PD-L1 upregulation is associated with activation of the DNA double-strand break repair pathway in patients with colitic cancer

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Abstract

Ulcerative colitis (UC) is a DNA damage-associated chronic inflammatory disease; the DNA double-strand break (DSB) repair pathway participates in UC-associated dysplasia/colitic cancer carcinogenesis. The DSB/interferon regulatory factor-1 (IRF-1) pathway can induce PD-L1 expression transcriptionally. However, the association of PD-L1/DSB/IRF-1 with sporadic colorectal cancer (SCRC), and UC-associated dysplasia/colitic cancer, remains elusive. Therefore, we investigated the significance of the PD-L1/DSB repair pathway using samples from 17 SCRC and 12 UC patients with rare UC-associated dysplasia/colitic cancer cases by immunohistochemical analysis. We compared PD-L1 expression between patients with SCRC and UC-associated dysplasia/colitic cancer and determined the association between PD-L1 and the CD8+ T-cell/DSB/IRF-1 axis in UC-associated dysplasia/colitic cancer. PD-L1 expression in UC and UC-associated dysplasia/colitic cancer was higher than in normal mucosa or SCRC, and in CD8-positive T lymphocytes in UC-associated dysplasia/colitic cancer than in SCRC. Moreover, PD-L1 upregulation was associated with γH2AX (DSB marker) and IRF-1 upregulation in UC-associated dysplasia/colitic cancer. IRF-1 upregulation was associated with γH2AX upregulation in UC-associated dysplasia/colitic cancer but not in SCRC. Multicolour immunofluorescence staining validated γH2AX/IRF-1/PD-L1 co-expression in colitic cancer tissue sections. Thus, immune cell-induced inflammation might activate the DSB/IRF-1 axis, potentially serving as the primary regulatory mechanism of PD-L1 expression in UC-associated carcinogenesis.

Introduction

Ulcerative colitis (UC) is a chronic autoinflammatory disease characterised by persistent inflammation in the colorectal mucosa, described as UC-associated dysplasia that can ultimately progress to UC-associated colorectal cancers (colitic cancer). This carcinogenetic sequence is designated as the “inflammation-dysplasia-carcinoma-sequence”.[1] Treatment of patients with UC-associated dysplasia/colitic cancer generally includes proctocolectomy with ileoanal anastomosis as metastatic or synchronic lesions are frequent.[2] In contrast, the pathogenesis of sporadic colon cancer (SCRC), which is sporadic or spontaneous colorectal cancer, is not associated with genetic factors or family history, and the associated carcinogenesis sequence is termed the “adenoma-carcinoma-sequence”.[3] Moreover, the carcinogenic sequences of colitic cancer and SCRC differ. Inflammatory responses initiated by the infiltrating immune cells and their secreted cytokines can lead to accumulation of DNA damage during UC.[1] The DNA double-strand break (DSB) repair pathway is, therefore, considered to play an important role in the carcinogenesis of both SCRC patients and rare UC patients with UC-associated dysplasia and colorectal cancers, the condition being referred to as colitic cancer.[4-6] Although DSBs are induced in the UC mucosa, rather than the normal colon mucosa,[7] the significance of DSBs in SCRC and UC-associated dysplasia/colitic cancer remains controversial.[6,8,9]

Recent advancements in immune checkpoint inhibitors (ICIs) targeting programmed cell death-1 (PD-1) and its ligand (PD-L1) have improved survival in several cancers, including SCRC, with high microsatellite

instability (MSI).[10] The interaction between PD-1 on cytotoxic T lymphocytes (CTLs) and PD-L1 on tumour cells inhibits proliferation, survival, and effector functions in immune cells, including the secretion of inflammatory cytokines.[11] Hence, PD-L1 levels are considered a marker of sensitivity towards ICIs in several cancers.[12] Furthermore, PD-L1 expression is upregulated in UC compared with that in the uninflamed colon mucosa,[13] while PD-L1 upregulation in SCRC is associated with cancer progression and a poor prognosis.[14] However, differences in PD-L1 expression profiles between SCRC and rare UC patients with UC-associated dysplasia/colitic cancer have not yet been investigated.

Several mechanisms underlying PD-L1 expression have been reported, including release of inflammatory cytokines from several immune cells such as CTLs, DNA damage responses, Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling, oncogenic pathways, hypoxic conditions, and microRNAs.[15] Among these, we focused on the DNA DSB repair pathway, which is important for colitis-associated carcinogenesis.[16,17] Moreover, Sato et al.[18] reported that DSBs can upregulate PD-L1, and the mechanism underlying PD-L1 expression is mediated via the activation of the DSB/interferon regulatory factor 1 (IRF-1) pathway. Indeed PD-L1 induction and overexpression by IRF-1 activation have been reported in several cancers,[19,20] with IRF-1 reportedly upregulated in UC compared with the normal colon mucosa in healthy volunteers.[21] However, limited information is available as to whether differences in PD-L1 expression profiles in SCRC and UC-associated dysplasia/colitic cancer are associated with the tumour-infiltrating immune cells/DSB/IRF-1 signalling axis in clinical samples from rare UC patients with UC-associated dysplasia/colitic cancer.

This study, therefore, aimed to investigate the associations among PD-L1 expression, immune cells, and the DSB repair pathway, and elucidate the potential mechanism underlying UC carcinogenesis. To this end, we performed immunohistochemistry for surgically resected samples from 17 SCRC patients and 12 UC patients with UC-associated dysplasia/colitic cancer.

Results

Clinical significance of PD-L1 expression in tissues from patients with SCRC and UC-associated dysplasia/colitic cancer

Membrane and cytoplasmic PD-L1 expression was detected in colorectal tissue samples harvested from patients with SCRC and those with UC (Figure 1). Membrane PD-L1 expression levels in tissues of UC and UC-associated dysplasia/colitic cancer were significantly higher than that in the tissues of SCRC and the corresponding normal mucosa ($P < 0.001$; Table 1). The association between PD-L1 expression and clinical and pathological factors of patients with SCRC and UC-associated dysplasia/colitic cancer is summarised in Table 2. Membrane PD-L1 expression levels differed significantly between male and female SCRC patients ($P = 0.028$; Table 2).

Correlation between PD-L1 and CD8+ CTLs, DSB marker γH2AX, and IRF-1 in SCRC and UC-associated dysplasia/colitic cancer

Figure 1 shows representative sections immunostained for γ-H2AX, IRF-1, and PD-L1 in the respective tissues. Figure 2 shows tumoural CD8 + CTLs in representative sections of SCRC and colitic cancer. PD-L1 expression in SCRC was significantly associated with IRF-1 expression (P = 0.035); however, no association was observed between PD-L1 expression in SCRC tissues and levels of CD8 or γH2AX (Table 3). Moreover, PD-L1 upregulation in UC-associated dysplasia/colitic cancer was significantly associated with γH2AX (P = 0.002) and IRF-1 (P = 0.002) upregulation but not with CD8 expression (P = 0.103; Table 3). IRF-1 upregulation in UC-associated dysplasia/colitic cancer was significantly associated with CD8 (P = 0.032) and γH2AX (P = 0.001) upregulation (Table 3). Furthermore, the expressions of CD8 as a CTL marker (P = 0.001), γH2AX as a DSB marker (P = 0.031), IRF-1 as a transcriptional factor for PD-L1 (P = 0.005), and PD-L1 (P = 0.003) in UC-associated dysplasia/colitic cancer were significantly higher than that noted for SCRC (Table 4). The expression patterns of target proteins in each UC patient with UC-associated dysplasia/colitic cancer are summarised in Table 5.

Associations among γH2AX, IRF-1, and PD-L1 expression in colitic cancer

Using multicolour immunofluorescence analysis, we were able to validate the co-expression of γH2AX, IRF-1, and membrane PD-L1 in seven UC with colitic cancer and dysplasia. Consequently, cancer cells expressing membrane PD-L1 showed nuclear γH2AX and IRF-1 expression, in contrast to those with low PD-L1 expression, which did not show co-expression of γH2AX, IRF-1, and PD-L1 (Figure 3). Furthermore, in SCRC tissue sections, γH2AX and IRF-1 were found to be co-expressed in cells expressing PD-L1 as well as those that did not, suggesting that PD-L1 regulation in SCRC may not be dependent on the DNA damage response/PD-L1 signalling axis (Supplementary Figure 1). These findings are consistent with the data presented in Table 3.

Discussion

This study showed that PD-L1 expression levels were significantly upregulated in UC and UC-associated dysplasia/colitic cancer tissues compared with that in SCRC tissues and the corresponding non-cancerous mucosa. Moreover, PD-L1 expression was higher in tumoural CD8-positive T lymphocytes in UC-associated dysplasia/colitic cancer tissues than that in SCRC tissues. PD-L1 upregulation was found to be associated with increased expression of γH2AX (a DSB marker) and IRF-1 (a PD-L1 inducer) in clinical UC-associated dysplasia and colitic cancer tissues but not in SCRC tissues or the corresponding non-cancerous UC mucosa.

Colitic cancer is caused by UC-induced persistent chronic inflammation, and the carcinogenic sequence of colitic cancer is suggested to differ from that of SCRC.[1] Chronic inflammation in UC frequently results in DSB through the generation of reactive oxygen species, with a dysfunctional DNA damage repair system suggested to lead to carcinogenesis during UC.[6-8] However, PD-L1 is regulated by various inflammation-associated transcription factors, including STAT1/3, IRF-1, and NF-κB.[19,22] Among these, DSB-induced-IRF-1 activates PD-L1.[18] The present findings showed that γH2AX expression was particularly associated with DSB in UC-associated dysplasia/colitic cancer and positively correlated with

IRF-1 expression. PD-L1 expression in UC-associated dysplasia/colitic cancer was also positively correlated with γH2AX and IRF-1 expression. Moreover, γH2AX, IRF-1, and PD-L1 were significantly upregulated in UC-associated dysplasia/colitic cancer compared with SCRC, suggesting activation of the DSB/IRF-1/PD-L1 signalling axis only in UC-associated dysplasia/colitic cancer and not in SCRC. These results indicated that PD-L1 expression in UC-associated dysplasia and colitic cancer was potentially associated with the activation of the DSB/IRF-1 signalling axis.

ICIs have recently attracted increasing attention as an innovative cancer treatment strategy. [23] However, the beneficial effects of ICI treatment in SCRC patients are often limited to MSI-high cancers with a high tumour burden,[24,25] and it remains unknown whether ICIs are effective in rare cases of UC with UC-associated dysplasia/colitic cancer. Studies have reported the tumour mutational burden as a promising biomarker for predicting the sensitivity to ICIs,[26] with tumours exhibiting high PD-L1/high CD8+ CTLs designated as “immune-inflamed tumours” or “hot tumours” displaying positive responses compared with ICI-resistant uninflamed tumours or ‘cold tumours’ without PD-L1 expression/CD8+ CTLs.[27] In contrast, enterocolitis is known as one of the most common adverse events associated with ICIs, and it is distinct with clinical and pathological characteristics similar to that of inflammatory bowel disease (IBD). [28,29] A recent study showed patients with pre-existing IBD to be at an increased risk for several gastrointestinal adverse events associated with ICIs, and the safety of ICI treatment for patients with pre-existing IBD is undetermined.[30] However, TNF-α blockade treatment in patients with IBD strongly inhibits inflammation in the colorectal mucosa by suppressing several pro-inflammatory pathways, including the TNF-α pathway[31], and immunosuppressive therapy, including TNF-α blockade and vedolizumab, which is an α4β7 integrin inhibitor blocking the migration of gut-specific lymphocytes into the gut, is administered for treating not only IBD, but also ICI-induced colitis.[32,33] Particularly, vedolizumab has been administered concurrently with ICIs in patients with ICI-induced colitis.[34] Furthermore, TNF-α blockade does not inhibit the antitumour effect of ICIs in experimentally induced melanoma.[35] Perez-Ruiz E et al.[36] reported the therapeutic effect of TNF-α blockade against ICI induced-colitis in tumour-bearing mouse models. Several studies have demonstrated that ICIs can be administered without occurrence of serious adverse events in a small number of patients with IBD,[37-39] and Frohne CC et al.[40] reported that the combination of vedolizumab and ICI could be successfully used in treating a patient having metastatic melanoma with active Crohn's disease and suppressed Crohn’s disease flares and ICI-induced colitis. This study showed that PD-L1 and IRF-1 expression levels, as well as CD8+ CTLs in UC-associated dysplasia/colitic cancer were significantly increased compared with that in the SCRC samples, suggesting that UC-associated dysplasia/colitic cancer, rather than SCRC, was potentially associated with the “hot tumour” phenotype. Furthermore, the tumour mutational burden in colitic cancer is greater than that in SCRC,[41] and patients with melanoma having high IRF-1 expression levels exhibit a significantly higher sensitivity towards ICI treatment than those with low IRF-1 expression.[42] These results suggested that ICI treatment might be more effective in patients with UC-associated dysplasia/colitic cancer with distant metastases after total colectomy that are not at a risk for ICI-induced colitis than in patients with SCRC. This is because, a combinatorial treatment with ICIs and TNF-α

blockade and vedolizumab might help control ICI induced-colitis in patients with UC-associated dysplasia/colitic cancer rather than in those with SCRC, with clinical characteristics including favourable ICI responses, “hot tumour” immune phenotypes including high PD-L1/high CD8+ CTLs, and high tumour mutational burden and IRF-1 expression.

Nonetheless, our study has several limitations. First, this was a retrospective study with a small patient cohort because UC-associated dysplasia and colitic cancer are rare types of cancer; this may have introduced a bias in our results. Hence, prospective studies with a larger patient cohort may be challenging for clearly elucidating the UC carcinogenesis mechanisms, considering the rarity of the disease. Currently, biological data on UC with UC-associate dysplasia/colitic cancer have been obtained from small-scale clinical cohort studies. This study provides further valuable insights into the importance of PD-L1 expression in patients with UC with UC-associated dysplasia/colitic cancer. Second, this study discussed the potential use of ICIs for colitic cancer treatment; however, we only enrolled patients that did not receive ICI treatment targeting the PD-1/PD-L1 signalling axis. Therefore, future studies are required to evaluate the potential of DSB/IRF-1/PD-L1 as a biomarker for rare cases of UC-associated dysplasia/colitic cancer to be treated with ICIs. Lastly, only three target markers were assessed, which may not be sufficient to clarify the complexity of the interactions between PD-L1 and the DSB repair response. Therefore, in the future we plan to examine the importance of and relationship among these three markers in an experimental animal model[43] of inflammatory colorectal carcinogenesis.

In conclusion, this study showed that PD-L1 expression was significantly higher in UC and UC-associated dysplasia/colitic cancer than in the normal mucosa and SCRC. Furthermore, PD-L1 expression was significantly associated with high levels of tumoural CD8+ CTLs, γH2AX as a DSB marker, and IRF-1 as a transcriptional factor for PD-L1 in UC-associated dysplasia/colitic cancer compared with SCRC. Therefore, our findings suggested that immune cell induced-chronic inflammation might activate the DSB/IRF-1 signalling axis, which might serve as the primary regulatory mechanism for regulating PD-L1 expression in UC carcinogenesis.

Methods

Patients and samples

Twelve patients (nine male and three female) with UC, who underwent surgical resection for UC-associated dysplasia/colitic cancer at Gunma University Hospital (Maebashi, Gunma, Japan), Maebashi Red Cross Hospital (Maebashi, Gunma, Japan), and Gunma Prefectural Cancer Center (Ohta, Gunma, Japan) between 1999 and 2014, were included in this retrospective study. The median age of the patients was 54 years (range, 37–76 years). One patient only had UC-associated dysplasia. Three patients harboured two or more tumours, and all dysplastic and cancerous lesions were evaluated. All dysplastic lesion samples were obtained from patients with high-grade dysplasia, while patients with low-grade dysplasia were not included in the study. Additionally, 17 SCRC patients (12 male and five female) who underwent partial colectomy at the Gunma University Hospital between 1999 and 2014 were randomly

selected and included in the study. Supplementary table 1 summarises the clinical characteristics of the patients. For an accurate pathological diagnosis of dysplastic and cancerous lesions among patients with UC, all histological cancer tissue sections were evaluated by a specialised pathologist, Dr. Yao T (Department of Human Pathology, Juntendo University Graduate School of Medicine). This study conformed to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board for Clinical Research at the Gunma University Hospital, Maebashi, Gunma, Japan (approved number: HS2018-092). Owing to the retrospective nature of this study, informed consent was waived, and participants were given the opportunity to decline participate in this study via opt-out method. All methods were performed in accordance with relevant guidelines and regulations.

Immunohistochemistry

Paraffin-embedded blocks of all surgically resected specimens obtained from the patients were cut into 4 μm thick sections and mounted on glass slides. Sections were deparaffinised with xylene and dehydrated in alcohol. Endogenous peroxidase was inhibited using 0.3% H_2O_2/methanol for 30 min at approximately 26 °C. After rehydration through a graded ethanol series, antigen retrieval was performed using Immunosaver (Nisshin EM, Tokyo, Japan) at 98–100 °C for 45 min, and PD-L1 was retrieved using Universal HIER antigen retrieval reagent (Abcam, ab208572) at 120 °C for 20 min in an autoclave. Nonspecific binding sites were blocked through incubation with Protein Block Serum-Free (Dako, Carpinteria, CA, USA) for 30 min. Thereafter, sections were probed with primary anti-PD-L1 (Abcam, 28-8 Rabbit mAb, 1:200), anti-γH2AX (Abcam, 9F3, Mouse mAb, 1:200; DSB marker) [44], anti-IRF-1 (Abcam, EPR 18301, Rabbit mAb, 1:300), and anti-CD8 (Dako C8/144B, Mouse mAb, 1:100) antibodies at 4 °C for 24 h. The slides were then stained using rabbit-specific IHC polymer detection kit HRP/DAB (Abcam, ab209101) containing the secondary antibody for PD-L1-stained sections, and with the Histofine Simple Stain MAX-PO (Multi) Kit (Nichirei, Tokyo, Japan) for other markers, in accordance with the manufacturer's instructions. The chromogen 3,3'-diaminobenzidine tetrahydrochloride was applied as a 0.02% solution, containing 0.005% H_2O_2 in ammonium acetate-citrate acid buffer (50 mM, pH 6.0). Finally, nuclear counterstaining was performed using Mayer's haematoxylin solution. In the negative control sections, primary antibodies were replaced with phosphate-buffered saline in 0.1% bovine serum albumin, thus confirming a lack of staining.

Assessment of PD-L1, γH2AX, IRF-1, and CD8 staining

Immunostained sections were evaluated by two experienced researchers blinded to the clinical data. We evaluated tumour cells and non-cancerous cells displaying membrane PD-L1 staining as positive when at least 1% of the cells were stained.[45] The staining intensity for γH2AX was scored as follows: 0, no staining; 1+, weak staining; 2+, moderate staining; and 3+, strong staining. The percentage of nuclear-stained cells was determined by examining three sections with the highest staining intensity. The percentage of nuclear γH2AX staining was scored as follows: 0, no staining; 1+, 1–25%; 2+, 26–50%; and 3+, 51–100%. The final score was defined as the percentage score multiplied by the intensity score (0, 1+, 2+, 3+, 4+, 6+, and 9+). Nuclear immunoreactivity of γH2AX was scored as 0–4+ and 6–9+, which were

defined as low and high nuclear expression, respectively.[46] We evaluated nuclear and cytoplasmic staining for IRF-1 using almost the same method as that for γH2AX described above. The staining percentage for IRF-1 was scored as follows: 0, no staining; 1+, 1–25%; 2+, 26–75%; and 3+, 76–100%.[47] The final score was defined as the percentage score multiplied by the intensity score (0, 1+, 2+, 3+, 4+, 6+, and 9+). IRF-1 immunoreactivity was scored as 0–4+ and 6–9+, which were defined as low and high expression, respectively. Supplementary Figure 2 shows the representative low or high expression of γH2AX, IRF-1, and PD-L1 in SCRC and UC-associated dysplasia/colitic cancer tissues. Tumoural CD8-positive CTLs, defined as CD8 cells infiltrating the cancer stroma, were scored as low or high when the mean number of cells in a microscopic field at ×200 magnification was <200 or ≥200, respectively.[48]

Multicolour immunofluorescence staining for PD-L1, γH2AX, and IRF-1

Multicolour immunofluorescence staining was performed for seven patients with UC on a total of seven colitic cancer and two dysplasia tissue sections, and three SCRC tissue sections using a PerkinElmer Opal kit (Catalogue# NEL810001KT, PerkinElmer, Hopkinton, MA, USA) in accordance with the manufacturer's instructions. γH2AX staining was visualised using the Opal 520 Fluorophore, IRF-1 staining with the Opal 690 Fluorophore, and PD-L1 staining with the Opal 570 Fluorophore. All sections were then counterstained with 4',6-diamidino-2-phenylindole (DAPI) and examined under an FV10i-LIV system (Olympus Life Science, Tokyo, Japan).

Statistical analysis

The JMP Pro 14.0 software package (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. Mann–Whitney U test and Fisher's exact test were performed to analyse the associations among PD-L1 expression, immune cells, and the DSB repair pathway. All differences were considered statistically significant at $P < 0.05$.

Declarations

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

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Additional Information

The authors declare no competing interests.

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Tables

Table 1. Expression of PD-L1 in respective colon tissues.

Factors	PD-L1		P-value
	Low (%)	High (%)	
[a]Normal mucosa	14 (100)	0 (0)	P<0.001
[b]UC	2 (17)	10 (83)	
SCRC	11 (65)	6 (35)	
Dysplasia/colitic cancer	4 (17)	19 (83)	

UC, ulcerative colitis; SCRC, sporadic colorectal cancer

[a]Normal mucosa indicates the corresponding non-cancerous tissue in sections with SCRC.

[b]UC indicates the corresponding non-cancerous tissues in sections with dysplasia/colitic cancer.

Table 2. Association between the expression of PD-L1 and clinicopathological factors in SCRC, dysplasia/colitic cancer tissues.

Factors	SCRC (n = 17)		P-value	Dysplasia/colitic cancer (n = 23)		P- value
	PD-L1			PD-L1		
	Low	High		Low	High	
	11	6		4	19	
Sex						
Female	10	2	0.028	0	3	1.000
Male	1	4		2	7	
Age						
<65	6	2	0.619	1	8	0.455
>65	5	4		1	2	
Location						
Right	6	4	1.000	3	4	0.067
Left	5	2		1	15	
Differentiation						
Dysplasia/Well/Moderate	11	5	0.353	3	18	0.324
Poor	0	1		1	1	
T factor						
m, sm, mp	5	1	0.333	2	14	0.557
ss,se, si	6	5		2	5	
N factor						
Absent	8	4	1.000	2	17	0.125
Present	3	2		2	2	
M factor						
Absent	11	5	0.353	2	9	1.000
Present	0	1		0	1	
TNM stage						
0, I, II	8	3	0.600	0	8	0.091
III,IV	3	3		2	2	

Age, sex, M factor, and TNM stage data for dysplasia/colitic cancer are shown for a total of 12 patients, and not for the total 23 lesions analyzed in the study.

Table 3. Association between the expression of PD-L1, IRF-1 and that of other proteins in SCRC, dysplasia/colitic cancer tissues.

Factors		SCRC PD-L1		P-value	Dysplasia/colitic cancer PD-L1		P-value
		Low	High		Low	High	
		11	6		4	19	
CD8	Low	10	5	0.596	3	5	0.103
	High	1	1		1	14	
γH2AX	Low	4	4	0.247	2	0	0.024
	High	7	2		2	19	
IRF-1	Low	9	1	0.035	3	0	0.002
	High	2	5		1	19	
Factors		SCRC IRF-1		P-value	Dysplasia/colitic cancer IRF-1		P-value
		Low	High		Low	High	
		10	7		3	20	
CD8	Low	9	6	0.669	3	5	0.032
	High	1	1		0	15	
γH2AX	Low	4	4	0.419	3	0	0.001
	High	6	3		0	20	

Table 4. Difference in the expression of immune-related factors between SCRC and dysplasia/colitic

Factor		SCRC	Dysplasia/colitic cancer	P-value
CD8	Low	15	8	0.001
	High	2	15	
γH2AX	Low	8	3	0.031
	High	9	20	
IRF-1	Low	10	3	0.005
	High	7	20	
PD-L1	Low	11	4	0.003
	High	6	19	

Table 5. Clinicopathological characteristics of UC patients with UC-associated dysplasia/colitic cancer.

Pathological diagnosis	Location	CD8	γH2AX	IRF-1	PD-L1	Differentiation	T factor	N factor	M factor	Stage
Case 1										
UC	S	+	-	+	+	-	-	-	-	
Cancer	S	-	+	+	+	Poor	4b	0	0	IIC
Case 2										
UC	D	+	-	+	+	-	-	-	-	
Cancer	D	+	+	+	+	Well	1	0	0	I
Cancer	Rb	+	+	+	+	Well	2	0	0	
Case 3										
UC	S	-	-	+	+	-	-	-	-	
Dysplasia	Ce	-	+	+	+	-	-	-	-	
Dysplasia	A	-	+	+	+	-	-	-	-	
Dysplasia	S	-	+	+	+	-	-	-	-	
Cancer	Ce	+	+	+	+	Moderate	3	0	0	IIB
Cancer	A	+	+	+	+	Moderate	4a	1a	0	
Cancer	S	-	+	+	+	Moderate	2	0	0	
Case 4										
UC	S	-	-	+	+	-	-	-	-	
Dysplasia	S	+	+	+	+	-	-	-	-	
Cancer	S	+	+	+	+	Well	2	0	0	I
Case 5										
UC	S	+	-	+	+	-	-	-	-	
Dysplasia	S	+	+	+	+	-	-	-	-	
Cancer	S	+	+	+	+	Well	4a	2a	M1b	IVB
Case 6										
UC	T	+	-	+	-	-	-	-	-	
Dysplasia	T	+	+	+	-	-	-	-	-	
Cancer	T	-	-	-	-	Moderate	3	1a	0	IIB
Case 7										
UC	D	-	-	+	-	-	-	-	-	
Cancer	A	-	-	-	-	Poor	4a	2a	0	IIC
Cancer	D	-	-	-	-	Moderate	1b	0	0	
Case 8										
UC	S	+	-	+	+	-	-	-	-	
Cancer	S	+	+	+	+	Moderate	4a	0	0	IIB
Case 9										
UC	Ra	-	-	-	+	-	-	-	-	
Cancer	Ra	+	+	+	+	Well	Tis	0	0	0
Case 10										
UC	Rb	+	-	+	+	-	-	-	-	
Cancer	Rb	+	+	+	+	Well	T1b	0	0	I
Case 11										
UC	D	+	-	-	+	-	-	-	-	
Dysplasia	D	+	+	+	+	-	-	-	-	
Cancer	D	+	+	+	+	Well	Tis	0	0	0
Case 12										
UC	RS	+	-	-	+	-	-	-	-	
Dysplasia	RS	+	+	+	+	-	-	-	-	0*

Ce, cecum; A, ascending colon; T, transverse colon; D, descending colon; S, sigmoid colon; RS, rectosigmoid; Ra, upper rectum; Rb, lower rectum.

UC indicates the corresponding noncancerous tissues in sections with dysplasia/colitic cancer.

*One case with no colitic cancer and only dysplasia was included in Stage 0.

Figures

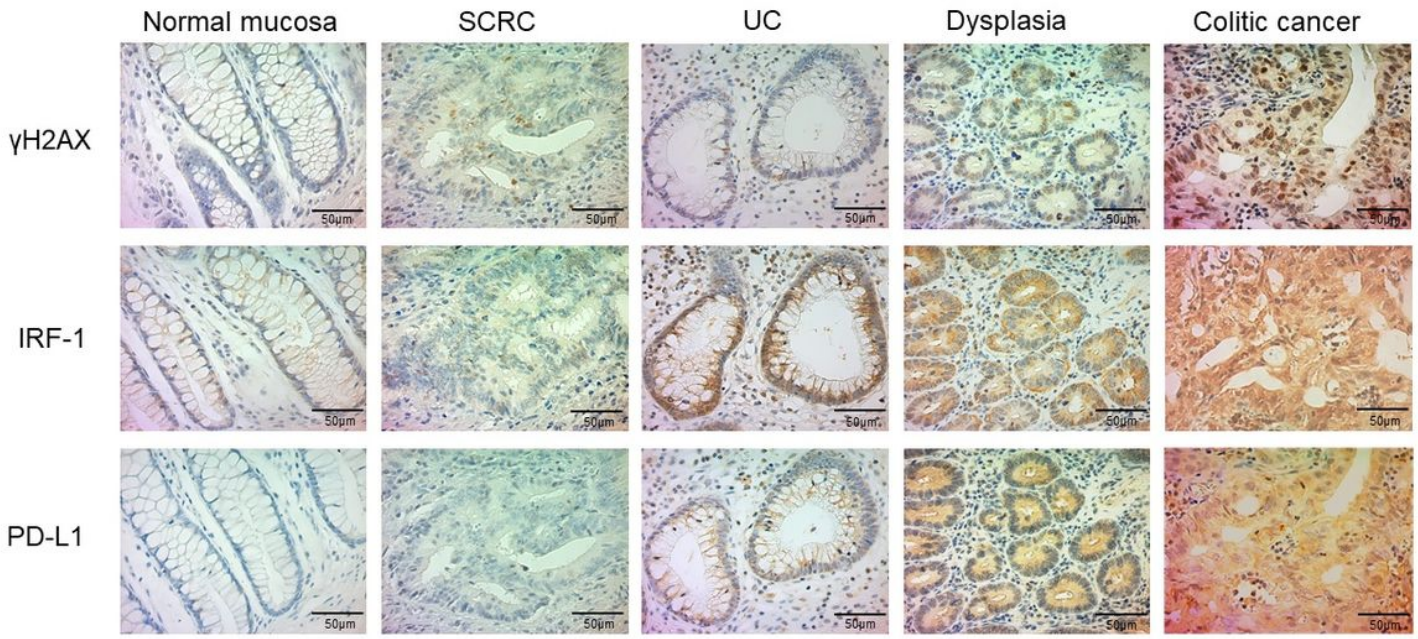

Figure 1

Immunohistochemistry for γH2AX, IRF-1, and PD-L1 expression in the respective colon tissues. The upper panel displays γH2AX expression in the normal mucosa, SCRC, UC, UC-associated dysplasia and colitic cancer. The middle panel displays IRF-1 expression in the normal mucosa, SCRC, UC, UC-associated dysplasia and colitic cancer. The lower panel shows the expression of PD-L1 in the normal mucosa, SCRC, UC, UC-associated dysplasia and colitic cancer. Scale bar, 50 μm (original magnification, ×400). SCRC, sporadic colorectal cancer; UC, ulcerative colitis; γH2AX, H2A.X variant histone; IRF-1, interferon regulatory factor 1; PD-L1, programmed cell death ligand 1.

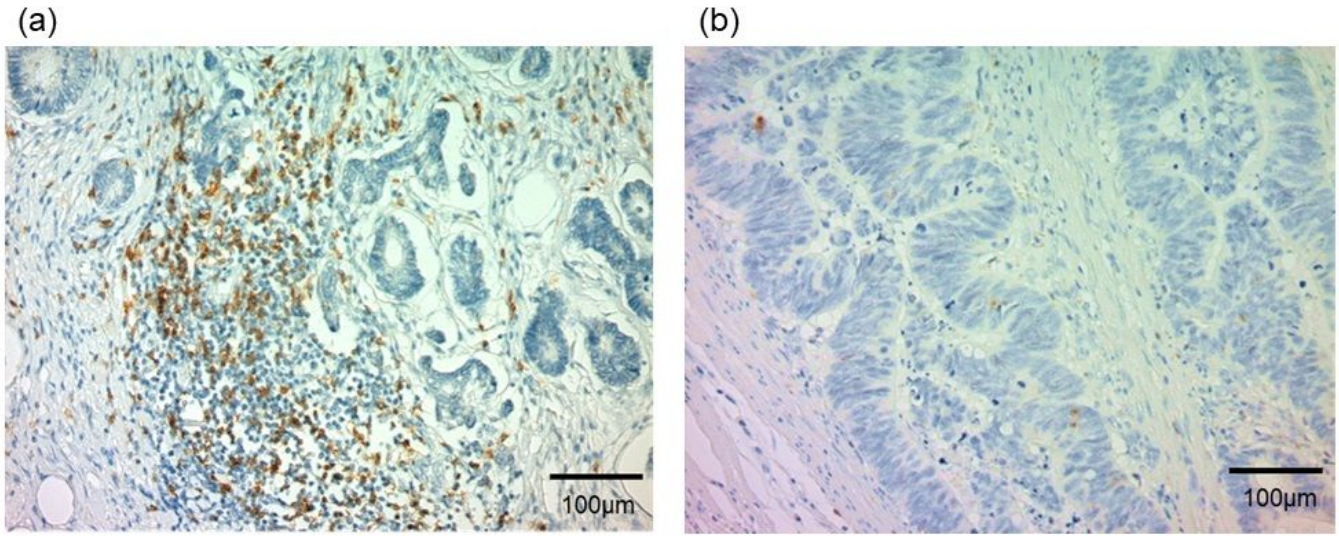

Figure 2

Immunohistochemistry for CD8 expression in colitic cancer and sporadic colorectal cancer. (a) High levels of tumoural CD8 cytotoxic T lymphocytes (CTLs) in a representative colitic cancer tissue. (b) Low levels of tumoural CD8 CTLs in a representative sporadic colorectal cancer (SCRC) tissue.

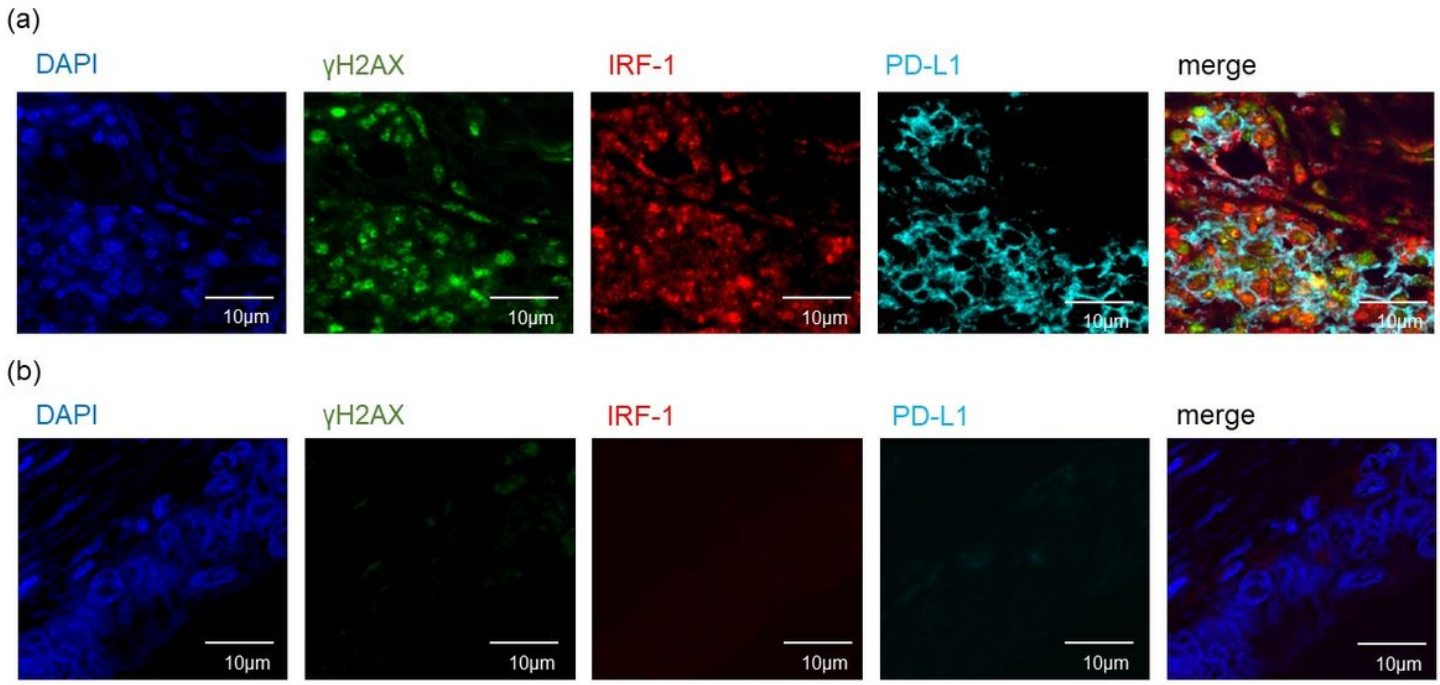

Figure 3

Immunofluorescence analysis for γH2AX, IRF-1, and PD-L1 expression in colitic cancer tissues. Colitic cancer tissues with high (a) or no (b) PD-L1 expression were immunostained with anti-γH2AX (green), anti-IRF-1 (red), and anti-PD-L1 (cyan) antibodies. All sections were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue). Scale bar, 10 μm (original magnification, ×60). γH2AX, H2A.X variant histone; IRF-1, interferon regulatory factor 1; PD-L1, programmed cell death ligand 1.

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