CD133 expression: a potential prognostic marker for non-small cell lung cancers.

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ABSTRACT

Background: CD133 is a membrane glycoprotein containing five transmembrane loops. Previous reports suggest that a CD133-positive subpopulation of multipotent cells with extensive proliferative and self-renewal characteristics has biological features of a cancer stem cell. In addition, the presence of CD133-positive cells was associated with a significantly poorer prognosis for some solid tumors, compared to those with CD133-negative cells. However, little is known about the relationships between CD133 expression and clinical features of lung cancer.

Methods: We conducted immunohistochemical assessment of 161 non-small cell lung cancers (NSCLCs) surgically resected at Hokkaido University Hospital between 1982 and 1994 to evaluate correlations between CD133 expression and various clinicopathological features.

Results: CD133 expression was significantly correlated with pathologic stages (pStages) II, III and IV for the various NSCLC types analyzed, and was an independent factor for unfavorable prognosis in this population (hazard ratio = 3.157, P = 0.015).

Conclusions: CD133 expression was correlated with pStage and was predictive of unfavorable prognosis in patients with pStages II, III, and IV NSCLC; these results suggest the possibility of using CD133 as a novel prognostic marker in these patients.
**Key words:** CD133, non-small cell lung cancer, immunohistochemical assessment, prognostic marker
BACKGROUND

Lung cancer is a leading cause of cancer death worldwide, and non-small cell lung cancer (NSCLC) accounts for more than 80% of all lung cancer cases. Despite some advances in early detection and recent improvements in treatment, prognosis for patients with NSCLC remains poor [1]. The current challenge is to identify new therapeutic targets and strategies, and to incorporate them into existing treatment regimens with the goal of improving therapeutic gain. Identifying reliable markers predictive of clinical outcome is also desirable in order to establish therapeutic strategies and select suitable treatment options for individual NSCLC patients.

CD133 is a membrane glycoprotein containing five transmembrane loops [2]. Previous reports have suggested that a CD133-positive subpopulation of multipotent cells with extensive proliferative and self-renewal characteristics has the biological features of a cancer stem cell [3-4]. In addition, it has been reported that the presence of CD133-positive cells compared with CD133-negative cells in some solid tumors was associated with a significantly poorer prognosis [5-7].

Accumulated evidence points that CD133-positive cells were increased in NSCLC and had tumorigenic potential [8-9]. However, little is known about the relationships between CD133 expression and the clinical and clinicopathological characteristics of NSCLC, especially survival outcomes. Here we report results of a comprehensive analysis of CD133 expression and its correlation with clinical features and survival
outcomes in a large series of NSCLC patients.

METHODS

Tumor specimens and survival data

This study was approved by the Medical Ethics Committee of Hokkaido University School of Medicine. Informed consent was obtained from all patients. The current study included 161 consecutive patients (109 men and 52 women) with adequate archival primary tumor specimens obtained during radical surgery at Hokkaido University Medical Hospital between 1982 and 1994. Histological diagnoses and tumor grades were determined in accordance with the 1982 World Health Organization criteria. Specimens included 85 adenocarcinomas, 66 squamous cell, 2 large cell, and 8 adenosquamous cell carcinomas. The pathologic stage (pStage) was based on the American Joint Committee on Cancer guidelines for postoperative tumor-lymph node-metastasis (TNM) classification [10]. Study specimens represented pStage I (n = 94), II (n = 23), III (n = 40), and IV (n = 4) tumors. Survival data were updated in May 2006; the median follow-up period for surviving patients was 110.0 months (range, 3.5-191.5 months).

Immunohistochemical analysis

CD133 expression was analyzed by immunohistochemistry. Archived sections (4
µm) were deparaffinized with xylene and rehydrated with graded concentrations of ethanol. For antigen retrieval, sections were placed in 1 mmol/L ethylenediaminetetraacetic acid (EDTA) solution (pH 8.0) and heated in a pressure cooker. Immunohistochemical staining of CD133 antibody was performed using ab19898, a rabbit polyclonal IgG antibody (1:100) (Abcam plc, Cambridge, UK), and visualized with a standard labeled streptavidin-biotinylated antibody method on an automated immunostainer (NexES, Inc; Ventana Medical Systems, Tucson, AZ, USA). One CD133-positive sample was included as a positive external control with each staining batch. CD133-positive tumor cells were counted under high magnification (×400) in five random and non-overlapping fields (100 tumor cells per field, total of 500 tumor cells per specimen). CD133-positivity was defined as moderate-to-strong staining intensity in the cytoplasm, ‘moderate’ being of similar intensity to the positive control and ‘strong’ being even more intense. Immunohistochemical evaluations were performed three times by one investigator (H.M.) who was blinded to the status of other immunohistological and clinical data, using a BX 40 microscope (Olympus, Tokyo, Japan).

**Statistical analysis**

Associations between CD133 expression and categorical variables were analyzed using chi-square tests or Fisher exact tests, as appropriate. Survival curves were
estimated using the Kaplan-Meier method, and differences in survival distributions were evaluated using the log-rank test. Univariate analyses using Cox proportional hazards modeling was applied to determine correlations between various factors and overall survival. The level of significance was set at \( P < 0.05 \). Statistical analyses were done using SPSS software (version 11.0; SPSS Inc., Chicago, IL, USA).

RESULTS

CD133 expression in NSCLC

Normal alveoli were used as negative controls and Bowman’s capsule basement membrane as positive controls [11]. CD133 immunoreactivity was present predominantly in cytoplasm of tumor cells (Figures 1A and B). Distribution of the degree of CD133 expression in the study cohort is shown in Figure 1C (range 0% - 95%). The median CD133-positive staining rate in NSCLC tumors was 33%. On the histogram of CD133 expression indices, there was a peak with 0%. The CD133 expression indices of the other tumors were distributed evenly between 5 and 95%. We defined the CD133 expression between 5 and 95% as the CD133 positive group (a total of 124 cases, 77 %), while we defined the cases with 0% as the CD133 negative group (37 cases, 23 %).

Correlation between CD133 expression and clinical and clinicopathological
characteristics

Chi-squared tests revealed that CD133-positive expression was significantly more prevalent in tumors from pStage II, III and IV patients than in those from pStage I patients (P < 0.05). There was no correlation between CD133 expression and other clinicopathological characteristics, including age, sex, smoking history and other histological diagnoses (Table 1).

Prognostic value of CD133 expression in NSCLC

Patients of all pStages with positive CD133 expression tended to experience a significantly shorter survival compared to those lacking expression (P = 0.052). In subgroup analysis of pStage II, III, and IV patients, those with positive CD133 expression had poorer prognosis compared to those with negative CD133 expression (P = 0.0098) (Figure 3). This prognostic stratification did not remain significant for stage I patients (P = 0.66). Univariate Cox’s regression analysis revealed that positive CD133 expression alone was an independent unfavorable prognostic factor for this population (hazard ratio = 3.157, P = 0.015) (Table 2).

DISCUSSION

The results of this study solidify our understanding of CD133 antigen expression in NSCLC. CD133 expression is correlated with pStage II, III and IV disease and also with
poorer prognosis in the study population. These data are consistent with previous reports demonstrating that CD133 expression is correlated with recurrence, aggressiveness, metastatic potential and poor survival in patients with various other solid tumors [5-7, 12].

There are a few reports of studies investigating associations between CD133 expression and clinical features or survival in NSCLC, but use of CD133 expression as a prognostic factor remains controversial. Hilbe et al. have noted that increased numbers of CD133-positive endothelial cells might contribute to tumor vasculature in NSCLCs [13]. In addition, a significant association between survival and CD133 expression in NSCLC has been observed [14-15]. In sharp contrast, Salnikov et al. reported that CD133 was indicative of a resistant phenotype, but was not useful as a prognostic marker for patients with NSCLC [16]. Li et al. reported that CD133 was not associated with survival nor any clinicopathological features studied [17]. In the current study, patients with positive CD133 expression tended to have a shorter survival compared to those with negative CD133 expression at all pStages. We suggest that use of a scoring system based on the intensity of immunohistochemical staining, as well as differing distributions of the stages included, might have contributed to the discrepancies seen in the results of these various studies.

The current results concerning the prognostic impact of CD133 expression in advanced-stage NSCLC seem consistent with other CD133-positive cell features,
including resistance to chemotherapy or radiotherapy. Considering previous reports and our results together, we suggest that CD133 may be useful for assessing the risk of disease progression or survival in NSCLC patients.

A small subpopulation of tumor cells comprises cancer stem cells (CSC), which are self-renewing and undergo multipotent differentiation [18-19]. CD133 was initially identified as an important marker of this subset in colorectal cancer, brain tumors, hepatocellular carcinoma, and prostate cancer [3-4, 20-21]. In lung cancer, CD133-positive cells demonstrating CSC characteristics, such as self-renewal potential and extensive proliferation, were identified [22]. CD133-positive lung cancer cells were also shown to display stem cell-like features and were resistant to cisplatin treatment [9]. In the current study, the population of CD133-positive cells among the NSCLCs analyzed was relatively large (median 33%, range 0% - 95%). Not all of these cells possessed stem cell features; CSC are usually thought to be rare [3, 22-23]. Recent studies have raised the question of the validity of CD133 as a marker for CSC and its functional relevance in tumor-initiating cells [24]. Shmelkov et al. reported that CD133 is widely expressed in human primary colon cancer and that both CD133-positive and -negative colon cancers exhibit phenotypic features of CSC [25]. In the lung cancer cell lines H460 and A549, similar data have been reported, suggesting that CD133 alone might not be useful as a CSC marker. Therefore, co-expression in NSCLC of CD133 and the ATP-binding cassette superfamily G member 2 (ABCG2), and also expression of the
combination of CD133 and nestin were investigated for usefulness as a CSC marker [17, 26]; these and other combinations warrant further investigation in the future.

CONCLUSIONS

CD133 expression was correlated with pStage and was an independent factor for unfavorable prognosis in patients with pStage II, III, and IV NSCLC. Thus, CD133 may prove useful as a novel prognostic marker for NSCLC.

DISCLOSURE OF POTENTIAL COMPETING INTEREST

All authors report that they have no financial conflicts of interest.

ACKNOWLEDGEMENTS

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AUTHORS’ CONTRIBUTIONS

HM was responsible for the study design, the pathological analysis, drafting the manuscript and performing immunohistochemical staining. JK and EK carried out sample collections and coordination. JM performed immunohistochemical staining. KK and YM provided direction for pathological evaluation. JSK, IK, SO and HAD provided
general support. MN supervised all of this research. All authors read and approved the final manuscript.
Reference


Figure Legends

Figure 1. (A) Positive and negative controls for CD133 staining. Normal alveoli were used as negative controls and Bowman’s capsule basement membrane as positive controls. (B) Immunohistochemical staining patterns for CD133 in various non-small cell lung cancer (NSCLC) types. (C) Distribution of CD133 expression rates in NSCLC.

Figure 2. Kaplan-Meier survival curves for radically-resected NSCLC patients. CD133 expression is correlated with shorter survival in pStage II, III and IV.
Table 1. Relationship between CD133 expression and clinical and clinicopathological characteristics in 161 surgically resected NSCLCs*

<table>
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<td></td>
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<td>Positive</td>
<td>P value</td>
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<td>60</td>
</tr>
<tr>
<td></td>
<td>≥65</td>
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<td>pStage §§</td>
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<td></td>
<td>II - IV</td>
<td>10</td>
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</table>

*non-small cell lung cancer
†large cell carcinoma and adenosquamous cell carcinoma
‡pathologic tumor classification
§pathologic lymph node status
§§pathologic disease stage
Table 2. Univariate analyses of features associated with survival in 67 pathological Stage II, III and IV patients.

<table>
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<th>Variables</th>
<th>Hazard Ratio (95% CI*)</th>
<th>P value</th>
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<td>Sex</td>
<td>Female vs. Male</td>
<td>0.912 (0.502 - 1.655)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>≥65 vs. &lt;65</td>
<td>1.124 (0.657 - 1.924)</td>
</tr>
<tr>
<td>Smoking history (pack years)</td>
<td>≥20 vs. &lt;20</td>
<td>1.462 (0.791 - 2.703)</td>
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<tr>
<td>Histology</td>
<td>Adenocarcinoma vs. Non-adenocarcinoma</td>
<td>1.353 (0.785 - 2.332)</td>
</tr>
<tr>
<td>Differentiation</td>
<td>Well/Moderate vs. Poor</td>
<td>0.997 (0.514 - 1.934)</td>
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<td>pT†</td>
<td>T2-4 vs. T1</td>
<td>1.140 (0.408 - 3.179)</td>
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<tr>
<td>pN‡</td>
<td>N1-3 vs. N0</td>
<td>1.157 (0.592 - 2.237)</td>
</tr>
<tr>
<td>CD133</td>
<td>Positive vs. Negative</td>
<td>3.157 (1.252 - 7.964)</td>
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*confidence interval
†pathologic tumor classification
‡pathologic lymph node status
Figure 1

(A) Positive control vs. Negative control

(B) CD133 positive rate (%)

(C) CD133 positive rate (%) for Squamous cell carcinoma and Adeno carcinoma
Figure 2

All pStage, n=161

pStage I, n=94

pStage II,III,IV, n=67

Overall Survival (days)

Cumulative Survival Rate

p=0.0523

p=0.6624

p=0.0098

CD133 positive

CD133 negative