The significance of E-cadherin and α -, β -, and γ -catenin expression in surgically treated non-small cell lung cancers of 3 cm or less in size

Yung-Chie Lee, MD, PhD^a

Chen-Tu Wu, MD^b

Chin-Shin Chen, MD^a

Hsao-Hsun Hsu, MD^a

Yih-Leong Chang, MD^b

Objectives: Expression of the cell-cell adhesion molecules E-cadherin and α -, β -, and γ -catenin seems closely related to tumor invasiveness. The relationship between the expression and clinicopathologic characteristics in surgically resected non-small cell lung cancers of 3 cm or less in size was studied. The relationship to patient survival was analyzed.

Methods: A total of 115 patients with surgically resected lung cancers of 3 cm or less in size were enrolled in this study. Expression of E-cadherin and α -, β -, and γ -catenin was immunohistochemically measured. The χ^2 test was used to correlate this expression with clinicopathologic parameters. Their influence on patient survival was evaluated with the Cox proportional hazards model.

Results: There was a positive correlation between E-cadherin and catenin expression in lung cancers. In general, E-cadherin and catenin expression were greater in tumors that were either bronchioloalveolar carcinomas or adenocarcinomas, well differentiated, early stage, peripheral, and without vascular or pleural invasion. By using multivariate analysis of patient survival, only early-stage and peripheral tumors were significantly favorable prognostic factors. Further analysis of the group of patients with early-stage disease showed that higher α -, β -, or γ -catenin expression was a favorable prognostic indicator.

Conclusion: Expression of α -, β -, or γ -catenin can be used as a prognostic indicator in patients with surgically resected stage I non-small cell lung cancers of 3 cm or less in size.

From the Departments of Surgery^a and Pathology,^b National Taiwan University Hospital, Taipei, Taiwan.

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Address for reprints: Yih-Leong Chang, MD, 6F-1, 99, Section 3, Roosevelt Rd, Taipei 106, Taiwan (E-mail: damu@ha.mc.ntu.edu.tw).

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Lung cancer is the most common cause of cancer death in Taiwan. Although a significant proportion of non-small cell lung cancers (NSCLCs) are detected when the tumor is less than 3 cm in size, the results of surgical treatment in this category of patients are not uniformly favorable.^{1,2} Small-sized lung cancers can present at an early or late stage of tumor development, and survival depends on the biologic characteristics of the tumor. Various investigators have shown that pathologic staging with the TNM system is the most significant prognostic indicator. Other histopathologic features, such as histologic type, degree of differentiation, vascular invasion, and mitotic index, can also help to predict the patient's outcome. Nevertheless, even at the same stage and with similar pathologic characteristics,

Figure 1. Normal bronchial epithelium used as a positive internal control. (Original magnification $\times 66$.)

Figure 2. Immunohistochemical study of a moderately differentiated adenocarcinoma shows diffuse cell membrane staining for β -catenin in the lung cancer. (Original magnification $\times 66$.)

surgically treated, 3-cm or smaller NSCLCs can have a variable prognosis. Recent advances in tumor cell biology will help to shed light on the associated mechanics of tumor invasion and metastasis.^{3,4}

Tumor invasion and metastasis are complex processes involving an interaction between the biologic characteristics of the tumor cells and the competence of host defenses. Alteration of cell-cell adhesion molecules may play a role in tumor-cell detachment in the early stages of tumor invasion and metastasis.⁵⁻⁸ E-cadherin is a subclass of the cadherin family that plays a major role in the maintenance of intercellular junctions in epithelial tissues. This function also depends on an interaction between the cytoplasmic domain of the cadherin molecule with the cytoskeleton through E-cadherin-associated cytoplasmic proteins known as α -, β -, and γ -catenin, which form an E-cadherin-catenin complex.⁹⁻¹¹ It has been reported that underexpression of E-cadherin; α -, β -, and γ -catenin; or both may be closely related to dedifferentiation and invasiveness in many types of human epithelial cancers.¹²⁻¹⁵ In the present study we investigated the clinicopathologic significance of E-cadherin and α -, β -, and γ -catenin expression in terms of their association with patient prognosis after surgical treatment of NSCLCs of 3 cm or less in size.

Materials and Methods

During the period from January 1990 to December 1997, 115 patients who had small-sized (≤ 3 cm) NSCLCs and had undergone complete surgical resection at the National Taiwan University Hospital were consecutively enrolled in the study. All of the resected tumor specimens and dissected lymph nodes were formalin fixed, sectioned, and stained with hematoxylin and eosin for microscopic examination. Histologic diagnoses and pathologic features were obtained, including histologic type, degree of differentiation, regional lymph node metastasis, microscopic tumor emboli, and the presence of direct invasion to the pleura or chest

wall. Pathologic staging was performed according to the International Union Against Cancer (1997),¹⁶ which is based on tumor size, location and involvement, and the presence of lymph node metastases or distant metastases.

The study group included 66 male and 49 female patients, and the mean age of the group was 59.2 years (range, 32-81 years). Clinical data for each of these patients were collected and included sex, age, smoking history, symptoms, signs, and location of the tumor. *Smoker* was defined as more than 1 pack-year of smoking history. The survival status of the patients was followed at regular outpatient clinics or by communication. The follow-up period ranged from 30 to 110 months. The clinical and pathologic data were analyzed with reference to the expression of E-cadherin and α -, β -, and γ -catenin in each tumor.

Immunohistochemistry

For immunohistochemical study of E-cadherin and α -, β -, and γ -catenin expression in the tumor tissue, 4- μ m-thick sections from each formalin-fixed, paraffin-embedded tissue block were treated with 0.3% hydrogen peroxide in methanol and heated in a microwave oven for 20 minutes to block endogenous peroxidase. The tissue sections were then incubated with normal nonimmune goat serum. After excessive goat serum had been blotted, the slides were incubated separately with 4 specific mouse monoclonal antibodies, including anti-E-cadherin (Takara Shuzo Co Ltd, Kyoto, Japan), anti- α -catenin, anti- β -catenin, and anti- γ -catenin (Transduction Laboratories, San Diego, Calif) for 1 hour at room temperature. After being washed with phosphate-buffered solution (PBS) 3 times, the sections were incubated with bionylated goat anti-mouse antibody for 20 minutes at room temperature. The sections were again washed 3 times with PBS and then incubated with peroxidase-conjugated streptavidin for 15 minutes at room temperature. After a third triple washing with PBS, the sections were stained with 0.05% (3',3')-diamino-benzidine tetrachloride freshly prepared in 0.05 mol/L Tris-HCl (pH 7.6) containing 0.01% hydrogen peroxide. Finally, the sections were counterstained with methylene green and mounted.

TABLE 1. E-cadherin and α -, β -, and γ -catenin expression in NSCLCs of 3 cm or less in size with relation to clinical parameters

Parameter	Proportion of E-cadherin <50% (%)	Proportion of α -catenin <50% (%)	Proportion of β -catenin <50% (%)	Proportion of γ -catenin <50% (%)
Age (y)				
<60	13/37 (35.1)	9/37 (24.3)	5/36 (13.9)	10/35 (18.6)
≥ 60	36/75 (48.0)	21/77 (27.3)	13/78 (16.7)	21/74 (28.4)
Sex				
Male	34/63 (54.0)*	23/66 (34.8)*	13/65 (20.0)	18/62 (29.0)
Female	15/49 (30.6)*	7/48 (14.6)*	5/49 (10.2)	13/47 (27.7)
Status of smoking				
Smoker	28/43 (65.1)*	19/45 (42.2)*	12/45 (26.7)*	12/42 (28.6)
Nonsmoker	18/62 (29.0)*	10/62 (16.1)*	6/63 (9.5)*	17/60 (28.3)
Tumor location				
Central	26/31 (83.9)*	15/32 (46.9)*	10/32 (31.2)*	12/31 (38.7)
Peripheral	23/81 (28.4)*	15/82 (18.3)*	8/82 (9.8)*	19/78 (24.4)

* $P < .05$.**TABLE 2. E-cadherin and α -, β -, and γ -catenin expression in NSCLCs of 3 cm or less in size with relation to pathologic characteristics**

Pathologic characteristics	Proportion of E-cadherin <50% (%)	Proportion of α -catenin <50% (%)	Proportion of β -catenin <50% (%)	Proportion of γ -catenin <50% (%)
Histologic type				
Squamous cell	16/22 (72.7)*	13/24 (54.2)*	8/23 (34.8)*	8/21 (38.1)
Adenocarcinoma	29/54 (53.7)*	13/54 (24.1)*	6/55 (10.9)*	17/54 (31.5)
Bronchioloalveolar	3/34 (8.8)*	3/34 (8.8)*	3/34 (8.8)*	5/32 (15.6)
Grade of differentiation				
Well	6/41 (14.6)*	6/41 (14.6)	4/41 (9.8)	9/39 (23.1)
Moderate	30/56 (53.6)*	17/58 (29.3)	11/58 (19.0)	17/56 (30.4)
Poor	12/13 (92.3)*	6/13 (46.2)	2/13 (15.4)	4/12 (33.3)
Tumor stage				
Stage I	24/73 (32.9)*	17/75 (22.7)	10/75 (13.3)	18/70 (25.7)
Stage II	2/10 (20.0)*	2/10 (20.0)	0/10 (0)	1/10 (10.0)
Stage III	23/29 (79.3)*	11/29 (37.9)	8/29 (27.6)	12/29 (41.4)
Vascular invasion or emboli				
+	9/12 (75.0)*	5/13 (38.5)	3/13 (23.1)	3/12 (25.0)
—	39/99 (39.4)*	24/100 (24.0)	14/100 (14.0)	27/96 (28.1)
Pleural invasion				
+	10/13 (76.9)*	5/12 (41.7)	5/13 (38.5)*	6/13 (46.2)
—	38/98 (38.8)*	34/101 (23.8)	12/100 (12.0)*	24/95 (25.3)

* $P < .05$.

By use of normal bronchial epithelium adjacent to the tumor as an internal positive control (Figure 1), the expression of E-cadherin and α -, β -, and γ -catenin for each tumor was assessed. The expression of these 4 markers was considered as positive if the intensity of stained tumor cells was comparable with that of normal epithelial cells. For each marker, 500 tumor cells per 10 high-power fields were counted. After observation by 2 pathologists, the percentage of positive-stained tumor cells in each tumor was recorded. The lung tumors studied were divided into 2 groups. A lung tumor was categorized as strongly positive if 50% or more of the tumor cells were stained (Figure 2) or categorized as weakly positive or negative if less than 50% of the tumor cells were stained.

Statistical Analysis

Correlation of various clinical or pathologic parameters with the expression of E-cadherin and α -, β -, and γ -catenin was performed with the χ^2 test. Survival curves were estimated by using the Kaplan-Meier method, and differences in survival were evaluated with the general Wilcoxon test. The multivariate relationships were analyzed with the Cox proportional hazards general linear model.

Results

In the present study the rate of high E-cadherin expression ($\geq 50\%$) in NSCLCs of less than 3 cm was 56.3%. The rates

Figure 3. Survival curves in patients with stage I, surgically resected, small-sized NSCLCs with higher ($\geq 50\%$) or lower ($< 50\%$) E-cadherin (*E-cad*) expression ($P = .26$).

Figure 5. Survival curves in patients with stage I, surgically resected, small-sized NSCLC with higher ($\geq 50\%$) or lower ($< 50\%$) β-catenin (*β-cat*) expression ($P < .05$).

Figure 4. Survival curves in patients with stage I, surgically resected, small-sized NSCLC with higher ($\geq 50\%$) or lower ($< 50\%$) α-catenin (*α-cat*) expression ($P < .05$).

Figure 6. Survival curves in patients with stage I, surgically resected, small-sized NSCLC with higher ($\geq 50\%$) or lower ($< 50\%$) γ-catenin (*γ-cat*) expression ($P < .05$).

of high α-, β-, and γ-catenin expression ($\geq 50\%$) were 73.7%, 84.2%, and 71.6%, respectively. There was a positive correlation between E-cadherin expression and α-, β-, and γ-catenin expression. Expression among the 3 catenins was also significantly correlated.

The relationship between various clinical parameters in these patients with the extent of E-cadherin and α-, β-, or γ-catenin expression is shown in Table 1. Higher E-cadherin and α-catenin expression was noted in female patients, nonsmokers, and patients with peripheral tumors. Higher β-catenin expression was also noted in nonsmokers and in patients with peripheral tumors. With reference to the pathologic characteristics of the tumor, higher E-cadherin expression was noted in tumors with histologic characteristics, including bronchioloalveolar carcinoma (BAC), adenocarcinoma, higher grades of differentiation, early tumor

stage, absence of vascular invasion, or absence of pleural invasion. Higher α-catenin and β-catenin expression was also noted in tumors with histologic characteristics of BAC or adenocarcinoma. Higher β-catenin expression was noted in tumors without pleural invasion (Table 2).

The influence of various clinicopathologic parameters on patient survival was analyzed, including the expression of E-cadherin and α-, β-, or γ-catenin. By using single-variate analysis of survival, significantly better prognostic indicators included BAC, stage I and stage II tumors, peripheral tumors, tumors without pleural invasion, and increased levels of E-cadherin and α-, β-, or γ-catenin expression. By using multiple covariate analysis, only early-stage and peripheral tumors remained as independent prognostic factors indicating significantly better survival. These patients were then divided according to their tumor stage, and a total

of 75 patients had stage I tumors. An analysis of the effect of various clinicopathologic factors on patient survival was performed for these patients with stage I disease. By using single-variate analysis, female sex and increased expression of E-cadherin and α -, β -, or γ -catenin were found to be significantly better prognostic indicators (Figure 3). By using multiple covariate analysis, increased expression of α -, β -, and γ -catenin remained as an independent, favorable prognostic indicator (Figures 4-6). No significant prognostic factors could be found for the other stages of lung cancer, which may be due to the limited sample size. Further investigation of prognostic indicators for the other stages of lung cancer is required.

Discussion

Small-sized lung tumors are frequently encountered by thoracic surgeons. The associated prognosis after surgical intervention is quite variable and usually dependent on the tumor pathology, namely the tumor's grade and stage.^{1,2} In the present study multivariate analysis showed that in addition to tumor stage, the location of the tumor was also a significant prognostic indicator.

It has been recognized that reduced expression of the cell-to-cell adhesion molecule E-cadherin is closely related to the dedifferentiation and invasiveness of a tumor.¹⁷ The association of catenins with E-cadherin is an essential requirement for the correct function and maintenance of cell-cell adhesions within a tissue.⁹⁻¹¹ In the present study the percentages of reduced expression of E-cadherin and α -, β -, and γ -catenin were 43.7%, 26.4%, 15.8%, and 28.4%, respectively. These results were comparable with those of other studies of NSCLC with the same criteria for assessment.^{14,18} A positive correlation between reductions in E-cadherin and α -, β -, and γ -catenin were also noted in this study, which was similar to the results of studies of gastric carcinoma, hepatocellular carcinoma, and breast cancer.^{12,19,20} The mechanism of simultaneous reduction of E-cadherin and catenins is unclear. A mutual influence of underexpression of this group of molecules may occur at the gene, transcription, or protein level.

A positive correlation between reduced E-cadherin, α -catenin, or β -catenin expression and patients who smoked or had central tumors was identified in this study. This phenomenon may be partly explained by the fact that most of the tumors in smokers or centrally located tumors were of the non-BAC type. BAC exhibited significantly higher expression of E-cadherin and catenins when compared with non-BAC tumors, which was similar to the results of the study by Hidaka and colleagues.¹⁸ In this study reduced expression of E-cadherin correlated significantly with dedifferentiation, advanced stage, and vascular and pleural invasion. With catenins, there was also a tendency toward reduced expression in tumors that were poorly differenti-

ated, of advanced stage, or invasive, but this tendency was not statistically significant. These results suggest that when compared with catenins, a reduction in E-cadherin expression was more prominent when defects of cell-cell adhesion were present in NSCLC. The invasiveness of the tumor may largely be influenced by these cell-cell adhesion molecules, particularly E-cadherin.

The association between E-cadherin and catenin expression on patient survival has been reported for various types of human cancers. Reduced expression of α -catenin influences patient survival in prostate, ovarian, colorectal, and bladder cancer.^{15,21-23} Reduced β -catenin expression is predictive of a poorer prognosis in gastric cancer.²⁴ In NSCLC the prognostic significance of E-cadherin and catenin expression has remained unclear to date. In our previous study of surgically treated NSCLC, E-cadherin expression was shown to have no prognostic value as a whole, and the preliminary data showed that E-cadherin was a significant prognostic factor between large (>3 cm) and small ($\leq$ 3 cm) lung tumors by means of univariate analysis.¹⁶ In the present study neither E-cadherin nor catenin expression in small-sized NSCLCs were shown to have prognostic significance after multivariate survival analysis. Nevertheless, if only patients with early-stage disease are included in the analysis, reduced expression of α -, β -, and γ -catenins became a significant prognostic factor indicative of a poorer outcome. These results are not dissimilar to those of previous studies of prostate and ovarian cancer.^{15,21} These results suggest that loss of α -, β -, or γ -catenin expression in early-stage NSCLC indicates a higher potential for malignancy.

In conclusion, the results of the present study demonstrated a close correlation between reduced E-cadherin expression and tumor dedifferentiation, advanced tumor stage, and tumor invasion in NSCLCs of 3 cm or less in size. Reduced expression of α -, β -, or γ -catenin indicates a poorer prognosis in stage I NSCLCs of 3 cm or less in size.

References

1. Koike T, Terashima M, Takizawa T, Watanabe T, Kurita Y, Yokoyama A. Clinical analysis of small-sized peripheral lung cancer. *J Thorac Cardiovasc Surg.* 1998;115:1015-20.
2. Yokose T, Suzuki K, Nagai K, Nishiwaki Y, Sasaki S, Ochiai A. Favorable and unfavorable morphological prognostic factor in peripheral adenocarcinoma of the lung 3 cm or less in diameter. *Lung Cancer.* 2000;29:179-88.
3. Harpole GH JR, Marks JR, Richards WG, Herndon JE II, Sugarbaker DJ. Localized adenocarcinoma of the lung: oncogene expression of erb B-2 and P53 in 150 patients. *Clin Cancer Res.* 1995;1:659-64.
4. Lee YC, Chang YL, Luh SP, Lee JM, Chen JS. Significance of P53 and Rb protein expression in surgically treated non-small cell lung cancer. *Ann Thorac Surg.* 1999;68:343-8.
5. Shimoyama Y, Hirohashi S, Hirano S, Noguchi M, Shimosato Y, Takeichi M, et al. Cadherin cell adhesion molecules in human epithelial tissues and carcinomas. *Cancer Res.* 1989;49:2128-33.
6. Frixen Uh, Behrens J, Sachs M, Eberle G, Voss B, Warda A, et al. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. *J Cell Biol.* 1991;113:173-85.

7. Bracke ME, Van Roy FM, Mareel MM. The E-cadherin/catenin complex in invasion and metastasis. *Curr Top Microbiol Immunol*. 1996; 213/I:123-61.
8. Takeichi M. Cadherins in cancer: implication for invasion and metastasis. *Curr Opin Cell Biol*. 1993;5:806-11.
9. Ozawa M, Baribault H, Kemler R. The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO J*. 1989;8:1711-7.
10. Jou JS, Stewart DB, Stappert J, Nelson WJ, Marrs JA. Genetic and biochemical dissection of protein linkage in the cadherin catenin complex. *Proc Natl Acad Sci U S A*. 1995;92:5067-71.
11. Kadowahi T, Shiozaki H, Inoue M, Tamura S, Oka H, Doki Y, et al. E-cadherin and α -catenin expression in human esophageal cancer. *Cancer Res*. 1994;54:291-6.
12. De Leeuw WJF, Berx G, Vos CBJ, Peterse JL, Van De Vijver MJ, Litvinov S, et al. Simultaneous loss of E-cadherin and catenins in invasive lobular breast cancer and lobular carcinoma in situ. *J Pathol*. 1997;183:404-11.
13. Hiscox S, Jiang WG. Expression of E-cadherin, α , β and γ catenin in human colorectal cancer. *Anticancer Res*. 1997;17:1349-54.
14. Shibamura H, Hirano T, Tsuji K, Qing Fang W, Shrestha B, Konaka C. Influence of E-cadherin dysfunction upon local invasion and metastasis in non-small cell lung cancer. *Lung Cancer*. 1998;22:85-95.
15. Aaltomaa S, Lipponen P, Ala-Opas M, Eskelinen M, Kosma VM. Alpha-catenin expression has prognostic value in local and locally advanced prostate cancer. *Br J Cancer*. 1999;80:477-82.
16. International Union Against Cancer (UICC) Lung. In: Sobin LH, Wittekind CB, editors. TMN classification of malignant tumors. 5th ed. New York: Wiley-Liss; 1997.
17. Lee YC, Wu CT, Chen CS, Chang YL. E-cadherin expression in surgically-resected non-small cell lung cancers-a clinicopathological study. *Thorac Cardiovasc Surg*. 2000;48:294-9.
18. Hidaka N, Nagao T, Asoh A, Kondo Y, Nagao K. Expression of E-cadherin, α -catenin, β -catenin and γ -catenin in bronchioloalveolar carcinoma and conventional pulmonary adenocarcinoma: an immunohistochemical study. *Mod Pathol*. 1998;11:1039-45.
19. Nuruki K, Toyoyama H, Ueno S, Hamanoue M, Tanabe G, Aikou T, et al. E-cadherin but not N-cadherin expression is correlated with the intracellular distribution of catenins in human hepatocellular carcinomas. *Oncol Rep*. 1998;5:1109-14.
20. Xiangming C, Hokita S, Nuruki K, Toyoyama H, Tanabe G, Baba M, et al. The expression of cadherin-catenin complex in association with the clinicopathological features of early gastric cancer. *Surg Today*. 1998;28:587-94.
21. Anttila M, Kosma VM, Ji H, Wei-ling X, Puolakka J, Juhola M, et al. Clinical significance of alpha-catenin, collagen VI, and Ki-67 expression in epithelial ovarian cancer. *J Clin Oncol*. 1998;16:2591-600.
22. Raftopoulos I, Davaris P, Karatzas G, Karayannacos P, Kouraklis G. Level of alpha-catenin expression in colorectal cancer correlates with invasiveness, metastatic potential, and survival. *J Surg Oncol*. 1998; 68:92-9.
23. Mialhe A, Louis J, Montlevier S, Peoch M, Pasquier D, Bosson JL, et al. Expression of E-cadherin and alpha-, beta- and gamma-catenins in human bladder carcinomas: are they good prognostic factors? *Invasion Metastasis*. 1997;17:124-37.
24. Ramesh S, Nash J, McCulloch PG. Reduction in membranous expression of beta-catenin and increased cytoplasmic E-cadherin expression predict poor survival in gastric cancer. *Br J Cancer*. 1999;81:1392-7.