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CD133 Expression Predicts Relapse in Patients With Locally Advanced Rectal Cancer Treated With Neoadjuvant Chemotherapy

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Abstract. *Aim: The aim of the present study was to explore the association between CD133 expression and postoperative relapses in patients with locally advanced rectal cancer (LARC) who received neoadjuvant chemotherapy (NAC). Patients and Methods: We retrospectively examined 52 patients with LARC (cT3-4, Nany, M0) who received oxaliplatin-based NAC before surgery. CD133 expression was evaluated using immunohistochemistry and divided into low and high expression groups. Results: High CD133 expression was observed in 22 patients (42.3%). Patients with high CD133 expression had more frequent vessel invasion and relapse than those with low CD133 expression (p=0.013 and p=0.036, respectively). Comparing the low with high CD133 expression groups, the 4-year relapse-free survival rates were 82.2% vs. 46.3% (p=0.009). Multivariate analysis indicated that CD133 expression was an independent risk factor for relapse (HR=3.138; 95%CI=1.046-9.412; p=0.041). Conclusion: CD133 expression may be a predictive biomarker for postoperative relapse in patients with LARC who received NAC before surgery.*

In Western countries, the combination of neoadjuvant chemoradiotherapy (NACRT), total mesorectal excision (TME) surgery and adjuvant chemotherapy is the current standard of care in the management of locally advanced rectal cancer (LARC) (1). These treatments reduce local relapse rates to less than 10% (2-4). However, distant relapse rates for rectal cancer treated with NACRT have still been consistently >25% (2, 3). On the other hand, chemoradiotherapy for rectal cancer is known to be associated with several complications such as intraoperative complications, urinary and sexual dysfunction, intestinal and defecation problems, and secondary carcinogenesis (5, 6). Therefore, it has been suggested that in order to improve the rate of distant relapse, therapeutic strategies need to introduce earlier systemic treatments to prevent dissemination of micrometastasis (7). Neoadjuvant chemotherapy (NAC) without radiation therapy for LARC has recently been tried in order to improve distant control and avoid radiation toxicities without compromising local control (7, 8).

Recent studies have demonstrated that a small population of cancer cells, known as cancer stem cells (CSCs), may be the main initiation of the metastasis and recurrence (9). In addition, investigation of the CSCs characteristics, and other convincing evidence have suggested that CSCs play an important role in the resistance to chemo- and radio-therapies (10, 11). Although the functional role of CD133 in tumor progression and the CSC phenotype remains controversial, recent investigations have actively examined the significance of CD133 as a CSC marker (12, 13). Among CSC markers, CD133 is widely used to address malignant potential and prognostic biomarker in several solid cancers (14-16). Moreover, CD133-positive cells have been shown to be more resistant to chemo- and radio-therapies compared to CD133-negative tumor cells in cancer (10, 17).

Although it has been reported that there is a large number of predictive biomarkers associated with postoperative outcome in patients with LARC who underwent neoadjuvant therapy, the most important predictors of the postoperative outcome remain the pathological findings, which include the degree of bowel wall penetration and nodal involvement (18, 19). Other recent reports have also demonstrated that CD133 expression is closely associated with the postoperative outcome in LARC patients who received NACRT (20-22). To the best of our knowledge, there have yet to be any reports describing CD133 expression in patients with LARC who received NAC. Thus, the present study, evaluated CD133 expression, for the first time, in patients with LARC who received NAC.

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Key Words: CD133 Expression, rectal cancer, relapse, neoadjuvant chemotherapy.

Patients and Methods

Patients and specimens. A total of 52 patients who were diagnosed LARC (clinical-stage T3/4 and/or Nany, and M0) and received surgical resection after NAC at Saitama Medical Center, Dokkyo Medical University in April 2010 and June 2018, were evaluated in this retrospective study. Thirty-one patients who treated with NACRT followed by surgical resection were excluded within the above period in the present study. This study was approved the Institutional Review Board at the Saitama Medical Center, Dokkyo Medical University (No.1534).

All patients were preoperatively assessed for accurate diagnosis using colonoscopy, pelvic magnetic resonance imaging (MRI), Chest and abdominopelvic computed tomography. The Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation was evaluated by the preoperative biopsy tissue. The clinical lymph node metastasis was judged as lymph node-positive (N-positive) when there were perirectal or lateral pelvic lymph nodes with a diameter ≥7mm. The improvement of T (tumor) and N (lymph node) states after the surgical resection as compared to the pretreatment state based on the MRI before surgery, was defined as T- and N-downstaging, respectively.

Tumors were staged according to the eighth edition of the TMN classification of malignant tumors (23). Histological effect of tumor regression was classified according to the Japanese Society for Cancer of the Colon and Rectum: Grade 0, no regression; Grade 1a, minimal effect (necrosis less than one-third of the lesion); Grade 1b, mild effect (necrosis less than two-thirds but one-third or more of the lesion); Grade 2, moderate effect (necrosis more than two-thirds of the lesion); Grade 3, absence of residual tumor cells (24). Tumors were divided into a non-responder Group (Grade0, 1a and 1b) and a responder Group (Grade 2 and 3).

Neoadjuvant therapy. The NAC regimen which was decided based on the KRAS state, was scheduled as previously described (25). If patients had proven wild-type KRAS, 2 cycles of NAC-SOX+Cetuximab: S-1 (80 mg/m^2) from days 1 to 14, oxaliplatin (85 mg/m^2) on day 1, plus cetuximab (400 mg/m^2 as the initial dose, followed 250 mg/m^2/week) were given. If patients had proven non-wild-type KRAS, 1-2 cycles of SOX, 2-9 cycles of NAC-mFOLFOX6: in 2-week cycles (5-fluorouracil bolus 400 and 2400 mg/m^2 continuous infusion over 46h, L-leucovorin 200 mg/m^2, oxaliplatin, 85 mg/m^2), or 2-3 cycles of NAC-XELOX: capecitabine (1,000 mg/m^2) twice daily on days 1-14 and oxaliplatin (130 mg/m^2) on day 1 were given. Surgical resection was planned 4-6 weeks after completion of NAC.

Surgery. Total mesorectal excision (TME) or tumor-specific mesorectal excision with/without sphincter preservation was performed by the standardized techniques. Laparoscopic surgery was performed in 44 patients (84.6%), and a conventional open approach was performed in 8 patients (15.4%). A sphincter-preserving operation, an abdominoperineal resection, and a Hartmann operation were performed in 28 patients (53.8%), in 21 patients (40.4%), and in 3 patients (5.8%), respectively. The choice of surgical procedure and creation of a temporary diverting ostomy were depended on the surgeon's discretion. Lateral pelvic lymph-node dissection was performed in 11 patients (21.1%), as there was suspicious node-positivity on the MRI prior to the NAC.

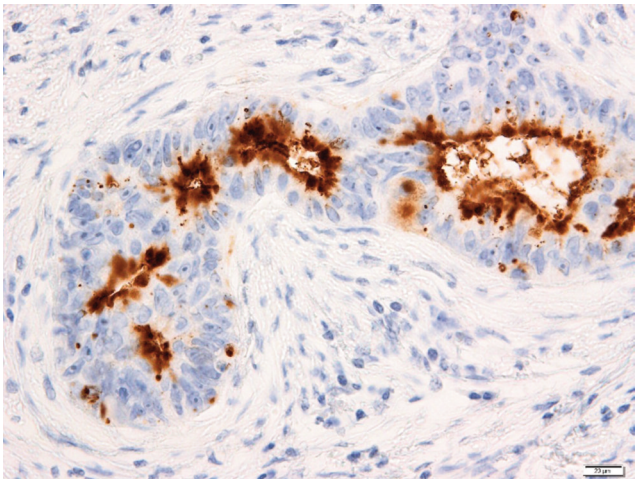

Figure 1. *Immunohistochemical analysis of CD133 expression in the invasive front of primary tumors. CD133-expressing cancer cells were observed on the glandular luminal surface of rectal cancer gland and the intraglandular cellular debris. (Magnification ×400).*

Postoperative follow-up and adjuvant chemotherapy. Postoperative follow-up including laboratory and physical examinations was carried out every 3 months, and CT scan of chest, abdominal and pelvis was carried out every 6 months for until 5 years after surgery. Colonoscopy were performed every 1 year. Adjuvant chemotherapy (AC) was performed to patients with ypT4 or ypN+ for 6 months using a regimen including 5FU-leucovorin, capecitabine, SOX or FOLFOX. The choice of regimen was depended on the attending physician.

Immunohistochemical staining of CD133. Tumor specimens were fixed in 10% neutral-buffered formalin for 48 h, embedded in paraffin, cut into 4-μm-thick sections, and then mounted on silane-coated glass slides. Sections were deparaffinized and immersed in a 0.3% hydrogen peroxide solution in methanol for 15 min to inhibit endogenous peroxidase activity. After washing with phosphate-buffered saline (PBS), the sections were placed in ethylenediaminetetraacetic acid buffer at pH8.0 for CD133 staining. For antigen retrieval, slides were heated at 95˚C for 20 min in a waterbath and allowed to cool at room temperature. Each slide was incubated for 1 h at room temperature with the primary anti-CD133 antibody (AC133; Miltenyi Biotec, Auburn, CA, USA) at a dilution of 1:100. After washing the slides three times with PBS, they were incubated with EnVision complex (Dako A/S, Glostrup, Denmark) for 20 min at room temperature, and after three washes with PBS, each slide was incubated for 3 min in 2% 3, 3'-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin.

Evaluation of CD133 expression. CD133 expression was evaluated in terms of the staining intensity, percentage of positive cancer cells, and staining pattern. Staining of CD133 was observed on the apical luminal surface of the rectal cancer glands, with most of the CD133-positive tumors showing CD133-positive cellular debris in the glandular lumen (Figure 1) (26). CD133-positive cancer cells were counted at the apical luminal surface with/without intraluminal cell debris, with the rate of positive cancer cells in the invasive front

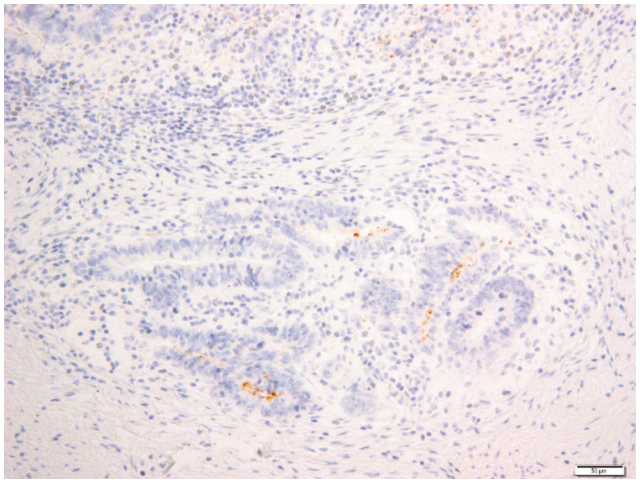

Figure 2. *The cancer cell staining of CD133 in surgically resected specimens. Low CD133 expression (magnification ×200). Staining of CD133 marks a low expression group (<50%).*

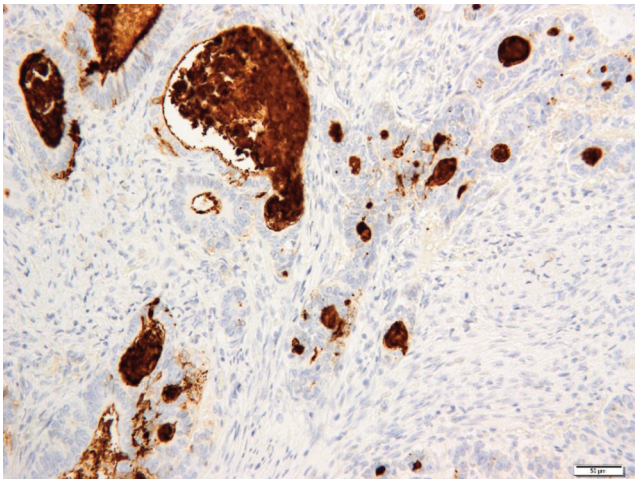

Figure 3. *Cancer cell staining of CD133 in surgically resected specimens. High CD133 expression (magnification ×200). Staining of CD133 marks a high expression group (≥50%).*

determined for the primary tumor (Figure 2 and 3). Cancer cell staining of CD133 was divided into a low expression group (<50%) and high expression group (≥50%). Specimens were evaluated by two independent observers, with one an experienced pathologist (YO), having no knowledge of the outcomes and other clinical information, and the other a surgeon (TO). Their inter-observer agreement was calculated using *k*-statistics. The inter-observer agreement coefficient *k* was 0.72.

Statistical analysis. Categorical variables were compared using χ^2 and Fisher's exact tests. Continuous variables are presented as medians and were analyzed with the Mann-Whitney *U*-test. Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test. Uni- and multivariate analyses to identify the significant factors for RFS were performed using Cox proportional hazard regression models. In the multiple analyses, a force entry method was used to identify risk factors for RFS. A *p*-value less than 0.05 was considered significant. Statistical analysis was performed using SPSS version 25 (IBM Japan Ltd., Tokyo, Japan).

Results

Baseline clinicopathological characteristics. This study evaluated 36 men (69.2%) and 16 women (30.8), with a median age (IQR) of 67 years (range=60-72 years). The median follow-up period (IQR) was 53 months (range=25-79). For the NAC regimens, which included SOX+cetuximab, SOX, XELOX and mFOLFOX6, there were a total of 14 (26.9%), 21 (40.4%), 6 (11.5%) and 11 (21.2%) patients who underwent administration of the therapy before surgical resection, respectively. The median pretreatment serum CEA levels (IQR) were 4.3 (range=2.2-8.4). The clinical tumor stage was cT3 in 45 patients (86.5%) and cT4 (T4a and T4b) in 7 patients (13.5%). There was clinical evidence of lymph node metastasis in 49 patients (94.2%). Using the pathological tumor response criteria, 12 patients (23.1%) were responders (Grades 2-3) and 40 patients (76.9%) were non-responders (Grades 0-1b), and 1 patient (2%) had a pathological complete response. Concerning the improvement of the T- and N- categories, T-downstaging occurred in 15 patients (28.8%) and N-downstaging in 33 patients (63.5%). The percentage of patients with ≥12 examined lymph nodes was 76.9% (40/52). Surgical site infection occurred in 11 patients (21.2%). Adjuvant chemotherapy was administered in 20 patients (38.5%). There were 17 (32.7%) out of 52 patients who developed tumor relapses, which included lung (8 patients), liver (3 patients), peritoneum (1 patient), paraaortic lymph node (1 patient), pelvic lymph node (2 patients), and local (2 patients). High CD133 expression was observed in 22 patients (42.3%). Table I presents the baseline clinicopathological characteristics of the 52 patients.

Association between CD133 expression and clinicopathological characteristics. Tumors with high CD133 expression were significantly associated with more frequent vessel invasion and tumor relapse than those with low CD133 expression (p=0.013 and p=0.036, respectively). There was a significant difference found in CD133 expression between patients who had T- and N-downstaging and those who did not (p=0.003 and p=0.012, respectively). None of the other clinicopathological characteristics were associated with CD133 expression. Table II summarizes the correlations between the CD133 expression and the clinicopathological characteristics.

Table I. *The baseline clinicopathological characteristics of patients*.

Characteristics	Number (%)	Characteristics	Number (%)
Gender		Vessel invasion	
Male	36 (69.2%)	Absent	25 (48.1%)
Female	16 (30.8%)	Present	27 (51.9%)
Age (yr, median, IQR)	67 (60-72)	CRM	
Oxaliplatin based NAC		Negative	46 (88.5%)
SOX+Cetuximab	14 (26.9%)	Positive	6 (11.5%)
SOX	21 (40.4%)	Pathological tumor response	
XELOX	6 (11.5%)	Non-responder (Grade0, 1a, 1b)	40 (76.9%)
mFOLFOX6	11 (21.2%)	Responder (Grade2, 3)	12 (23.1%)
Serum CEA level [median, IQR, (ng/ml)]	4.3 (2.2-8.4)	Responder (Grade 3)	2 (1/52, 2%)
Tumor size [median, IQR, (mm)]	45 (31-50)	T-downstaging	
Differentiation		No	37 (71.1%)
Well, Mod, pap	49 (94.2%)	Yes	15 (28.9%)
Por, Muc, Sig	3 (5.8%)	N-downstaging	
Clinical Tumor depth (cT)		No	19 (36.5%)
T3	45 (86.5%)	Yes	33 (63.5%)
T4a	6 (11.5%)	Examined lymph node	
T4b	1 (2%)	≥12	40 (76.9%)
Clinical Lymph node metastasis (cN)		<12	12 (22.1%)
N0	3 (5.8%)	Surgical approach	
N1	33 (63.5%)	Laparotomy	8 (15.4%)
N2	5 (9.5%)	Laparoscopic surgery	44 (84.6%)
N3	11 (21.2%)	SSI	
Pathological tumor depth (pT)		Absent	41 (78.8%)
CR	1 (2%)	Present	11 (21.2%)
T1	4 (7.2%)	Adjuvant chemotherapy	
T2	15 (28.8%)	No	32 (61.5%)
T3	31 (60%)	Yes	20 (38.5%)
T4	1 (2%)	Postoperative relapse (n=17, 32.7%)	
Pathological Lymph node metastasis (pN)		Lung	8
N0	35 (76.3%)	Liver	3
N1	10 (19.2%)	Peritoneum	1
N2	6 (11.5%)	Paraaortic lymph node	1
N3	1 (2%)	Pelvic lymph node	2
Lymphatic invasion		Local relapse	2
Absent	30 (57.7%)	CD133	
Present	22 (42.3%)	Low	30 (55.7%)
		High	22 (42.3%)

NAC: Neoadjuvant chemotherapy; SOX: S-1+oxaliplatin; XELOX: capecitabine+oxaliplatine; mFOLFX: 5-fluorouracil+L-leucovorin+oxaliplatin; CRM: circumferential resection margin; SSI: surgical site infection.

CD133 expression as a risk factor for relapse. The 4-year relapse-free survival (RFS) rate for the entire study population were 67.4%. Figure 4 shows the Kaplan-Meier survival curves for the RFS in the patients according the grade of CD133 expression. Patients with a low CD133 expression had a significantly better RFS rate than those with a high CD133 expression (82.2% *vs.* 46.3%, 95% CI=63.203-88.246; p=0.009). Uni- and multi-variate analyses undertaken in order to identify factors that were related the RFS were performed using a Cox proportional hazard model (Table III). The univariate analyses showed that a more frequent lymphatic invasion (HR=6.127; 95% CI=1.986-18.903; p=0.002), circumferential resection margin (CRM)-positive (HR=13.117; 95% CI=4.478-38.416; p<0.001), presence of lymph node metastasis (HR=4.929; 95% CI=1.852-13.118; p=0.001), performance of adjuvant chemotherapy (HR=0.190; 95% CI=0.067-0.541; p=0.002), N-downstaging (HR=0.373; 95% CI=0.143-0.970; p=0.043) and a high CD133 expression (HR=3.475; 95% CI=1.274-9.478; p=0.015) were significantly associated with a shorter RFS. Furthermore, multivariate analysis showed that CRM-positive and a high CD133 expression were factors that were significantly associated with a shorter RFS (HR=12.654; 95% CI=2.668-60.015; p=0.001 and HR=3.138; 95% CI=1.046-9.412; p=0.041, respectively). Using multivariate analyses excluding patients with CRM positive, a more frequent lymphatic invasion (HR=2.390; 95%

Table II. *The relationships between CD133 expression and clinicopathological patient characteristics.*

	CD133				CD133		
	Low (%)	High (%)	*p*-Value		Low (%)	High (%)	*p*-Value
Gender				Vessel invasion			
Female	8 (33.3)	8 (36.4)	0.55	Absent	19 (63.3)	6 (27.2)	0.013
Male	22 (66.7)	14 (73.6)		Present	11 (36.7)	16 (72.8)	
Age			0.85*	CRM			
Serum CEA level				Negative	28 (93.3)	18 (81.8)	0.38**
≤5	20 (66.7)	16 (72.7)	0.77	Positive	2 (6.7)	4 (18.2)	
>5	10 (33.3)	6 (27.3)		Pathological tumor response			
Tumor size (cm)			0.79*	Responder	10 (33.3)	2 (9.0)	0.051**
Differentiation				Non-responder	20 (66.7)	20 (91.0)	
Well, moderately, pap	28 (93.3)	21 (95.5)	1**	T-Downstage			
Por, Muc, Sig	2 (6.7)	1 (4.5)		Yes	13 (43.3)	2 (9.0)	0.003**
pT				No	17 (56.7)	20 (91.0)	
T0-1	5 (16.7)	0 (0)	0.065**	N-Downstage			
T2-4	25 (83.3)	22 (100)		Yes	21 (70.0)	12 (54.5)	0.012
pN				No	9 (30.0)	10 (45.5)	
Absent	23 (76.7)	12 (54.5)	0.136	Cetuximab			
Present	7 (23.3)	10 (45.5)		Yes	10 (33.3)	4 (18.2)	0.38**
Lymphatic invasion				No	20 (66.7)	18 (81.8)	
Absent	20 (66.7)	10 (45.5)	0.16	Tumor relapse			
Present	10 (33.3)	12 (54.5)		Absent	24 (80.0)	11 (50.0)	0.036
				Present	6 (20.0)	11 (50.0)	

Por: Poorly; Muc: mucinous; Sig: signet; CRM: circumferential resection margin. *Mann-Whitney U analysis. **Fisher's exact test.

Table III. *Uni-and multi-variate analyses to identify clinicopathological characteristics related to relapse-free survival (n=52).*

	Univariate analysis			Multivariate analysis		
Predictor	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Gender (Male)	1,234	0.456-3.337	0.68			
Age	0.968	0.968-1.012	0.15			
Serum CEA level	0.998	0.989-1.007	0.63			
Differentiation (Por, Muc, Sig)	1,505	0.199-11.387	0.69			
Tumor size	0.986	0.957-1.015	0.34			
pT (pT2-4)	0.616	0.217-1.751	0.36			
Lymphatic invasion (Present)	6,127	1.986-18.903	0.002	4,742	0.956-23.528	0.057
Vessel invasion (Present)	0.52	0.192-1.406	0.2			
CRM (Positive)	13,117	4.478-38.416	<0.001	12,654	2.668-60.015	0.001
Lymph node metastasis (Present)	4,929	1.852-13.118	0.001	2,626	0.339-20.360	0.356
Histological grade (Responder)	2,617	0.598-11.458	0.2			
Surgical approach (Laparoscopic)	0.658	0.150-2.885	0.58			
Adjuvant chemotherapy (Yes)	0.19	0.067-0.541	0.002	0.503	0.112-2.270	0.372
Cetuximab (Yes)	0.539	0.155-1.880	0.33			
T-downstaging (Yes)	0.301	0.069-1.316	0.11			
N-downstaging (Yes)	0.373	0.143-0.970	0.043	0.35	0.076-1.617	0.18
BMI (High)	1,021	0.293-3.561	0.97			
CD133 (High)	3,475	1.274-9.478	0.015	3,138	1.046-9.412	0.041

Por: Poorly; Muc: mucinous; Sig: signet; CRM: circumferential resection margin; BMI: body mass index.

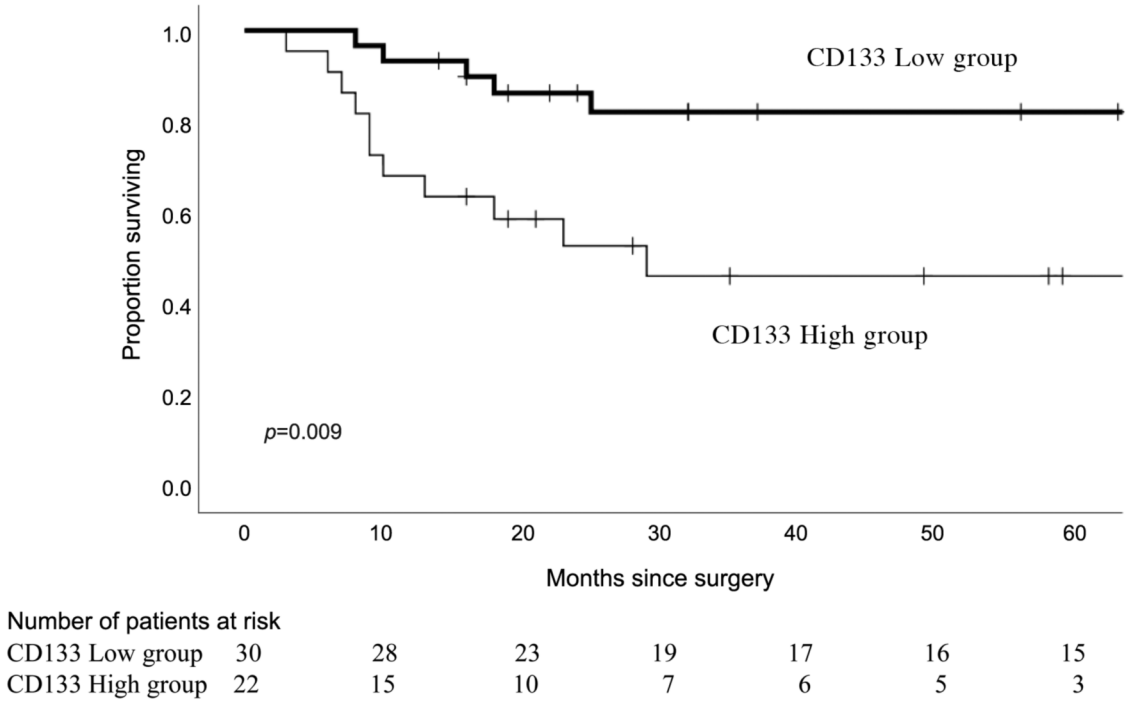

Figure 4. *Kaplan-Meier survival curves for RFS in patients with a low or high CD133 expression.*

CI=1.356-4.212; p=0.003) and a high CD133 expression (HR=3.106; 95% CI=1.132-8.528; p=0.028) were significantly co-factors associated with a shorter RFS (Table IV).

Discussion

In the present study, we showed that tumors with a high CD133 expression were significantly correlated with a more frequent venous invasion and postoperative relapse. In addition, there was a significant difference in CD133 expression between patients who had T- and N-downstaging *versus* those who did not. Patients with a low CD133 expression had better RFS compared to those with a high CD133 expression. Moreover, multivariate analysis for RFS showed that CD133 expression was a significant risk factor for postoperative relapse. Based on these results, the present data suggest that CD133 expression may be predictive of postoperative relapse in patients who received NAC followed by surgical resection.

Chemo- or radio-therapy resistance is a major problem that has an influence on the survival of patients with LARC. Difference in the mechanism regarding the role of CD133 to traditional therapy resistance has yet to be fully explained at the molecular or cellular level (10, 11). With regard to chemotherapy, an *in vitro* study by Dallas *et al*. reported that as compared to HT29 human cancer cells with low CD133 expression, those with a high CD133 expression were able to obtain resistance to chemotherapy using 5-FU (10). It has been proposed that the mechanism of this chemoresistance was related to antiapoptotic proteins, including IL-4, and that HCC cancer cells with high CD133 expression are enriched in anti-apoptotic proteins (13, 27). Cancer cells with high CD133 expression are regulated by activation or suppression of signaling pathways, including Notch, TGF-β, P13k/AKT, Wnt/β-catenin, and Hedgehog (28). CD133 can promote angiogenesis by activating Wnt signal pathway and increasing the expression of VEGF-A and interleukin-8. In addition, CD133 can accelerate the cell growth, proliferation and survival by activating the P13k/AKT signaling pathway and increasing the level of phosphorylated-Akt (28). However, there have yet to be any studies that have investigated CD133 expression in patients with LARC after NAC.

Cancer cells in rectal cancer specimens after NACRT were reported to be located close to the invasive front of the tumor (29). Similarly, RCCs in the present study were mostly observed at the invasive front of the tumor. Karamitopoulou *et al*. reported that there are differences in the protein expression profiles between the invasive front and the other regions of the tumor (30). Genetic instability that results in accumulation of gene and epigenetic alternations is responsible for causing the intratumor heterogeneity (31). Marusyk *et al*. suggested that the CSCs, which may regulate the interaction between cancer

Table IV. *Uni-and multi-variate analyses to identify clinicopathological characteristics related to relapse-free survival excluding patients with CRM positive (n=46).*

	Univariate analysis			Multivariate analysis		
Predictor	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Gender (Male)	1,349	0.395-4.612	0.63			
Age	0.672	0.196-2.300	0.53			
Serum CEA level	2,414	0.736-7.918	0.15			
Differentiation (por, muc)	0.047	0.000-33307.159	0.66			
Tumor size	0.462	0.135-1.584	0.22			
pT (pT2-4)	24,405	0.008-72,674.234	0.43			
Lymphatic invasion (Present)	5,211	1.374-19.756	0.015	2.39	1.356-4.212	0.003
Vessel invasion (Present)	1,261	0.384-4.140	0.7			
Lymph node metastasis (Present)	3,185	0.966-10.500	0.057			
Lymph node harvest	0.368	0.111-1.215	0.1			
Histological grade (Responder)	0.519	0.358-7.681	0.52			
Surgical approach (Laparoscopic)	0.467	0.060-3.658	0.47			
Adjuvant chemotherapy (Yes)	3,084	0.938-10.141	0.064			
Cetuximab (Yes)	4,195	0.536-32.837	0.17			
T-downstaging (Yes)	0.469	0.101-2.174	0.33			
N-downstaging (Yes)	0.487	0.148-1.606	0.24			
BMI (High)	1,732	0.458-6.547	0.42			
CD133 (High)	3,524	1.020-12.180	0.046	3,106	1.132-8.528	0.028

CRM: Circumferential resection margin; Por: poorly; Muc: mucinous; Sig: signet; BMI: body mass index.

cells and the surrounding microenvironment, might also contribute to intratumor heterogeneity (32). On the other hand, several recent studies have demonstrated that CSCs promote tumor invasion and metastasis by inducing EMT processes (33, 34). Thus, the evaluation of CSCs at the invasive front of the tumor may predict the postoperative relapse in patients with LARC who received neoadjuvant therapy.

The selection of patients who need adjuvant chemotherapy after neoadjuvant therapy and TME is controversial (35, 36). The National Comprehensive Cancer Network (NCCN) guidelines recommended the routine use of AC in LARC patients who were treated with NACRT followed by surgery, whereas the European Society for Medical Oncology (ESMO) guidelines recommend AC for all stage III patients and for stage II patients with high risk factors including lymphatic and vascular invasion, pT4 lesion, and perforation (18, 19). In the present study, the administration of AC was determined in accordance with the criteria presented in the ESMO guidelines. In our present study, 5 out of 32 patients (16.5%) who did not receive AC, did develop tumor relapses during the observation period, with 3 out of these 5 patients found to have high CD133 expression. Based on these results, the examination of CD133 may be helpful when selecting patients who have a high risk for postoperative relapse.

Recently, several reports have shown that NAC could potentially have an effect on the possibility of micrometastases and reduction of distant metastases in LARC (7, 8). Since it has been shown that advanced rectal cancers (cT3-4 and N-any) are associated with an increased risk of distant metastasis, which is the essential prognostic determinant in these patients, NAC could be a potential option for preventing postoperative relapses in these patients (7). In order to confirm a rectal cancer treatment strategy that incorporates a selective radiation therapy, the PROSPECT trial (ClinicalTrials.gov identifier: NCT01515787) is currently assessing this therapeutic approach in North America (37). On another recent study, the FOWARC trial in China showed that no significant difference in the 3-year disease-free survival between neoadjuvant mFOLFOX6 with or without radiation and fluorouracil plus radiation for LARC (38). Although the long-term outcomes of these studies need to be evaluated with regard to introduction of NAC without radiation therapy as a standard treatment option, we speculate that this strategy could be an effective therapy for selective patients with LARC.

There were several limitations to the present study. First, due to its retrospective design, the present study had an inherent selection bias. Second, the number of patients was not large enough to attain a sufficient power in order to reach any definitive conclusions. Therefore, a further study that includes a sufficient number of patients will need to be undertaken in order to explored the use of the CD133 expression as a useful maker for postoperative relapse.

Conclusion

In conclusion, the present data suggest that CD133 expression in patients with LARC who have received oxaliplatin-based NAC may be useful predictive marker for postoperative relapse. Patients with a high CD133 expression therefore would be candidate for AC, as well as requiring careful follow-up plans.

Conflicts of Interest

The Authors have no conflicts of interest to declare.

Authors' Contributions

HO, TO and MO designed the study. HO, TO, SM and MO reviewed the clinical records. YO, TO and HO reviewed the pathological records. All authors participated in the study design, data interpretation, and critical discussion. HO, TO, and MO wrote the manuscript. All Authors read and approved the final manuscript.

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