Prognostic significance of E-cadherin and β-catenin in resected stage I non-small cell lung cancer

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Abstract

Objectives: E-cadherin and its associated intracellular molecules, catenins, are important for cell–cell adhesion. Impaired expression of these molecules are frequently observed in several cancers. E-cadherin and β-catenin are often expressed in non-small cell lung cancers. The aim of this study was to investigate the expressions of E-cadherin and β-catenin and their significance as prognostic markers in pathological stage I non-small cell lung cancer.

Methods: Paraffin embedded tumor tissue blocks were obtained from 141 patients who underwent resection without preoperative radiotherapy or chemotherapy with pathological stage I non-small cell lung cancer. Tumor samples were prepared in tissue microarrays and they were stained by immunohistochemistry with antibodies against E-cadherin and β-catenin. The expressions of E-cadherin and β-catenin were analyzed with relation to the clinico-pathological data. The median follow-up period of the patients was 41 months (range, 2–88 months).

Results: Preserved expressions of E-cadherin and β-catenin were observed in the membrane and the cytoplasm of normal epithelial cells and tumor cells. Absent or reduced expression for E-cadherin and β-catenin were observed in 60% and 45% of all the patients, respectively. There was a significant positive correlation between E-cadherin and β-catenin expression (P < 0.01). Absent or reduced expression of E-cadherin was observed in 72.5%, 36.6%, and 60.0% of squamous cell carcinoma, adenocarcinoma, and bronchioloalveolar carcinoma, respectively. There was a significant decrease of E-cadherin expression in squamous cell carcinoma compared to adenocarcinoma (P < 0.01). Patients with reduced expression of β-catenin had poor recurrence free survival in adenocarcinoma, but not in squamous cell carcinoma. Conclusion: Decreased expressions of E-cadherin and β-catenin were closely correlated in resected stage I non-small cell lung cancer. Reduced expression of E-cadherin and β-catenin indicates tumor cell dedifferentiation and reduced expression of β-catenin had poor recurrence free survival in adenocarcinoma of the resected stage I non-small cell lung cancer.

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1. Introduction

Lung cancer has become the leading cause of cancer death in Korea as well as in Western countries. Death from lung cancer is mainly due to metastatic tumor spread. Patients with stage I lung cancer are considered to have the best prognosis after resection. However, more than 40% of these patients will have recurrent lung cancer within 2 years after resection and finally die of metastatic spread [1,2]. Therefore, stage I non-small cell lung cancers under current TNM staging have variable prognosis even in the same stage. Recent advances in molecular biology may provide genetic and molecular information useful for predicting long-term outcome of lung cancer [3].

Alteration of cell-to-cell adhesion molecules may play an important role in tumor cell detachment in the early stages of tumor invasion and metastasis. Cadherins are single transmembrane proteins that mediate cell-to-cell adhesion and include E-(epithelial), N-(neuronal), and P-(placental) cadherins [4]. E-cadherin is the basic mediator of
intercellular adhesion in epithelial cells. This transmembrane glycoprotein is mainly localized in adherence junctions and its function is mediated by extracellular calcium-dependent homotypic interactions. Its cytoplasmic domain is associated with a set of cytoplasmic molecules named catenins (α-catenin, β-catenin, γ-catenin, and p120ctn). Catenins bind the intracellular domain of the E-cadherin to the actin cytoskeleton.

Loss or reduction of the E-cadherin and β-catenin expression is known to play an important role in tumor progression and metastasis and has also been reported to be associated with a poor prognosis in many carcinomas such as esophagus, stomach, colon, liver, prostate, and pancreas cancers [5–9]. Moreover, recent clinical studies in patients with non-small cell lung cancer have shown that reduced E-cadherin, α-catenin, β-catenin, γ-catenin, and p120ctn are associated with dedifferentiation, increased local invasion, regional and distant metastasis, and survival [10–11].

The aim of this study was to investigate the expression of E-cadherin and β-catenin and their significance as independent prognostic markers in pathological stage I non-small cell lung cancer. In the present study, the expression of E-cadherin and β-catenin was analyzed by tissue microarrays in the resected non-small cell lung cancer.

2. Materials and methods

2.1. Patients and tissue specimens

The study population included 141 patients who underwent resection of tumor for pathological stage I non-small cell lung cancer between March 1995 and December 1999 in Samsung Medical Center. None of the patients received chemotherapy or radiotherapy before the operation. Preoperative staging methods included chest CT, bronchoscopy, percutaneous needle biopsy, and routine mediastinoscopy prior to thoracotomy. At thoracotomy, mediastinal lymph nodes were dissected as completely as possible including ipsilateral paraatracheal, lower mediastinal, and subcarinal, as well as N1 areas to get an accurate pathological staging. Patients with positive tumor resection margin were excluded from the study. Clinical data including sex, age, survival, and cancer recurrence were collected retrospectively. The median follow-up period of the patients was 41 months (range, 2–88 months). 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 and presence of regional lymph node metastasis. Pathologic staging was performed according to the International Union Against Cancer (1997), which is based on primary tumor size, location and involvement, and the presence of lymph node metastases or distant metastases [2].

2.2. Tissue microarray construction and immunohistochemistry

For the construction of tissue microarrays, two representative tumor areas were carefully selected and marked on the hematoxylin eosin slide. Forty holes in a recipient block were created by using thin-walled stainless biopsy needle. The cylindrical core tissue samples were retrieved from the selected region in the donor blocks and extruded directly into the recipient block with a solid stylet. Considering the tumor heterogeneity, a large-diameter stylet (2 mm) was used and the specimens were sampled with two core samples of the tumor. Normal bronchial epithelial area adjacent to the tumor was included in each tissue array block for internal positive control.

For immunohistochemical study of E-cadherin and β-catenin expression in the tumor tissue, 4-μm thick sections were cut with a Leica microtome from formalin fixed paraffin embedded tumor blocks. First, hematoxylin and eosin slides were made from tumor blocks in all the patients. Sections were transferred to polyl-lysine coated slides. The sections were deparaffinized in xylene three times for 5 min each and placed in a graded series of ethanol (100%, 95%, 80%, 70%, and 50%) for rehydration and then washed in distilled water. To enhance antigen retrieval, the sections were pretreated in a microwave oven for 5 min in 0.01 mol/l citrate buffer pH 6.0 and then cooled to room temperature. Thereafter, to block the endogenous peroxidase activity, the sections were processed using 3% H2O2 in methanol for 20 min and then rinsed in phosphate buffered saline (PBS) three times for 5 min each. The sections were incubated with 10% rabbit normal serum for 10 min at room temperature and then incubated overnight at 4 °C with primary antibodies at a 1:100 dilution that consisted of mouse monoclonal antibody against human E-cadherin and β-catenin (Transduction Laboratories, Lexington, KY, USA). The sections were rinsed three times with PBS for 5 min and then sequentially incubated with 0.1% Triton-X 100 for 10 min, incubated with biotinylated secondary antibodies for 30 min at room temperature, and incubated with streptavidin–biotin-peroxidase for 30 min at room temperature. The peroxidase reaction was visualized by staining with 0.05% diaminobenzidine chromogen supplemented with 0.2% hydrogen peroxidase in PBS. Finally, the sections were counterstained with hematoxylin and mounted (Fig. 1). We undertook the immunohistochemical staining of the E-cadherin and β-catenin in non-neoplastic lung diseases (six patients with tuberculosis) and samples of a region of the lung distant from the tumor (four patients) as negative controls as a pilot study.

2.3. Immunohistochemical assessment

Tissue microarray slides were assessed under the light microscopy. Staining for E-cadherin and β-catenin was graded by three degrees as compared to the staining...
intensity of positive control core specimens; grade 0 (no expression or minimal expression with less than 10% of tumor cells stained), grade 1 (reduced expression or heterogeneous expression), and grade 2 (preserved expression with more than 90% of the tumor cells stained) for each antibody. Necrotic areas were not taken into consideration. All samples were evaluated and scored randomly by two independent readers who had no knowledge of the patients’ clinical data. In the case of disagreement, the slides were reviewed again and a consensus was reached.

2.4. Statistical analysis

Statistical analyses was performed with SPSS 10.0 (SPSS, Inc, Chicago, IL, USA). Chi-square test was used to analyze the relation between pathologic characteristics and immunohistochemical expression. Differences were considered significant when P values were less than 0.05. Survival curves were estimated by Kaplan–Meier method, and differences in survival were evaluated by log-rank test.

3. Results

3.1. Clinicopathologic variables

Demographic, clinical, and histopathologic data were reviewed retrospectively. The patients consisted of 98 men and 43 women, ranging in age from 20 to 80 years of age (median, 62 years). There were seven cases of pneumonectomy and 134 cases of lobectomy. The cell type of seven patients who underwent pneumonectomy was all squamous cell carcinoma. None of the 141 patients had mediastinal lymph node metastasis. According to the revised staging classification, 40 patients were stage IA and 101 were stage IB. Histologically, 80 patients were squamous cell carcinoma type, 41 were adenocarcinoma type, and 20 were bronchioloalveolar carcinoma type. In the degree of differentiation, 31 patients were well differentiated, 87 were moderately differentiated, and 23 were poorly differentiated.

Cancer recurrences were found in 46 patients (33%) during the follow-up period. The recurrence free period ranged from 2 to 56 months (mean 21 months). Distant metastases comprised 80% of cancer recurrence cases. There was no significant difference of cancer recurrence with relation to cell types, differentiation, or tumor stage.

3.2. Expression of E-cadherin and β-catenin with relation to pathology

We confirmed that expression of the E-cadherin and β-catenin is well preserved in all the 10 samples of non-neoplastic diseases or tissues of non-primary tumor lobe as we expected. The expression of E-cadherin and β-catenin was primarily membranous and cytoplasmic in normal and tumor cells of the lung (Figs. 2–4). Nuclear expressions of E-cadherin and β-catenin were very rare. Absent or reduced expression for E-cadherin and β-catenin were observed in...
60%, 45% of 141 patients, respectively. There was a significant positive correlation between E-cadherin and β-catenin expression ($P < 0.01$).

The relationship between pathologic parameters and the expression of E-cadherin and β-catenin is shown in Table 1. Absent or reduced expression of E-cadherin was observed in 72.5%, 36.6%, and 60.0% of squamous cell carcinoma, adenocarcinoma, and bronchioloalveolar carcinoma, respectively. There was a significant decrease of E-cadherin expression in squamous cell carcinoma versus adenocarcinoma ($P < 0.01$). The grade of differentiation showed a trend in which absent or reduced expression of E-cadherin was more common in poorly differentiated (78.3%) than in well-differentiated group (54.8%) ($P = 0.135$). There was no differential expression of E-cadherin between stage IA and stage IB.

Absent or reduced expression of β-catenin was found in 52.5%, 39%, and 25% of squamous cell carcinoma,

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Fig. 3. High magnification of tissue microarray section stained with monoclonal anti-E-cadherin. Absent (A), reduced (B), preserved (C) expression in squamous cell carcinoma and absent (D), reduced (E), preserved (F) expression in adenocarcinoma.
adenocarcinoma, and bronchioloalveolar carcinoma, respectively. There was a significant decrease of β-catenin expression in squamous cell carcinoma versus adenocarcinoma \((P = 0.05)\). There was a significant decrease of β-catenin expression in poorly differentiated (65.2%) than in well-differentiated group (25.8%, \(P < 0.01)\). There was no differential expression of β-catenin between stage IA and stage IB.

In the subgroup of 80 squamous cell carcinoma patients, poorly differentiated tumors showed more frequently absent or reduced expression of E-cadherin than in moderate \((P = 0.09)\) and well-differentiated tumors \((P < 0.01)\). In the case of β-catenin in squamous cell carcinoma subgroup, there was also a tendency in which dedifferentiation was more commonly associated with the decreased expression of β-catenin but lacked in statistical significance \((P > 0.05)\). In the subgroup analysis of adenocarcinoma or bronchioloalveolar carcinoma, there was no statistical
significance between dedifferentiation and expressions of E-cadherin/β-catenin.

3.3. Recurrence free survival with relation to expression of E-cadherin and β-catenin

There was no significant association between the expression of E-cadherin/β-catenin and cancer recurrence as shown in Table 2. Recurrence free survival also showed no difference with relation to the expression of E-cadherin/β-catenin (Fig. 5A–C.). In the subgroup analysis, there was a significant decrease only in the recurrence free survival of 40 adenocarcinoma patients with the absent expression of β-catenin as shown in Fig. 5D ($P = 0.01$). There was no different recurrence free survival between cancer recurrence and the expression of E-cadherin/β-catenin in the subgroup of squamous or bronchioloalveolar cell carcinoma.

4. Discussion

The prognosis for resected non-small cell lung cancer patients is so variable even within early non-small cell lung cancers. There is increasing evidence that modulation of the E-cadherin/catenin cell-to-cell adhesion complex is an important step in the initiation and progression of human malignancies [12]. In several types of solid malignancies, reduced expressions of E-cadherin or catenins are associated with invasiveness, metastasis, and poor prognosis [5–9]. Recently, several clinical studies have suggested that dedifferentiation, metastasis, and reduced survival in non-small cell lung cancer result from the reduced expression of E-cadherin and catenins [10,11]. In this study, tissue microarray was applied to evaluate the prognostic role of E-cadherin and β-catenin in patients with stage I non-small cell lung cancer. Tissue microarray is an innovative tool in the research of prognostic markers, but there have been few reports on E-cadherin and β-catenin expression by tissue microarray. We found that 2-mm cores used in microarrays were not difficult to work with and histologic findings were easily and reproducibly evaluated on limited regions of the tumor. Compared with normal tissues, malignant tumors generally show less and more heterogeneous expression of E-cadherin and catenin proteins [13,14]. Hoos validated tissue microarrays for immunohistochemical profiling of cancer specimens using the example of human fibroblastic tumors [15]. Their data showed a concordance for staining of three antibodies between tissue microarrays with trireplicate cores per tumor and the full sections. In this study, bireplicate cores per tumor were used and we obtained the concordance by the preliminary study. We were able to make more than 50 slides from each tissue microarray block. Since we could place at least 40 cores in each array, all of the 141 specimens could be accommodated on seven slides. The common availability of formalin fixed paraffin embedded tumor blocks and

<table>
<thead>
<tr>
<th>Pathologic characteristics</th>
<th>Expression of E-cadherin</th>
<th>Expression of β-catenin</th>
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<tr>
<td></td>
<td>Absent</td>
<td>Reduced</td>
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<td>Histology</td>
<td></td>
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<tr>
<td>SCC (n = 80)</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>ADC (n = 41)</td>
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<td>15</td>
</tr>
<tr>
<td>BAC (n = 20)</td>
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<td>6</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well (n = 31)</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Moderate (n = 87)</td>
<td>16</td>
<td>34</td>
</tr>
<tr>
<td>Poor (n = 23)</td>
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<td>15</td>
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<tr>
<td>Tumor stage</td>
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<tr>
<td>Stage IA (n = 40)</td>
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<td>16</td>
</tr>
<tr>
<td>Stage IB (n = 101)</td>
<td>14</td>
<td>45</td>
</tr>
</tbody>
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SCC, squamous cell carcinoma; ADC, adenocarcinoma; BAC, bronchioloalveolar carcinoma.

Table 2

The relationship between cancer recurrence and expression of E-cadherin/β-catenin

<table>
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<tr>
<th>Follow-up status</th>
<th>Expression of E-cadherin</th>
<th>Expression of β-catenin</th>
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<tbody>
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<td></td>
<td>Absent</td>
<td>Reduced</td>
</tr>
<tr>
<td>No-recurrence</td>
<td>18</td>
<td>42</td>
</tr>
<tr>
<td>Recurrence</td>
<td>6</td>
<td>19</td>
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The cost-effectiveness of microarray method could provide much advantages in the field of clinical service.

Well preserved expression of E-cadherin and β-catenin was observed in the benign disease and in the non-tumor lobe of the lung cancer as we expected. The percentages of reduced expression of E-cadherin and β-catenin were 60% and 45%, respectively, in the whole study population. These results were comparable with those of other studies of non-small cell lung cancer [16]. A positive correlation between the reductions in E-cadherin and β-catenin were also noted in this study, which was similar to gastric carcinoma, hepatocellular carcinoma, and breast cancer [17–19]. The mechanism of simultaneous reduction of E-cadherin and catenins is not clear. Mutual influence of the under-expression of these molecules may occur at the gene, transcription, or protein level. It is expected from the studies that β-catenin, first discovered as a link between cadherins and the cytoskeleton, provided a complement to those of cadherins.

In general, E-cadherin and catenins are strongly expressed in well differentiated cancers that maintain cell adhesiveness and are less invasive, whereas their expressions are reduced in poorly differentiated tumors which have lost their intercellular adhesion and strong invasive behavior [12]. Also in non-small cell lung cancer, well differentiated bronchioalveolar carcinomas have the highest level of E-cadherin and catenins proteins, while undifferentiated large cell carcinomas have low levels [20]. In this study, there was a significant decrease of E-cadherin and β-catenin expression in squamous cell carcinoma as compared with adenocarcinoma. Data from several non-small cell lung cancer studies have demonstrated that the expression of E-cadherin and catenins correlates significantly with the differentiation of malignant cells [10,11,20–22]. We also found that there was a significant decrease of E-cadherin and β-catenin expression in poorly differentiated than in well differentiated tumors. The reduced expression of E-cadherin and β-catenin indicates tumor cell dedifferentiation. In a supposedly well-differentiated tumor as pure bronchioalveolar carcinoma, the prevalence of expression of the molecules was higher than in adenocarcinoma, which is a poorer differentiated neoplasm in our study.

Fig. 5. Kaplan—Meier’s survival curves. Statistical analyses were reached by log–rank test. (A) Recurrence free survival of total patients. (B) Recurrence free survival of total patients with relation to E-cadherin expression ($P > 0.05$). (C) Recurrence free survival of total patients with relation to β-catenin expression ($P > 0.05$). (D) Recurrence free survival of adenocarcinoma patients with relation to β-catenin expression ($P = 0.01$ in preserved versus absent expression). E-cad, E-cadherin; β-cat, β-catenin.
population of bronchioloalveolar carcinoma in our study is not exactly the pure type of BAC but an adenocarcinoma with bronchioloalveolar carcinoma components. We confirmed that there was no pure bronchioloalveolar carcinoma by pathological review.

Increased regional lymph node metastasis correlates to the reduced expression of E-cadherin, α-catenin, β-catenin, and γ-catenin [10,22]. This study included only stage I non-small cell lung cancer patients to obtain the homogeneity of the population, thus the association between lymphatic invasion and reduced expression of E-cadherin and β-catenin could not be evaluated. The association between E-cadherin and catenin expression on patient survival has been reported for various types of human cancers. In non-small cell lung cancer, the prognostic significance of E-cadherin and catenin expression has remained unclear to date. Some recent studies have found that E-cadherin as well as each catenin has prognostic significance in non-small cell lung cancer patients [23–25]. In this study, the recurrence free survival was evaluated with relation to the expression of E-cadherin and β-catenin. We were not able to confirm the prognostic significance of E-cadherin and β-catenin in the entire cohort of 141 patients. However, there was a significant difference in the recurrence free survival of 40 adenocarcinoma patients with relation to the expression of β-catenin. The adenocarcinoma-specific association between β-catenin expression and survival does not correspond to the observed association between this marker expression and tumor cell type. It may be postulated that although under-expression of β-catenin is more common in squamous cell carcinoma, under-expression of β-catenin have a poorer prognosis than normal expression in adenocarcinoma patients. These findings may indicate that different cellular mechanisms are responsible for the progression of adenocarcinomas versus squamous cell carcinoma of the lung.

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