

The Expression of CD146 Predicts a Poor Overall Survival in Patients with Adenocarcinoma of the Lung

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Abstract. *Background: The epithelial-mesenchymal transition (EMT) is also involved in cancer progression and metastasis. The expression of mesenchymal marker genes might be associated with a more aggressive form of the disease. CD146 is a marker of mesenchymal cells, a potentially metastasis-promoting cell adhesion molecule, and its expression has been described in various types of solid tumors. We, herein, evaluated the expression of CD146 in patients with adenocarcinoma of the lung by using immunohistochemistry to clarify its clinical significance. Materials and Methods: Tumor specimens were collected from 183 consecutive patients who underwent complete resection for lung adenocarcinoma from 2003 to 2007 in our Department. We analyzed the CD146 expression levels in the primary lung adenocarcinoma by immunohistochemistry. Results: Positive expression of CD146 was identified in 16 (8.7%) patients. A significant association was only observed between the CD146 expression level and male patient gender ($p=0.03$); other factors were not associated with CD146 expression. The 5-year overall survival (OS) rate in patients with tumors that were negative and positive for CD146 expression was 84.4% and 50.0%, respectively ($p<0.01$). A positive CD146 expression was found to be associated with the OS based on a univariate survival analysis ($p=0.013$). Conclusion: CD146 expression was more frequently detected in males than in females. The positive expression of CD146 was associated with a poorer OS according to the survival analysis. CD146 may be a useful marker for predicting poor prognosis in patients with NSCLC following complete resection.*

Lung cancer is the leading cause of cancer-related death worldwide (1), and non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer (2). The

incidence of adenocarcinoma, one of the major histological subtypes of NSCLC is increasing (3). Even when it is possible to perform surgery, which is the major curative treatment, a certain population of patients nevertheless develops tumor recurrence (4). As a result, it is important to evaluate the biological characteristics of NSCLC to identify the factors related to prognosis following surgery. However, no useful markers for predicting clinical recurrence exist at present.

Cancer progression has been shown to be related to the epithelial-mesenchymal transition (EMT) (5). Therefore, mesenchymal markers might be more closely related to the progression of cancer than epithelial markers. Aberrant expression of cell adhesion molecules is frequently observed during tumor development and progression, probably because of increased cell motility, allowing or enhancing tumor invasion and metastasis (6). CD146, also known as melanoma cell adhesion molecule or MUC18, is a transmembrane glycoprotein belonging to the immunoglobulin superfamily that functions as a Ca^{2+} independent adhesion molecule (7). CD146 is also recognized as a marker of mesenchymal cells (8). An increased CD146 expression has been shown to be closely associated with advanced stage malignant melanoma, prostate cancer, and ovarian cancer (8-12). However, there has been only one study that investigated the relationship between the expression of CD146 and adenocarcinoma of the lung (13). The purpose of the present study was to evaluate the clinical utility of the CD146 expression level in the adenocarcinoma of the lung.

We therefore examined the relationship between the expression of both this molecule and the tumor aggressiveness with regards to poor prognosis. The identification of a molecule that is a marker of a poor prognosis may allow patients expected to have a poor prognosis to receive treatment with intensive therapy, such as chemotherapy.

Materials and Methods

Patients, clinical features, and follow-up. The Institutional Review Board approved this study, and informed consent for the use of the tumor specimens was obtained from either the patients or from their legal guardians. Tumor samples were obtained from 296 patients with primary lung adenocarcinoma who had undergone a surgical resection between 2003 and 2007 in our Department. Nine of these patients had

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stage IV disease, and 25 underwent an incomplete resection. The tumor samples from 79 patients were too small to evaluate by immunohistochemical (IHC) staining to determine the CD146 status. As a result, 113 patients were excluded from further analysis. Therefore, 183 tumor specimens were evaluated.

The patients were followed-up every month within the first postoperative year and at approximately 2- to 4-month intervals thereafter. The evaluations included a physical examination, chest roentgenography, an analysis of blood chemistry, and measurements of tumor markers. Chest and abdominal computed tomography, brain magnetic resonance imaging, and a bone scintiscan were performed every 6 months for 3 years after surgery. Additional examinations were performed if any symptoms or signs of recurrence were detected. A follow-up was conducted of all 183 patients. The median follow-up period was 53.7 months. Twenty-seven (15.3%) patients received adjuvant chemotherapy as follows: carboplatin plus paclitaxel (n=18), carboplatin plus gemcitabine (n=7), and tegafur-uracil (UFT) (n=2) (14, 15). At the last follow-up, 144 patients were alive and free of cancer, while 11 patients had died of other causes without evidence of cancer, 8 patients were alive with recurrent cancer, and 20 patients had died of cancer. In total, 28 (15.3%) of the 183 patients demonstrated disease recurrence after surgery.

Immunohistochemical (IHC) staining of paraffin-embedded tumor samples. IHC staining was conducted using serial sections from the same paraffin-embedded blocks, according to previously described methods (16, 17). Briefly, all tissue specimens were formalin-fixed and processed similarly, according to the standard histology practices. A 3-micron-thick formalin-fixed, paraffin-embedded tissue section was prepared from each specimen. All specimens were stained with hematoxylin-eosin for the histological diagnosis. The sections were briefly immersed in citrate buffer [0.01 mol/l citric acid (pH 6.0)] and then were incubated twice for 10 min at 121°C in a high pressure sterilization oven for antigen retrieval. They were then incubated with the CD146 antibody (Abcam, Tokyo, Japan) (18) diluted at 1:500 in phosphate-buffered saline for 60 min at room temperature. Thereafter, IHC staining was performed by the labeled polymer method (Histofine Simple Stain MAX-PO kit, Nichirei, Tokyo, Japan) according to the manufacturer's instructions (19). The positive controls for CD146, were processed melanomas cells. The negative control used Rabbit IgG (Dako, Denmark) instead of the primary antibody.

Evaluation of the stained specimens. Following the IHC detection of the protein expression in each specimen, the percentage of immunoreactive tumor cells in five $\times 400$ fields, selected randomly on one slide was recorded and then the final value of the positive tumor cells was determined as the average of the positively immunostained cells. To determine whether there were any correlations with the clinicopathological characteristics, these protein expression scores were divided into positive or negative groups. The cut-off level for CD146 staining (positive/negative) was defined as the cellular membrane staining $\geq 10\%$. The slides were independently examined by two of the investigators (S.O. and Y.C.) who were blinded to the patients' clinicopathological data. When a discrepancy was found between the two investigators, a consensus was reached *via* their simultaneous examination using a double-headed microscope.

Statistical analyses. Statistical significance was evaluated using the chi-square test or Fisher's exact test. The Kaplan-Meier method was used to estimate the probability of survival, and survival differences

were analyzed by the log-rank test. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated for each variable. Differences were considered to be statistically significant for p -values < 0.05 . The data were analyzed using the Stat View software package (Abacus Concepts, Inc., Berkeley, CA, USA).

Results

Detection of the CD146 expression and correlation with clinicopathological factors. All of the patients were Japanese, consisting of 102 males and 81 females in this series, with a median age of 70 years (range 23-88 years). The tumor stage was classified according to the new TNM Classification for Lung Cancer (20). According to the pathological stage, 106 patients had tumors of stage IA, 39 of IB, 13 of IIA, 6 of IIB, 16 of IIIA and 3 of stage IIIB. CD146 expression was mainly localized in the cell membrane (Figure 1A). CD146 expression was identified in 16 (8.7%) patients. A significant association was only observed between the CD146 expression and male patient gender ($p=0.03$), and no other factors were associated with the CD146 expression (Table I).

Influence of CD146 expression on the overall survival. The 5-year OS rate in patients with negative and positive CD146 expression was 84.4% and 50.0%, respectively ($p=0.009$) (Figure 2). Positive CD146 expression was also found to be associated with the OS according to a univariate survival analysis ($p=0.013$) (Table II).

Discussion

In this study, we uncovered two important findings. Firstly, male NSCLC patients have a higher frequency of CD146 expression than females. The gender frequency of CD146 expression in NSCLC has not been previously studied. There might be distinct mechanism(s) responsible for the gender difference. In fact, a large number of studies have reported that the prognosis of female patients with NSCLC was more favorable than the one of males (21, 22).

The other finding is that the patients with positive CD146 expression had a worse overall survival than patients without a positive expression. Thus, CD146 expression was a worse prognostic factor in patients with adenocarcinoma of the lung. These findings are consistent with a small former study (13). Moreover, these findings suggest that CD146 expression might be a suitable biomarker to identify candidate patients who would benefit most from adjuvant chemotherapy for adenocarcinoma following a complete resection.

This investigation was also unique for three reasons. Firstly, it employed surgical specimens after complete resection. Secondly, this is the first molecular analysis of the CD146 expression in adenocarcinoma of the lung for a Japanese population (homogeneous group) and included the highest number of Japanese subjects evaluated. Thirdly, the

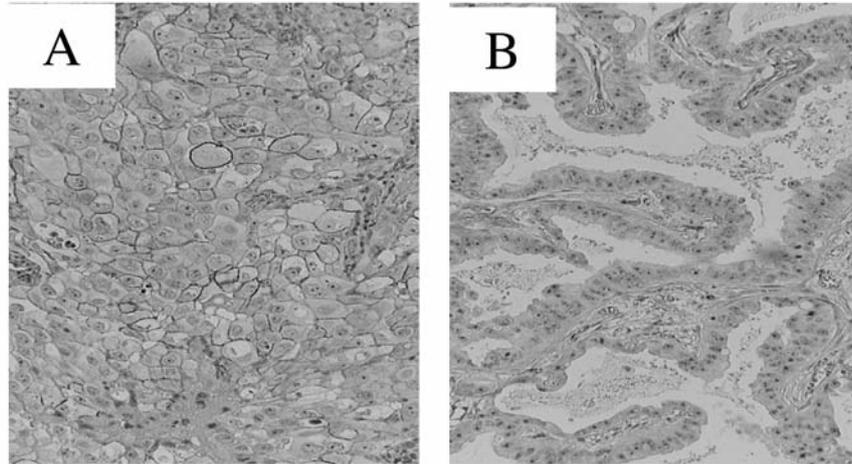


Figure 1. Kaplan-Meier curves stratified by the CD146 expression. The 5-year OS rates in patients negative and positive for CD146 expression were 84.4% and 50.0%, respectively ($p=0.009$).

Table I. Relationship between CD146 expression and clinicopathological characteristics.

Variables Category	No. of patients n=183	CD146		p-Value	
		Positive 16	Negative 167		
Gender	Male	102	13	89	0.03
	Female	81	3	78	
Age (years)	≤70	90	8	82	0.95
	>70	93	8	85	
Pathologic stage	IA	106	7	99	0.23
	IB-III	77	9	68	
T Status	T1a	76	4	72	0.16
	T1b-4	107	12	95	
N Status	Negative	152	12	140	0.37
	Positive	31	4	27	
Smoking history	Never	74	3	71	0.06
	F/C	108	13	96	
Vimentin	Negative	151	14	137	0.583
	Positive	32	2	30	

F/C: Former + current.

Table II. Univariate analysis using a proportional hazard model for the overall survival.

Variable	Characteristic		95% CI	HR	p-Value
	Unfavorable	Favorable			
Gender	Male	Female	1.045-5.848	2.475	0.039
Age (years)	≤70	>70	0.472-2.141	1.006	0.980
Smoking history	F/C	Never	0.899-5.025	2.128	0.086
T status	1b-4	1a	1.577-13.16	4.566	0.051
N status	Positive	Negative	1.934-9.009	4.184	<0.001
CD146	Positive	Negative	1.269-7.813	3.155	0.013

CI: Confidence Interval, HR: hazard ratio, F/C: former + current.

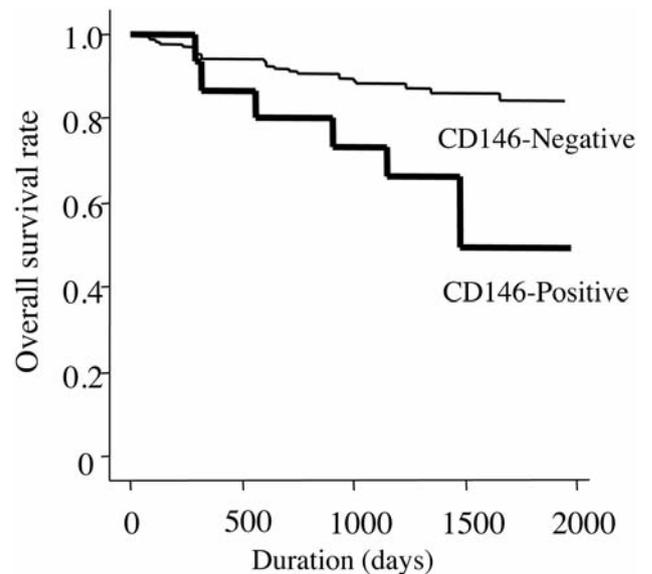


Figure 2. Results of the immunohistochemical analysis of CD146 expression. Positive reaction was mainly localized in the cell membrane. A: Positive CD146 expression in adenocarcinoma of the lung (original magnification $\times 200$). B: Negative CD146 expression in adenocarcinoma of the lung.

method is based on simple IHC staining, which has the advantage of maintaining the morphology of the tissue, and thereby minimizing sampling error.

Conclusion

The current results indicate that the CD146 expression may be a useful marker for predicting a poor prognosis in patients with NSCLC following complete resection. Further investigations will be necessary to examine the optimal method of detecting

CD146 expression, to both validate the method and to determine the efficacy of adjuvant chemotherapy for the patients identified using this marker.

Conflict of Interest Statement

None declared.

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