Tumor-associated macrophage infiltration is highly associated with PD-L1 expression in gastric adenocarcinoma

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Abstract

Background Programmed death ligand 1 (PD-L1) is a key protein upregulated by tumor cells to suppress immune responses. Tumor-associated macrophages (TAMs) play a major role in this immunosuppression, but the relationship between PD-L1 expression and TAMs remains unclear in gastric adenocarcinoma (GAC). We simultaneously examined expression of PD-L1 and TAMs in GAC.

Methods We performed immunohistochemical staining for PD-L1, CD68 (pan-macrophage), and CD163 (M2-like macrophage) in 217 GAC samples using a tissue microarray. Expression of PD-L1 and CD68- and CD163-positive cells was evaluated using the Cytoplasmic V2.0 algorithm in

Aperio ImageScope software, and logistic regression analysis was used to compare expression patterns between groups. **Results** Thirty-one samples (14%) were positive for PD-L1 expression. The mean ($\pm$ standard error) rates of infiltration were $6.83 \pm 0.38\%$ for CD68-positive cells and $6.16 \pm 0.29\%$ for CD163-positive cells. The mean rate of CD163-positive cell infiltration was significantly higher in diffuse GAC than in intestinal GAC (diffuse $n = 111$, 6.91%; intestinal $n = 91$, 5.26%; $p = 0.006$), but the mean rate of CD68-positive cell infiltration was similar between these types ($p = 0.38$). The mean infiltration rates of CD68- and CD163-positive cells in PD-L1-positive GAC were significantly higher than in PD-L1-negative GAC (CD68 $p = 0.0002$; CD163 $p < 0.0001$). In multivariate logistic regression analyses, CD163-positive cell infiltration was associated with PD-L1 expression (odds ratio 1.13; 95% confidence interval 1.02–1.25; $p = 0.021$). **Conclusion** M2-like macrophage infiltration is highly associated with PD-L1 expression in GAC cells, suggesting that macrophage infiltration can serve as a potential therapeutic target.

Keywords Gastric adenocarcinoma · Programmed death protein 1 · Tumor microenvironment · Macrophages

Introduction

Gastric adenocarcinoma (GAC) is the fifth most common cancer (951,000 cases per year) and the third leading cause of cancer-related mortality worldwide (723,000 deaths per year) [1]. Despite the development of multimodality therapies such as surgery, systemic therapy, and radiation therapy, the mortality rates for GAC remain high [2]. Immune checkpoint blockade has recently received much attention for prolonging patient survival in GAC.

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Programmed death protein 1 (PD-1) in T cells and its ligands (PD-L1/PD-L2) in tumor cells inhibit the response of T cells to tumor cells. Therefore, PD-1/L1 inhibitors have been developed for clinical application and shown to be efficacious in several cancers [3–5]. Many ongoing clinical trials are evaluating the benefit of PD-1/L1 inhibitors in GAC, and early results are promising [6–10]. Importantly, GAC is more sensitive to PD-1/L1 inhibitors when PD-L1 expression is present, and some trials have enrolled only patients with PD-L1 expression [8–10]. Thus, discovering the mechanisms and the predictive markers of PD-L1 expression can further refine selection of patients with GAC most likely to benefit from PD-1/L1 inhibitors.

The tumor microenvironment, especially immune cell infiltration, appears to be associated with PD-L1 expression. For instance, in the beginning, infiltrating T cell and B cells may be targeting tumor cells and subsequently facilitate PD-1 pathway [11]. Moreover, tumor-associated macrophages (TAMs), especially M2 macrophages, are also promoting an immunosuppressive microenvironment [12, 13]. Several reports have shown that TAMs overexpress PD-L1 themselves and suppress cytotoxic T lymphocyte function against tumor cells [14]. However, the relationship between PD-L1 expression in tumor cells and TAMs remains unclear.

In the current study, we performed immunohistochemical staining of PD-L1, CD68 (pan-macrophage), and CD163 (M2-like macrophage) in 217 GAC samples using a tissue microarray (TMA) and evaluated the relationship between PD-L1 expression in GAC cells and macrophage infiltration as well as the clinicopathologic features.

Materials and methods

Study subjects

This study retrospectively evaluated 217 GAC samples (using TMA) from patients who underwent resection at The University of Texas MD Anderson Cancer Center (Houston, USA) or at St. Luke's International Hospital (Tokyo, Japan) between March 1999 and September 2011. The analysis excluded patients who underwent palliative resection and those whose tissue samples were unavailable for TMA. Tumors were staged according to the American Joint Committee on Cancer Staging Manual (6th edition) [15]. Overall survival was defined as the time between the treatment start date and the date of death. All procedures were followed in accordance with the ethical standards of institutional and national committees on human experimentation and with the Helsinki Declaration of 1964 and later versions. Informed consent or a substitute for it was obtained from all patients included in the study.

TMA construction

Representative areas of the tumors were marked by a gastrointestinal pathologist (J.S.E.) on hematoxylin-eosin-stained sections of formalin-fixed, paraffin-embedded archival GAC or normal (control) gastrointestinal tissue. Using an automated tissue arrayer (Beecher ATA-27; Estigen OU, Tartu, Estonia), we extracted duplicate 1-mm-diameter cores from the designated areas of the corresponding paraffin blocks and placed the cores in the TMA blocks according to prearranged maps. Staggering of cores was used to ensure proper orientation and identification of each case. Sections that were 5 µm thick were cut from the TMA blocks for immunohistochemical analysis.

Immunohistochemistry

TMA slides were dewaxed in xylene and ethanol and steamed in a 2100 Retriever (Aptum Biologics, Southampton, UK) with pH 6.0 sodium citrate buffer to retrieve antigen epitopes. Once the samples had cooled, endogenous peroxidase activity was blocked using 3% hydrogen peroxide, and biotin was blocked using a biotin blocking kit. The slides were incubated at 4 °C overnight with a primary antibody: PD-L1 with an anti-human PD-L1 rabbit monoclonal antibody (dilution 1:100; clone SP142; Spring Bioscience, Pleasanton, CA, USA), CD68 with an anti-human CD68 mouse monoclonal antibody (dilution 1:200; KP-1; Agilent, Santa Clara, CA, USA), or CD163 with an anti-human CD163 mouse monoclonal antibody (dilution 1:200; NCL-L-CD163; Leica, Nussloch, Germany). The secondary antibody was a ready-to-use VECTASTAIN ABC HRP Kit (Vector Laboratories, Burlingame, CA, USA).

Evaluation of immunohistochemistry

The density of CD68- and CD163-positive cells was evaluated using computer-assisted image analysis and the Cytoplasmic V2.0 algorithm in Aperio ImageScope software (Leica, Nussloch, Germany). Density was calculated as the area with positive staining divided by the total area analyzed. Fifteen patient samples could not be evaluated for the CD163-positive cell area owing to sample conditions.

PD-L1 expression on tumor cells, not stroma, was immunohistochemically analyzed by an experienced pathologist (X. Dong) without knowledge of the clinical data or CD68- or CD163-positive cell density. Samples were considered PD-L1 positive if >1% of tumor cells expressed PD-L1. One patient sample could not be evaluated for PD-L1 expression owing to the sample condition. Cancer and stromal cells were distinguished by cell morphology, structures, and nucleus status.

Statistical methods

IBM SPSS Statistics Software, version 23, was used for all statistical analyses. All p values were two-sided, and the statistical significance cutoff was $p \leq 0.05$. Means were compared using the independent-sample Kruskal-Wallis test. Association between categorical variables was assessed using the Pearson chi-square test or Fisher's exact test, accordingly. We performed univariate and multivariate logistic regression analyses to assess the predictors of associated with positive PD-L1 and estimated the odds ratios with 95% confidence intervals. Factors with $p \leq 0.25$ in the univariate analyses were entered into the multivariate analyses. To assess the interaction between the variables, we cross-correlated the intratumoral density of CD163-positive cells with another variable of interest in the odds ratio for PD-L1 expression in GAC cells, and the interaction was evaluated. The hazard ratio (HR) of mortality was assessed by the Cox regression model.

Results

PD-L1 expression and CD68+ and CD163+ macrophage infiltration in GAC

We performed immunohistochemical staining for PD-L1, CD68, and CD163 in 217 GAC tissue samples using a TMA (Fig. 1). Thirty-one of the 216 evaluable GAC samples (14%) stained positive for PD-L1 expression in cancer cells and 24 samples (11%) stained positive in stroma

(Table 1). None of the clinicopathologic variables was associated with PD-L1 expression (all $p > 0.05$).

The intratumoral density of CD68-positive cells in the 217 GAC samples was distributed as follows: mean 6.83; median 5.24; standard deviation 5.65; range 0.48–32.4; interquartile range 2.88–8.73 (Table 2). The intratumoral density of CD163-positive cells in the 202 evaluable GAC samples was distributed as follows: mean 6.16; median 5.06; standard deviation 4.24; range 0.37–22.7; interquartile range 3.28–7.94 (Table 2). The intratumoral density of CD163-positive cells in diffuse GAC was significantly higher than in intestinal GAC ($p < 0.006$; Table 2). However, the intratumoral density of CD68-positive cells was not associated with the Lauren classification.

Relationship between PD-L1 expression and CD68- or CD163-positive cell intratumoral density in GAC

We then examined the relationship between PD-L1 expression and the intratumoral density of CD68- and CD163-positive cells (Fig. 2). The density of CD68- and CD163-positive cells in PD-L1-positive GAC samples was significantly higher than in PD-L1-negative GAC samples (CD68 $p = 0.0002$; CD163 $p < 0.0001$). These results indicate that CD163 density was more strongly associated with PD-L1 expression in GAC cells than CD68 density was.

PD-L1 expression in stroma was also significantly associated with higher CD68- or CD163-positive cell intratumoral density (CD68 $p \leq 0.0001$; CD163 $p = 0.0227$).

Fig. 1 Immunohistochemistry staining for PD-L1, CD68, and CD163 expression in gastric adenocarcinoma tissues. The *left panel* shows negative staining for PD-L1 and low macrophage density. The *right panel* shows positive staining for PD-L1 and high macrophage density

Table 1 PD-L1 expression (via immunohistochemistry) and clinical features in 216 evaluable gastric adenocarcinoma patient samples

Clinical or pathologic feature	Total no.	PD-L1 expression in cancer cell			PD-L1 expression in stroma cell		
		Positive	Negative	<i>p</i> value	Positive	Negative	<i>p</i> value
All cases	216	31 (14)	185 (86)	–	24 (11)	192 (89)	–
Age				0.11			0.59
<70 years	119	13 (11)	106 (89)		12 (10)	107 (90)	
≥70 years	97	18 (19)	79 (81)		12 (12)	85 (88)	
Sex				0.27			0.34
Male	134	22 (16)	112 (84)		17 (13)	117 (87)	
Female	82	9 (11)	73 (89)		7 (9)	75 (91)	
Race/ethnicity				0.091			0.28
White	33	2 (6)	31 (94)		2 (6)	31 (94)	
Japanese	151	27 (18)	124 (82)		20 (13)	131 (87)	
Hispanic	32	2 (6)	30 (94)		2 (6)	30 (94)	
Lauren classification				0.60			0.09
Intestinal	100	13 (13)	87 (87)		15 (15)	85 (85)	
Diffuse	116	18 (16)	98 (84)		9 (8)	107 (92)	
Preoperative therapy				0.097			0.80
None	165	27 (16)	138 (84)		17 (10)	148 (90)	
Chemotherapy	29	4 (14)	25 (86)		4 (14)	25 (86)	
Chemoradiotherapy	22	0 (0)	22 (100)		3 (14)	19 (86)	
Tumor depth				0.99			0.34
T1	31	4 (13)	27 (87)		6 (19)	25 (81)	
T2	104	16 (15)	88 (85)		13 (13)	91 (87)	
T3	66	9 (14)	57 (86)		4 (6)	62 (94)	
T4	14	2 (14)	12 (86)		1 (7)	13 (93)	
TX	1	1 (100)	0 (0)		0 (0)	1 (100)	
LN involvement				0.25			0.83
N0	67	11 (16)	56 (84)		8 (12)	59 (88)	
N1	84	7 (8)	77 (92)		10 (12)	74 (88)	
N2	27	5 (19)	22 (81)		3 (11)	24 (89)	
N3	33	6 (18)	27 (82)		2 (6)	31 (94)	
NX	5	2 (40)	3 (60)		1 (20)	4 (80)	
Stage				0.64			0.35
I	67	11 (16)	56 (84)		11 (16)	56 (84)	
II	49	4 (8)	45 (92)		3 (6)	46 (94)	
III	38	6 (16)	32 (84)		5 (13)	33 (87)	
IV	59	10 (17)	49 (83)		5 (8)	54 (92)	
X	3	0 (0)	3 (100)		0 (0)	3 (100)	

LN lymph node

Predictive factors for PD-L1 expression in GAC

To identify factors predicting positive PD-L1 expression in GAC cells, we performed univariate and multivariate logistic regression analyses. In the univariate analysis, PD-L1 expression in GAC cells was associated with the density of CD68- and CD163-positive cells (Table 3). In the multivariate analysis, which included features with $p < 0.2$ in the univariate analysis (age, preoperative therapy, and density of CD68- and CD163-positive cells), only the density of

CD163-positive cells was associated with PD-L1 expression in GAC cells (odds ratio 1.13; 95% confidence interval 1.02–1.25; $p = 0.021$; Table 3).

PD-L1 expression and density of CD163-positive cells in various strata

Additionally, we examined whether the influence of CD163-positive cell density on PD-L1 expression in GAC cells was associated with any of the clinical or

Table 2 Density of CD68- and CD163-positive cells in the tumor field and clinical features in 217 samples of gastric adenocarcinoma

Clinical or pathologic feature	CD68			CD 163		
	Total no.	CD68 (%) (Mean $\pm$ SE)	<i>p</i> value	Total no.	CD 163 (%) (Mean $\pm$ SE)	<i>p</i> value
All cases	217	6.83 $\pm$ 0.38	–	202 ^a	6.16 $\pm$ 0.30	–
Age			0.41			0.17
<70 years	120	6.43 $\pm$ 0.47		111	5.71 $\pm$ 0.37	
$\geq$ 70 years	97	7.32 $\pm$ 0.63		91	6.72 $\pm$ 0.48	
Sex			0.82			0.41
Male	135	6.81 $\pm$ 0.46		128	6.14 $\pm$ 0.40	
Female	82	6.85 $\pm$ 0.68		74	6.21 $\pm$ 0.44	
Race/ethnicity			0.06			0.44
White	33	4.43 $\pm$ 0.68		28	4.87 $\pm$ 0.64	
Japanese	152	7.35 $\pm$ 0.46		145	6.47 $\pm$ 0.37	
Hispanic	32	6.82 $\pm$ 1.13		29	5.90 $\pm$ 0.74	
Lauren classification			0.43			0.006
Intestinal	100	6.47 $\pm$ 0.56		91	5.26 $\pm$ 0.37	
Diffuse	117	7.14 $\pm$ 0.53		111	6.91 $\pm$ 0.44	
Preoperative therapy			0.56			0.58
None	166	6.99 $\pm$ 0.44		158	6.31 $\pm$ 0.34	
Chemotherapy	29	6.75 $\pm$ 1.19		26	5.86 $\pm$ 0.82	
Chemoradiotherapy	22	5.79 $\pm$ 0.98		18	5.29 $\pm$ 0.95	
Tumor depth			0.98			0.29
T1	32	6.66 $\pm$ 0.82		27	5.82 $\pm$ 0.70	
T2	104	6.89 $\pm$ 0.54		100	5.86 $\pm$ 0.44	
T3	66	6.61 $\pm$ 0.71		64	6.74 $\pm$ 0.49	
T4	14	8.26 $\pm$ 2.18		10	7.07 $\pm$ 1.92	
TX	1	0.81		1	0.67	
Lymph node involvement			0.37			0.14
N0	68	7.10 $\pm$ 0.65		63	6.41 $\pm$ 0.60	
N1	84	6.46 $\pm$ 0.65		78	5.42 $\pm$ 0.41	
N2	27	5.98 $\pm$ 0.80		25	6.27 $\pm$ 0.73	
N3	33	8.13 $\pm$ 1.14		31	7.56 $\pm$ 0.86	
NX	5	5.40 $\pm$ 2.53		5	5.43 $\pm$ 1.88	
Stage			0.51			0.28
I	68	7.19 $\pm$ 0.68		62	6.13 $\pm$ 0.58	
II	49	5.92 $\pm$ 0.69		47	5.24 $\pm$ 0.54	
III	38	7.12 $\pm$ 1.09		37	6.32 $\pm$ 0.60	
IV	59	7.17 $\pm$ 0.74		53	6.98 $\pm$ 0.63	
X	3	3.27 $\pm$ 1.23		3	5.18 $\pm$ 2.90	

SE standard error

^a Fifteen samples could not be evaluated for CD163 owing to sample conditions

pathologic variables, including age, sex, Lauren classification, preoperative therapy, tumor depth, lymph node involvement, and tumor stage. The relationship between CD163-positive cell density and PD-L1 expression in GAC cells was not modified by any clinical or pathologic variables (all *p* for interactions >0.05 ; Fig. 3).

Prognosis

Among the 217 evaluated patients, 89 patients had died at the last follow-up period. The median follow-up time for censored patients was 5.54 years. We performed a Cox regression analysis of overall survival. All immunohistochemical statuses, such as PD-L1 expression in GAC and

Fig. 2 Associations between PD-L1 expression and the infiltration area of CD68 and CD163 positive cells. **a** PD-L1 expression in GAC cells and CD68. **b** PD-L1 expression in GAC cells and CD163. **c** PD-L1 expression in stroma and CD68. **d** PD-L1 expression in stroma and CD163

intratumoral density of CD68- and CD163-positive cells (continuous variable), was not associated with overall mortality (all univariate analysis $p \geq 0.05$; Table 4).

Discussion

We evaluated immunohistochemical staining of PD-L1, CD68, and CD163 in 217 GAC samples from a TMA and found that PD-L1 expression in GAC is independently associated with CD163-positive cell infiltration, suggesting that M2-like macrophages potentially facilitate PD-L1 expression in GAC tumor cells. These results also suggest that M2 macrophage infiltration could be used as a predictive marker for PD-L1 expression and M2 macrophages could serve as a potential therapeutic target.

Several prior reports have established that PD-L1 expression can range from 25 to 65% in gastric cancers; in our TMA, it was only 14% [16–20]. This rate may vary among studies for many reasons, including heterogeneity and phenotypic differences in the samples, patient differences in cancer stage or prior therapy, and the use of different PD-L1 antibodies. To date, four PD-L1 antibodies (22C3, 28-8, SP142, and SP263) have been studied and are in widespread use. A phase 1 study of atezolizumab showed

that the PD-L1-positive rate was 5/141 (3.5%) in GAC cells and 18/141 (12.8%) in stromal cells, using the SP142 antibody and PD-L1 positivity defined as >5% of cells [21]. The KEYNOTE-012 study showed that the PD-L1-positive rate in both GAC cells and stromal cells was 40%, using the 22C3 antibody and PD-L1 positivity defined as >1% of cells. In a study of 35 patients whose tissue samples were positive for PD-L1 expression, 6 (17%) had PD-L1-positive tumor cells, which is similar to our findings [6]. Given these variations in the literature, we consider the PD-L1-positive rate in our study is valid.

The relationship between TAMs and PD-L1 expression in tumor cells is not well understood. There is some evidence that TAMs, especially M2 macrophages, express PD-L1 on their surface and directly suppress T cell responses. This is suggested by clinical trial results showing that PD-L1 expression in tumor stroma is a predictive marker for patient outcomes after immune checkpoint blockade therapy [6, 12]. TAMs have been found to release several factors, such as cytokines, chemokines, polypeptide growth factors, hormones, matrix remodeling proteases, and metabolites. TAMs have also been shown to directly or indirectly promote many important features of tumor progression, such as angiogenesis, invasion, proliferation, and the tumor microenvironment [22].

Table 3 Univariate and multivariate analysis of factors associated with PD-L1 expression in cancer cells

Clinical or pathologic feature	Univariate		Multivariate	
	Odds ratio (95% CI)	<i>p</i> value	Odds ratio (95% CI)	<i>p</i> value
Age				
<70 years	1 (reference)		1 (reference)	
≥70 years	1.86 (0.86–4.02)	0.12	1.58 (0.66–3.86)	0.30
Sex				
Male	1 (reference)			
Female	0.63 (0.26–1.40)	0.26		
Race/ethnicity				
White	1 (reference)	0.46		
Japanese	3.37 (0.94–21.6)			
Hispanic	1.03 (0.12–9.06)			
Lauren classification				
Intestinal	1 (reference)			
Diffuse	1.22 (0.57–2.70)	0.60		
Preoperative therapy				
None	1 (reference)		1 (reference)	
Present	0.43 (0.12–1.18)	0.11	1.03 (0.19–4.77)	0.97
Tumor depth				
T1–2	1 (reference)			
T3–4	0.91 (0.50–2.49)	0.45		
Lymph node involvement				
None	1 (reference)			
Present	0.72 (0.59–3.07)	0.45		
Stage				
Stage I–II	1 (reference)			
Stage III–IV	1.33 (0.62–2.88)	0.46		
CD68-positive cell density ^a	1.11 (1.04–1.18)	0.0007	1.05 (0.98–1.13)	0.17
CD163-positive cell density ^a	1.17 (1.08–1.29)	0.0001	1.13 (1.02–1.25)	0.021

CI confidence interval

^a Continuous variable

For instance, M2 macrophages secrete epidermal growth factor or transforming growth factor- β , which stimulate epithelial-mesenchymal transition [23, 24]. Sugihara et al. showed that M2 macrophages upregulated expression of Bmi1, which may be a stem cell marker, through micro-RNA-30e* suppression in GAC [25]. Canan et al. found that TAMs receptor (Tyro3, Ax1, and Mertk) upregulated PD-L1 expression in a breast cancer cell line, supporting the idea that M2 macrophages might activate PD-L1 expression in tumor cells [26]. Meanwhile, interferon- γ (IFN- γ), which is secreted by inflammatory cells and is associated with differentiation of macrophages, was found to facilitate PD-L1 expression in tumor cells [27–29]. Therefore, IFN- γ might influence the relationship between M2 macrophage infiltration and PD-L1 expression in tumor cells. However, no previous reports have addressed M2 macrophage stimulation of PD-L1 expression in GAC. The current study demonstrates a novel relationship between M2 macrophages and PD-L1 expression in GAC

cells. The mechanism by which TAMs activate PD-L1 expression in tumor cells requires further research, and we are now evaluating this mechanism in our laboratory.

Our findings suggest that adding macrophage-targeting therapy, such as CSF1/CSF1R blockade, to immune checkpoint blockade therapy may be a viable strategy in GAC. In several types of solid tumors, CSF1/CSF1R blockade reduces M2 macrophage infiltration into tumor tissue and increases the CD8+/CD4+ T cell ratio [30]. Zhu et al. showed that adding CSF1R inhibition to T cell checkpoint immunotherapy increased the effectiveness of treatment by remodeling macrophage and other immune cell infiltration mechanisms and functions in pancreatic cancer [31]. Currently, a clinical trial evaluating the combination of CSF1R antagonists and checkpoint blockade is ongoing (NCT02323191). Our findings suggest that CSF1/CSF1R blockade might reduce PD-L1 expression in tumor cells and suppress direct inhibition of cytotoxic T cell response by the tumor cells.

Fig. 3 Interaction between PD-L1 expression in CD163-positive cells in various strata. Shown is the $\log_e$ (odds ratio) plot of PD-L1 expression in the infiltration area of CD163-positive cells for each variable, along with 95% confidence intervals

Table 4 Univariate analysis of survival

Clinical or pathologic feature	Univariate	
	Hazard ratio (95% CI)	p value
PD-L1 expression (cancer cell)		
Negative	1 (reference)	
Positive	0.68 (0.31–1.28)	0.25
PD-L1 expression (stroma)		
Negative	1 (reference)	
Positive	0.78 (0.35–1.53)	0.50
PD-L1 expression (cancer cell or stroma)		
Negative	1 (reference)	
Positive	0.65 (0.36–1.11)	0.13
CD68-positive cell density ^a	0.97 (0.93–1.02)	0.25
CD163-positive cell density ^a	0.99 (0.94–1.05)	0.84

CI confidence interval

^a Continuous variable

PD-L1 expression was marginally more frequent in Japanese than in Whites or Hispanics. In addition, CD68 infiltration is also marginally higher in Japanese. Lin et al. assessed gene signature differences between Asian and non-Asian GACs and showed that inflammation- and immunity-related T cell pathways were enriched in non-Asian GACs [32]. Although the molecular biology might differ between Asian and non-Asian GACs, importantly, the contribution of CD163 infiltration to PD-L1 expression was not modified by ethnicity (p for interactions = 0.35; Fig. 3).

No relationship between PD-L1 and prognosis was found in the current study. This results consistent with a previous study [17]. Some reports showed that PD-L1 expression correlated with favorable prognosis [19, 33]; on the other hand, some reports showed the opposite results [18, 34]. Recently, a meta-analysis indicated that PD-L1 expression was associated with an unfavorable prognosis [35]. Further studies are needed to confirm the prognostic value of PD-L1.

PD-L1 expression has intratumoral heterogeneity. This is well evaluated in lung cancer [36–38]. A study in GAC showed intratumoral heterogeneity of PD-L1 expression [19]. Boger et al. reported that 33% PD-L1-positive GACs have PD-L1 expression cells only in the invasion frontal area or tumor center [19]. In the current study, PD-L1 expression was evaluated in the one point lesion where TMA was made. Thus, multi-point evaluation is needed in further studies.

Recently, molecular classifications, especially microsatellite instability status, were found to be associated with PD-L1 expression [39, 40]. In the current study, the relationship between M2 macrophage infiltration and PD-L1 expression in GAC cells was not influenced by any clinicopathologic variables. However, we did not examine the genetic subtypes of GAC according to The Cancer Genome Atlas.

In conclusion, we found that TAM infiltration is associated with the upregulation of PD-L1 expression in GAC cells. This suggests that macrophage infiltration can serve as a potential therapeutic target.

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Compliance with ethical standards

Conflict of interest The authors have no potential conflicts of interest to disclose.

Human rights statement and informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964 and later versions. Informed consent or a substitute for it was obtained from all patients included in the study.

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