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Association Between CD133 Expression and Prognosis in Human Lung Adenocarcinoma

NAOKI YAMASHITA[1,2], TSUNEHIRO OYAMA[1,3], TETSUYA SO[2], TAKEAKI MIYATA[4], TAKASHI YOSHIMATSU[5], RYUJI NAKANO[6], WATARU MATSUNAGA[1] and AKINOBU GOTOH[1]

[1]*Laboratory of Cell and Gene Therapy, Hyogo College of Medicine, Nishinomiya, Japan;*
[2]*Department of Thoracic Surgery, Shinkomonji Hospital, Kitakyushu, Japan*
[3]*Imamitsu Home Care Clinic, Kitakyushu, Japan;*
[4]*Department of Thoracic Surgery, Shinkuki General Hospital, Kuki, Japan;*
[5]*Department of Thoracic Surgery, Fukuoka-Wajiro Hospital, Fukuoka, Japan;*
[6]*Department of Pathology, Fukuoka-Wajiro Hospital, Fukuoka, Japan*

Abstract. *Background/Aim: CD133 is a promising candidate marker for cancer stem cells. However, clinical studies on CD133 expression in human lung adenocarcinoma have not yet been conducted. We hypothesized that CD133 expression in lung adenocarcinoma is a poor prognostic factor. Patients and Methods: CD133 expression in lung adenocarcinoma was examined clinicopathologically. Then, clinicopathological parameters and patient prognosis were investigated. Moreover, CD133 expression was examined via immunohistochemical staining, and the relationship between CD133 expression and clinicopathological parameters was explored. Results: Approximately 48.0% (49/102) of patients had CD133-positive cells. Based on a subgroup analysis, the CD133-positive group with pStage I+II disease had a significantly worse disease-free interval than the CD133-negative group (p<0.05). Conclusion: CD133 expression may be a poor prognostic factor in lung adenocarcinoma.*

Cancer accounts for approximately 30% of all causes of mortality, and it is considered a leading cause of death. Moreover, the incidence of cancer-related death is increasing in the aging society (1). In particular, lung cancer is one of the most common cancers with poor prognosis worldwide, and the 5-year lung-cancer survival rate is <15% (2). This finding may be attributed to metastasis, drug resistance to therapy and lack of target-based drugs (3).

In recent years, the concept of cancer stem cells (CSCs) in the pathophysiology of cancer has attracted attention (4). Cancer tissues were previously considered to be a population of tumor cells that have acquired different properties by random growth, such as development and maintenance of malignancy and resistance to treatment. Later, they were found to be controlled by stem cells that form the basis of cancer tissues (5, 6). In 1997, Bonnet *et al*. demonstrated that cells expressing cell surface markers such as CD34 and CD38 are CSCs in acute myeloid leukemia. Moreover, they also demonstrated that CSCs is at the top in terms of hierarchy (7). In 2003, Al-Hajj *et al*. revealed that CD44+CD24–/low cells were CSCs in breast cancer, and CSCs in solid cancers have been a topic of interest (8). To date, the presence of CSCs has been reported in colorectal, breast, ovarian, bladder cancers and other types of malignancies (9-12). In addition, CSCs can satisfy the following properties (6, 13): [1] a minor population of cancer cells with tumorigenicity, [2] the ability to produce cell populations with low or lacking tumorigenicity and [3] self-renewal ability. Hence, they can replicate themselves, thereby producing identical cells, and differentiate into several types of cells. While they maintain cells identical to themselves by self-renewal in cancer tissues, CSCs can produce most surrounding cancer cells by differentiation. Moreover, CSCs can cause recurrence and metastasis through resistance to anticancer drugs and radiation therapy (14). Hence, the biological characteristics of cancer stem cells can be a new therapeutic target.

The CD133 antigen, also called prominin 1, is a 5-transmembrane domain glycoprotein (15). Its function is currently unknown. However, it may be a specific cell surface marker of CSC in several malignancies, including

Correspondence to: Naoki Yamashita, Institute for Advanced Medical Sciences, Hyogo College of Medicine, Laboratory of Cell and Gene Therapy, 1-1, Mukogawa-cho, Nishinomiya-City, Hyogo 663-8501, Japan. Tel: +81 798456808, Fax: +81 798456806, e-mail: naoooook1988@gmail.com

Key Words: Lung adenocarcinoma, CD133, cancer stem cell.

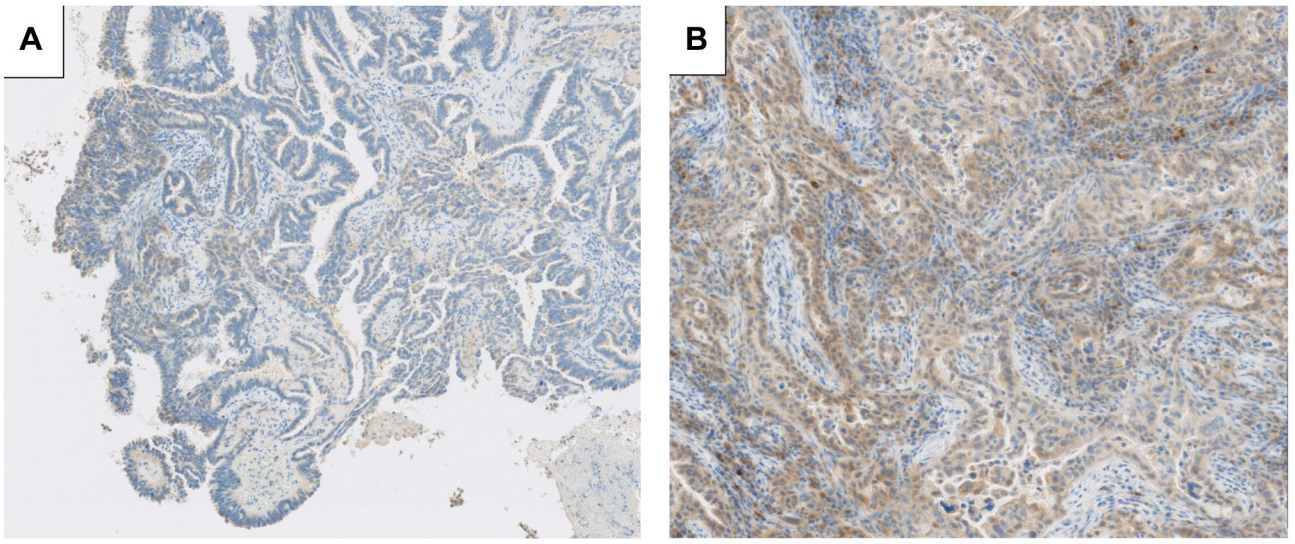

Figure 1. *The CD133 expression score was defined as the proportion of cells with strong expression levels on membranous staining. The percentage of positive cells was graded as 0% –100%. A) 5%, negative. B) 100%, positive.*

those of the central nervous system and colon, breast, prostate and ovarian cancers (8, 16-19). CD133 is a promising candidate marker for CSCs because tumor cells expressing CD133 have high tumorigenicity and resistance to differentiation and radiation therapies (20, 21). Currently, some studies have demonstrated that CD133 expression in a subpopulation of lung-cancer cells should identify them as a CSC. However, this remains controversial (22). Therefore, we investigated the relationship between CD133 expression and clinical parameters in lung adenocarcinoma.

Patients and Methods

In total, 186 patients with lung adenocarcinoma underwent complete or partial resection from 2010 to 2018 at Shinkomonji Hospital. Pathological specimens were used to assess clinicopathological parameters. The mean age at the time of surgery was 72 (range=43-89 years). None of the patients received chemotherapy or radiation before surgery. Tumor staging was performed in accordance with the 8th edition of TNM staging for lung-cancer guidelines. The clinicopathological parameters included age, sex, surgical procedure, smoking history, pathological stage and tumor marker levels (CEA: Carcinoembryonic antigen, SLX: Sialyl Lewis X-i antigen and CYFRA: Cytokeratin 19 fragment). Sectioning of lung adenocarcinoma was performed for immunohistochemical staining for CD133 using a standard immunoperoxidase technique, as described previously (23). CD133 staining was performed on 4-μm-thick paraffin sections using a mouse monoclonal antibody (anti-CD133; Millipore Inc., Temecula, CA, USA) at 1:200 dilution. The CD133 expression score was defined as the proportion of cells with strong membranous staining in tumor sections. The percentage of positive cells was graded as 0%-100% (Figure 1). CD133 positivity was defined as staining of more than 20% of the tumor cells (negative: ≤20%, positive: >20%). The survival curves were evaluated using the Kaplan–Meier method. *p*-Values ≤0.05 were considered statistically significant. This study received ethical approval for human subjects from Hospital Research Ethics Committee. Informed consent was obtained from each patient.

Results

Of 186 patients with lung adenocarcinoma, 102 (54.8%) were eligible for this study and were examined. Approximately 48.0% (49/102) of patients had CD133-positive cells, with a cut-off value of 20%. Next, the relationship between the tumor markers and prognosis was examined, and the results are presented in Table I. The prognosis for overall survival (OS) was poor in the elderly (≥75 years) ($p<0.05$), but there was no difference in gender. Smokers had a significantly worse OS and disease-free interval (DFI) than non-smokers ($p<0.05$). As to the surgical procedure, others (partial resection, *etc.*) (n=14, 13.7%) had a worse prognosis than lobectomy (n=88, 86.3%). There were 79 (77.4%) patients with pathological stage I+II and 23 (22.5%) patients with stage III+IV cancer. Patients with advanced-stage lung adenocarcinoma had a worse prognosis than patients with early-stage disease ($p<0.05$) (Figure 2). Next, in terms of tumor markers, patients with high serum CEA levels (>5 ng/ml) had significantly worse OS and DFI compared to those with normal CEA levels. Patients with high serum SLX levels (>38 U/ml) had significantly worse OS than those with normal SLX levels. However, OS and DFI were not significantly different between patients with high (>3.5 ng/ml) serum CYFRA levels and those with normal

Table I. *CD133 expression and its relationship with clinical parameters.*

	Total			CD133 expression			
Parameters	Number (%)	*p*-Value (OS)	*p*-Value (DFI)	Positive (%)	Negative	*p*-Value (OS)	*p*-Value (DFI)
Total	102			49 (48.0)	53		
Age							
<75years	63 (64.7)	**0.0002**	0.5962	30 (47.6)	33	0.6623	0.1537
≥75years	39 (35.3)			19 (48.7)	20		
Gender							
Female	45 (44.1)	0.3959	0.9152	24 (53.3)	21	0.5495	0.1026
Male	57 (55.9)			25 (43.8)	32		
Smoking hystoly							
Never and former	69 (67.6)	**0.0487**	**0.0215**	32 (46.3)	37	0.9824	0.2148
Current	33 (32.3)			17 (51.5)	16		
Surgical procedure							
Lobectomy	88 (86.3)	**0.0011**	0.6481	40 (45.5)	48	0.9340	0.1164
Others	14 (13.7)			9 (64.2)	5		
Pathological stage							
I+II	79 (77.4)	**<0.0001**	**0.0009**	36 (45.6)	43	0.8271	0.2449
III+IV	23 (22.5)			13 (56.5)	10		
Serum CEA							
Negative	63 (61.8)	**0.0102**	**<0.0001**	28 (44.4)	35	0.6994	0.4430
Positive	39 (38.2)			21 (53.8)	18		
Serum SLX							
Negative	87 (85.3)	**0.0093**	0.1687	38 (43.7)	49	-	0.1975
Positive	15 (14.7)			11 (73.3)	4		
Serum CYFRA							
Negative	70 (68.6%)	0.1039	0.1089	31 (44.3)	39	0.9693	0.1100
Positive	32 (31.4%)			18 (56.3)	14		

Significant *p*-Values are shown in bold.

serum CYFRA levels. From a CSC viewpoint, there was no significant difference in the CD133 levels and other clinical parameters. Nevertheless, the CD133-positive group had worse OS than the CD133-negative group (Figure 3). In particular, the CD133-positive group with pStage I+II disease (43/79) had a significantly worse DFI ($p<0.05$) (Figure 4).

Discussion

Lung cancer is a preventable cancer, but once it starts, it is one of the cancers with the poorest prognosis. Lung cancer mortality shows a slight downward trend that is mainly due to smoking control and improved early detection and treatment. However, the 5-year survival rate is low because lung cancer has few symptoms, many metastases and recurrences, and a low rate of curative treatment. As in this case, elderly and smokers had a significantly poorer prognosis (Table I). Currently, only a small proportion of lung cancer is detected early, and more effective methods are needed to reduce lung cancer morbidity and mortality. Understanding the etiology of lung cancer, as we did in our study of CSC, may allow the development of novel therapies.

CSCs are commonly associated with resistance to anticancer drugs. However, this is easy to understand if the presence of CSCs is considered (24-26). These cells have similar properties to those of tissue stem cells and a slow division rate, and they can remain in the quiescent phase (G0 phase) for a long period. However, radiation/chemotherapy targets cancer cells with a high division rate. Therefore, CSCs are resistant to chemotherapy because of their characteristics. To date, CSCs often express multidrug-resistant genes, which can lead to metastasis and recurrence.

The biological function of CD133 remains unknown. After isolating CD133-positive cells from human glioma, Singh *et al*. transplanted 100 CD133 cells in NOD/SCID mice. Then, tumor development was observed (10). In contrast, mice transplanted with CD133-negative cells did not develop tumors even after transplanting ≥105 cells. Furthermore, Ricci-Vitiani *et al*. revealed that in colorectal cancer, the CD133-positive cells account for approximately 2.5% of tumor cells in a densely populated region. This is associated with the development of a tumor similar to the primary lesion in immunodeficient mice (27). O'Brien *et al*. reported that CSCs were concentrated in the CD133-positive fraction

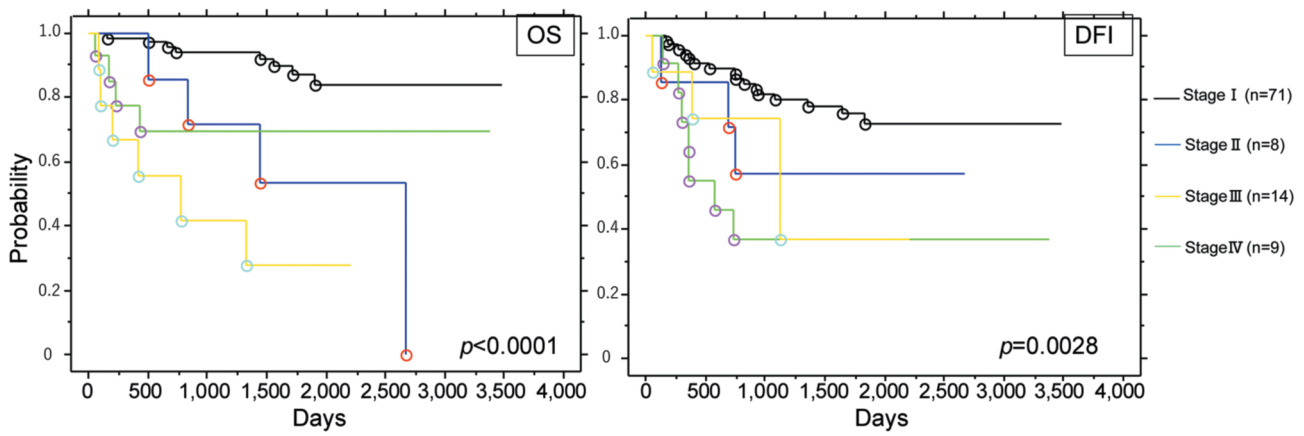

Figure 2. *The Kaplan–Meier analysis of overall survival (OS) and disease-free interval (DFI) in patients with lung adenocarcinoma. In total, 102 patients presented with lung adenocarcinoma. Among them, 71, 8, 14 and 9 had stage I, II, III and IV disease, respectively.*

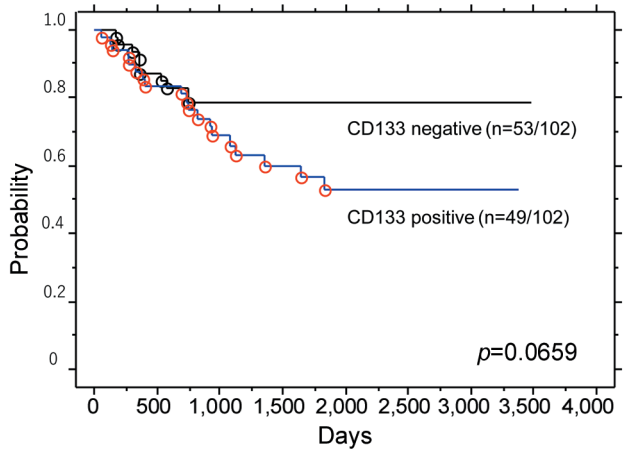

Figure 3. *Kaplan–Meier analysis of DFI in patients with lung adenocarcinoma according to CD133 expression.*

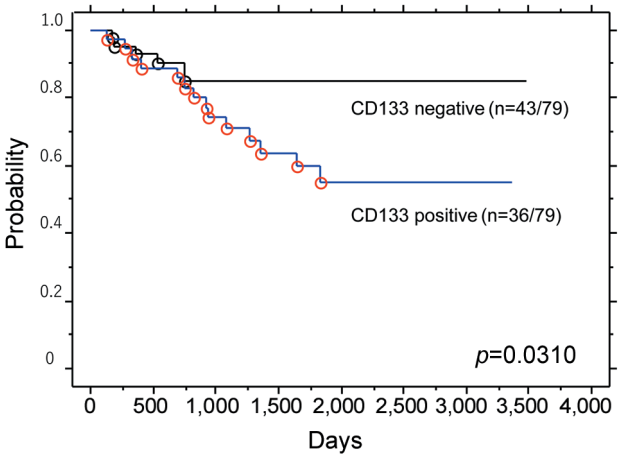

Figure 4. *Survival curves of DFI according to CD133 expression in pStage I+II disease.*

in colorectal cancer (17). However, the potential prognostic value of CD133 when used as a marker of CSCs in solid tumors remains controversial (28-30). Our study revealed that immunohistological CD133 expression was correlated with the pathological stage of human adenocarcinoma, particularly with stage I+II disease. In the study of Engeng *et al.*, only Asian patients with non-small cell lung cancer who had high CD133 expression had a short OS (22). These results suggest that investigating CD133 cells by isolating samples from Asian patients with early-stage lung adenocarcinoma may be a good therapeutic strategy. For example, drug therapy is used as postoperative adjuvant therapy instead of surgery alone for lung adenocarcinoma expressing CD133. However, the mechanism by which CD133-expressing cells are associated with recurrence and metastasis remains unclear. Hence, further research should be conducted.

These findings indicate that targeting CSCs can facilitate cancer treatment. Therefore, studies on important CSC markers should be carried out, and novel therapeutic strategies should be developed.

In future studies, we will investigate the relationship between CD133 and other CSC markers. Moreover, unfavorable prognostic factors will be assessed and used for the multidisciplinary treatment of early-stage lung adenocarcinoma.

Conflicts of Interest

The Authors declare that there are no conflicts of interest in relation to this study.

Authors' Contributions

Tsunehiro Oyama designed the study, data analysis and acquisition of funding. Tetsuya So analysis data. Takeaki Miyata, Takashi Yoshimatsu, Wataru Matsunaga, Akinobu Gotoh revised it critically for important intellectual content.

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