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Expression of CD133 is associated with poor prognosis in stage II colorectal carcinoma

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Abstract

CD133 is currently believed to be one of the best colorectal cancer stem cell markers. This study aimed to evaluate prognostic significance of CD133 expression in colorectal cancer patients.

A total of 303 patients with stage I to III colorectal cancer who underwent curative surgical resection from 2003 to 2008 at a single institution were included. CD133 expression was evaluated using immunohistochemical staining, and clinicopathological data were retrospectively reviewed. The patients were dichotomized after scoring CD133 expression (0 to 2+: low CD133 expression vs 3+ to 4+: high CD133 expression) according to the extent of area of CD133 positive tumor cells (<50% vs ≥50%) and pattern of staining (membranous staining of the luminal surface and/or staining of cellular debris in the tumor glands and cytoplasm).

The 5-year overall survival (OS) (61.9% vs 80.2%, $P = .001$) and disease-free survival (64.8% vs 75.8%, $P = .026$) were poorer in the high CD133 expression group than the low CD133 expression group. In the multivariate analysis for risk factors of OS in the whole population, higher nodal stage (N2 compared to N0: hazard ratio [HR] 3.141; 95% confidence interval [CI] 1.718–5.744, $P < .001$), perineural invasion (HR 2.262; 95% CI 1.347–3.798, $P = .002$) and high CD133 expression (HR 1.929; 95% CI 1.221–3.048, $P = .005$) were independent poor prognostic factors of OS. Subgroup analyses according to each TNM stage revealed that CD133 expression was associated with OS only within the stage II patients (HR 3.167 95% CI 1.221–8.216, $P = .018$). Furthermore, the stage II patients demonstrating the high CD133 expression showed survival benefit of adjuvant chemotherapy, regardless of high-risk feature positivity (HR 0.201 95% CI 0.054–0.750, $P = .017$).

High CD133 expression is correlated with poor prognosis in colorectal cancer patients after radical resection. The CD133 expression may serve as a more potent and informative biomarker for prognosis than conventional high-risk features in the stage II colorectal cancer patients.

Abbreviations: AJCC = American Joint Committee on Cancer, ASA Classification = American Society Of Anesthesiologists Classification, CEA = carcinoembryonic antigen, CI = confidence interval, CRC = colorectal cancer, CSCs = cancer stem cells, CT = computed tomography, DFS = disease-free survival, HR = hazard ratio, IHC = immunohistochemical, LN = lymph node, OS = overall survival, PBS = phosphate-buffered saline, PD = poorly-differentiated, SD = standard deviation.

Keywords: cancer stem cell, CD133, colorectal cancer, immunohistochemistry, prognosis, recurrence

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide and a leading cause of morbidity and mortality.[1] Tumor stage, based on the International Union Against Cancer (Union for International Cancer Control-TNM)[2] and American Joint Committee on Cancer (AJCC) classifications,[3] remains the most reliable prognostic factor in newly diagnosed CRC. Tumor budding, tumor grade, and the assessment of lymphovascular invasion are considered important pathological prognostic factors. Recently, various molecular analyses and protein markers have been investigated as a means of improving the identification of patients who are likely to experience poor clinical outcomes and may benefit from adjuvant therapy.

A model of cancer development involving cancer stem cells (CSCs) has been proposed as a potential explanation for tumor hierarchy. CSCs constitute a small minority of tumor cells that can maintain the malignant population.[4] CSCs are characterized by self-renewal capacities, the capability to develop into multiple lineages and the potential to proliferate extensively, and these cells present the typical marker profile consisting of CD44 and CD133 expression.[5] Moreover, the CSC hypothesis for cancer development and heterogeneity may explain chemotherapy and radiotherapy resistance and early tumor relapse.[6–9]

CD133 is a 120-kDa 5-transmembrane glycoprotein[4,10] that localizes to membrane protrusions. This molecule was first reported to be expressed in hematopoietic stem and progenitor cells[11] and has also been found on endothelial and lymphangiogenic progenitors. Although the function of CD133 remains unclear, CD133 has been suggested to be a CSC surface marker in various

Editor: Victor C. Kok.

The authors have no conflicts of interest to disclose.

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Medicine (2019) 98:32(e16709)

Received: 29 October 2018 / Received in final form: 10 July 2019 / Accepted: 11 July 2019

http://dx.doi.org/10.1097/MD.0000000000016709

tumors, including cancers of the pancreas,[12] bone,[13] kidneys,[14] lungs,[15] endometrium,[16] brain,[17] ovaries,[18] and liver.[19]

The majority of previous research supports the hypothesis that CD133 expression is predictive of survival. However, some investigators have reported that CRC patient survival is not related to CD133 expression. The present study aimed to investigate CD133 expression using immunohistochemical (IHC) staining and analyze the prognostic significance of CD133 expression in CRC.

2. Methods

2.1. The study was CD133 research based on consecutive patients which underwent a curative surgery for CRC

2.1.1. Patients. CRC specimens were selected from patients who underwent intentional curative surgical resection from 2003 to 2008 at the single institution in a university training hospital. We included all patients who were diagnosed with stage I-III CRC and underwent curative resection. We excluded patients who died within 1 month after surgical resection to exclude the death of acute surgical complication and excluded specimens with unavailable paraffin blocks. The final case collection included CRC specimens from 303 patients.

Clinicopathological parameters, including age, gender, American Society of Anesthesiologists (ASA) classification, cancer location, AJCC stage, tumor depth, lymph node (LN) metastasis, tumor differentiation, lymphatic invasion, perineural invasion, venous invasion, chemotherapy, and carcinoembryonic antigen (CEA) level, were collected retrospectively from the CRC Patients Registry of our center. High-risk factors for stage II patients included defined cancer obstruction, perforation, lymphovascular invasion, poor differentiation, and T4 stage. The pathological staging of all cases was performed according to the 7th edition of the AJCC TNM classification.[3] Patients with clinically high-risk stage II and stage III CRC received adjuvant chemotherapy, which based of 5-fluorouracil and leucovorin.

2.1.2. Follow-up. All patients were prospectively followed at 3-month intervals for the first 2 years, 6-month intervals for the next 3 years and yearly after that. The follow-up examinations included a physical examination, CEA, chest X-rays, colonoscopy, and abdominal computed tomography (CT). If recurrence was suspected, further studies, such as chest CT scans, whole-body bone scans, or whole-body positron emission tomography-CT scans, were conducted to clarify the site of recurrence. The definition of recurrence included a recurrent lesion proven by pathological confirmation or progressively increasing size in imaging studies. Patients with tumor recurrence were treated using chemotherapy, radiation therapy, or surgical resection, when possible.

The median follow-up period was 39 months (6–157 months). This study was approved by the Institutional Review Board of the Ethical Committee of the College of Medicine, Catholic University of Korea (UC14SISI0024).

2.2. CD133 immunohistochemistry

2.2.1. CD133 IHC stain. Formalin-fixed and paraffin-embedded human CRC tissues were sectioned at 4 mm for IHC staining. The slides were deparaffinized in xylene 3 times for 10 minutes each and then rehydrated in a graded series of ethanol before incubation for 15 minutes with a 3% hydrogen peroxide solution in methanol to inhibit endogenous peroxidase activity. For antigen retrieval, the slides were treated with 10 mM citrate buffer (pH 6.0) at 98°C for 15 minutes in a microwave oven and allowed to cool for 1 hour at room temperature. After incubation for 15 minutes in a blocking solution (Histo-Plus kit, Zymed, San Francisco, CA) containing 10% normal serum in phosphate-buffered saline (PBS), the sections were incubated at 4°C overnight in a humidified chamber with a rabbit polyclonal anti-CD133 primary antibody (diluted 1:200; Abnova., Taipei, Taiwan). A biotinylated secondary antibody (Histo-Plus kit, Zymed) was used to detect the primary antibody, and the slides were incubated for 10 minutes at 45°C. The sections were rinsed 3 times in PBS and incubated with a streptavidin-horseradish peroxidase complex (Histo-Plus kit, Zymed) for 10 minutes. Each slide was incubated for 2 minutes in 3,3′-diaminobenzidine tetrahydrochloride, and 50 mM tris-buffer (pH 7.6) containing 0.3% hydrogen peroxide as a chromogen and then counterstained with hematoxylin (Vector Laboratories, Inc, Burlingame, CA).

2.2.2. Evaluation of CD133 expression. We evaluated 1 slide per case under 20×10 magnification for both patterns and extent of CD133 staining. Regard to patterns, using previously published criteria,[20] CD133 immunostaining was evaluated as the membranous staining of the luminal surface and the staining of cellular debris in the tumor glands and cytoplasm. Regard to extent of area of CD133 positive tumor cells,[21] we divided the patients into 2 categories; $<50\%$ of stained tumor cells in the entire tumor area and $\geq 50\%$ of stained tumor cells in the entire tumor area. We scored CD133 expression by using patterns and extent as follows; a score of 0 corresponding to no staining; a score of 1+ indicating luminal staining only with extent of less than 50%; a score of 2+ indicating luminal and cytoplasmic staining with extent of less than 50%; a score of 3+ assigned to luminal staining only with extent of greater than 50%; and a score of 4+ assigned to luminal and cytoplasmic staining with extent of greater than 50%. Finally, we dichotomized the patients into low and high CD133 expression groups. The 0, 1+, and 2+ groups were considered to express CD133 weakly, whereas the 3+ and 4+ groups were considered to express CD133 highly. The specimens were evaluated by 3 researchers who did not know patient prognosis or other clinicopathological variables. Cases that were scored differently were discussed until a consensus was reached.

2.3. Statistical analyses

For quantitative variables, the 2 groups were compared using independent t tests, and the results were expressed as the means ± standard deviation. Categorical variables were analyzed using a χ^2 test or Fisher exact test. Survival analysis was performed using the Kaplan–Meier method with a log-rank test. A Cox proportional hazards regression model was used to estimate the hazard ratio (HR) and 95% confidence interval (CI). After univariate analyses for overall survival (OS) and disease-free survival (DFS), subsequent multivariate analyses were performed using the variables showing $P < .05$ in the univariate analyses. All statistical analyses were performed using SPSS version 18.0 for Windows (SPSS, Inc, Chicago, IL) and differences were considered to be statistically significant when the P-values were $<.05$.

3. Results

3.1. Clinicopathologic features

In the present study, 179 male and 124 female patients were included. The mean age of the patients was 63.6 ± 12.50, and the

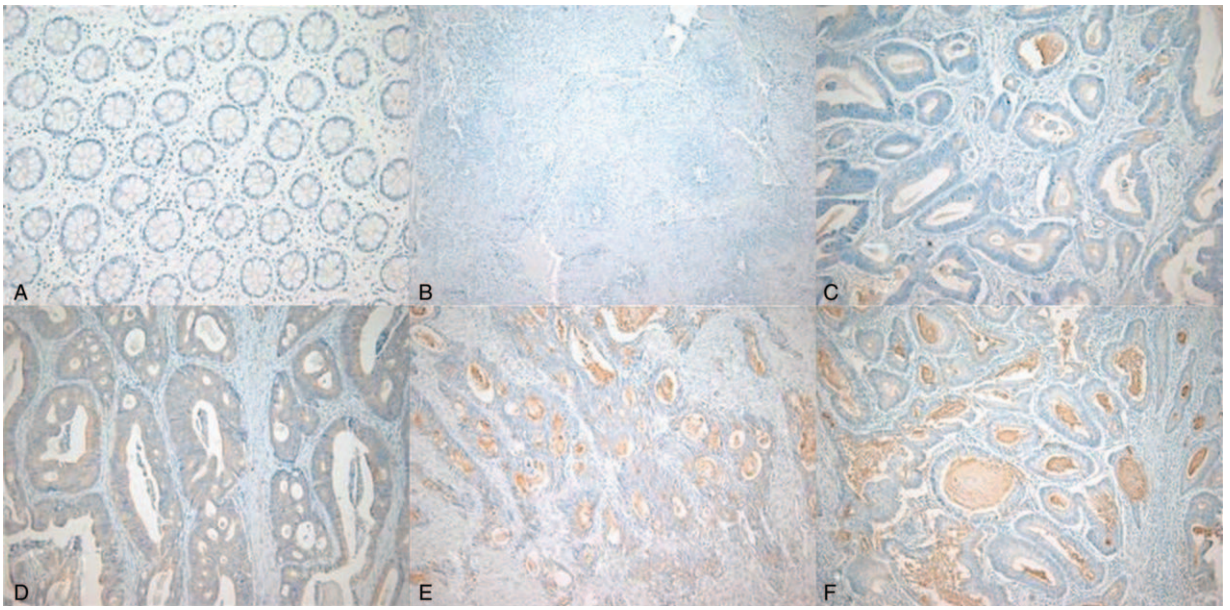

Figure 1. CD133 scoring in CRC specimens at 20 × 10 magnification. (A) Nontumor tissue was negative for CD133 expression. (B) Tumor tissue displayed no staining. (C) Only luminal staining <50%. (D) Luminal and cytoplasmic staining <50%. (E) Only luminal staining ≥50%. (F) Luminal and cytoplasmic staining ≥50%. CRC = colorectal cancer.

mean CEA value for all patients was 7.38 ± 28.76 ng/ml. There were 175 (57.8%) patients with colon cancer and 128 (42.2%) patients with rectal cancer. There were 43 (14.2%) patients with stage I, 107 (35.3%) with stage II and 153 (50.5%) with stage III cancer. Two hundred sixty-three (86.8%) patients underwent adjuvant chemotherapy.

3.2. CD133 expression in CRC

CD133 expression was mainly detected on the luminal surface membranes of epithelial tumor cells. CD133 was partially expressed in the cytoplasm of cancer cells and the intraglandular deposits of cancer lesions (Fig. 1). No staining was observed in 18 patients, and 285 patients demonstrated positive CD133 tumor cells. Regard to the patterns of CD133 expression, 48 patients showed only luminal membranous staining. There was no cytoplasm-only expression without luminal membranous staining, and cytoplasmic staining tumor cells always coexisted with luminal membranous staining tumor cells. High CD133 expression (3+ and 4+ groups) was detected in 100 of the 303 tumors (33%), and each frequency is shown in the Table 1.

3.3. Association between CD133 expression and clinicopathological factors

All clinicopathologic variables except nodal stage showed no differences between the low and the high CD133 expression groups. CD133 expression was not significantly correlated with TNM stage, histologic differentiation, lymphatic invasion, or perineural invasion. However, higher nodal stage was associated with high expression of CD133 with statistical significance ($P = .01$) (Table 2). Among 153 patients with stage III cancer, significant differences in LN metastases were observed ($P = .006$); the low CD133 expression group displayed N1 (77%) and N2 (23%) stage, while the high CD133 expression group displayed N1 (54.7%) and N2 (45.3%) stage.

3.4. CD133 expression as a prognostic factor

The 5- and 10-year OS rates of the high CD133 expression group were significantly poorer than those of the low CD133 expression (5-year: 61.9% vs 80.2%; 10-year: 51.1% vs 71.2%, $P = .001$). Moreover, the 5- and 10-year DFS rates of the CD133-high patients were also lower than those of the CD133-low patients (5-year: 64.8% vs 75.8%; 10-year: 32.4% vs 75.8%, $P = .026$ (Fig. 2).

Regard to prognostic factors of OS in the whole study population, the univariate analysis revealed that T stage, nodal stage, TNM stage, lymphatic invasion, perineural invasion, differentiation, adjuvant chemotherapy, and CD133 expression were significantly associated with OS. In the subsequent multivariate analysis, the patients who received adjuvant chemotherapy showed longer OS (HR 0.347; 95% CI 0.198–0.607, $P < .001$). Higher nodal stage (N2 compared to N0: HR

Table 1

Frequencies according to patterns and extent of CD133 expression.

			Pattern of staining	
		No staining	Luminal membranous staining only	Combined with cytoplasmic staining
Extent of CD 133 positive cells	<50%	18 (score 0)	15 (score 1+)	160 (score 2+)
	≥50%		33 (score 3+)	77 (score 4+)

Table 2

Clinicopathological parameters and CD133 expression in colorectal cancer patients (n = 303).

Variables		CD133 expression Low n (%)	CD133 expression High n (%)	*P*
Age, yr		64.23 ± 11.76	62.32 ± 13.85	.210
Sex	Male	121 (59.6)	58 (58.0)	.810
	Female	82 (40.4)	42 (42.0)	
ASA score	1–2	169 (83.3)	83 (83.0)	>.999
	≥3	34 (16.7)	17 (17.0)	
Location	Colon	120 (59.1)	55 (55.0)	.490
	Rectum	83 (40.9)	45 (45.0)	
CEA, ng/ml		6.0 ± 20.5	10.3 ± 41.0	.230
AJCC stage	Stage I	32 (15.8)	11 (11.0)	.340
	Stage II	71 (35.0)	36 (36.0)	
	Stage III	100 (49.3)	53 (53.0)	
Tumor depth	T1	5 (2.5)	2 (2.0)	.210
	T2	42 (20.7)	15 (15.0)	
	T3	132 (65.0)	64 (64.0)	
	T4	24 (11.8)	19(19.0)	
Lymph node metastasis	N0	103 (50.7)	47 (47.0)	.010
	N1	77 (37.9)	29 (29.0)	
	N2	23 (11.3)	24 (24.0)	
Differentiation	Well/moderate	180 (88.7)	85 (85.0)	.230
	Poor/mucinous	23 (11.3)	15 (15.0)	
Lymphatic invasion	absent	94 (46.3)	45 (45.0)	.460
	Present	109 (53.7)	55 (55.0)	
Perineural invasion	Absent	172 (84.7)	80 (80.0)	.190
	Present	31 (15.3)	20 (20.0)	
Chemotherapy	No	24 (11.8)	16 (16.0)	.370
	Yes	179 (88.2)	84 (84.0)	

AJCC = American Joint Committee on Cancer, ASA = American society of anesthesiologists classification, CEA = carcinoembryonic antigen.

3.141; 95% CI 1.718–5.744, $P < .001$), perineural invasion (HR 2.262; 95% CI 1.347–3.798, $P = .002$), and high CD133 expression (HR 1.929; 95% CI 1.221–3.048, $P = .005$) were independent poor prognostic factors of OS.

Meanwhile, regard to prognostic factors of DFS in the whole study population, rectal cancer, tumor depth, nodal stage, TNM stage, lymphatic invasion, perineural invasion, differentiation, and CD133 expression was significantly associated with DFS. However, the multivariate analysis revealed that only rectal cancer (HR 2.662; 95% CI 1.661–4.268, $P < .001$), advanced T stage (T3/4) (HR 2.185; 95% CI 1.034–4.617, $P = .041$), and higher nodal stage (N1 compared to N0: HR 2.609; 95% CI 1.496–4.548, $P = .001$, N2 compared to N0: HR 4.105; 95% CI 2.173–7.755, $P < .001$) were independent poor prognostic factors of DFS (Table 3).

In the subgroup analysis according to each pathologic stage, CD133 expression was not associated with survival in both stage I and stage III subgroups. However, in the stage II disease, the patients with high CD133 expression showed shorter OS than those with low CD133 expression with statistical significance (5-year OS: 70.0% vs 85.5%, 10 year OS: 42.0% vs 80.7%) (Fig. 3). After univariate analysis for the variables that might affect prognosis of stage II CRC including high-risk feature positivity, further multivariate analysis for prognostic factors of OS in the stage II subgroup patients was conducted. It revealed that T4 (HR 3.481; 95% CI 1.324–9.148, $P = .011$), high CD133 expression (HR 3.167; 95% CI 1.221–8.216, $P = .018$), and chemotherapy (HR 0.217; 95% CI 0.085–0.554, $P = .001$) were independent prognostic factors of OS (Table 4).

With consideration of possible selection bias for the patients who did not receive adjuvant chemotherapy due to poor physical performance after surgery, despite that preoperative ASA classification was not significantly different between the high and low CD133 expression groups in the study population, we further performed subgroup analysis within the patients who received adjuvant chemotherapy. Among 263 patients who received adjuvant chemotherapy, there were more patients with N2 stage in the high CD133 expression group (n = 84) than in the low CD33 1expression group (n = 179); however, the other variables were not different between the 2 groups. As for univariate analysis for prognostic factors of OS and DFS, rectal cancer, T stage, nodal stage, TNM stage, lymphatic invasion,

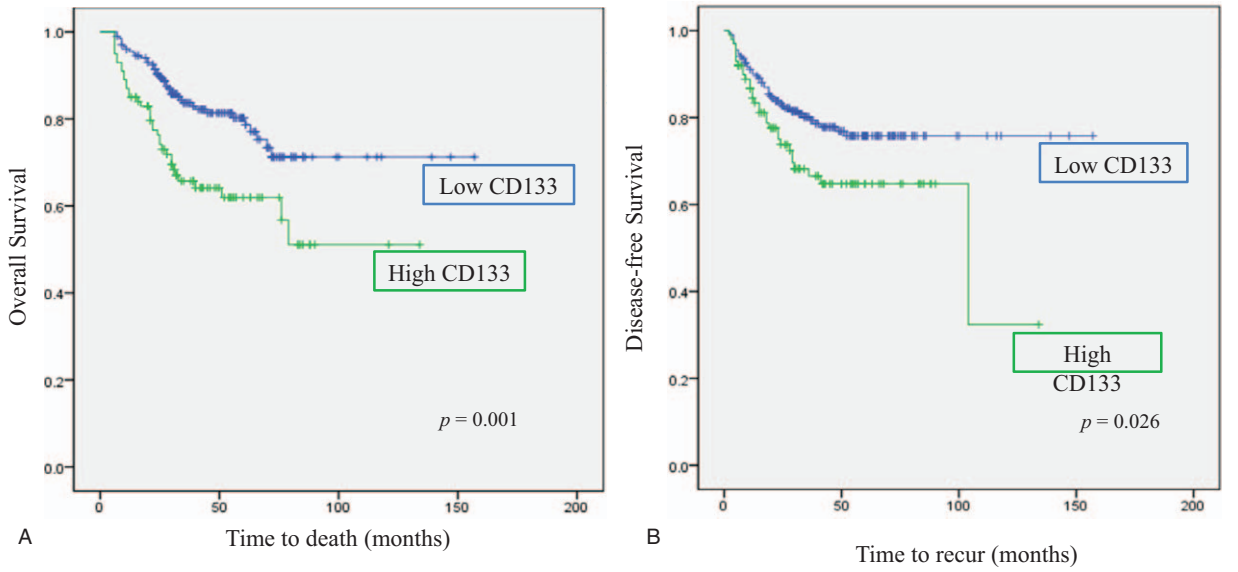

Figure 2. Kaplan–Meier curves for overall survival and disease-free survival in the whole population. (A) The high CD133 expression group displayed significantly lower overall survival. (B) The high CD133 expression group displayed significantly lower disease-free survival.

Table 3

Cox proportional hazard model of overall and disease-free survival in colorectal cancer patients (n = 303).

	OS				DFS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age	1.004 (0.985–1.023)	.706			0.991 (0.973–1.010)			
Sex (male)	1.030 (0.649–1.635)	.901			1.021 (0.641–1.625)	.931		
ASA ≥3	1.061 (0.583–1.930)	.846			0.677 (0.337–1.359)	.273		
Location (rectum)	1.546 (0.983–2.433)	.059			2.332 (1.461–3.723)	<.001	2.662 (1.661–4.268)	<.001
CEA, ng/ml	1.003 (0.998–1.008)	.259			0.999 (0.991–1.008)	.863		
Stage III	2.074 (1.288–3.339)	.003			3.102 (1.855–5.189)	<.001		
≥T3	2.510 (1.205–5.227)	.014			2.552 (1.225–5.316)	.012	2.185 (1.034–4.617)	.041
N stage								
0	1		1		1		1	
1	1.438 (0.830–2.490)	.195	1.236 (0.707–2.161)	.457	2.666 (1.533–4.638)	.001	2.609 (1.496–4.548)	.001
2	3.870 (2.219–6.750)	<.001	3.141 (1.718–5.744)	<.001	4.299 (2.308–8.006)	<.001	4.105 (2.173–7.755)	<.001
Lymphatic invasion	2.440 (1.483–4.016)	<.001			2.389 (1.449–3.937)	.001		
Perineural invasion	2.956 (1.813–4.819)	<.001	2.262 (1.347–3.798)	.002	2.493 (1.501–4.140)	<.001		
Differentiation (PD and mucinous)	2.253 (1.312–3.870)	.003			2.195 (1.258–3.831)	.006		
High CD133	2.170 (1.379–3.415)	.001	1.929 (1.221–3.048)	.005	1.680 (1.058–2.668)	.028		
Chemotherapy	0.394 (0.229–0.677)	.001			1.479 (0.641–3.413)	.358		

ASA = American society of anesthesiologists classification, CEA = carcinoembryonic antigen, CI = confidence interval, DFS = disease-free survival, HR = hazard ratio, OS = overall survival, PD = poorly-differentiated.

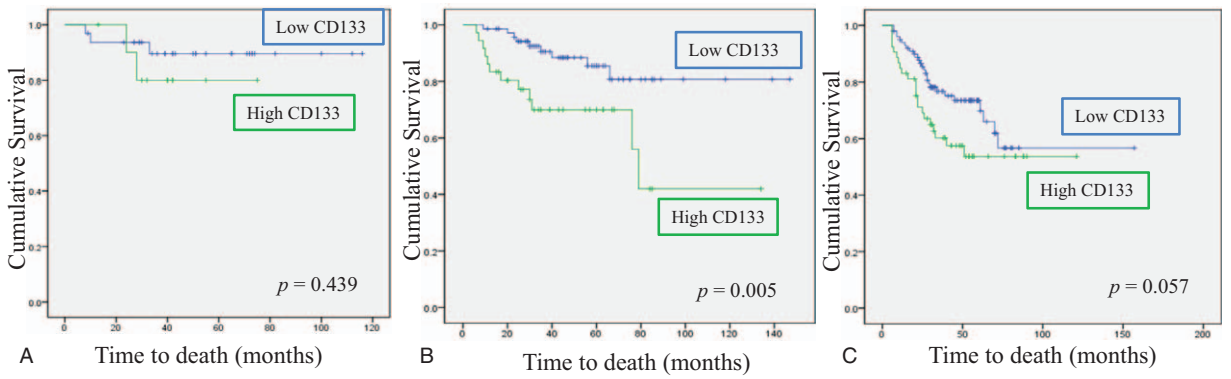

Figure 3. Kaplan–Meier curves for overall survival in each stage. (A) No significant difference in survival was shown in stage I patients. (B) The high CD133 expression group displayed significantly lower overall survival in stage II patients. (C) No significant difference in survival was shown in stage III patients.

Table 4

Subgroup analyses of overall and disease-free survival in the stage II patients (n = 107).

	OS				DFS	
	Univariate analysis		Multivariate analysis		Univariate analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age	1.060 (1.014–1.108)	.010			0.994 (0.956–1.033)	.760
Sex (male)	2.228 (0.859–5.774)	.099			1.482 (0.548–4.008)	.439
ASA ≥3	1.472 (0.538–4.026)	.452			0.616 (0.141–2.697)	.521
Location (rectum)	1.148 (0.483–2.726)	.754			1.823 (0.703–4.727)	.217
CEA, ng/ml	1.007 (0.999–1.014)	.069			0.997 (0.973–1.022)	.824
T4	2.544 (1.021–6.341)	.045	3.481 (1.324–9.148)	.011	2.400 (0.844–6.826)	.101
Lymphatic invasion	1.373 (0.532–3.544)	.512			1.006 (0.327–3.093)	.992
Perineural invasion	1.871 (0.541–6.477)	.323			0.566 (0.075–4.274)	.581
Differentiation (PD and mucinous)	1.239 (0.365–4.213)	.731			1.661 (0.631–4.370)	.304
High CD133	3.193 (1.343–7.589)	.009	3.167 (1.221–8.216)	.018	1.840 (0.684–4.949)	.227
Chemotherapy	0.163 (0.067–0.398)	<.001	0.217 (0.085–0.554)	.001	0.734 (0.209–2.574)	.629
High risk feature (+)	1.750 (0.724–4.231)	.214			2.573 (0.905–7.314)	.076

ASA = American society of anesthesiologists classification, CEA = carcinoembryonic antigen, CI = confidence interval, DFS = disease-free survival, HR = hazard ratio, OS = overall survival, PD = poorly-differentiated.

Table 5

Subgroup analyses of overall and disease-free survival in the patients who received adjuvant chemotherapy (n = 263).

	OS				DFS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age	0.989 (0.968–1.011)	.322			0.992 (0.972–1.012)	.410		
Sex (male)	0.972 (0.574–1.645)	.915			0.844 (0.521–1.366)	.489		
ASA ≥3	0.945 (0.448–1.993)	.881			0.758 (0.363–1.586)	.462		
Location (rectum)	1.732 (1.032–2.907)	.038			2.419 (1.482–3.950)	<.001	2.664 (1.625–4.367)	<.001
CEA, ng/ml	1.008 (1.002–1.014)	.005	1.008 (1.001–1.014)	.030				
Stage III	3.194 (1.773–5.755)	<.001			3.456 (1.993–5.995)	<.001		
≥T3	3.875 (1.403–10.703)	.009			3.100 (1.341–7.169)	.008		
N stage								
0	1				1		1	
1	2.060 (1.054–4.026)	.035	1.802 (0.883–3.678)	.106	2.913 (1.610–5.271)	<.001	2.671 (1.463–4.876)	.001
2	6.166 (3.211–11.840)	<.001	4.048 (1.934–8.469)	<.001	4.868 (2.544–9.315)	<.001	4.405 (2.205–8.799)	<.001
Lymphatic invasion	3.522 (1.928–6.434)	<.001			2.673 (1.585–4.509)	<.001		
Perineural invasion	4.309 (2.536–7.321)	<.001	2.590 (1.410–4.759)	.002	2.991 (1.783–5.017)	<.001	2.049 (1.182–3.552)	.011
Differentiation (PD and mucinous)	2.142 (1.155–3.972)	.016			1.816 (0.989–3.336)	.054		
High CD133	2.159 (1.288–3.619)	.004	1.938 (1.127–3.333)	.017	1.822 (1.126–2.948)	.015		

ASA = American society of anesthesiologists classification, CEA = carcinoembryonic antigen, CI = confidence interval, DFS = disease-free survival, HR = hazard ratio, OS = overall survival, PD = poorly-differentiated.

perineural invasion, differentiation, CEA level, and CD133 expression were associated with OS; rectal cancer, T stage, nodal stage, TNM stage, lymphatic invasion, perineural invasion, and CD133 expression were associated with DFS. The multivariate analyses revealed that higher nodal stage (N2 compared to N0: HR 4.048; 95% CI 1.934–8.469, $P < .001$), perineural invasion (HR 2.590; 95% CI 1.410–4.759, $P = .002$), high CD133 expression (HR 1.938; 95% CI 1.127–3.333, $P = .017$), and higher CEA level (HR 1.008; 95% CI 1.001–1.014, $P = .030$) were independent poor prognostic factors of OS; higher nodal stage (N1 compared to N0: HR 2.671; 95% CI 1.463–4.876, $P = .001$, N2 compared to N0: HR 4.405; 95% CI 2.205–8.799, $P < .001$), and perinueral invasion (HR 2.049; 95% CI 1.182–3.552, $P = .011$) were independent poor prognostic factors of DFS (Table 5).

We further investigated whether adjuvant chemotherapy benefited the patients in terms of OS according to the CD133 expression status. Adjuvant chemotherapy, as a result of multivariate analyses, had survival benefit in both within the high CD133 subgroup patients (Table 6) and within the low CD133 subgroup patients (HR 0.382; 95% CI 0.171–0.852, $P = .019$). Interestingly, the stage II CRC patients with high CD133 expression had a survival benefit of adjuvant chemotherapy, regardless of high-risk feature positivity (5-year OS in those with adjuvant chemotherapy vs without adjuvant chemotherapy: 81.5% vs 43.6%) (Table 7), but not within the stage III patients (HR 2.144; 95% CI 0.288–15.960, $P = .456$). On the contrary, the stage II CRC patients with low CD133 expression were not benefited from adjuvant chemotherapy (HR 0.299; 95% CI 0.062–1.451, $P = .134$). The 5-year OS in those with adjuvant

Table 6

Subgroup analysis of overall survival in the patients with high CD133 expression (n = 100).

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age	0.995 (0.970–1.020)	.677		
Sex (male)	0.767 (0.398–1.476)	.427		
ASA ≥3	0.921 (0.383–2.215)	.854		
Location (rectum)	1.403 (0.728–2.703)	.312		
CEA, ng/ml				
Stage III	1.247 (0.730–2.791)	.298		
≥T3	2.690 (0.824–8.782)	.101		
N stage				
0	1		1	
1	0.500 (0.180–1.392)	.185	0.465 (0.166–1.308)	.147
2	3.099 (1.524–6.304)	.002	3.669 (1.483–9.074)	.005
Lymphatic invasion	1.950 (0.974–3.905)	.059		
Perineural invasion	3.718 (1.843–7.501)	<.001	2.339 (1.091–5.012)	.029
Differentiation (PD and mucinous)	1.204 (0.500–2.896)	.679		
Chemotherapy	0.439 (0.205–0.939)	.034	0.222 (0.089–0.556)	.001

ASA = American society of anesthesiologists classification, CEA = carcinoembryonic antigen, CI = confidence interval, HR = hazard ratio, PD = poorly-differentiated.

Table 7

Subgroup analysis of overall survival in stage II patients with high CD133 expression (n = 36).

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age	1.055 (0.995–1.117)	.072		
Sex (male)	2.514 (0.747–8.461)	.137		
ASA ≥3	1.230 (0.333–4.549)	.756		
Location (rectum)	0.908 (0.285–2.887)	.870		
CEA, ng/ml	1.016 (0.926–1.114)	.738		
T4	7.763 (2.134–28.231)	.002	7.763 (2.134–28.231)	.002
Lymphatic invasion	1.989 (0.522–7.581)	.314		
Perineural invasion	1.350 (0.166–10.995)	.779		
Differentiation (PD and mucinous)	0.328 (0.042–2.546)	.287		
Chemotherapy	0.158 (0.045–0.550)	.004	0.201 (0.054–0.750)	.017
High risk feature (+)	1.274 (0.403–4.026)	.680		

ASA = American society of anesthesiologists classification, CEA = carcinoembryonic antigen, CI = confidence interval, HR = hazard ratio, PD = poorly-differentiated.

chemotherapy and without adjuvant chemotherapy was 87.9% and 58.3%, showing no statistically significant difference in survival curves ($P = .112$) in them.

4. Discussion

CSCs are well known for their tumor progression capacity and role in early recurrence, metastasis, and resistance to chemoradiotherapy. Various markers have been found to be expressed on the surface of CSCs, among which CD133 has garnered much attention and importance. In the present study, we investigated the expression and prognostic significance of the CSC marker CD133 in CRC using an IHC approach. CD133 was highly expressed in 33% of CRC patients, which is consistent with the results of other studies.[20,22] This study also analyzed the association between CD133 expression and CRC survival during each stage and revealed that high CD133 expression was associated with lower survival.

Previous studies have revealed controversial findings regarding the pattern (cytoplasmic vs membranous) and distribution of CD133 IHC staining in CRC.[9,22,23] In the present study, CD133 expression was defined as membranous staining of the luminal surface or staining of cellular debris in the tumor glands as well as cytoplasmic staining in the tumor. At our institution, we previously investigated CD133 expression in gastric cancer using similar methods, with CD133 deposits in tumor glands and cytoplasmic staining considered to represent positive expression.[24] Recent studies have shown that CD133 expression at different locations within the cell (cytoplasm or membrane) indicates different cellular functions of CD133[25]; for example, apical/endoluminal membranous CD133 staining was characteristic of well-oriented, polarized, and differentiated cells, whereas cytoplasmic CD133 staining was observed in a minor population of cells, suggesting that cytoplasmic CD133 staining in cancer cells may be indicative of putative CSCs.

Many studies have demonstrated that CD133 expression is correlated with survival, recurrence, metastases and chemotherapy resistance, and most studies support the hypothesis that high CD133 expression is a poor prognostic marker.[8,20,26] However, Choi et al[27] investigated 523 CRC patients with various tumor stages using an IHC approach and reported that survival was not significantly related to CD133 expression. In addition, Kijima et al[28] analyzed samples from 189 patients with different stages of CRC using IHC and found that CD133 overexpression was not correlated with recurrence-free survival but was associated with significantly poorer OS.

Differences in the patient cohort, antibodies used for IHC, tissue samples (cell lines vs human tissue), tissue sampling method (individually mounted tissue slides vs tissue microarrays), methods of detection (IHC expression vs polymerase chain reaction-based techniques)[29] and methods used to score positive CD133 IHC expression may have generated the inconsistent results reported in previous studies. Moreover, in studies involving IHC, the evaluation criteria are dependent upon the research conducted, as some studies evaluate IHC results as positive or negative, while others evaluate IHC results as a high or low expression.

In the present study, stage IV patients, who may present a significant statistical confounding bias, were excluded, and individually mounted tissue slides were used. The correlation between CD133 expression and patient survival based on CRC stage differed significantly among the total patient group, as patients who expressed low CD133 levels displayed better OS rates than those who expressed high CD133 levels, especially for cases of stage II disease. However, stage I and III patients who expressed low CD133 levels showed a trend of better OS rates, although the differences were not statistically significant.

According to the National Comprehensive Cancer Network guidelines, obstruction, perforation, lymphovascular invasion, poor differentiation, and T4 status are considered clinical high-risk features for stage II CRC, and adjuvant chemotherapy is recommended for stage II CRC patients with any high-risk features. In our study, high CD133 expression more accurately predicted prognosis than positivity of any clinical high-risk features traditionally used to predict prognosis

Stage II CRC is very heterogeneous, showing wide range of differences in survival rates among stage IIa to IIc. In that sense, it is important to select patients who might benefit from adjuvant chemotherapy. Microsatellite stability or mismatch repair (MMR) status has been suggested as a prognostic factor and one of the valuable predictive biomarkers for efficacy of adjuvant chemotherapy in the stage II CRC patients.[30] As regard to the subgroup analyses to evaluate benefit of adjuvant chemotherapy in the stage II CRC patients in this study, benefit of adjuvant chemotherapy was demonstrated only in the patients with high CD133 expression. This study showed good prognosis of stage II CRC patients with low CD133 expression and no impact of adjuvant chemotherapy for further increase of OS in them.

Meanwhile, prognosis of the stage II CRC patients with high CD133 expression was much poorer than those with low CD133 expression; but they were benefited from adjuvant chemotherapy revealing comparable 5-year OS rate to those in the stage II CRC patients with low CD133 expression. This result resembles those in the report by Sargent et al,[31] demonstrating poorer prognosis of CRC patients with proficient MMR (pMMR) compared to those with defective MMR (dMMR) and beneficial outcome after adjuvant chemotherapy in CRC patients with pMMR, contrary to no benefit in CRC patients with dMMR in stage II/III CRC. Recently, Cheah et al[32] reported that pMMR correlated with high CD133 expression, whereas dMMR with low CD133 expression. They hypothesized that CRC stem cells may benefit from pMMR system promoting continued self-perpetuation. Based on these findings, CD133 expression may serve as a possible predictive biomarker for adjuvant chemotherapy. However, the number of high CD133 patients in each subgroup was too limited to draw concrete evidence; therefore, further study conduction is needed; such as randomized controlled trials on benefit of adjuvant chemotherapy in stage II CRC with high CD133 expression or on possible induction of resistance to chemotherapeutic agents in advanced CRC patients with high CD133 expression.

This study might have pitfalls due to its retrospective nature such as selection bias. Nevertheless, this study gives an important implication that there is a room for use of CD133 expression not only as a prognostic factor but also as an additional guidance to consider candidates who might benefit from adjuvant chemotherapy in stage II CRC patients in clinical setting, although standardization of evaluating method and determination of cut-off value are still remained problem for practical use. In that sense, further randomized controlled study to evaluate survival benefit of adjuvant chemotherapy in stage II CRC patients are highly suggested.

5. Conclusion

In conclusion, CD133 expression in CRC is correlated with prognosis and survival in CRC patients after curative radical resection. Moreover, the CD133 expression may serve as a more potent and informative biomarker for prognosis than conventional high-risk features in the stage II CRC patients

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