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Association between poorly differentiated clusters and efficacy of 5-fluorouracil-based adjuvant chemotherapy in stage III colorectal cancer

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Abstract

Objective: Although poorly differentiated cluster has been reported to be a useful grading system for predicting prognosis in colorectal cancer, its relationship to chemotherapy efficacy has not been demonstrated. We aimed to investigate the association between poorly differentiated cluster and the efficacy of 5-fluorouracil-based adjuvant chemotherapy in stage III colorectal cancer.
Methods: This retrospective study enrolled 131 patients with stage III colorectal cancer who underwent curative resection: 72 received 5-fluorouracil-based adjuvant chemotherapy (chemotherapy group) and 59 did not (surgery-alone group). Poorly differentiated cluster was defined as a cancer cluster of ≥5 cancer cells without gland-like structure, and was classified into poorly differentiated cluster G1, G2 and G3 according to the number of clusters. The benefit of 5-fluorouracil-based adjuvant chemotherapy was evaluated based on poorly differentiated cluster grade.
Results: Thirty-nine, 40 and 52 patients were classified as poorly differentiated cluster G1, G2 and G3, respectively. Significant differences in the 5-year cumulative recurrence rate and relapse-free survival were observed between poorly differentiated cluster G1/G2 and G3 (26.7% vs. 47.5%, $P = 0.010$; 66.0% vs. 43.9%, $P = 0.004$). A comparison of cumulative recurrence rate and relapse-free survival between the chemotherapy and surgery-alone groups showed a significant benefit of adjuvant chemotherapy in poorly differentiated cluster G1/G2 patients (cumulative recurrence rate: 17.4% vs. 37.3%, $P = 0.035$; relapse-free survival: 79.5% vs. 51.9%, $P = 0.002$), but not in poorly differentiated cluster G3 patients (cumulative recurrence rate: 48.6% vs. 44.8%, $P = 0.885$; relapse-free survival: 51.4% vs. 32.7%, $P = 0.068$).
Conclusions: In stage III colorectal cancer, poorly differentiated cluster G1/G2 predicts a significant benefit from 5-fluorouracil-based adjuvant chemotherapy, whereas poorly differentiated cluster G3 predicts a poor response to it.

Key words: poorly differentiated cluster, colorectal cancer, adjuvant chemotherapy

Introduction

In developed countries, colorectal cancer (CRC) is the second most common malignancy (1). Despite curative surgery, nodal metastatic disease still leads to death in ~30% of cases (2). Since the 1990s, adjuvant chemotherapy has been administered to decrease the risk of tumor recurrence and improve survival in CRC (3). It is well established that adjuvant therapy with 5-fluorouracil (5-FU) improves disease-free survival by 10–15% in stage III colon cancer (4–6) plus an additional 4–6% with 5-FU-based regimen plus oxaliplatin (oxaliplatin-based regimen) (7–9). Although TNM staging remains the most important determinant of CRC prognosis and treatment including adjuvant chemotherapy, there are other independent prognostic factors in addition to TNM staging.

Histopathological grading is one of the prognostic factors for CRC, independent of TNM stage (10–12). The most widely accepted histopathological grading is based on the degree of tumor differentiation. When a carcinoma has heterogeneity in differentiation, histopathological grading is determined based on the least differentiated component, not including the advancing edge of the tumor (13). Multivariate analysis has shown that histopathological grading of tumor differentiation is a TNM stage-independent prognostic factor, but there is significant interobserver variability (14–16).

Recently, poorly differentiated cluster (PDC) was reported to be a useful grading system for predicting prognosis in CRC patients (17). According to the original definition, PDC is composed of five or more cancer cells with no gland formation, and is found at the advancing edge of the tumor (18). On the basis of the count of PDCs, PDC was classified as grade (G) G1, G2 and G3, respectively (18). PDC affects the outcome independent of T and N categories (18), and in a recent multicenter study analyzing 3243 CRC patients, the quantification of PDCs to grade tumors was expected to be more objective than conventional histopathological grading and more informative for predicting prognosis than TNM staging (17).

The prognosis of CRC patients with PDC G3 is worse than those with PDC G1 or G2 (17,18). We hypothesized that tumors with PDC G3 are more tolerant of adjuvant chemotherapy compared with those with PDC G1 or G2. The aim of this study was to investigate the association between PDC grade and the efficacy of 5-FU-based adjuvant chemotherapy in stage III CRC.

Patients and methods

Patients

The institutional review board approved and issued a waiver of informed consent for this retrospective study. A total of 164 patients diagnosed with stage III CRC according to the AJCC seventh edition staging classification (19) who had curative surgery between 2000 and 2010 at Niigata University Medical and Dental Hospital, Niigata, Japan, were identified. We selected patients from our colorectal database using the following inclusion and exclusion criteria (Fig. 1): (i) patients diagnosed with adenocarcinoma were included, while (ii) patients who received endoscopic mucosal resection before operation (unclear PDC status), (iii) patients who received neoadjuvant therapy, (iv) patients who received adjuvant chemotherapy with '5-FU only' or 'oxaliplatin-based regimen' and (v) patients who withdrew from adjuvant chemotherapy before completion were excluded. According to the criteria, 131 of 164 patients were included for further investigation. Among the 131 patients, 72 underwent adjuvant chemotherapy (chemotherapy group) and 59 did not because of age, comorbidity and/or patient preference (surgery-alone group). Among the 131 patients, the median follow-up period was 61 (1–150) months.

'5-FU-based' regimens

In this study, we classified adjuvant chemotherapy into three categories: '5-FU only', '5-FU-based' or 'oxaliplatin-based' regimens. '5-FU only' refers to oral 5-FU drugs alone, such as oral tegafur–uracil (UFT). 'Oxaliplatin-based' regimen refers to a 5-FU-based plus oxaliplatin regimen, such as FOLFOX. '5-FU-based' regimens included the Roswell Park Memorial Institute (RPMI) (20), oral UFT/leucovorin (LV) (21,22), capecitabine (4) and tegafur–gimeracil–oteracil potassium (S-1) regimens (23). The RPMI regimen comprised one cycle of 600 mg/m^2 5-FU and 250 mg/m^2 LV weekly for 6 weeks, with cycles repeated every 8 weeks for three cycles (20). The UFT/LV regimen comprised one cycle of 300 mg/m^2/day UFT and 75 mg/day LV for 28 consecutive days, with cycles repeated every 5 weeks for five cycles (21,22). The capecitabine regimen comprised one cycle of 2500 mg/m^2 capecitabine for 14 consecutive days, with cycles repeated every 3 weeks for eight cycles (4). The S-1 regimen comprised one cycle of S-1 (80 mg/day with body surface area [BSA] <1.25 m^2, 100 mg/day with BSA 1.25–1.50 m^2, 120 mg/day with

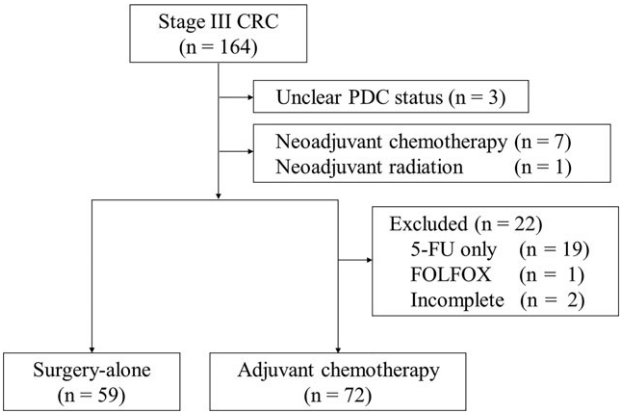

Figure 1. Flow chart diagram of inclusion and exclusion criteria in this study. CRC, colorectal cancer; PDC, poorly differentiated cluster.

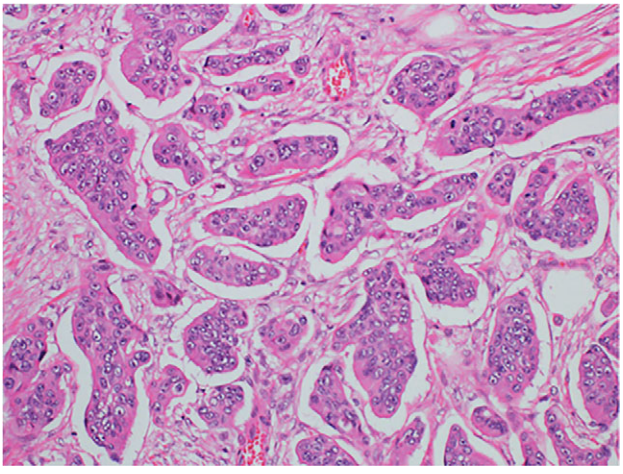

Figure 2. Poorly differentiated clusters (PDCs). Cancer cell clusters located in the stroma, comprising ≥5 cancer cells and lacking glandular formation are classified as PDCs. H&E staining, ×20 objective lens.

BSA >1.50 m^2) for 28 consecutive days, with cycles repeated every 6 weeks for four cycles (23). The choice of regimen was based on each physician's preference.

In this study, we focused on the association between PDC G3 and the '5-FU-based' regimen, and excluded patients who received the '5-FU only' or 'oxaliplatin-based' regimen. We excluded patients who received the '5-FU only' regimen because there is little evidence for the effectiveness of this regimen (24). If we include this regimen, we may underestimate the efficacy of '5-FU-based' adjuvant chemotherapy in this study. We also excluded patients who received the 'oxaliplatin-based' regimen to avoid the additive effect of oxaliplatin. We speculate that the mechanism of oxaliplatin resistance may be different from that of 5-FU resistance (25).

Definitions of PDC and histopathological grading

PDC was defined as cancer clusters in the stroma composed of ≥5 cancer cells that lack a gland-like structure (17). To quantify PDCs, the entire tumor including its advancing edge was first viewed at low-power magnification to identify the area containing the greatest number of PDCs. The clusters were then counted under a microscope using a ×20 objective lens (Fig. 2). Tumors with <5, 5–9 and ≥10 clusters were classified as G1, G2 and G3, respectively (18). With regard to assessment for mucinous carcinoma, malignant clusters with the above-mentioned features infiltrating the stroma with minimal extracellular mucin formation were classified as PDCs (17). In contrast, cancer cell clusters within a large mucin pool (i.e. mucinous lake) were not classified as PDCs (17). On the other hand, histopathological grading was determined on the basis of the least differentiated component, and the invading edge was regarded as suboptimal to evaluate histopathological grade according to the World Health Organization (WHO) classification (13). Two independent surgical pathologists (Y.S. and T.O.) blinded to all clinical details assessed each section. Any differences in assessment between the surgical pathologists were resolved by a double review using a multi-head microscope.

Prognostic factors

In this study, we assessed the association between PDC and the efficacy of 5-FU-based adjuvant chemotherapy by using cumulative recurrence rate (CRR) and relapse-free survival (RFS). To elucidate factors influencing CRR and RFS, 11 clinicopathological variables were tested in all 131 patients: age (<65 vs. ≥65 years), sex, American Society of Anesthesiologists physical status (ASA-PS; 1–2 vs. 3–4), tumor location (colon vs. rectum), tumor size (<50 vs. ≥50 mm), T category (T1–T3 vs. T4), histopathological grading (G1, G2 vs. G3), lymphatic invasion (absence vs. presence), venous invasion (absence vs. presence), N category (N1 vs. N2), PDC (G1, G2 vs. G3) and adjuvant chemotherapy (absence vs. presence).

Table 1. Association between poorly differentiated cluster and other clinicopathological characteristics

	PDC		*P* value
	G1/G2 (*n* = 79)	G3 (*n* = 52)	
Age			
<65	30	21	0.855
≥65	49	31	
Sex			
Male	49	29	0.585
Female	30	23	
ASA-PS			
1/2	66	48	0.331
3/4	13	4	
Tumor location			
Colon	31	18	0.712
Rectum	48	34	
Tumor size (mm)			
<50	39	23	0.595
≥50	40	29	
Tumor stage			
T1–T3	66	43	0.999
T4	13	9	
Histopathological grading			
G1/G2	74	39	0.004
G3	5	13	
Lymphatic invasion			
Absence	39	19	0.156
Presence	40	33	
Venous invasion			
Absence	39	27	0.859
Presence	40	25	
Nodal involvement			
N1	60	32	0.083
N2	19	20	
Adjuvant chemotherapy			
Absence	38	21	0.473
Presence	41	31	

PDC, poorly differentiated cluster; ASA-PS, American Society of Anesthesiologists physical status.

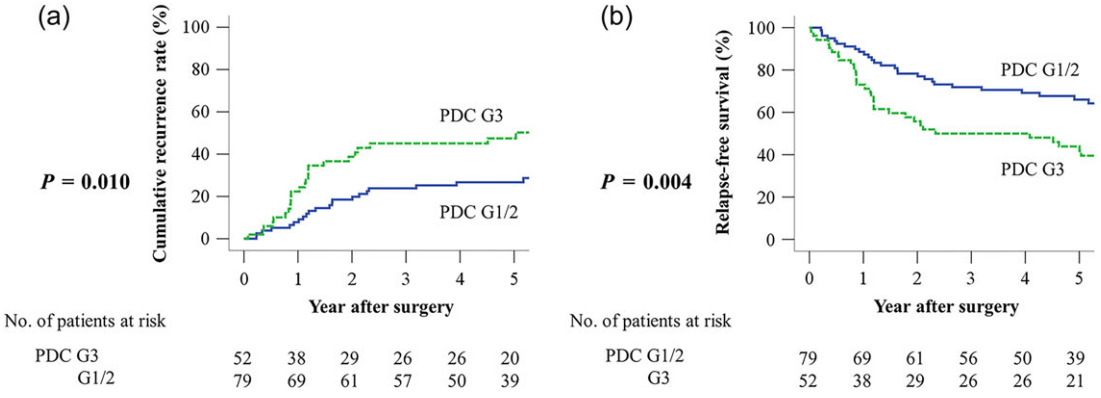

Figure 3. Comparative cumulative recurrence rate (CRR) and relapse-free survival (RFS) curves of PDC G1/G2 and G3 groups in stage III CRC. (a) CRR and (b) RFS.

Table 2. Univariate and multivariate analyses of prognostic factors for cumulative recurrence rate

Variable	Modality	*n*	Univariate		Multivariate	
			5-y CRR (%)	*P* value	HR (95% CI)	*P* value
Age (years)	<65	51	34.0	0.853		
	≥65	80	35.3			
Sex	Male	78	32.8	0.593		
	Female	53	37.9			
ASA-PS	1/2	114	33.5	0.565		
	3/4	17	44.4			
Tumor location	Colon	49	27.5	0.105		
	Rectum	82	39.5			
Tumor size (mm)	<50	62	36.3	0.903		
	≥50	69	33.6			
T category	T1–T3	109	32.4	0.146		
	T4	22	47.5			
Histopathological grading	G1/G2	113	31.2	0.006	1.00	
	G3	18	58.3		1.43 (0.98–2.07)	0.063
Lymphatic invasion	Absence	58	35.4	0.745		
	Presence	73	34.8			
Venous invasion	Absence	66	30.5	0.220		
	Presence	65	39.8			
N category	N1	92	29.6	0.034	1.00	
	N2	39	47.5		1.40 (0.74–2.65)	0.302
PDC	G1/G2	79	26.7	0.010	1.00	
	G3	52	47.5		1.85 (1.01–3.38)	0.048
Adjuvant chemotherapy	Absence	59	39.8	0.282		
	Presence	72	31.3			

CRR, cumulative recurrence rate; HR, hazard ratio; CI, confidence interval; ASA-PS, American Society of Anesthesiologists physical status; PDC, poorly differentiated cluster.

Table 3. Univariate and multivariate analyses of prognostic factors for relapse-free survival (RFS)

Variable	Modality	*n*	Univariate		Multivariate	
			5-y RFS (%)	*P* value	HR (95% CI)	*P* value
Age (years)	<65	51	62.1	0.298		
	≥65	80	54.1			
Sex	Male	78	55.4	0.633		
	Female	53	59.3			
ASA-PS	1/2	114	58.6	0.154		
	3/4	17	47.1			
Tumor location	Colon	49	68.3	0.018	1.00	
	Rectum	82	50.4		1.78 (0.99–3.16)	0.051
Tumor size (mm)	<50	62	55.5	0.698		
	≥50	69	58.4			
T category	T1–T3	109	58.5	0.445		
	T4	22	50.0			
Histopathological grading	G1/G2	113	61.0	0.011	1.00	
	G3	18	32.4		1.37 (0.99–1.89)	0.058
Lymphatic invasion	Absence	58	58.6	0.624		
	Presence	73	55.7			
Venous invasion	Absence	66	62.4	0.258		
	Presence	65	51.3			
N category	N1	92	61.1	0.139		
	N2	39	47.1			
PDC	G1/G2	79	66.0	0.004	1.00	
	G3	52	43.9		2.00 (1.20–3.33)	0.008
Adjuvant chemotherapy	Absence	59	44.9	0.001	1.00	
	Presence	72	67.1		2.20 (1.31–3.71)	0.003

RFS, relapse-free survival; HR, hazard ratio; CI, confidence interval; ASA-PS, American Society of Anesthesiologists physical status; PDC, poorly differentiated cluster.

Statistical analyses

Statistical analyses were performed with IBM SPSS Statistics 22 (IBM Japan, Inc., Tokyo, Japan). The relationships between each clinicopathological variable and PDC (G1, G2 vs. G3) were analyzed using Fisher's exact test. CRR and RFS were estimated using the Kaplan–Meier method. The log-rank test was used to assess significant differences between the subgroups by univariate analysis. *P* values <0.05 were considered statistically significant. To assess the potential prognostic factors for CRR and RFS, those with *P* values <0.05 in the univariate analyses were entered into the multivariate analysis. We used the Cox proportional hazards regression model to identify factors that were independently associated with CRR and RFS after surgery. In the PDC G1/G2 and G3 groups, the efficacy of adjuvant chemotherapy was evaluated by comparing CRR and RFS between the surgery-alone group and the chemotherapy group.

Results

Tumor grading based on PDCs and other clinicopathological characteristics

According to the number of PDCs, 39, 40 and 52 tumors were classified as G1, G2 and G3, respectively. Compared with PDC G1/G2, PDC G3 was significantly associated with histopathological grading G3 ($P = 0.004$), while there were no significant associations between PDCs and other clinicopathological characteristics (Table 1).

Clinical significance of PDC grade

Significant differences were observed between PDC G1/G2 and G3 in the 5-year CRR (26.7% vs. 47.5%, $P = 0.010$) (Fig. 3a) and RFS (66.0% vs. 43.9%, $P = 0.004$) (Fig. 3b). Multivariate analysis identified that PDC G3 was an independent prognostic factor for CRR [hazard ratio (HR): 1.85, 95% confidence interval (CI): 1.01–3.38, $P = 0.048$] (Table 2) and RFS (HR: 2.00, 95% CI: 1.20–3.33, $P = 0.008$) (Table 3).

First sites of recurrence and PDC grade

The first recurrence was detected in the lung in 16 patients, liver in 13, local site in 9, extraregional lymph node in 9, peritoneum in 4, adrenal gland in 1 and brain in 1. In nine patients, more than two organs were involved. PDC G3 was significantly associated with cumulative extraregional lymph node metastasis ($P = 0.007$), while PDC G1/G2 was not associated with first sites of recurrence.

Adjuvant chemotherapy status and other clinicopathological characteristics

Compared with the chemotherapy group, the surgery-alone group was significantly associated with Age ≥65 ($P < 0.001$) and ASA-PS 3,4 ($P = 0.035$), while there were no significant associations between adjuvant chemotherapy status and other clinicopathological characteristics (Table 4).

CRR according to 5-FU-based adjuvant chemotherapy in PDC G1/G2 and PDC G3 patients

Among PDC G1/G2 patients, a significant difference in the 5-year CRR was observed between the chemotherapy group and surgery-alone group (17.4% vs. 37.3%, $P = 0.035$) (Fig. 4a). Conversely, among PDC G3 patients, no significant difference in 5-year CRR was observed between the chemotherapy group and surgery-alone group (48.6% vs. 44.8%, $P = 0.885$) (Fig. 4b).

Table 4. Association between adjuvant chemotherapy status and other clinicopathological characteristics

	Chemotherapy group ($n = 72$)	Surgery-alone group ($n = 59$)	*P* value
Age			
<65	39	12	<0.001
≥65	33	47	
Sex			
Male	39	39	0.211
Female	33	20	
ASA-PS			
1, 2	67	47	0.035
3, 4	5	12	
Tumor location			
Colon	29	20	0.474
Rectum	43	39	
Tumor size (mm)			
<50	36	26	0.598
≥50	36	33	
Tumor stage			
T1–T3	59	50	0.815
T4	13	9	
Histopathological grading			
G1, G2	62	51	1.000
G3	10	8	
Lymphatic invasion			
Absence	30	28	0.596
Presence	42	31	
Venous invasion			
Absence	32	34	0.161
Presence	40	25	
Nodal involvement			
N1	48	44	0.344
N2	24	15	
PDC			
G1, G2	41	38	0.473
G3	31	21	

PDC, poorly differentiated cluster; ASA-PS, American Society of Anesthesiologists physical status.

RFS according to 5-FU-based adjuvant chemotherapy in PDC G1/G2 and PDC G3 patients

Among PDC G1/G2 patients, a significant difference in the 5-year RFS was observed between the chemotherapy group and surgery-alone group (79.5% vs. 51.9%, $P = 0.002$) (Fig. 4c). Conversely, among PDC G3 patients, no significant difference in 5-year RFS was observed between the chemotherapy group and surgery-alone group (51.4% vs. 32.7%, $P = 0.068$) (Fig. 4d).

Discussion

The importance of the PDC was first highlighted by Ueno et al. in 2008 (26). Recently, several studies showed that PDC predicted prognosis in CRC more accurately than other histopathological parameters such as histopathological grading, venous or lymphatic invasion, tumor depth and nodal status (17,18). Furthermore, the PDC grading system is associated with a more 'proportionate' distribution of CRC tumors in each PDC grade (18). In our study,

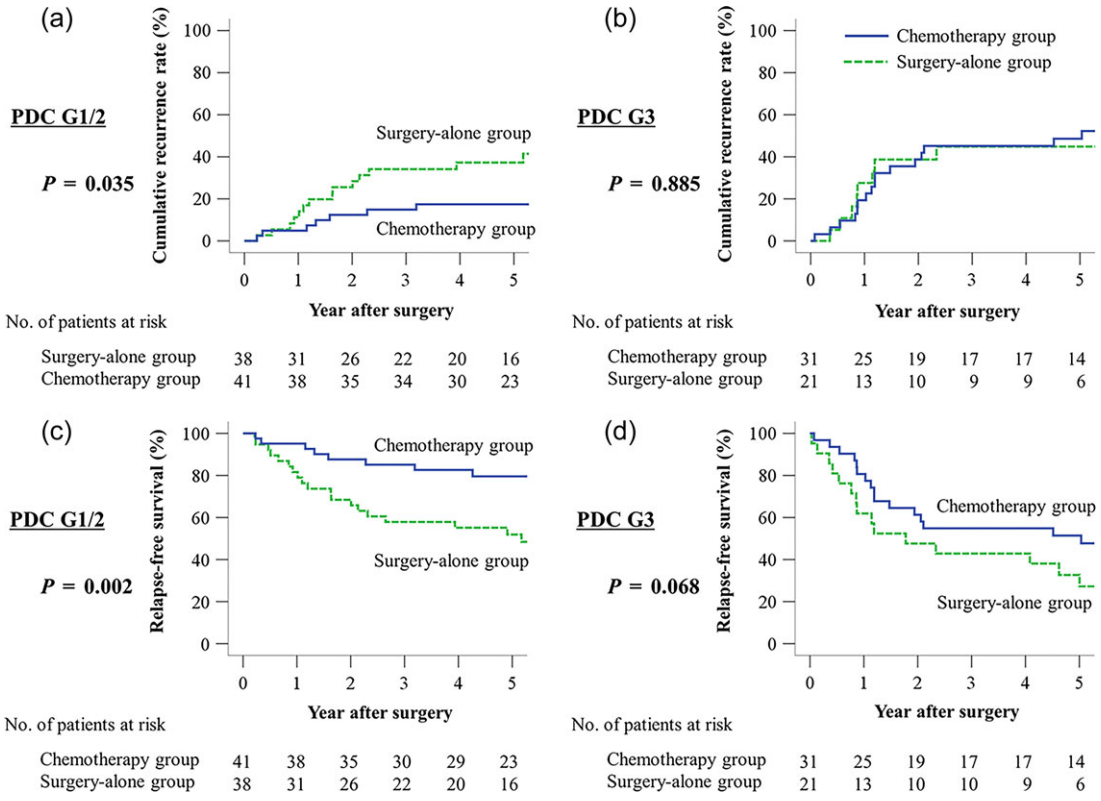

Figure 4. Comparative CRR and RFS curves of patients with or without 5-fluorouracil (5-FU)-based adjuvant chemotherapy in stage III CRC. (a) CRR of PDC G1/G2 patients, (b) CRR of PDC G3 patients, (c) RFS of PDC G1/G2 patients, (d) RFS of PDC G3 patients.

39 (29.8%), 40 (30.5%) and 52 (39.7%) tumors were classified as PDC G1, G2 and G3, respectively. On the other hand, histopathological grading is associated with a more 'disproportionate' distribution of CRC tumors (18). In our study, 20 (15.3%), 93 (71.0%) and 18 (13.7%) tumors were classified as G1, G2 and G3. Therefore, we suggest that PDC grading identifies high-risk patients for recurrence and it stands for malignant biology.

To date, 5-FU-based adjuvant chemotherapy is universally recommended for patients with stage III CRC (27,28). However, Ueno et al. reported that the PDC enables the selection of a group of advanced CRC patients with very favorable survival outcome, thereby preventing unnecessary post-operative adjuvant chemotherapy and intensive surveillance in these patients (26). Few valuable predictors of efficacy of adjuvant chemotherapy have been investigated in CRC patients. The NCCN guidelines state that there is no evidence of predictive value of any of the available multigene assays in terms of the potential benefit of adjuvant chemotherapy (27). In this study, we demonstrated that PDC G1/G2 may be a useful predictor of response to 5-FU-based adjuvant chemotherapy in stage III CRC patients. Conversely, we speculate that CRC with PDC G3 may be resistant to 5-FU-based adjuvant chemotherapy.

Nowadays, the oxaliplatin-based regimen is the standard adjuvant chemotherapy regimen for stage III CRC (7,29). However, oxaliplatin is associated with significant side effects such as peripheral neuropathy or allergic reactions (7,29). Among the PDC G3 patients in this study, no significant differences in 5-year CRR and RFS were observed between the chemotherapy group and surgery-alone group ($P = 0.885$ and $P = 0.068$, respectively). These results indicate that the 5-FU-based regimen may not improve the prognosis of stage III CRC patients with PDC G3. Among those with PDC G1/G2, on the other hand, significant differences in 5-year CRR and RFS were observed between the 5-FU-based chemotherapy and the surgery-alone groups ($P = 0.035$ and $P = 0.002$, respectively). Therefore, the removal of oxaliplatin from adjuvant chemotherapy may be possible in stage III CRC patients with PDC G1/G2 to avoid these side effects. Thus, we consider that the PDC grade may play an important role in the selection of adjuvant chemotherapy regimen for stage III CRC.

In this study, we showed no significant differences in both 5-year CRR and RFS of the PDC G3 patients between the chemotherapy group and surgery-alone group. However, RFS of the PDC G3 patients in the surgery-alone group tended to be worse ($P = 0.068$). We speculate that the death caused by other diseases may make the RFS of the surgery-alone group worse. In Table 4, we demonstrated the differences of clinicopathological characteristics between the two groups: compared with the chemotherapy group, the surgery-alone group was significantly associated with Age ≥65 ($P < 0.001$) and ASA-PS 3,4 ($P = 0.035$).

Based on several recent studies, we think that PDC is associated with drug resistance, in which epithelial mesenchymal transition (EMT) is involved. Both PDC and cancer cells undergoing EMT have lost expression of E-cadherin and are closely associated with an upregulated Wnt/β-catenin signaling pathway (30–33). In their study of CRC progression, Brabletz et al. found that tumor cells at the tumor–host interface expressed EMT-associated and stemness-associated genes, which suggests a relationship between EMT and cancer stem cells (CSCs) in CRC (34). Despite limited understanding of the mechanisms causing CSC-related drug resistance, one of the hypotheses is that drug resistance is caused by overexpression of drug transporters and DNA repair enzymes in CSCs (35–37).

Further studies such as immunohistochemical assays to identify markers of EMT or CSC in PDC are needed to elucidate the mechanism of resistance to 5-FU-based adjuvant chemotherapy in CRC patients with PDC G3.

This study has some potential limitations. This was a non-randomized, retrospective study performed at one institution. This study included the small sample size, and the selection bias due to limited inclusion criteria. A variety of 5-FU-based chemotherapy regimens such as infusional 5-FU/LV, UFT/LV, capecitabine or S-1 were administered in the chemotherapy group. Furthermore, 'oxaliplatin-based' regimens were excluded. A randomized controlled study comparing the oxaliplatin-based regimen with the 5-FU-based regimen is needed to clarify the efficacy of oxaliplatin as adjuvant chemotherapy in stage III CRC with PDC G3.

In conclusion, the efficacy of 5-FU-based adjuvant chemotherapy in stage III CRC differs according to PDC grade. The presence of PDC G1/G2 predicts a significant benefit from 5-FU-based adjuvant chemotherapy, whereas the presence of PDC G3 predicts a poor response to this regimen.

Authors' contributions

Dr Tajima and Dr Shimada contributed to the conception and design of the study. Dr Tajima and Dr Shimada contributed to analysis and interpretation of data. Dr Tajima, Dr Shimada, Dr Kameyama, Dr Yagi, Dr Okamura and Dr Kobayashi contributed to collection and assembly of data. Dr Tajima contributed to drafting of the article. Dr Tajima, Dr Shimada and Dr Kosugi contributed to critical revision of the article for important intellectual content. Dr Wakai gave final approval for submission of the article.

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Conflict of interest statement

The authors declare no conflict of interest.

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