rabbit, mouse immunoglobulins) from LSAB kit (DAKO, CA, U.S.A.) for 30 min at room temperature. After washing, the sections were incubated

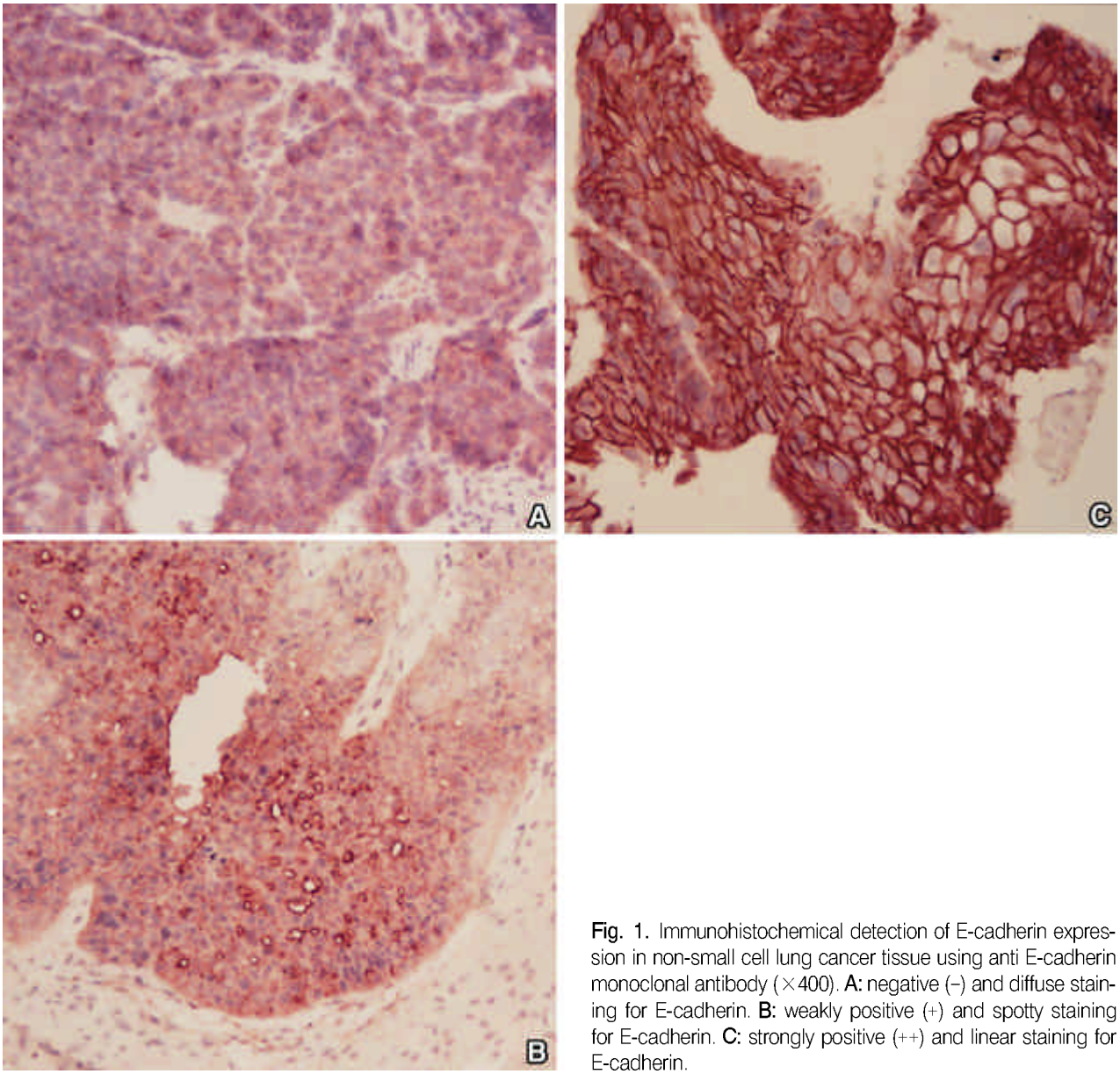

Fig. 1. Immunohistochemical detection of E-cadherin expression in non-small cell lung cancer tissue using anti E-cadherin monoclonal antibody (×400). **A:** negative (−) and diffuse staining for E-cadherin. **B:** weakly positive (+) and spotty staining for E-cadherin. **C:** strongly positive (++) and linear staining for E-cadherin.

for 30 min with streptavidin conjugated with horseradish peroxidase. They were incubated for 5 min with 3-amino-9-ethyl-carbazole solution as the chromogenic substrate, and were counterstained with Meyer's hematoxylin. As negative controls, sections were incubated with nonimmunized sera instead of primary antibody. All slides were examined using standard light microscopy, and scored independently by two observers in quartiles as percentage of positive tumor cells. When clear staining was present in less than 5% of the tumor cells, the result was defined as negative (−). When more than 5% but less than 50% of the tumor cells stained for E-cadherin, the result was defined as weakly positive (+). When more than 50% of the tumor cells stained positive, the result was defined as strongly positive (++). Strongly positive (++) E-cadherin tumors were classified as a type of preserved E-cadherin expression (Pr type), while the others (+, − tumors) were classified as a type of reduced E-cadherin expression (Rd type). E-cadherin expression pattern was classified as linear when staining was uninterrupted and confined to the cell borders, as spotty when it was interrupted but confined to the cell borders, and as diffuse when it was not confined to the cell borders (Fig. 1).

Statistical analysis

For statistical analysis frequency table, chi-squared test and Pearson's correlation coefficient were used. Statistical significance was defined as a two tailed p value of less than 0.05.

RESULTS

Characteristics of this study population are detailed in Table 1. One hundred and seventy-five patients (162 males and 13 females) with histologically proven bronchogenic cancer were included. Their age ranged from 36 to 81 years with a mean age of 61.6±8.7 years. Histologic types consisted of 139 squamous cell carcinomas and 36 adenocarcinomas.

Table 1. General characteristics of patients

Number		175
Gender (M/F)		162/13
Age (yr)		61.6±8.7 (36-81)
Smoking (yes/no)		144/31
Histology		
squamous cell carcinoma		139
adenocarcinoma		36
Stage	I	9 (5.1%)
	II	15 (8.7%)
	IIIa	44 (25.1%)
	IIIb	77 (44.0%)
	IV	30 (17.1%)
T stage	T1	7 (4.0%)
	T2	46 (26.3%)
	T3	50 (28.6%)
	T4	72 (41.1%)
N stage	N0	21 (12.0%)
	N1	71 (40.6%)
	N2	49 (28.0%)
	N3	34 (19.4%)
M stage	M0	145 (82.9%)
	M1	30 (17.1%)

There was a significant correlation between E-cadherin expression and histological types. In squamous cell carcinomas, the frequency of Pr type was higher (59.0%) than Rd type (41.0%). In adenocarcinomas, however, the frequency of Rd type was higher than Pr type (Table 2).

The relationship between E-cadherin expression pattern and histological classification is shown in Table 3. The frequency of linear pattern (72.7%) in squamous cell carcinomas was significantly higher than spotty (15.0%)

Table 2. E-cadherin expression by histologic types in patients with non-small cell lung cancer

	Total	Pr type	Rd type			p value
		++	+	−	Subtotal	
SQC	139	82 (59.0%)	40	17	57 (41.0%)	
ADC	36	11 (30.6%)	10	15	25 (69.4%)	0.002

Pr type, preserved E-cadherin expression; Rd type, reduced E-cadherin expression; SQC, squamous cell carcinoma; ADC, adenocarcinoma

Table 3. E-cadherin expression patterns by histologic types in patients with non-small cell lung cancer

		Linear	Spotty	Diffuse	Absent	p value
SQC	(n=139)	101 (72.7%)	21 (15.1%)	16 (11.5%)	1 (0.7%)	
ADC	(n= 36)	12 (33.3%)	10 (27.8%)	11 (30.6%)	3 (8.3%)	<0.001

SQC, squamous cell carcinoma; ADC, adenocarcinoma

Table 4. E-cadherin expression by degree of cell differentiation in patients with non-small cell lung cancer

Differentiation	Total	Pr type	Rd type			*p* value
		++	+	-	Subtotal	
Well	80	48 (60.0%)	17	15	32 (40.0%)	
Moderate	68	38 (55.9%)	23	7	30 (44.1%)	
Poorly	27	7 (25.9%)	12	8	20 (74.1%)	0.008

Table 5. E-cadherin expression by stage of lymph node metastasis in patients with non-small cell lung cancer

Lymph node stage	Total	Pr type	Rd type			*p* value
		++	+	-	Subtotal	
≤N1	92	61 (66.3%)	23	8	31 (33.7%)	
≥N2	83	32 (38.6%)	27	24	51 (61.4%)	<0.001

or diffuse pattern (11.5%) ($p<0.001$).

E-cadherin expression was significantly correlated with increasing tumor differentiation (Pearson correlation coefficient -0.204, $p=0.007$). E-cadherin expression of well-differentiated tumors was more frequently preserved than that of poorly differentiated tumors (60.0% vs. 25.9%) (Table 4).

E-cadherin expression pattern was significantly correlated with status of cell differentiation (Pearson correlation coefficient 0.394, $p<0.001$). Diffuse or spotty pattern of E-cadherin expression was more frequently observed in undifferentiated cancers, while linear expression pattern was observed in well-differentiated cancer cells.

In non-small cell lung cancers, the percentage of tumors with Pr type E-cadherin expression declined from 66.3% (≤N1) to 38.6% (≥N2) (Pearson correlation coefficient -0.278, $p<0.001$) (Table 5).

DISCUSSION

The invasive behavior of cells critically differentiates between benign and malignant tumor cells. Acquisition of invasive potential by malignant cancer cells results from an accumulation of characteristics, including increased cell motility, secretion of proteolytic enzyme, and alterations of cell-substrate and cell-cell adhesion (1, 14). The molecular mechanism responsible for this latter process and altered cell-cell adhesion in invasive cancer cells are partially understood. In particular, the role of cadherin, a family of Ca^{2+}-dependent cell adhesion molecules, has been documented (7, 10-12, 15) as an important modulator of these processes.

Cadherins are the family of transmembrane glycoproteins responsible for calcium-dependent intercellular adhesion. Cadherins produce strong intercellular connections by hemophilic interaction in the presence of calcium, and the inactivation of other adhesion systems has little effect on cell-cell adhesion when cadherin functions (16, 17). Cadherins are divided into subclasses, all of which share a common basic structure. Several subclasses are well characterized at the molecular level. They are E-cadherin (epithelial cadherin or uvomorulin) (18), P-cadherin (placental cadherin) (19), N-cadherin (neural cadherin) (20), L-CAM (liver cell adhesion molecule) (21), and others (22).

E-cadherin molecules, which are distributed mainly in epithelial tissues, have shown to be responsible for organogenesis and morphogenesis of tissues (23). Taking into account the significant guiding role played by E-cadherin in normal development, various authors suggested that impaired function of this molecule could be important in the acquisition of invasive potential (7, 11, 24). Frixen et al. (25) found that noninvasive cell lines expressed E-cadherin protein, whereas invasive carcinoma cell lines lost E-cadherin expression.

In the present study, we found various patterns of E-cadherin expression in lung cancer tissues. E-cadherin expression had significantly correlated with a degree of histological differentiation. E-cadherin expression was preserved more frequently in well-differentiated tumors, while poorly differentiated tumors observed significant reduction of E-cadherin expression. The E-cadherin level was strong positive and expression pattern was linear in squamous cell carcinomas. The E-cadherin level was reduced and expression pattern was diffuse or spotty in adenocarcinomas. These observations indicate that E-cadherin expression on tumor cells could play a role to some degree in tumor differentiation in lung cancer cells. Recent studies, showing that E-cadherin expression was significantly correlated with increasing tumor differentiation in non-small cell lung cancers (26), are consistent with our results. Bohm et al. (27) reported on the differences of E-cadherin expression levels and patterns in human lung cancer. E-cadherin level was reduced and the expression pattern was spotty or diffuse in moderately

and poorly differentiated squamous cell carcinomas. Oka et al. (28), who used the monoclonal antibody for human E-cadherin (HECD-1), showed a correlation between E-cadherin expression and grade of tumor differentiation in gastric cancer cells. E-cadherin expression was frequently (70%) preserved in differentiated tumors, while most undifferentiated tumors (85%) showed reduced expression. On the other hand, Shimoyama et al. (29) could not find any correlation between E-cadherin expression and differentiation of primary gastric carcinomas also using HECD-1.

In this study concerning the correlation between E-cadherin expression and stage of lymph node metastasis, the percentage of tumors with Pr type E-cadherin expression declined from 66.3% (≤N1) to 38.6% (≥N2). This is consistent with the results of other studies (10, 26, 28) that show the loss of E-cadherin expression is responsible for acquisition of invasive potential. Behrens et al. (10) reported that inhibition of cadherins with antibodies promotes the invasiveness of the tumor cells. Sulzer et al. (26) found that tissue sections with the highest proportion of E-cadherin positive tumor cells were most common in patients without lymph node metastasis (N0, 63%).

From these results, we concluded that E-cadherin might play an important role in the genesis of histological differentiation and affect lymph node metastasis of lung cancer. Further studies are needed to clarify the significance of E-cadherin and evaluate whether this marker will help predict prognosis in lung cancer. Identifying relevant prognostic factors in lung cancer is important in predicting clinical outcome. Also, in the future it might enable us to find the most optimal treatment regimen for the individual patient.

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