Prognostic Impact of CD133 Expression in Gastric Carcinoma

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Abstract. *Background and Aim: CD133 expression in cancer cells has been recognised as a putative cancer stem cell (CSC) marker in epithelial malignancies. CD133 expression was evaluated in gastric cancer and the clinical impact of CD133-positive gastric cancer was clarified. Patients and Methods: Ninety-seven gastric cancer patients who received curative gastrectomy were enrolled. CD133 expression in cancerous tissue was evaluated by immunohistochemistry. Results: CD133 expression positively correlated with tumour extension and the degree of nodal involvement. CD133 expression significantly affected patient postoperative outcome. Multivariate analysis revealed CD133 positivity as an independent prognostic factor superior to the depth of invasion and similar to nodal involvement in gastric cancer ($p<0.05$). Conclusion: Even slight CD133 expression in gastric cancer patients may be a useful prognostic marker via CSC. Further examination of CD133 with respect to CSC markers can enable prediction of the recurrence risk of gastric cancer.*

Genetic and immunological approaches have recently shown the marked clinical impact of stem cells in cancer patients (1). It has been reported that like embryonic stem cells, cancer stem cells (CSCs) are immortal, can self-renew and can differentiate into all cell types of the body (2). These CSCs are a small population of cells with unique self-renewal ability and metastatic potential that have been detected in myeloma (3), brain tumours (4), pancreatic cancer (5), hepatocellular carcinoma (6), colorectal cancer (7) and prostatic carcinoma (8). CSCs are believed to work to maintain tumour growth, and cells bearing CSCs may be an integral part of the development and perpetuation of various types of human cancer. In this context, CSCs are not only associated with tumour initiation and growth, but also play a crucial role in distant metastasis. CD133 is a five transmembrane cell-surface glycoprotein, a specific surface marker for bone marrow-derived circulating endothelial progenitors (9, 10). CD133 expression in cancer cells has been focused on as a CSC marker of solid tumours since Singh *et al*. reported that CD133-positive cells were putative CSC in glioma (11).

Immunohistochemical analysis of cancerous tissues has revealed that CD133-positive malignancies show a more aggressive character than the CD133-negative group in colon and prostatic cancers (7, 8); however, no report has detailed the clinical impact of CD133 positivity in gastric cancer. The current study aimed to investigate CD133 positivity of 97 gastric cancer samples retrospectively and to clarify the clinical implication of CD133-positive cells in gastric cancer as a marker of CSCs.

Patients and Methods

Patients and samples. A series of 97 consecutive gastric cancer patients who received curative gastrectomy in Kagoshima University Hospital between January 2001 and September 2003 were enrolled in the current study. Their median age was 65 years (range 40 to 85 years). All patients received gastrectomy and at least D1 lymph node dissection. Fifty-seven patients received distal, 28 total and the remaining 12 proximal gastrectomy. Tissue samples were obtained from paraffin-embedded resected specimens of the stomach after histopathological diagnosis. The final pathological examination disclosed that 27, 20, 27, and 23 patients were stage I, II, III and IV, respectively (Table I).

None of the patients had received preoperative adjuvant therapy, but all stage IV patients received postoperative adjuvant chemotherapy. All patients were followed up for survival and recurrence-free survival. Clinical factors were assessed by the Japanese Classification of Gastric Carcinoma (12). The study was approved by the Institutional Review Board of Kagoshima University and performed according to the Helsinki Declaration.

Detection and evaluation of CD133-positive gastric cancer. To visualise CD133 positivity, avidin biotin complex (ABC) immunohistochemistry was performed according to a previous report (14). Namely, paraffin-embedded sections, including tumour tissue, were obtained from the 97 gastric cancer patients,

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Table I. *Patient information.*

		n
Gender	Male	69
	Female	28
Age (years)	Mean (range)	63 (40-85)
Operation	Total gastrectomy	28
	Distal	57
	Proximal	12
Stage	I	27
	II	20
	III	27
	IV	23
Histology	Differentiated	46
	Undifferentiated	51

deparaffinised and soaked in PBS. For retrieval of CD133 antigen, sections were submerged in 1% citrate buffer at 2 atoms at 120˚C for 10 minutes. The sections were then treated with 1% H_2O_2 for 30 minutes to block endogenous tissue peroxidase. Rabbit serum diluted with PBS was then applied for 30 minutes to reduce nonspecific binding. Sections were rinsed in PBS and visualised using standard techniques for labeled avidin-biotin immunoperoxidase staining. Pretreated specimens were stained for CD133 using a mouse monoclonal anti-CD133 antibody (clone AC133; Miltenyi Biotec, Bergisch Gladbach, Germany) (7). Anti-CD133 antibody diluted to 1:100 with PBS was applied and incubated for 24 hours at room temperature. CD133 positivity on cancer tissue was visualised by the Avitin Biotin Complex (ABC) method. After rinsing three times in PBS, the sections were incubated with peroxidase-labeled antibodies for 20 minutes. The sections were then applied with streptavidin-peroxidase complex. Nerve tissue was used as a positive control of CD133. Tumour sections containing at least one CD133-positive cell were assigned to the CD133-positive group according to previous reports (7).

Evaluation of CD133 positivity of gastric cancer. CD133 positivity was evaluated according to the method used for colon cancer as previously reported (7). Namely, five medium power fields per section were observed, and the presence or absence of CD133-positive cells was examined in five high power fields. The positivity of glands was defined as either membrane staining or the presence of CD133 deposits in tumour glands. The specimens were independently evaluated by two observers (SI and SN) blinded to patient information.

Statistical analysis. Correlations between CD133 expression and clinicopathological parameters, including patient survival, were analysed using the χ^2 test. Survival curves were calculated by the Kaplan Meier method, and statistical differences were evaluated by generalised Wilcoxson methods. The Cox proportional hazard model was used in multivariate analysis. A p-value <0.05 was considered significant.

Results

CD133 positivity in gastric cancer. CD133 expression was found in the membrane of gastric cancer. As in previous reports (7), luminal expression of CD133 in the center of the cancer gland was partly identified, especially in well-differentiated adenocarcinoma (Figure 1). Even though the percentage of CD133-positive gastric cancer cells was less than 1%, they were regarded as being in the CD133-positive group. In contrast, CD133 positivity was found in the cellular membrane of gastric cancer (Figure 2). Twenty-seven (28%) of 97 patients had CD133-positive findings.

Table II. *Correlation between CD133 positivity and clinical factors.*

Clinical factors		CD133 positivity		
		Positive n=27	Negative n=70	*P*-value
Gender	Male	22	47	N.S.
	Female	5	23	
Tumor depth	T1	2	25	
	T2	9	31	0.01
	T3-	16	14	
Nodal involvement	Yes	22	35	0.01
	No	5	35	
Clinical stage	I	2	26	
	II	3	17	0.01
	III 12	15		
	IV 10	13		
Lymphatic invasion	Yes	22	47	N.S.
	No	5	23	
Venous invasion	Yes	22	32	0.01
	No	5	38	
Histology	Differentiated	11	35	N.S.
	Undifferentiated	16	35	

N.S.: not significant.

Clinicopathological characteristics of CD133-positive gastric cancer (Table II). Patients with CD133-positive expression had significantly more advanced T stage ($p<0.01$) and nodal involvement ($p<0.01$) than those with CD133-negative expression. The incidence of venous invasion was also significantly higher in the CD133-positive group than in the CD133-negative group ($p<0.01$); however, there was no significant difference in histology between the two groups. Stage grouping was also significantly advanced in the CD133-positive group than in the CD133-negative group ($p<0.01$), because the stage included tumour depth and nodal metastasis.

Prognostic value of CD133-positive gastric cancer. The 5-year survival rate of the CD133-positive group was significantly

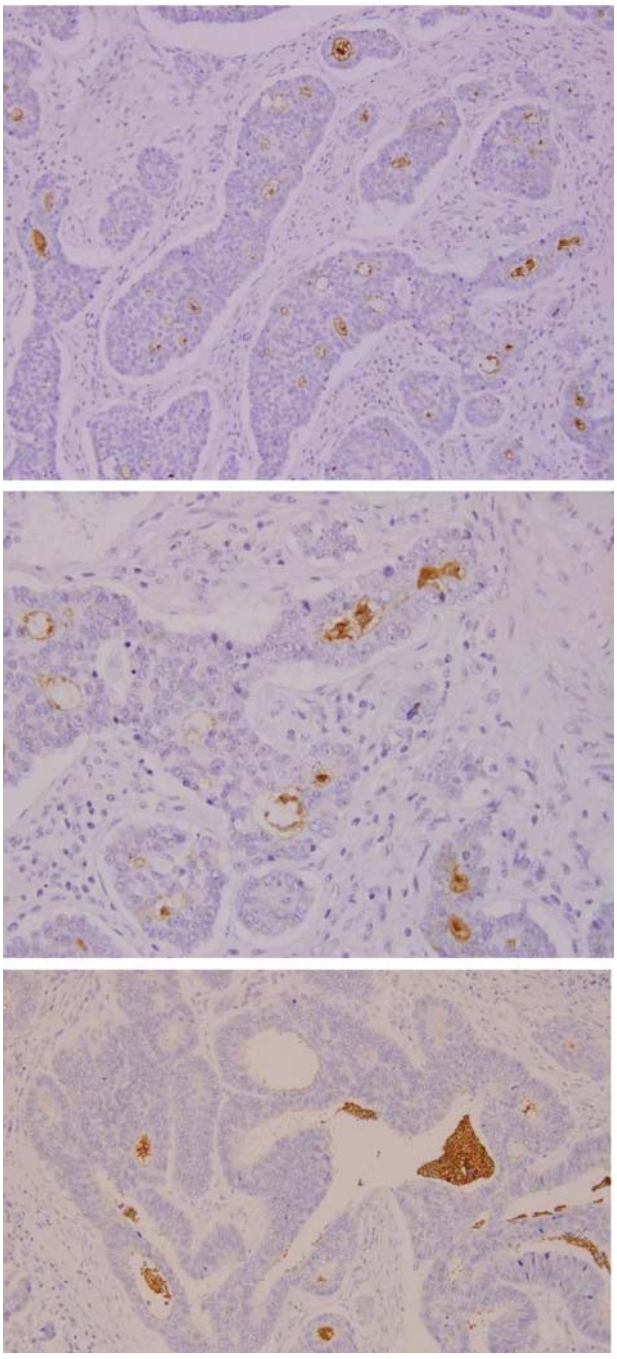

Figure 1. *CD133-positive gastric cancer cell (well-differentiated adenocarcinoma): Luminal expression of CD133 in the center of the cancer gland was partly identified.*

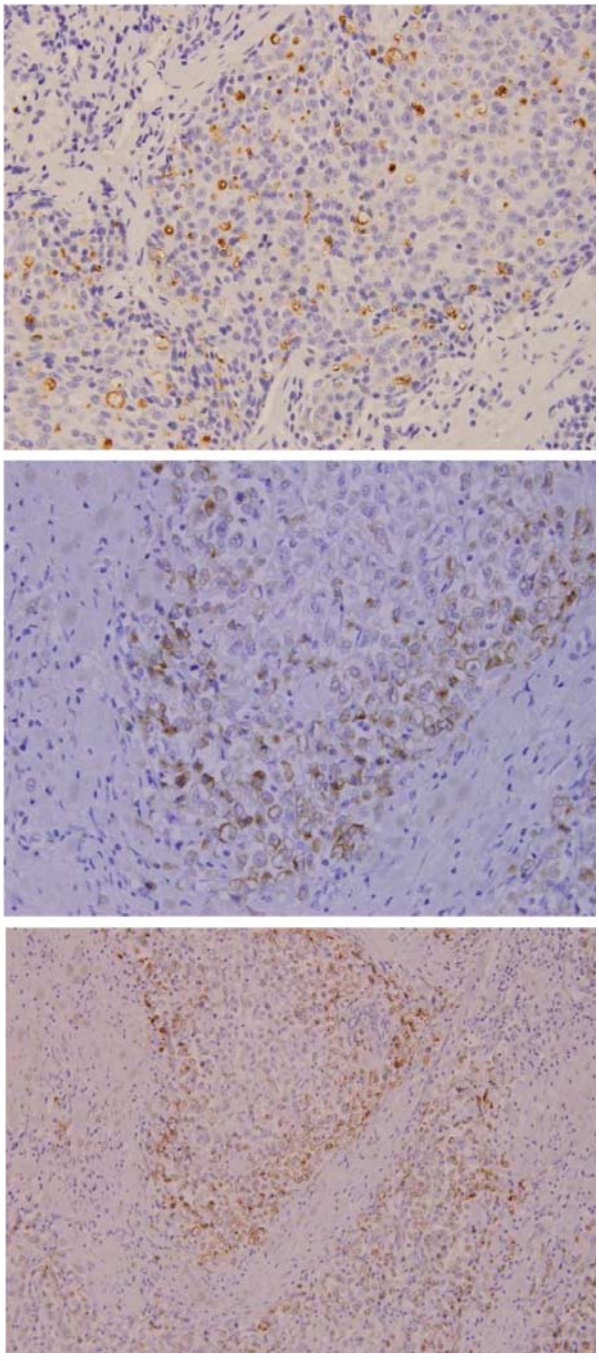

Figure 2. *CD133-positive gastric cancer cell (poorly differentiated adenocarcinoma): CD133 positivity was found in cytoplasm and cellular membrane.*

poorer than that of the CD133-negative group (29% *vs*. 78%, respectively; $p<0.01$, Figure 3). Using univariate analysis it was found that gender, age, nodal involvement and the depth of invasion significantly affected patient survival (data not shown).

When analysing these four clinical factors plus CD133 expression by multivariate analysis, CD133 expression was found to be an independent prognostic factor of gastric cancer (Table III).

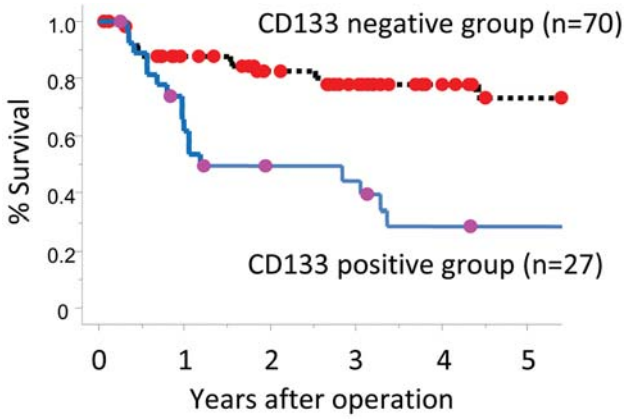

Figure 3. *Survival curves of 97 gastric cancer patients according to CD133 positivity. The CD133-positive group had significantly poorer survival than did the CD133-negative group (p<0.01).*

Table III. *Multivariate analyses of prognostic factors in gastric cancer.*

Independent factor	Multivariate *P*-value	Hazard ratio	95% Confidence interval
pT			
1, 2/3, 4	<0.0126	6.2	0.1-0.8
pN			
Negative/positive	<0.05	3.8	0.1-1.00
Age			
<70/≥70	0.6	1.3	0.3-1.3
CD133 positivity			
Yes/no	0.046	3.9	0.2-0.9

Discussion

CD133-positive expression has been detected in several types of cancer. In the current study, the positive rate in gastric cancer was 28%, which was similar to colon cancer (13), but less than pancreatic cancer (5) and hepatocellular carcinoma (14). Although positive expression was found in the cellular membrane up to 10%, the CD133-positive cells occupying the cancerous tissue comprised less than 1%. In the current study the CD133-positive group was defined as the presence of CD133-positive cells, similar to the evaluation of colon cancer (7). The minor population of CD133-positive cells can have significant clinical impact and may be compatible with one of the characteristics of CSC. This rarity also accorded with colon and pancreatic cancer (5,13) but not with glioma (11). Zeppernick *et al*. showed that not only the presence but also the volume of CD133-positive cells was a significant prognostic factor in glioma patients (15). The percentage and volume of CD133-positive cancer cells varied according to the tumour type.

Regarding the distribution of CD133-positive cells, positive cells were found only sparsely in cellular cytoplasm; moreover, CD133-positive material was found at the glandular-luminal surface. Cells with CD133-positive deposits were also identified in colon (13) and pancreatic cancer (16) and such cells were defined as CD133-positive cells. It has been reported that CD133-positive cancer cells simultaneously expressed other molecules. Maeda *et al*. (16) demonstrated a close relationship between VEGF-C and CD133 positivity in pancreatic cancer and they showed that cancerous CD133-positive cells lost CK20 positivity, suggesting high proliferative and non-epithelial potentiality. These properties make CD133-positive cells aggressive, with a metastatic potential.

A well-known characteristic of CSCs is undifferentiated carcinoma; however, in the present study the correlation between CD133 positivity and undifferentiated histology could not be clarified in gastric cancer. This may be partly because this study included only 21 cases of CD133 positivity and gastric cancer showed histological diversity. In tumour with a high percentage of CD133-positive cells, histology became undifferentiated in glioma. In contrast, Horst *et al*. (13) demonstrated that CD133 expression was not correlated with budding in colon cancer. CSCs of gastrointestinal cancer may not affect distinct histology. Further investigation of a large number of patients with CD133-positive expression is necessary.

This study is the first to clarify the prognostic significance of CD133 positivity in gastric cancer. The presence of CD133-positive cells is a significant prognostic factor in gliomas (15), and in colon (13), pancreatic (5) and prostatic cancer (17), which was in accordance with our results. Moreover, CD133 positivity in gastric cancer was selected as an independent prognostic factor following the depth of invasion. It is well-known that CSCs are resistant to chemotherapy (18) and radiotherapy (19). CSCs have the ability to resist treatment with anticancer agents because of slow cell cycling (15) and a minor population of CSCs, which were hard to kill by chemotherapy, may affect postoperative relapse.

CD133 can be a useful marker of CSCs to predict the risk of tumour recurrence; however, prognostic markers cannot be used preoperatively. Because of the minute population of CD133-positive cancer cells, cancerous CD133-positive cells could not be detected using biopsy specimens.

CD133 positivity in itself can be a target of antibody therapy. Smith *et al*. (20) showed that anti-CD133 antibody-drug conjugates eradicated $CD133^+$ gastric and hepatocellular tumours in SCID mice. This stem cell-targeted antibody therapy may improve the clinical outcome of these patients.

In conclusion, CD133 positivity may be a useful tool to predict surgical outcome in gastric cancer as a marker of CSC. Moreover, CD133-targeting antibody therapy is a promising treatment to overcome CSC-positive gastric cancer.

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