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Prognostic value of programmed death-ligand 1 expression in patients with stage III colorectal cancer

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Key words

CD8-positive T-lymphocytes, colonic neoplasms, immune microenvironment, immunohistochemistry, tumor infiltrating mononuclear cells

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The programmed death-1/programmed death-ligand 1 (PD-L1) pathway is a negative feedback pathway that suppresses the activity of T cells. Previous studies reported that high PD-L1 expression on tumor cells (TC) was associated with poor survival in patients with colorectal cancer; however, the prognostic evaluation of these studies was limited because they included patients at various disease stages. The purpose of the present study was to evaluate the relationship between PD-L1 status in the immune microenvironment and the clinicopathological features of stage III colorectal cancer. Two hundred and thirty-five patients were included in the analysis. PD-L1 expression on TC and tumor-infiltrating mononuclear cells (TIMC) was evaluated by immunohistochemistry. The median follow-up of thisi study was 52.9 months. A total of 8.1% of stage III colorectal cancer showed high PD-L1 expression on TC and 15.3% showed high PD-L1 expression on TIMC. Patients with high PD-L1 expression on TC had significantly shorter disease-free survival (DFS) than patients with low expression (hazard ratio [HR] 2.36; 95% confidence interval [CI], 1.21–4.62; *P* = 0.012). In addition, patients with high PD-L1 expression on TIMC were associated with longer DFS than patients with low expression (HR 0.40; 95% CI, 0.16–0.98; *P* = 0.046). These findings suggest that PD-L1 expression status may be a new predictor of recurrence for stage III colorectal cancer patients and highlight the necessity of evaluating PD-L1 expression on TC and TIMC separately in the tumor microenvironment.

Colorectal cancer is a major cause of cancer-related death worldwide.[1] In Japan, there were 124 921 new cases of colorectal cancer in 2011 and 48 485 deaths from the disease in 2014, respectively.[2] Multimodality therapy consisting of surgery, chemotherapy and radiotherapy is the main treatment for operable cancer;[3–5] however, the recurrence rate in patients with stage III colorectal cancer remains high.[6]

Previous studies have shown that molecular markers, including BRAF, KRAS and tumor mismatch repair (MMR) status, are useful for predicting recurrences in patients with stage III colorectal cancer.[7,8] In addition, the prognostic effect of the immune microenvironment has become increasingly recognized.[9–11] The programmed death 1 (PD-1)/programmed death 1-ligand 1 (PD-L1) signaling pathway is a negative feedback mechanism that suppresses the activity of T cells.[12] PD-L1 expression has been reported on tumor cells or tumor-infiltrating immune cells in several malignancies, including colorectal cancer.[13] Furthermore, it has been suggested that high PD-L1 expression on tumor cells (TC) and/or tumor infiltrating immune cells is associated with prognosis across different tumor types, including esophageal cancer[14,15] and urothelial cancer.[16] However, the prognostic value of PD-L1 expression in patients with stage III colorectal cancer has not yet been established.

The aim of the present study was to evaluate the relationship between PD-L1 status in the immune microenvironment and the clinicopathological features of stage III colorectal cancer.

Methods

Patients and samples. Formalin-fixed paraffin-embedded block specimens from surgical resection of the primary tumor were obtained from 318 patients with stage III colorectal cancer who underwent curative surgery and adjuvant chemotherapy at our institution between January 2009 and July 2012. We excluded 83 patients who received radiotherapy or chemoradiotherapy before surgery. A total of 235 patients were included in this study, and all of their samples were evaluable for immunohistochemistry (IHC). PD-L1 expression on TC and tumor-infiltrating mononuclear cells (TIMC) as well as the number of CD8 positive T cells were evaluated by IHC. TIMC were distinguished from TC by their morphological features following HE staining. Baseline clinicopathological characteristics and clinical outcome data were retrospectively collected from the Colorectal Cancer Database of the Department of Gastroenterological Surgery and the Pathological Diagnosis Database in the Department of Pathology. The Institutional Review Board of Toranomon Hospital approved the following

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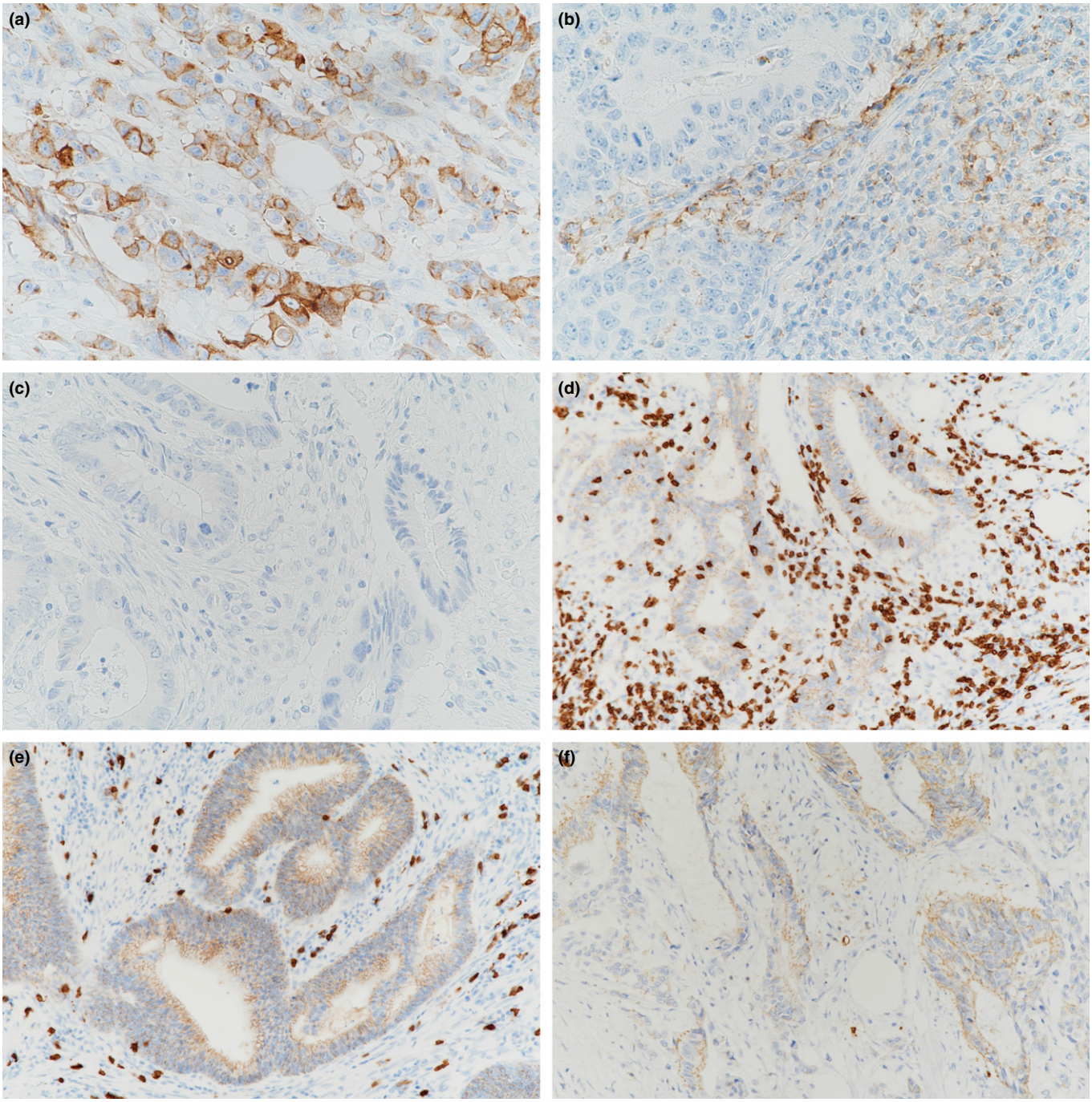

Fig. 1. Representative photomicrographs of PD-L1 expression on tumor cells (TC) and tumor infiltrating mononuclear cells (TIMC) (a), and CD8 positive T cell infiltration in the tumor microenvironment (b). High PD-L1 expression on TC and low PD-L1 expression on TIMC (a), low PD-L1 expression on TC and high PD-L1 expression on TIMC (b), and low PD-L1 expression on TC and low PD-L1 expression on TIMC (c). High CD8 expression in intratumoral and peritumoral cells is present (d), low CD8 expression in intratumoral cells and high CD8 expression in peritumoral cells (e), and low CD8 expression in intratumoral and peritumoral cells (f).

data acquisition and tumor staining. The data cutoff date of clinical outcome for this analysis was July 2015.

Scoring for PD-L1 expression. PD-L1 expression was evaluated using an anti PD-L1 rabbit monoclonal antibody (clone SP142; Spring Bioscience, Pleasanton, CA, USA). Two pathologists (N.I. and Y.M.) independently examined PD-L1 expression. Specimens were scored as IHC low or high when <5% or ≥5% of cells were PD-L1 positive, respectively. This criteria was validated in various types of cancer (Fig. 1a).[17–19]

Scoring for CD8 positive T cell numbers. CD8 positive T cells were identified using an anti-CD8 rabbit monoclonal antibody (clone SP57; Roche Tissue Diagnostics, Mannheim, Germany). Intratumoral CD8-positive T cell (intra CD8) density, defined as CD8 cells that infiltrated into cancer nests, were scored IHC low or high when their mean number was <50 or ≥50, respectively. This criteria was validated in colorectal cancer.[20,21] Peritumoral CD8-positive T cell (peri CD8) density, defined as CD8 cells that infiltrated into the cancer stroma or were

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distributed along the invasive margin of cancer, were scored IHC low or high when the mean number was <200 or ≥200, respectively. CD8-positive T cell numbers were counted twice in a microscopic field at a magnification of ×200 (Fig. 1b).

Statistical analysis. The primary objective of the present study was to correlate the levels of PD-L1 expression with disease-free survival (DFS). DFS was defined as the duration between surgery and disease relapse, any cause of death before disease relapse, or the last follow-up. Survival analysis was conducted using the Kaplan–Meier method and Cox proportional hazard regression. The multivariate Cox model included all variables with $P < 0.10$ in the univariate model. Fisher's exact test was used to assess the association of PD-L1 expression with clinicopathological features. Statistical analyses were performed by SPSS software, version 23.0; IBM Corp, Armonk, NY, USA. Statistical significance was determined when as $P < 0.05$.

Results

Patients and tumor characteristics. A total of 235 patients were included in the analysis. A summary of patients and tumor characteristics is presented in Table 1.

PD-L1 expression on tumor cells or tumor-infiltrating mononuclear cells. High and low PD-L1 expression on TC was observed in 19 (8.1%) and 216 patients (91.9%) out of 235 patients, respectively. High and low PD-L1 expression on TIMC was observed in 36 (15.3%) and 199 patients (84.7%) out of 235 patients, respectively. No patients had high PD-L1 expression on both TC and TIMC (Suppl. Table S1).

Association of PD-L1 expression with disease-free survival. The median follow-up time of this study was 52.9 months (range 4.6–78.8). The total number of DFS events was 67. Kaplan–Meier survival analysis stratified by PD-L1 expression is shown in Figure 2. Patients with high PD-L1 expression on TC had a significantly shorter DFS than those with low expression (Fig. 2a), while patients with high PD-L1 expression on TIMC showed the opposite result (Fig. 2b).

Table 1. Patient characteristics

	Number (%)
Median age, years (range)	63 (32–84)
Male/female	140/95 (59.6/40.4)
Primary tumor site	
Right	63 (26.8)
Left	172 (73.2)
T status†	
T1	25 (10.6)
T2	28 (11.9)
T3	129 (54.9)
T4	53 (22.6)
Stage†	
IIIa	49 (20.9)
IIIb	141 (60.0)
IIIc	45 (19.1)
Histological type	
Well-moderately differentiated	217 (92.3)
Poorly differentiated and mucinous	18 (7.7)
Chemotherapy	
Fluoropyrimidine alone	174 (74.0)
Fluoropyrimidine with oxaliplatin	61 (26.0)

†UICC TNM classification of malignant tumors, 7th edition.

Patients with a high PD-L1 expression on TC had a significantly shorter DFS than those with a low expression in both univariate (hazard ratio [HR] 2.36; 95% confidence interval (CI): 1.21–4.62; $P = 0.012$] and multivariate analysis (HR 2.45; 95% CI: 1.24–4.85, $P = 0.010$). In contrast, patients with high PD-L1 expression on TIMC had a significantly longer DFS than those with low expression in univariate analysis (HR 0.40; 95% CI: 0.16–0.98; $P = 0.046$) but not in multivariate analysis (HR 0.55; 95% CI: 0.23–1.52; $P = 0.28$) (Table 2).

Association of PD-L1 expression with clinicopathological features. As shown in Table 3), tumors with high PD-L1 expression were associated with a more distant spread of lymph node metastasis ($P = 0.008$) and exhibited advanced TNM stage ($P < 0.001$). However, there was no relationship between PD-L1 expression on TIMC and lymph node metastasis or TNM stage. Of note, tumors with high PD-L1 expression on TIMC were significantly associated with increased intra CD8-positive T cells ($P < 0.001$) (Table 3).

Association of CD8-positive T cell density with disease-free survival. Of 235 patients, 40 (17.0%) and 195 (83.0%) patients had high and low intra CD8-positive T cell densities, respectively. Peri CD8-positive T cell density was high in 73 patients (31.1%) and low in 162 patients (68.9%) (Suppl. Table S2).

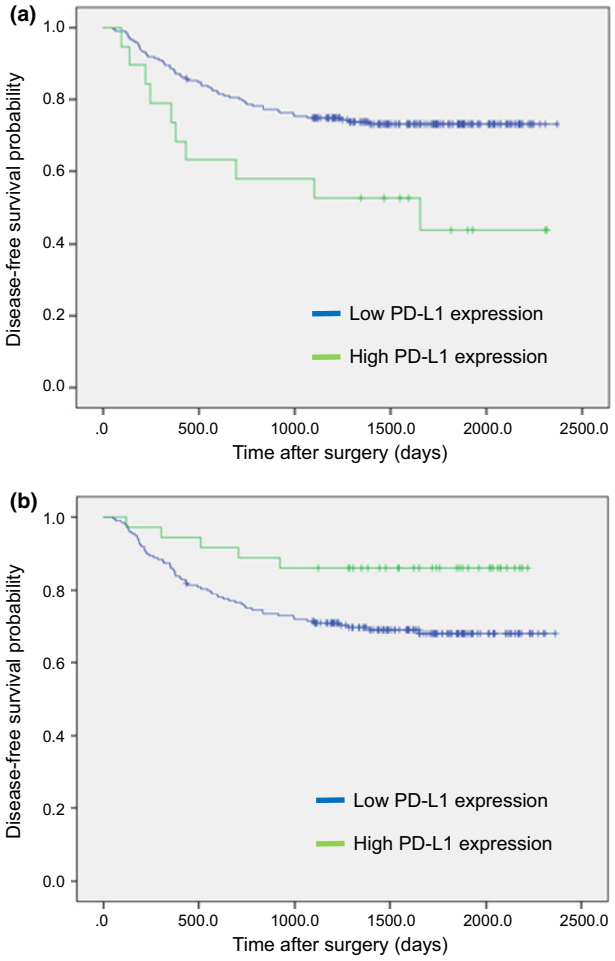

Fig. 2. Kaplan–Meier survival analysis of disease-free survival (DFS) stratified by PD-L1 expression on tumor cells (TC) (a) and tumor infiltrating mononuclear cells (TIMC) (b).

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Table 2. Univariate and multivariate analyses of prognostic factors associated with DFS

	Univariate		Multivariate	
	DFS		DFS	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age, years (≤65 *vs* >65)	0.988 (0.608–1.605)	0.960		
Sex (male *vs* female)	0.882 (0.537–1.447)	0.619		
Right versus left	0.854 (0.506–1.441)	0.555		
wel versus por/muc	2.804 (1.431–5.498)	0.003	2.607 (1.325–5.131)	0.006
PD-L1 expression (TC) low versus high	2.361 (1.205–4.624)	0.012	2.450 (1.239–4.847)	0.010
PD-L1 expression (TIMC) low versus high	0.395 (0.159–0.983)	0.046	0.549 (0.233–1.518)	0.277
Intra CD8 low versus high	0.352 (0.141–0.875)	0.025	0.395 (0.156–1.004)	0.051
Peri CD8 low versus high	0.932 (0.553–1.574)	0.793		
Chemotherapy (without *vs* with oxaliplatin)	1.382 (0.825–2.318)	0.219		

CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; intra CD8, intratumoral CD8-positive T cell; peri CD8, peritumoral CD8-positive T cell; por/muc, poorly differentiated/mucinous; TC, tumor cells; wel, well-moderately differentiated; TIMC, tumor-infiltrating mononuclear cells.

Table 3. Association of clinicopathological features with PD-L1 expression

		PD-L1 expression (TCs)¶			PD-L1 expression (TIMCs)¶		
		Low	High	*P*-value	Low	High	*P*-value
Age, years	≤65	120	14	0.151	107	27	0.018
	>65	96	5		92	9	
Sex	Male	128	12	0.811	119	21	0.856
	Female	88	7		80	15	
Primary site	Right	60	3	0.417	53	10	0.841
	Left	156	16		146	26	
Histological type	wel†	200	17	0.645	182	35	0.322
	por/muc†	16	2		17	1	
T status‡	T1	25	0	0.090	20	5	0.239
	T2	28	0		22	6	
	T3	116	13		108	21	
	T4	47	6		49	4	
N status‡	N1	157	8	0.008	140	25	1.000
	N2	59	11		59	11	
Stage‡	IIIa	49	0	<0.001	40	9	0.193
	IIIb	132	9		117	24	
	IIIc	35	10		42	3	
Intra CD8 positive T cells¶	Low	181	14	0.334	175	20	<0.001
	High	35	5		24	16	
Peri CD8 positive T cells¶	Low	151	11	0.305	140	22	0.328
	High	65	8		59	14	
Chemotherapy	FU alone§	162	12	0.279	146	28	0.682
	FU with Oxaliplatin	54	7		53	8	

†wel, well-moderately differentiated, por/muc, poorly differentiated/mucinous; ‡UICC TNM classification of malignant tumors, 7th edition; §FU, fluoropyrimidine, ¶definition of TCs, TIMCs, intra CD8, and peri CD8 are shown in methods.

Patients with high intra CD8-positive T cell density had a significantly longer DFS than patients with low density in univariate analysis (HR 0.35; 95% CI: 0.14–0.88; $P = 0.025$) but not in multivariate analysis (Table 2). In addition, there was no correlation between peri CD8-positive T cell density and DFS in both univariate and multivariate analyses (Table 2). Figure 3 shows the DFS Kaplan–Meier curve stratified by intra CD8-positive T cell and PD-L1 expression on TC (a), and by peri CD8-positive T cell and PD-L1 expression on TC (b). Patients with low PD-L1 expression on TC and high intra CD8-positive T cell numbers had the best prognosis (Fig. 3a), whereas patients with high PD-L1 expression on TC and low peri CD8-positive T cell numbers had the worst prognosis (Fig. 3b), although the relatively low number of patients in each group made it difficult to draw definitive conclusions.

Discussion

The current study showed that high PD-L1 expression on TC was significantly associated with a poor prognosis, whereas high PD-L1 expression on TIMC was associated with a good prognosis. To the best of our knowledge, this is the first study to demonstrate the prognostic significance of PD-L1 expression on stage III colorectal cancer.

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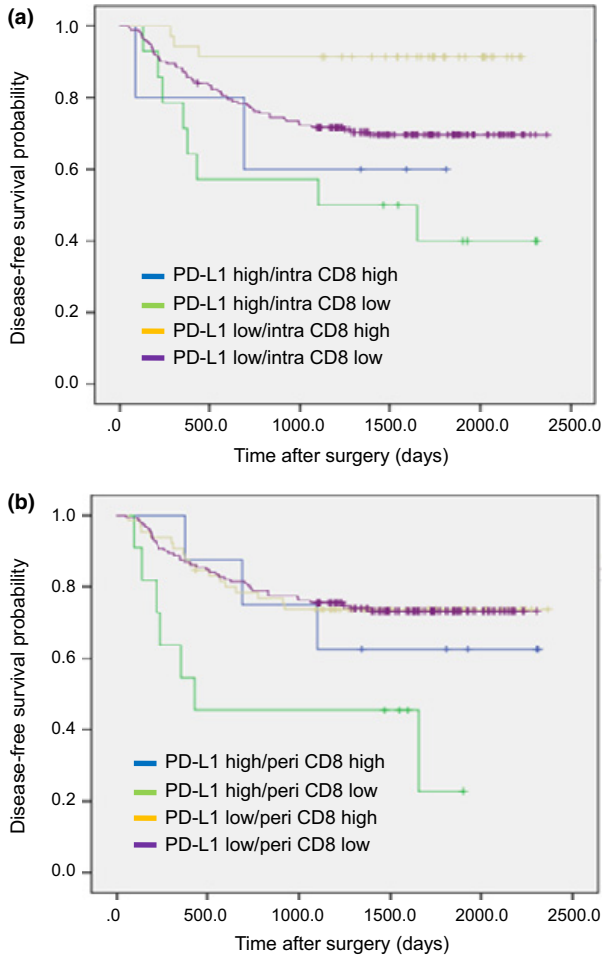

Fig. 3. Kaplan–Meier survival analysis of disease-free survival (DFS) stratified by intra CD8-positive T cells/PD-L1 expression on tumor cells (TC) (a), and peri CD8-positive T cells/PD-L1 expression on TC (b).

PD-L1 expression on TC may indicate immune evasion.[12] Previous studies have reported that high PD-L1 expression on TC is associated with advanced tumor stage and poor survival in patients with colorectal cancer.[22–24] However, the prognostic evaluation of these studies has been limited because they included patients at various disease stages. In the current study, we focused on stage III colorectal cancer and demonstrated that high PD-L1 expression on TC correlated with distant spread of lymph node metastasis, advanced tumor stage and poor prognosis. In addition, high infiltration of intra CD8 positive T cells was associated with longer DFS compared with those with low infiltration in the subset of low PD-L1 expressing TC, but there was no DFS difference in subsets of high PD-L1 expression (Fig. 3a). These results suggested that PD-L1 expression on TC might make intra CD8 positive T cells inefficient, leading to stage progression and/or poor survival in patients with stage III colorectal cancer.

The function of PD-L1 expression on TIMC has not been elucidated. The association of PD-L1 expression on TIMC with tumor aggressiveness and prognosis has not been determined across various tumor types. Thompson *et al.*[25] report that high PD-L1 expression on TIMC is associated with poor prognosis in renal carcinoma patients, but the opposite has been reported in urothelial cancer patients.[16] Here, we showed that high PD-L1 expression on TIMC was not correlated with tumor aggressiveness but with longer survival, in contrast to PD-L1 expression on TC. These findings highlight the need to evaluate these cell types separately within tumor microenvironments. In addition, we also showed the association of high PD-L1 expression on TIMC with increased intra CD8 positive T cell infiltration. These findings suggest that PD-L1 expression on TIMC may induce tumor antigen-specific CD8-positive T cells in tumor sites, which translates to a good prognosis in stage III colorectal cancer patients.

To date, several studies have reported that oncogenic pathway, such as BRAF, KRAS, phosphatidylinositol 3-kinase, catalytic, alpha (PIK3CA) and MMR status, might be associated with prognosis in patients with colorectal cancer. However, no consistent association has been observed between those molecular markers and DFS in stage III colorectal cancer.[8,26,27] In addition, the frequency of MMR deficient, which may be associated with PD-L1 expression,[28,29] was less than 5% in stage III colorectal cancer patients,[30] so that it may not influence our results.

Our study has several limitations. First, this study was a retrospective, single-institution study; therefore, there was the potential for selection bias. Second, we evaluated PD-L1 expression using only one antibody (SP142 clone). Previous study demonstrated the comparison of four different PD-L1 IHC assays for lung cancer.[31] They showed that the concordance rate of PD-L1 positivity in all four assays was 50% (19 of the 38 cases).[31] Importantly, SP142, the assay we used in our study, exhibited fewer stained tumor cells. Further investigation using other antibodies and cutoff values to validate our results is warranted. Third, we did not evaluate immune cell components in TIMC-expressed PD-L1 in our trial. It has become apparent that various myeloid cells express PD-L1 in colorectal cancer,[32] even though the prognostic value remains unclear. Further investigations are needed to identify the characteristics of PD-L1-expressed immune cells and reveal the function of PD-L1 molecules in those cells.

Recently, a new therapeutic approach using immune checkpoint inhibitors has achieved breakthrough results across various tumor types.[33–36] In addition, immune microenvironment status, such as PD-L1 expression on TC,[37] TIMC[17] and tumor infiltrating T cells, and MMR status[38] may be a potential predictive biomarkers in cancer immunotherapy. We believe that our analysis might enhance the development of novel treatment approaches for stage III colorectal cancer.

In conclusion, we demonstrated that high PD-L1 expression on TC negatively affected patient survival, whereas high PD-L1 expression on TIMC was associated with a favorable prognosis of patients with stage III colorectal cancer. Further prospective investigation is warranted to verify these prognostic roles in stage III colorectal cancer patients.

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Disclosure Statement

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. The number of patients with high (H) and low (L) PD-L1 expression on TC/TIMC.

Table S2. The number of patients with high (H) and low (L) intra/peri CD8 positive T cells.

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