Expression of CD44 Variant Exon 6 in Stage I Non-Small Cell Lung Carcinoma as a Prognostic Factor

Toshiki Hirata, Tatsuo Fukuse, Hironobu Naiki, Shigeki Hitomi, and Hiromi Wada

Department of Thoracic Surgery, Chest Disease Research Institute, Kyoto University, Kyoto 606 [T. H., T. F., S. H., H. W.], and Second Department of Pathology, Fukui Medical College, Fukui 910-11 [H. N.], Japan

Abstract

Specific CD44 isoforms are the surface adhesion molecules that have been shown to be associated with metastasis. In the present study, the role of the expression of standard and variant CD44 isoform (CD44s and CD44v6) as prognostic indicators in pathological stage I non-small cell lung carcinoma was investigated immunohistologically using monoclonal antibodies. The results showed that the expression of CD44v6 correlates with adverse prognosis in stage I non-small cell lung carcinoma but not CD44s. The 5-year survival rate of the patients with CD44v6-positive tumors was 50%, which was significantly lower than that of CD44v6-negative patients (88%; \( P = 0.001 \)). The incidence of recurrent distant metastasis in the CD44v6-positive patients (45%; 9 of 20 patients) was significantly higher than that in the CD44v6-negative patients (20%; 10 of 49 patients; \( P = 0.038 \), suggesting the involvement of CD44v6 in hematogeneous metastasis of lung carcinoma. Although the incidence of the expression of CD44v6 in squamous cell carcinoma was significantly higher than that in adenocarcinoma, histological type was not a significant prognostic factor. No significant correlation was found between the expression of CD44v6 and lymphatic or vascular vessel invasion, although lymphatic vessel invasion was found to be an independent prognostic factor in the multivariate analysis. To investigate the relationship between the expression of CD44v6 and proliferative activity of tumor cells, the expression of proliferating cell nuclear antigen (PCNA) was examined. The expression of CD44v6 was more frequently observed in PCNA-positive patients than in PCNA-negative patients (\( P = 0.019 \)), but the expression of PCNA was not a statistically significant prognostic indicator. The results of multivariate analysis by the Cox proportional hazards model showed that CD44v6 was an independent prognostic indicator. We concluded that the expression of CD44v6 is a useful prognostic factor in stage I non-small cell lung carcinoma.

Introduction

CD44 is a transmembrane glycoprotein that can be expressed as isoforms generated by the alternative splicing of variant exons (1). CD44s is formed by alternative splicing of exons 1–5 and 6–10 s, and CD44v is formed when exon 1v–10v is inserted between 5 s and 6 s. CD44 has been known to be a lymphocyte homing receptor, and recent studies have shown that CD44 is involved in cell-to-cell and cell-to-extracellular matrix interactions. Also, it has been reported that CD44v6 (encoded by variant exon 6) is strongly associated with metastasis (2).

Of the various malignant tumors, lung carcinoma is the most common cause of death due to carcinoma among both men and women. Because the 5-year survival rate of stage I NSCLC with surgical treatment remains only ~70% (3), it is necessary to treat lung carcinoma as a systemic disease. Therefore, it is important to identify stage I NSCLC patients with adverse prognosis and reevaluate the treatment strategy for these patients. Reliable prognostic indicators of NSCLC, however, have yet to be found.

Therefore, we investigated the role of the expression of CD44s and CD44v6 as prognostic factors of stage I NSCLC immunohistologically using monoclonal antibodies. Our results demonstrated that CD44v6 is a useful prognostic indicator of stage I NSCLC.

Patients and Methods

Patients. Formalin-fixed, paraffin-embedded tumor samples were available for study in 69 patients with pathological stage I NSCLC. All patients underwent complete resection of tumors and complete excision of regional lymph nodes at the Chest Disease Research Institute, Kyoto University, between January, 1981 and December, 1990.

The pathological characteristics of the surgical specimens were classified according to WHO criteria (4) and the Tumor-Node-Metastasis staging system (5). N0 was defined as being free of metastasis from the intrapulmonary lymph node to the mediastinal lymph node. The disease status of each patient was determined at the date of last follow-up. The characteristics of the 69 patients are shown in Table 1. Of the 69 patients, 40 received chemotherapy before or after surgery (main regimen: mitomycin C + 1-B-d-arabinofuranosyl-3-ethyl-1-carbonucleoside + 5-fluorouracil), and the remaining 29 patients underwent surgery only. After surgery, a physical examination (chest X-ray and computed tomography) was performed every 2–3 months.

Histopathology. Formalin-fixed, paraffin-embedded tumor samples were retrieved, and sections were stained with H&E. Vascular vessel invasion was defined as the presence of tumor cell infiltration to the vascular walls or tumor cells in the vascular lumen, and lymphatic vessel invasion was defined as the presence of tumor cells in the endothelium-lined space (6).

Immunohistochemistry. Immunohistochemical staining was performed according to the peroxidase method by the avidin-biotin complex technique (DAKO, Carpinteria, CA; Ref. 7). The expression of CD44s and CD44v6 was detected using monoclonal antibodies (SPF-2 and VPF-18, respectively: Bender MedSystem, Vienna, Austria). Proliferative activity was detected using monoclonal antibody (PC-10; DAKO) against PCNA. Mouse serum (DAK A/S, Denmark) was used for the negative control, and positive controls were tissues that were strongly stained previously by anti-CD44s and anti-CD44v6 antibodies. Human tonsil (DAKO) was used as the positive control for PCNA. The immunoreactivities of the antibodies against CD44s, CD44v6, and PCNA were determined by two observers (T. H. and T. F.), who were blinded to the clinical outcome. At least 1000 tumor cells were counted under a microscope, and all stained cells were considered positive, regardless of the intensity of the stain. The number of positive cells was then counted and expressed as a percentage. When the percentage of positive cells was <20%, the specimen was diagnosed as negative, and when ≥20%, the specimen was diagnosed as positive.

Statistical Analysis. The interrelationships among different clinicopathological variables were analyzed using \( \chi^2 \) test. For the analysis of survival rates of the subgroups, Kaplan-Meier curves were constructed, and the log-rank test was performed. Univariate and multivariate analyses were performed by the Cox proportional hazards model.

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CD44v6 IN STAGE I NON-SMALL CELL LUNG CARCINOMA

Table 1 Profiles of p-stage I non-small cell lung carcinoma patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
<th>Range</th>
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<tbody>
<tr>
<td>Age at surgery</td>
<td>62.6 ± 9.3</td>
<td>40-79</td>
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<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td></td>
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<tr>
<td>pT-Factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>1</td>
<td></td>
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<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Performed</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Not performed</td>
<td>29</td>
<td></td>
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</tbody>
</table>

* TNM staging.

Table 2 Correlation of CD44v6 with histopathological and prognostic indices

<table>
<thead>
<tr>
<th>CD44v6 expression</th>
<th>pT-Factor*</th>
<th>Histological type</th>
<th>Blood vessel invasion</th>
<th>Lymphatic vessel invasion</th>
<th>PCNA expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (n=13)</td>
<td>T1</td>
<td>Adenocarcinoma</td>
<td>Present</td>
<td>Present</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>Squamous cell carcinoma</td>
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<td>Present</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Large cell carcinoma</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
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<tr>
<td>Negative (n=24)</td>
<td></td>
<td></td>
<td>Present</td>
<td>Absent</td>
<td>Negative</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Present</td>
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<td>Absent</td>
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The correlation of CD44v6 with histopathological and prognostic indices was shown in Table 2. The expression of CD44v6 was detected in 13 of 25 patients with squamous cell carcinoma (52%) more frequently than in patients with adenocarcinoma (8 of 43 patients; 19%; \(P = 0.001\)). The expression of CD44v6 was more frequently observed in 17 of 44 PCNA-positive patients (39%) than in PCNA-negative patients (3 of 25; 12%; \(P = 0.019\)). However, neither the expression of PCNA nor histological type was a statistically significant prognostic indicator. No significant correlations were found between CD44v6 expression and the tumor diameter, vascular vessel invasion, or lymphatic vessel invasion.

Table 3 shows the results of the multivariate analysis using the Cox proportional hazards model. It was found that the expression of

Results

**Immunohistological Staining.** Of the 69 NSCLC cases, positivity for CD44v6 was observed in 20 cases (29%; Fig. 1), and 44 (64%) tumors stained positively for PCNA, but only 1 tumor stained positively for CD44s. Therefore, statistical analysis for CD44s was not performed. As an additional finding, the expression of CD44v6 was detected in the normal bronchial epithelium in some sections.

**Clinicopathological Characteristics.** The median follow-up period was 76 months, and of the 69 patients, 19 have died (28%) and 50 are still alive (72%). The estimated overall 5-year survival rate was 80%, and the chemotherapy described had no effect on the prognosis of these patients. Tumor recurrence was observed in 4 of the 50 surviving patients (3 cases of distant metastases and 1 case of local recurrence) and in 16 of the 19 patients who died (all distant metastases).

The 5-year survival rate of the CD44v6-positive patients (50%) was significantly lower than that of the CD44v6-negative patients (88%; \(P = 0.001\); Fig. 2). Distant hematogeneous metastases were detected in 9 of the 20 CD44v6-positive patients (45%) and in 10 of the 49 CD44v6-negative patients (20%), revealing a significant difference between the two groups (\(P = 0.038\)).

The results of the univariate analysis showed that only CD44v6 had a statistically significant influence on survival among the clinicopathological factors (\(P = 0.001\)), but any other factors did not: sex, \(P = 0.086\); pT-factor, \(P = 0.086\); histological type, \(P = 0.806\); chemotherapy, \(P = 0.560\); blood vessel invasion, \(P = 0.280\); lymphatic vessel invasion, \(P = 0.053\); and PCNA, \(P = 0.318\). The correlation of CD44v6 with histopathological and prognostic indices was shown in Table 2. The expression of CD44v6 was detected in 13 of 25 patients with squamous cell carcinoma (52%) more frequently than in patients with adenocarcinoma (8 of 43 patients; 19%; \(P = 0.001\)). The expression of CD44v6 was more frequently observed in 17 of 44 PCNA-positive patients (39%) than in PCNA-negative patients (3 of 25; 12%; \(P = 0.019\)). However, neither the expression of PCNA nor histological type was a statistically significant prognostic indicator. No significant correlations were found between CD44v6 expression and the tumor diameter, vascular vessel invasion, or lymphatic vessel invasion.

Table 3 shows the results of the multivariate analysis using the Cox proportional hazards model. It was found that the expression of

![Fig. 1. CD44v6 expression in a squamous cell carcinoma of the lung. The immunostaining is localized at the cell membrane.](image)

![Fig. 2. Kaplan-Meier survival curves of pathological stage I NSCLC patients based on the positive or negative of CD44v6 expression.](image)
CD44v6 and lymphatic vessel invasion were independent prognostic indicators.

Discussion

Previous research on CD44 has shown that CD44 is closely correlated with hematogenous metastasis, lymphatic metastasis, and peritoneal dissemination. Günther et al. (8) reported a possible contribution of CD44v6 to neoplasm metastasis by administration of antibodies that recognize the epitopes of the variant exon v6. In other studies, CD44 has been shown to be involved in the recirculation and activation of lymphocytes (9) and to act as a lymphocyte homing receptor. These findings suggest that CD44 plays an important role in lymphatic metastasis by mimicking the recirculation of lymphocytes (10). Furthermore, it has been shown that CD44 acts as a ligand in connecting carcinoma cells and mesothelial cells in ovarian carcinoma (11). Clinically, recent studies have demonstrated that CD44v6 is correlated with the prognosis of colorectal carcinoma (12), gastric carcinoma (13), and breast carcinoma (14).

As far as the role of CD44 in lung carcinoma is concerned, a previous study reported that isoforms including CD44v6 are expressed in cell lines established from lung carcinoma cells (15). Other studies indicated that CD44 is not expressed in lung small cell carcinoma or adenocarcinoma (except for alveolar cell carcinoma), and CD44v6 is not correlated with lung carcinoma metastasis (16,17). However, it has not been clarified whether the expression of CD44 was correlated with the prognosis of lung carcinoma.

The results of the present study showed that the expression of CD44v6 was of prognostic value in predicting clinical progression of patients with stage I NSCLC, whereas the expression of CD44v6 was not. The 5-year survival rate of the CD44v6-positive patients was significantly lower than that of the CD44v6-negative patients. Distant hematogenous metastasis was detected in CD44v6-positive patients more frequently than in the CD44v6-negative patients, showing that CD44v6 expression appeared to be strongly correlated with the prognosis of patients with stage I NSCLC mediated by hematogenous metastasis of lung carcinoma.

Regarding the prognostic indicators of lung carcinoma, pathological stage and histological type are thought to be independent predictors for the prognosis of lung carcinoma patients, and lymphatic vessel invasion (6), vascular vessel invasion, and altered p53 expression have been investigated as possible prognostic factors (18). Furthermore, in stage I lung carcinoma, recent studies have reported p53 mutation or tumor size (19) and PCNA (20) as prognostic indicators. Due to its characteristics, CD44 expression was presumed to be related to lymphatic or vascular vessel invasion. However, no correlation was found between the expression of CD44v6 and lymphatic or vascular vessel invasion, although lymphatic vessel invasion was found to be an independent prognostic factor in the multivariate analysis. The expression of CD44v6 in squamous cell carcinoma was significantly higher than in adenocarcinoma, but histological type was not a significant prognostic indicator in the present study. Moreover, the incidence of the expression of CD44v6 in the PCNA-positive patients was significantly higher than that in the PCNA-negative patients, but the expression of PCNA was not a statistically significant prognostic factor. The finding that CD44v6 was expressed at a high incidence in the patients with PCNA positivity, which reflects the proliferative activity of tumors, is comparable with the results of a previous study in which CD44v6 was shown to be expressed even in normal cells when they are activated and highly proliferating (1). Therefore, these findings support the contention that the prognostic role of the expression of CD44v6 is different from these previous prognostic indicators. The results of the multivariate analysis by the Cox proportional hazards model also showed that CD44v6 is an independent prognostic indicator.

The 5-year survival rate of stage I NSCLC patients who underwent surgery was ~70%, and hence about 30% of these patients do not live for more than 5 years because of distant metastases that could not be detected at surgery. Therefore, if chemotherapy can be selectively performed after surgery on stage I NSCLC patients with expression of CD44v6, the prognosis for these patients should improve.

Acknowledgments

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References

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