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Original Article

Prognostic value of CD133 expression in stage I lung adenocarcinomas

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Abstract: CD133 is one of the most representative cancer stem cell markers. This study evaluated the potential prognostic value of CD133 expression in stage I lung adenocarcinomas (ADC). Tumors from 177 patients were immunohistochemically examined for CD133 expression, and their associations with disease recurrence were analyzed. Also, the potential prognostic value of combining CD133 expression with proliferating activity measured by immunohistochemical expression of Ki-67 and vessel involvement was evaluated. CD133 high expressers showed a significantly higher risk of recurrence than CD133 low expressers: 5-year disease-free survival (DFS) rate 77.2% vs. 95.1% (p=0.004), adjusted Hazard ratio (HR) 4.37, 95% Confidence Interval (CI) 1.30–14.71 (p=0.017). CD133 high expressers having strong proliferating activity and/or with vessel invasion showed a higher risk of recurrence: 5-year DFS rate 66.5% in CD133 high/Ki-67 high expressers vs. 93.2% in the other types (p<0.001), adjusted HR 8.39, 95% CI 2.65–26.54 (p<0.001): 5-year DFS rate 51.0% in CD133 high expressers with vessel invasion vs. 92.9% in the other types (p<0.001), adjusted HR 4.50, 95% CI 1.51-13.34 (p=0.007): 5-year DFS rate 53.9% in CD133 high/Ki-67 high expressers with vessel invasion vs. 91.2% in the other types (p<0.001), adjusted HR 9.32, 95% CI 3.42–25.39 (p<0.001). In conclusion, the level of CD133 expression is an independent prognostic marker and its combination with proliferating activity and/or vessel invasion could have excellent prognostic value to predict postoperative recurrence in patients with stage I lung ADC.

Keywords: Lung adenocarcinoma, cancer stem cell, CD133, stage I, prognosis

Introduction

Lung cancer is one of the most common causes of cancer-related death in the developed world [1,2]. Adenocarcinoma (ADC) is the most common histological type comprising about 60% of non-small cell lung cancers (NSCLC) [1,2]. Even in patients with stage I NSCLC, a substantial proportion die due to recurrent disease (the 5-year survival rate is 66.0–83.9% in stage IA and 53.0–66.3% in stage IB) [3-5]. The vast majority of recurrences occur as metastasis [6]. Thus, it is quite important to select potentially metastatic tumors and treat them with appropriate adjuvant therapy.

To generate metastatic foci, vessel invasion in the primary locus and subsequent clonal expansion of neoplastic cells in metastatic sites are essential. Especially, to complete the latter process, the migrating neoplastic cells must have both clonogenic ability and proliferating activity.

In recent years, the cancer stem cell (CSC) concept has been proposed [7-9]. That is, only a certain percentage of CSC, but not all neoplastic cells, are clonogenic and contribute to tumor expansion and metastatic foci generation [7-9]. CD133, a 120kDa transmembrane glycoprotein, is one of the most representative and reliable molecular markers for CSC in a variety of malignant neoplasms [10-14], including lung cancers [15-17]. It is reasonable to consider that tumors with a higher percentage of neoplastic cells with high level CD133 expression are more aggres-

sive and will lead to a worse clinical outcome.

The present study examined lung ADCs from 177 patients with disease at stage I for CD133 expression immunohistochemically and analyzed its association with postoperative disease recurrence. In addition, the potential prognostic value of combining CD133 expression with other essential factors to generate metastatic foci, proliferating activity (Ki-67 labeling index) and vessel invasion, was also evaluated.

Materials and methods

Primary lung cancer

All 177 cases examined were patients with stage I ADC that underwent radical surgical resection at Kanagawa Cardiovascular and Respiratory Center (Yokohama, Japan) between January 2001 and December 2006. Tumor stage was determined according to the international TNM classification system (seventh edition of UICC) [18].

The median age was 68 year-old (range 45-85), 89 patients (50.3%) were men and 88 (49.7%) were women. Eighty-five patients (48.0%) had a smoking history (Brinkman Index, median 800, range 10-3200) and 92 (52.0%) were non-smokers. One-hundred-and-thirty-one patients (74.0%) were stage IA and 46 (26.0%) were stage IB. Lobectomy and segmentectomy were performed on 157 and 7 patients, respectively, which along with systemic lymphadenectomy, extended to the hilar and mediastinal lymph nodes. Thirteen patients (7.3%) underwent wedge resection along with intra-operative lymph node sampling. A follow-up evaluation was performed every 2 months for the first 2 years after the operation, every 3 months in the third year, and every 6 months thereafter. The evaluation included physical examinations and chest radiography. Screening for serum tumor markers, computed tomography (CT) of the thorax and upper abdomen, and magnetic resonance imaging (MRI) of the brain were obtained every 6 months for the first 3 years, and every 12 months thereafter. The median follow-up period was 35.9 months (range 1.1–82.5 months). Twelve patients (6.8%) died during the follow-up period, 7 died of lung cancer causes (range 10.4-45.1 months) and 5 died of non-lung cancer causes (range 1.1-12.9 months). The five-year overall survival (OS) rate was 91.5%. Disease recurrence was found in 18 out of 177 patients (10.2%), of whom 15 (8.5%) were affected by metastasis. The median disease-free span of patients with recurrence was 12.0 months (range 3.8–49.1 months). None of the patients received radiotherapy or chemotherapy preoperatively. None of patients with stage IA disease, and 18 of 46 patients with stage IB disease, received postoperative adjuvant chemotherapy (3 patients received cisplatin or carboplatin-based chemotherapy, and 15 received oral uracil-tegafur (UFT) chemotherapy). The 5-year disease-free survival (DFS) rate was 75.2% for the non-adjuvant IB patients vs. 75.0% for adjuvant IB patients (p=0.983; 4 patients who could not continue oral UFT treatment for more than 6 months were excluded). Informed consent for research use of the resected materials was obtained from all the subjects.

Immunohistochemistry

The largest tumor sections (4 um thick) were cut from formalin-fixed, paraffin-embedded tissue blocks. The sections were subjected to immunohistochemistry within 48 hours after being cut out to avoid loss of antigenicity [19-20]. The sections were deparaffinized, rehydrated, and incubated with 3% hydrogen peroxide, followed by 5% goat serum to block endogenous peroxidase activity and non-immunospecific protein binding. The sections were boiled in citrate buffer (0.01 M, pH 6.0) for 15 minutes to retrieve masked epitopes and then incubated with a primary antibody against either CD133 (working concentration of 1.0 μg/ml, clone W6B3C1 (IgG1), Miltenyi Biotec, Bergisch-Gladbach, Germany) or Ki-67 (working concentration of 1.0 μg/ml, clone MIB1 (IgG1), DAKO, Ely, UK) for 90 minutes at room temperature. Immunoreactivity was visualized using an Envision detection system (DAKO), and the nuclei were counterstained with hematoxylin and eosin (HE). To verify the specificity of the immunoreactivities, negative control experiments using mouse non-immunospecific immunoglobulin (IgG1, DAKO) to replace the primary antibodies were performed.

Evaluation of other biological properties

Proliferating activity of neoplastic cells was evaluated by a Ki-67 labeling index, which was calculated as the proportion of positive cells by counting 500–1000 neoplastic cells in the largest tumor section. The Ki-67 labeling indexes of

<10% and ≥10% were classified as low and high levels, according to the criteria of previous studies [21-22]. The vessel invasion was examined on the largest tumor section stained with Victoria blue-van Gieson's method.

Statistical analysis

The post-operative disease-free span was defined as the period ranging from the date of surgery to the date when the recurrence of disease was diagnosed. An observation was censored at the last follow-up if the patient was alive or had died of a cause other than lung cancer. The possible associations between disease recurrence and various parameters were analyzed by Chi-square test and Fisher's exact test. The recurrence curves were plotted using the Kaplan-Meier method, and the absolute risk of recurrence at five years was estimated from the curves. The differences in disease-free survival (DFS) span and rate were analyzed using the Log-rank test. Multivariate analyses were performed with adjustment for possible prognostic variables (CD133 expression, Ki-67 expression, vessel invasion, age, gender, smoking status, extent of operation). Hazard ratios and 95% CIs were calculated using Cox's proportional hazards model. p values less than 0.05 were considered significant. All statistical analyses were performed using SPSS software (SPSS for Windows Version 10.0; SPSS; Chicago, IL, USA).

Results

CD133 expression in normal and malignant lung epithelium

Immunohistochemical expression of CD133 was found not only in neoplastic cells, but also in non-neoplastic cells. Non-neoplastic bronchiolar epithelial cells expressed CD133 with exclusive plasma membrane localization at the apical and lateral sides (**Figure 1A**). Neoplastic cells expressed it at different levels and with various intracellular localizations. Some showed only a faint level with only membranous localization (**Figure 1B**), while others showed a level equivalent to that of bronchial epithelial cells (**Figure 1C**) or unequivocally strong levels with diffuse cytoplasmic localization (**Figure 1D**). Consistently, similar results for cytoplasmic expression of CD133 have been found in other diverse types of malignancies, such as gastric cancer [23], colon cancer [24-26], hepatocellular carcinoma [27], cholangiocellluar carcinoma [28], breast cancer [29], and ovarian cancer [30], including NSCLC [31-32]. Some studies reported that the diffuse cytoplasmic expression of CD133 could be a poor prognostic maker in diverse types of carcinomas [27-28]. According to observations in previous studies, unequivocally greater signal intensity with diffuse cytoplasmic staining than that of non-neoplastic bronchiolar epithelial cells was judged to be a strong level (**Figure 1D**). The CD133 expression score was defined as the proportion of cells with strong expression levels in the largest tumor section. The median CD133 expression score was 20.0%, ranging from 0.0 to 100.0%. A cut-off value was calculated from the receiver operating characteristic (ROC) curves for postoperative recurrence, and a score of <17.5% and ≥17.5% were classed as low and high, respectively (area under the curve 0.667, 95% confidence interval (CI) 0.556-0.775). Eighty-one tumors (45.8%) had CD133 high scores (high expressers), and 96 (54.2%) had CD133 low scores (low expressers) (**Table 1**).

Correlation between CD133 expression and disease recurrence

CD133 high expressers showed a significantly higher crude recurrence rate (17.3% vs. 4.2%, p=0.004) (**Table 1**), and also worse DFS than low CD133 expressers (5-year DFS rate 77.2% vs. 95.1%, p=0.004) (**Table 2** and **Figure 2A**).

Correlation between Other biological properties and disease recurrence

The median value of the Ki-67 labeling index was 7.3% (range 0.3-80.7%). 71 patients (40.1%) were high-expressers and 106 (59.9%) were low-expressers (**Table 1**). As for vessel invasion, 49 patients (27.7%) were positive, and 128 (72.3%) were negative. Both the proliferating activity measured by the Ki-67 level and vessel invasion were associated with a significantly higher crude recurrence rate (**Table 1**). Consistently, these biological properties were also associated with poorer DFS (**Table 2**). These findings supported previous observations [22,33-34].

Prognostic value of a combination of CD133 expression, proliferating activity (Ki-67 expression) and/or vessel invasion

Forty-three cases (24.3%) were CD133 high/Ki-67 high expressers and 134 (75.7%) were the

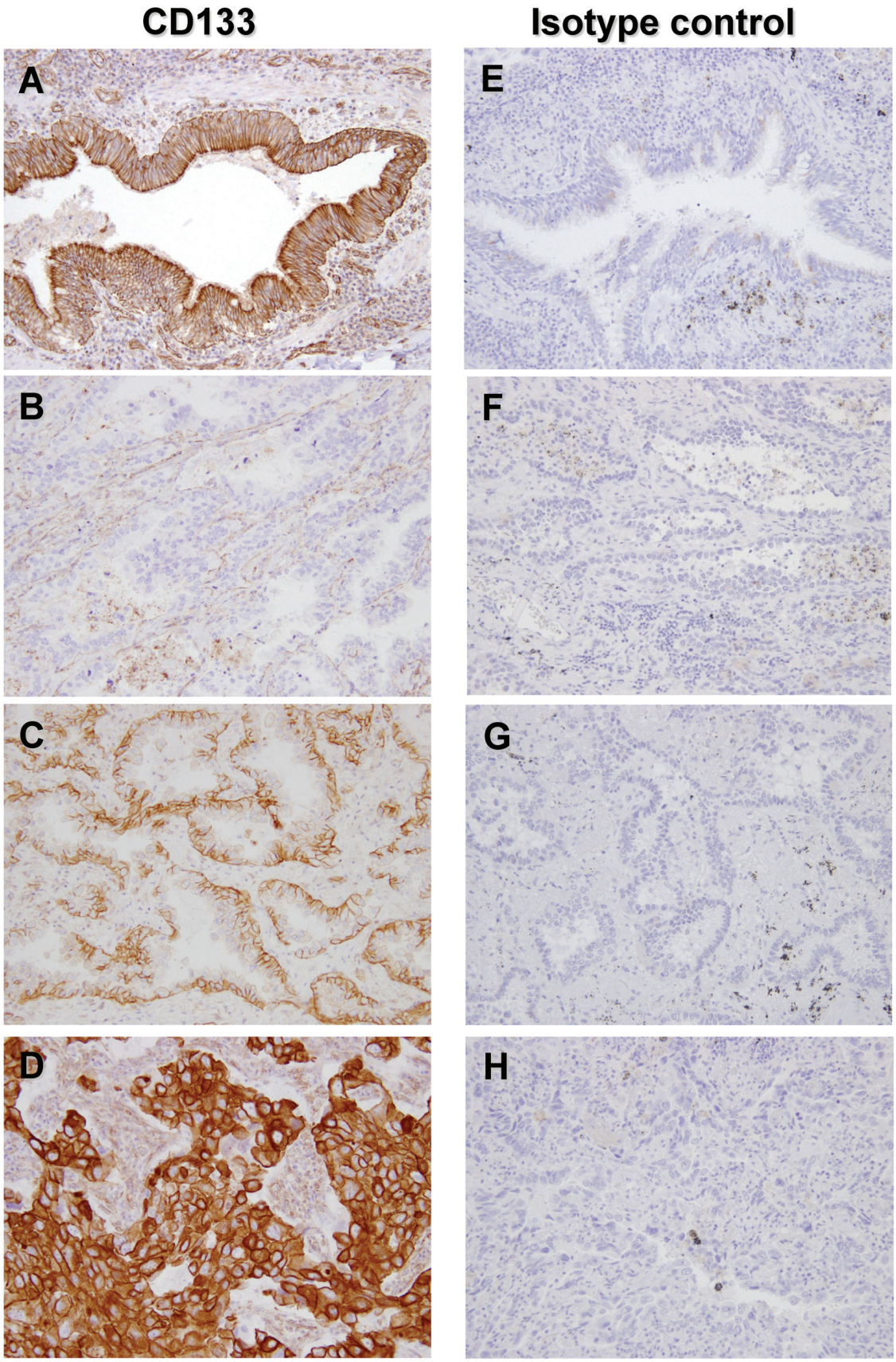
CD133
Isotype control
A
E
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Figure 1. Representative results of immunohistochemistry for CD133 (left panels) and a control antibody (right panels) are shown. **A**. Non-neoplastic bronchiolar epithelial cells expressed CD133 with exclusive plasma membrane localization. **B.** Bronchioloalveolar carcinoma did not express a detectable level of CD133. **C.** Acinar carcinoma expressed CD133 at a level almost the same as that of bronchiolar epithelial cells. **D.** Solid carcinoma expressed CD133 with diffuse cytoplasmic localization at an unequivocally stronger level compared to bronchiolar epithelial cells. **E**, **F**, **G**, **H**. Control antibody staining (isotype control) for each section. Magnification is ×200 for each.

other types (**Table 1**). The CD133 high/Ki-67 high expressers showed a significantly higher crude recurrence rate (27.9% vs. 4.5%, p<0.001) and also worse DFS compared to the other types (5-year DFS rate 66.5% vs. 93.2%, p<0.001) (**Table 2** and **Figure 2B**).

Twenty-seven cases (15.3%) were CD133 high expressers with vessel invasion and 150 (84.7%) were the other types (**Table 1**). CD133 high expressers with vessel invasion showed a significantly higher crude recurrence rate (33.3% vs. 6.0%, p<0.001) (**Table 1**) and also worse DFS in comparison with the other types (5-year DFS rate 51.0% vs. 92.9%, p<0.001) (**Table 2** and **Figure 2C**).

Twenty-two cases (12.4%) were CD133 high/Ki-67 high expressers with vessel invasion and 155 (87.6%) were the other types (**Table 1**). This group showed a significantly higher crude recurrence rate (36.4% vs. 6.5%, p<0.001) (**Table 1**) and also worse DFS than the other types (5-year DFS rate 53.9% vs. 91.2%, p<0.001) (**Table 2** and **Figure 2D**).

Multivariate analysis

Multivariate analyses revealed that CD133 high expression (HR 4.37, 95% CI 1.30-14.71,

Table 1. Associations between subjects and incidence of disease recurrence

Subjects	Total, No.(%)	Recurrence, No. (%)	Recurrence Rate (%)	p value
CD133				
Low (<17.5%)	96 (54.2)	4 (2.3)	4.2	0.004
High (≥17.5%)	81 (45.8)	14 (7.9)	17.3	
Ki-67 (MIB1 Labeling Index)				
Low (<10%)	106 (59.9)	3 (1.7)	2.8	<0.001
High (≥10%)	71 (40.1)	15 (8.5)	21.1	
Vessel invasion				
Negative	128 (72.3)	7 (4.0)	5.5	0.002
Positive	49 (27.7)	11 (6.2)	22.4	
CD133/Ki-67				
Others	134 (75.7)	6 (3.4)	4.5	<0.001
CD133 High/Ki-67 High	43 (24.3)	12 (6.8)	27.9	
CD133/Vessel invasion				
Others	150 (84.7)	9 (5.1)	6.0	<0.001
CD133 High/Vi Positive	27 (15.3)	9 (5.1)	33.3	
CD133/Ki-67/Vessel invasion				
Others	155 (87.6)	10 (5.6)	6.5	<0.001
CD133 High/Ki-67 High/Vi Positive	22 (12.4)	8 (4.5)	36.4	

No., number of cases; p, significant level for Chi-square test or Fisher's exact test; Vi, vessel invasion.

Table 2. Univariate analysis for disease-free survival

Subjects	No.(%)	5-year DFS (%)	Hazard ratio	95% CI	p value
CD133					
Low (<17.5%)	96 (54.2)	95.1	1.00		0.004
High (≥17.5%)	81 (45.8)	77.2	4.37	1.44-13.27	
Ki-67 (MIB1 Labeling Index)					
Low (<10%)	106 (59.9)	94.4	1.00		<0.001
High (≥10%)	71 (40.1)	75.1	8.51	2.46-29.41	
Vessel invasion					
Negative	128 (72.3)	93.5	1.00		<0.001
Positive	49 (27.7)	65.0	5.16	1.99-13.39	
CD133/Ki-67					
Others	134 (75.7)	93.2	1.00		<0.001
CD133 High/Ki-67 High	43 (24.3)	66.5	6.96	2.61-18.57	
CD133/Vessel invasion					
Others	150 (84.7)	92.9	1.00		<0.001
CD133 High/Vi Positive	27 (15.3)	51.0	6.57	2.60-16.61	
CD133/Ki-67/Vessel invasion					
Others	155 (87.6)	91.2	1.00		<0.001
CD133 High/Ki-67 High/Vi Positive	22 (12.4)	53.9	7.21	2.83-18.36	
Age					
<65	63 (35.6)	95.2	1.00		0.074
≥65	114 (64.4)	82.2	2.93	0.85-10.14	
Gender					
Female	88 (49.7)	84.2	1.00		0.667
Male	89 (50.3)	89.0	0.82	0.32-2.07	
Smoking status					
Non or light smoker	107 (60.5)	87.3	1.00		0.550
heavy smoker (B.I.≥500)	70 (39.5)	86.0	1.33	0.52-3.36	
Extent of operation					
Lobectomy	157 (88.7)	87.1	1.00		0.482
Limited Surgery (Segmentectomy or Wedge resection)	20 (11.3)	84.7	1.56	0.45-5.38	

No., number of cases; DFS, disease-free survival; 95% CI, 95% confidence interval; p, significant level for Log-rank test; Vi, vessel invasion; B.I., Brinkman Index.

p=0.017), Ki-67 high expression (HR 5.89, 95% CI 1.47-23.65, p=0.012), CD133 high/Ki-67 high expression (HR 8.39, 95% CI 2.65-26.54, p<0.001), CD133 high expression with vessel invasion (HR 4.50, 95% CI 1.51-13.34, p=0.007), and CD133 high/Ki-67 high expression with vessel invasion (HR 9.32, 95% CI 3.42 -25.39, p<0.001) had independent prognostic

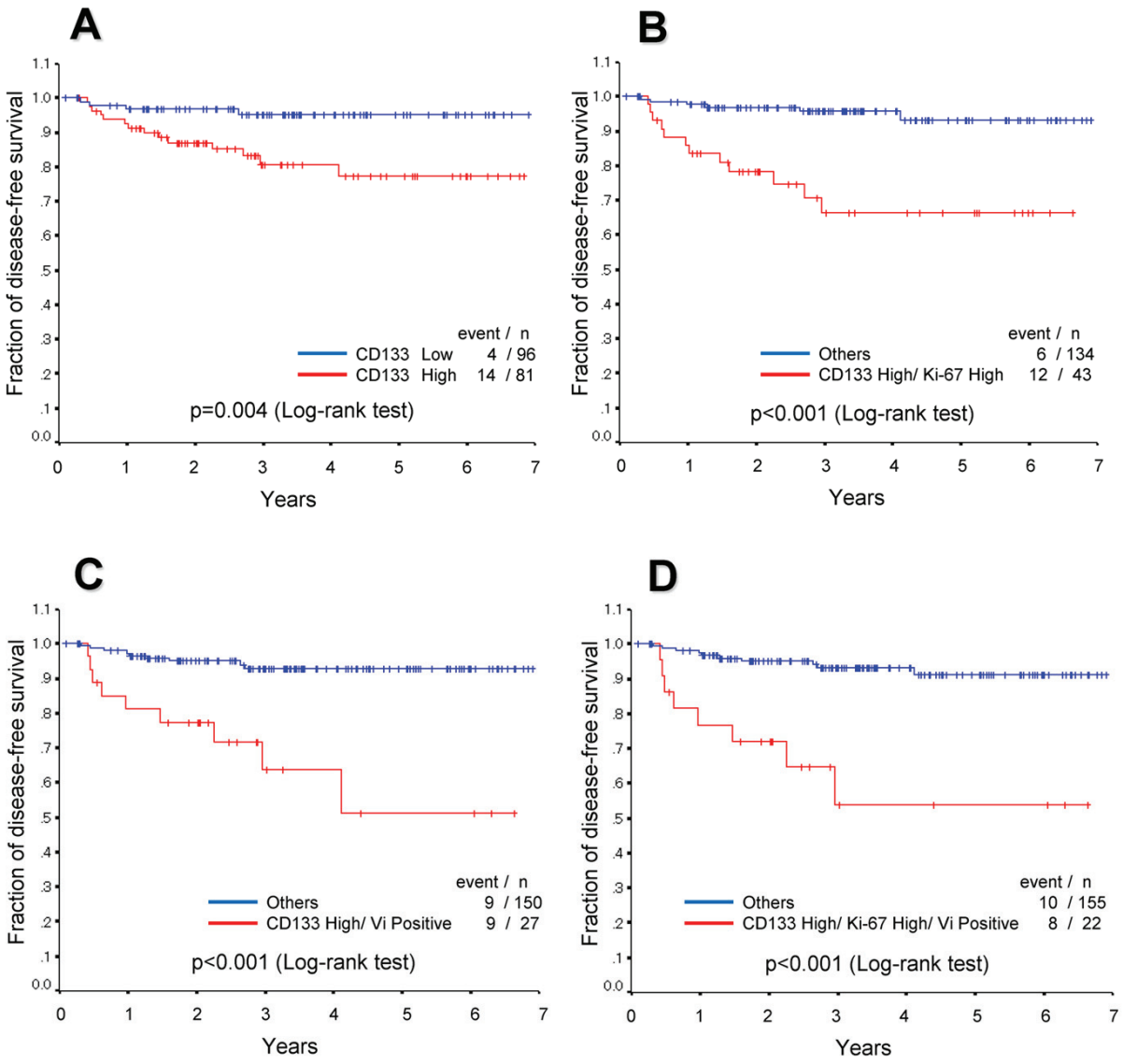

Figure 2. Five-year disease-free survival rates were calculated from Kaplan-Meier disease-free survival curves. **A**. CD133 high expressers (CD133 High, 77.2%) vs. CD133 low expressers (CD133 Low, 95.1%; Log-rank test, p=0.004) **B**. CD133 high expressers with Ki-67 high expression (CD133 High/Ki-67 High, 66.5%) vs. the other types (93.2%; Log-rank test p<0.001). **C**. CD133 high expressers with vessel invasion (CD133 High/Vi Positive, 51.0%) vs. the other types (92.9%; Log-rank test p<0.001). **D**. CD133 high/Ki-67 high expressers with vessel invasion (CD133 High/Ki-67 High/Vi Positive, 53.9%) vs. the other types (91.2%; Log-rank test p<0.001).

value to predict disease recurrence (**Table 3**).

Discussion

The present study immunohistochemically examined lung ADCs for CD133 expression. The proportion of CD133-strongly expressing cells observed here (median 20%, ranging 0 to 100%) was comparable with those in previous immunohistochemical studies on other diverse types of human cancers, such as gastric cancer [23], colon cancer [24], and breast cancer [29], including NSCLC [35]. Interestingly, the percentage of CD133-expressing cells detected by immunohistochemistry (mostly 20 to 50%), is found to be generally much higher than that by

Table 3. Multivariate analysis for disease-free survival

Subjects	Hazard Ratio	95% CI	p value
(A)			
CD133 (High)	4.37	1.30-14.71	0.017
Ki-67 (High)	5.89	1.47-23.65	0.012
Vessel invasion (Positive)	2.34	0.79-6.94	0.126
Age (≥65)	3.11	0.86-11.30	0.085
Gender (Male)	0.22	0.05-1.04	0.056
Smoking status (Heavy smoker, B.I.≥500)	2.84	0.66-12.14	0.160
Extent of operation (Limited surgery)	4.50	1.02-19.92	0.047
(B)			
CD133/Ki-67 (CD133 High/Ki67 High)	8.39	2.65-26.54	<0.001
Vessel invasion (Positive)	2.89	1.02-8.23	0.047
Age (≥65)	3.32	0.91-12.11	0.069
Gender (Male)	0.22	0.05-1.06	0.059
Smoking status (Heavy smoker, B.I.≥500)	3.40	0.78-14.78	0.103
Extent of operation (Limited surgery)	5.66	1.26-25.39	0.024
(C)			
CD133/Vi (CD133 High/Vi Positive)	4.50	1.51-13.34	0.007
Ki-67 (High)	6.16	1.60-23.66	0.008
Age (≥65)	3.33	0.93-11.88	0.064
Gender (Male)	0.25	0.06-1.05	0.059
Smoking status (Heavy smoker, B.I.≥500)	2.85	0.70-11.57	0.142
Extent of operation (Limited surgery)	3.89	0.90-16.73	0.068
(D)			
CD133/Ki-67/Vi (CD133 High/Ki-67 High/Vi Positive)	9.32	3.42-25.39	<0.001
Age (≥65)	3.40	0.95-12.11	0.060
Gender (Male)	0.28	0.06-1.28	0.101
Smoking status (Heavy smoker, B.I.≥500)	3.31	0.76-14.43	0.111
Extent of operation (Limited surgery)	3.19	0.80-12.74	0.100

95% CI, 95% Confidence Interval; p, significant level for Wald test; Vi, vessel invasion; B.I., Brinkman Index.

flow cytometry (mostly less than 5%) [12,15-16]. Immervoll et al. pointed out that using flow cytometric analysis required mechanical and/or enzymatic procedures to make tissues single-cell suspensions; these methods could disrupt the cellular microenvironment and affect protein expression [36]. On the other hand, Kemper et al. suggested that differences in the glycosylation status of specific epitopes could be a cause of the dissociation of the results between in vivo and in vitro studies [37]. Thus, the regulation of CD133 expression and/or modification, and also its function may differ between in vitro and in vivo studies.

Another noticeable result obtained here was that non-neoplastic bronchiolar epithelial cells, not only neoplastic cells, also expressed CD133. Although there are no studies showing CD133 expression in human non-neoplastic bronchiolar epithelial cells, results similar to ours were observed in previous studies on other tissues [36,38], such as salivary glands [36,38], lacrimal grands [36,38], sweat glands [38], the stomach [36], duodenum [36], colon [36], liver [36,38], pancreas [36], prostate [39], uterus

[38], and mammary glands [36]). The immunohistochemical expression of CD133 in non-neoplastic cells was found exclusively in plasma membranes at the apical and/or lateral sides [36,38], implying that it potentially has a specific physiological function other than maintenance of stemness.

Thus, it is interesting to investigate the potential differences in CD133's significance in vivo and in vitro, and also between neoplastic and non-neoplastic cells. In addition to such molecular biological matters, it is also important to evaluate its clinicopathologic significance. The main purpose of the present study was to verify the prognostic value of immunohistochemical expression of CD133 to predict the postoperative disease recurrence of lung ADCs at stage I. The results indicated that the level of CD133 expression had an independent prognostic value. Zhang et al. investigated 77 cases of NSCLC (47 ADC, 25 squamous cell carcinoma (SQC), 4 large cell carcinoma (LCC), 1 mucoepidermoid carcinoma), and demonstrated a significant association between CD133 expression and median survival span (10 months in the CD133-positive group versus 37 months in the CD133-negative group, $p<0.05$) [40]. Similarly, a high level of CD133 expression was also reported to be a poor prognostic factor in other types of malignant tumors such as glioma [41], gastric cancer [23], colon cancer [24-25,42], hepatocellular carcinoma [27], cholangiocarcinoma [28]. On the other hand, Salnikov et al. examined 88 cases of NSCLC (32 ADC, 43 SQC, 13 LCC), and showed contrary results to ours, that is, no significant difference in the survival time between a CD133-positive group and a CD133-negative group ($p=0.220$) [35]. A possible explanation for this discrepancy lies in the differences in the analytical subjects (i.e. patient number, histology, staging) and methods (i.e. antibody used, immunohistochemical protocols, definition of positive, scoring system, cut-off values). Some previous studies were small-scale (less than 90 patients) [35,40], and analyzed the patients collectively with different histological types [32,35,40] and disease stages [35,40]. Also, the antibody used for immunohistochemistry, positive criteria of CD133 expression and cut-off value determining whether the CD133 level was high or low (positive or negative) were different from that of our study [32,35,40]. In this study, we analyzed a larger number of cases (177 cases) with identical histological type (ADC) and disease stage. The expression level of CD133 in non-neoplastic bronchiolar epithelial cells in the same tissue section was used as an internal standard, and an unequivocally greater signal intensity with diffuse cytoplasmic staining was judged to be a strong level. The cut-off value was mathematically determined by the ROC analysis based on the proportion of neoplastic cells with strong expression levels. Thus, as our analytical subjects and methods were thought to be more objective, and we conclude that the CD133 level has an independent prognostic value.

We also examined the potential prognostic value of combining CD133 with other important factors to generate metastatic foci because most disease recurrence manifests as metastatic tumors. In addition to clonogenic activity (stemness), vessel invasion of neoplastic cells is generally accepted as one of the most important factors needed to initiate the metastatic process [43], while their proliferating activity is needed to complete this process [43]. Indeed, studies demonstrated that NSCLC with vessel invasion and/or strong proliferating activity measured by the Ki-67 labeling index was closely associated with occurrence of metastasis and worse outcome [21-22, 33-34]. As expected, the CD133 high expressers with vessel invasion exhibited a higher relative recurrence risk than the other types (**Table 2, 3** and **Figure 2C**). The CD133 high/Ki67 high expressers also showed a higher relative recurrence risk than the other types (**Table 2, 3** and **Figure 2B**). Similarly, Pallini et al. examined 44 cases of glioblastoma and demonstrated that the CD133 positive/Ki-67 high expressers had a significantly shorter survival span (OS; 6.75 months vs. 12.25 months, $p<0.007$, DFS; 2.5 months vs. 8 months, $p<0.001$) [44]. This finding is in good agreement with our results, supporting the utility of combining CD133 expression with Ki-67 expression.

In summary, the level of CD133 expression is an independent prognostic marker and its combination with proliferating activity and/or vessel invasion could have superior prognostic value to predict postoperative recurrence in patients with stage I lung ADC.

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