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Original Article

Programmed cell death ligand 1 (PD-L1) expression on gastric cancer and its relationship with clinicopathologic factors

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Abstract: Background: Targeting the immune checkpoints in solid tumors becomes hot recently. Programmed cell death ligand 1 (PD-L1) is ligand for programmed death 1 (PD-1), which is known to negatively regulate T-cell activation. In the present study, we investigated the expression of PD-L1 in tumor specimens of gastric cancer and its relationships with clinicopathological variables and survival. Methods: The expression of PD-L1 in 132 surgically resected specimens of stage II and III gastric cancer was evaluated by immunohistochemistry in microarray tissue. Results: Expression of PD-L1 was observed in 50.8% (67/132) of gastric cancer tumor specimens. Patients whose tumor size over 5cm had a higher positive rate of PD-L1 expression. There was no relationship between the expression of PD-L1 and other clinicopathological variables including age, gender, clinical stage, location as well as histological differentiation. PD-L1 positive patients had significantly poorer survival than negative patients. The 5-year survival rates was 83.1% in those with PD-L1 negative patients and 50.7% for PD-L1 positive patients (P<0.001). The multivariate analysis indicated that both PD-L1 positive and Tumor-node-metastasis stage were independent prognostic factors in gastric cancer patients (P=0.001 and 0.025, respectively). Conclusions: The expression of PD-L1 was found in half of stages II and III gastric cancer patients. Positive of PD-L1 expression indicated poor survival in Chinese stages II and III gastric adenocarcinoma patients. These results may provide the clue for immunotherapy in the adjuvant treatment setting of gastric cancer patients.

Keywords: Clinicopathologic features, gastric cancer, PD-L1, prognosis

Introduction

Gastric cancer is the second leading cause of cancer-related death worldwide [1]. The incidence of gastric carcinoma varies significantly from one part of the world to another and it is particularly common in Eastern Asia, especially in China [2]. In our previous report, we found out that at the time of diagnosis, stage II and III gastric cancer patients accounted for about 28% and 33% of the whole population respectively [3]. The five-year survival rate for the stage II and III gastric cancer patients was around 70% and 40% respectively [3, 4]. Stages II and III gastric cancer patients are a special group of patients. They have the potential to be cured, however, they are also under threaten to develop disease recurrent or distant metastasis [5]. To figure out the prognostic factors for this group of patients would be helpful to pick up patients who need close follow up.

Programmed cell death ligand 1 (PD-L1, CD274, B7-H1) is one of the members of the B7 superfamily [6, 7]. The B7 family members have been shown to down-regulate T-cell activation through receptor programmed death 1 (PD-1) [8, 9]. PD-L1 is more broadly expressed than the other B7 superfamily members. The PD-1/PD-L1 interaction serves as an important regulatory check against an excessive adoptive immune response to antigens and autoimmunity [10]. Thus, on T-cell receptor activation, PD-L1 acts as a negative regulator of the immune response [11, 12].

Table 1. Distribution of 132 patients according to PD-L1 expression status

Factor	PD-L1 expression		*P* value*
	Negative	Positive	
Gender			
Male	49	45	
Female	16	22	0.297
Age			
<62	37	28	
≥62	28	39	0.082
Differentiation			
Signet ring cell carcinoma	10	15	
Low differentiation	28	31	
Moderate differentiation	25	17	
High differentiation	2	4	0.346
Stage			
II	12	9	
III	53	58	0.430
Location			
Proximal	38	44	
Distal	25	22	
Whole	2	1	
Tumor size			0.627
<5 cm	40	29	
≥5 cm	25	38	0.036

*The fisher's exact test was used to evaluate the correlation between PD-L1 expression and the clinicopathologic characteristics. *P*<0.05 indicates statistical significance. PD-L1: Programmed cell death ligand 1.

In normal condition, PD-L1 is expressed on the endothelium in the thymus, heart, and placenta in both human and mice [9, 13, 14] in addition to lymphoid cells, such as activated T cells, B cells, macrophages, and dendritic cells on the protein level. PD-L1 is also abundant on tumor tissues, including lung carcinomas, ovarian carcinomas, breast carcinomas, glioblastoma, and squamous cell carcinoma of the head and neck [13-16]. In 2015 American Society of Clinical Oncology Gastrointestinal Cancers Symposium there was a poster about the relationship between PD-L1 expression and clinical outcomes in patients with advanced gastric cancer treated with the anti-PD-1 monoclonal antibody Pembrolizumab. In this study, they found that 40% of the advanced gastric cancer patients had PD-L1 positive tumors. They enrolled 39 patients to receive the monoclonal antibody treatment and the response rate was 22.2%. They also observed a trend toward improved outcomes with higher levels of PD-L1 expression. However, there is no data about the prognostic value of PD-L1 in gastric cancer. To better understand the role of PD-L1 in gastric cancer, we started this study. In the present study, using immunohistochemistry, we investigated the expression of PD-L1 in tumor specimens of gastric cancer, and we analyzed the relationship between the expression and clinic pathological variables as well as the postoperative survival.

Materials and methods

Patients

Between January 1996 and December 2004, the medical records of pathology-proven gastric adenocarcinoma patients who were diagnosed and received radical resection in the Cancer Center of Sun Yat-Sen University were retrospectively analyzed. We excluded patients who received neoadjuvant chemotherapy, or had a history of other primary cancer. All patients received gastrectomy and D1/2 lymphadenectomy. We identified 200 patients with gastric adenocarcinoma in our institution but excluded 32 patients because of missing baseline characteristics, 31 patients because of incomplete follow-up and 5 patients who died of non-malignant related reasons (car accidents and coronary heart diseases). The final study involved 132 patients: 94 males and 38 females with a median age of 62 years (range 24-80 years). The histological types of the patients were all adenocarcinoma, including 25 signet ring cells, 59 low differentiated, 42 moderate differentiated and 6 high differentiated. Staging was performed according to the tumor-node-metastasis (TNM) classification of the American Joint Committee on Cancer (AJCC, sixth edition, 2002). Twenty-one of them were classified as stage II tumors and the rest 111 were classified as stage III patients. No patient underwent radiation or chemotherapy before surgery. 63 (47.7%) patients received adjuvant chemotherapy. Forty (30.3%) patients received D1 resection and the rest 92 patients received D2 resection. The median follow-up was 66.0 months (range 3.0-153.0 months).

Tissue specimens were obtained from the histopathology archive in the Department of Pathology, Cancer Center of Sun Yat-sen

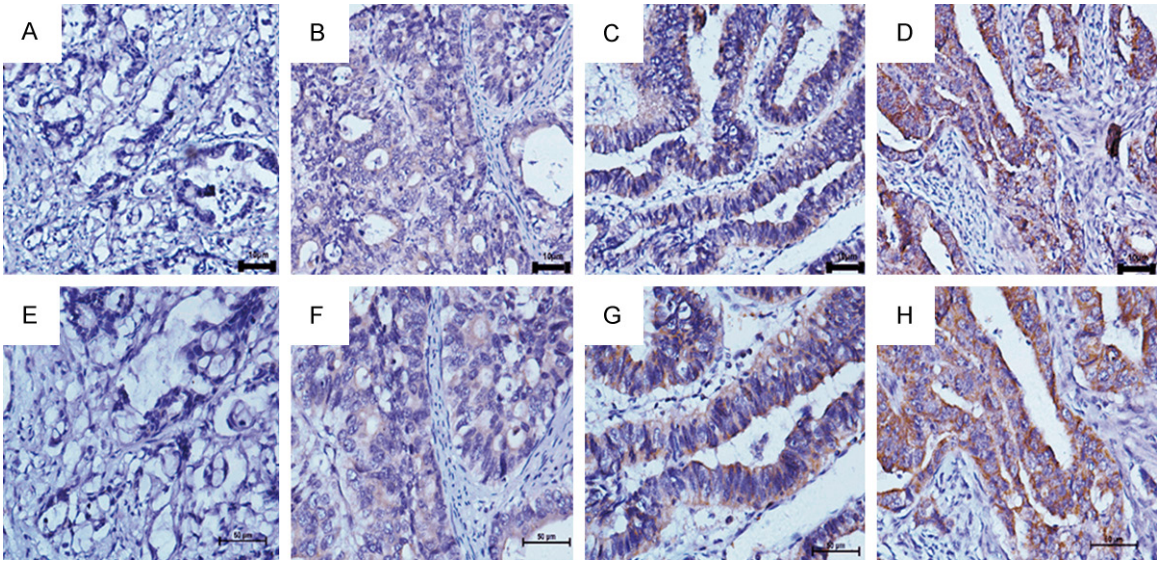

Figure 1. The expression of PD-L1 in gastric adenocarcinoma tissues by IHC. (A) Negative expression of PD-L1 protein (100×). (B) Weak expression of PD-L1 protein (100×), (C) Moderate expression of PD-L1 protein (100×), (D) Intense expression of PD-L1 protein (100×). (E-H) demonstrate the higher magnification (400×) from the area of the box in (A-D), respectively.

University. Histological examinations were carried out on tissue preparations with hematoxylin and eosin (H&E) staining. The best tissue sections that suited for immunohistochemistry were selected by the pathologist and the satisfying formalin-fixed paraffin-embedded resection specimens were obtained.

Tissue microarray construction

Formalin-fixed, paraffin-embedded tumor samples were taken from the selected cases. Tissue microarray construction was prepared as described by Kononen et al [17]. Three 1.0-mm tumor tissue cores were taken from each tumor sample. The arraying machine was from Beecher Instruments (Sun Prairie, WI). The array blocks were cut to produce 4-μm sections.

Immunohistochemistry

Slides were dewaxed and rehydrated in xylene and graded ethanol solutions for antigen retrieval. Slides were then blocked with 3% H_2O_2, goat serum, avidin solution, and biotin solution. Primary PD-L1 [(ab58810) Rabbit polyclonal, Abcam, 1:50] was added and then probed with biotinylated rabbit secondary antibody (Vector Laboratories) and high-sensitivity streptavidin-HRP conjugate. To visualize staining, slides were incubated in 3,3'-diaminobenzidine in 0.1% H_2O_2 in Tris-HCl buffer, and subsequently counterstained with Hematoxylin QS (Vector Laboratories). PDL1-positive samples were defined as those showing cytoplasmic staining pattern of tumor tissue. PD-L1 staining intensity was graded into four groups: no staining (0), weak staining (1+), moderate staining (2+), and intense staining (3+). Tumors without staining and weak staining were classified as negative while tumors which were moderate or intense staining were classified as positive. The immunostained slide was evaluated under the microscope. The staining intensity of cells showing positive membrane and cytoplasmic staining for PDL1 was calculated by reviewing the entire spot.

Analysis of immunohistochemistry was carried out by two independent observers (Shumei Yan and Lin Zhang) who were blinded to any prior information on clinic pathological features of the patients' samples. If there was difference between these two observers, these slides were reinvestigated by both investigators using a multiheaded microscope.

Statistical analysis

All statistical analyses were performed by Statistical Package of Social Sciences 16.0 software for Windows (SPSS, Inc., Chicago, IL). *P* value <0.05 was considered to be statistically

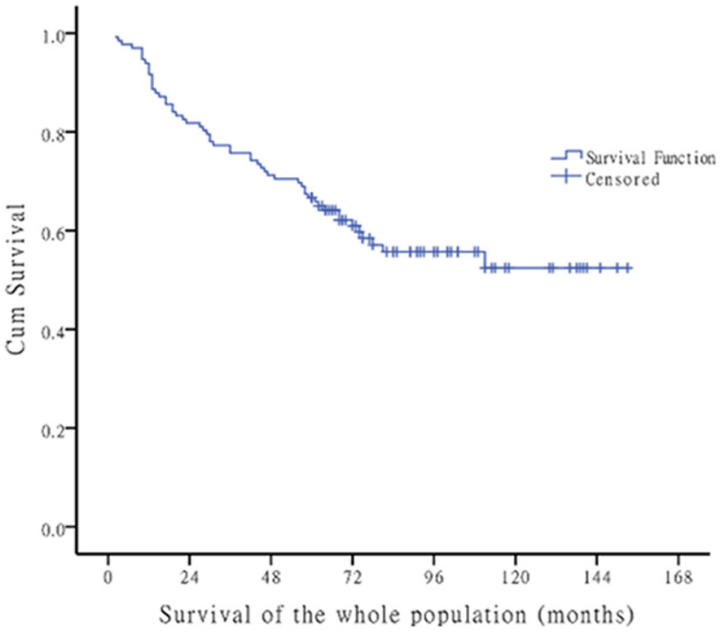

Figure 2. The overall survival of the whole population.

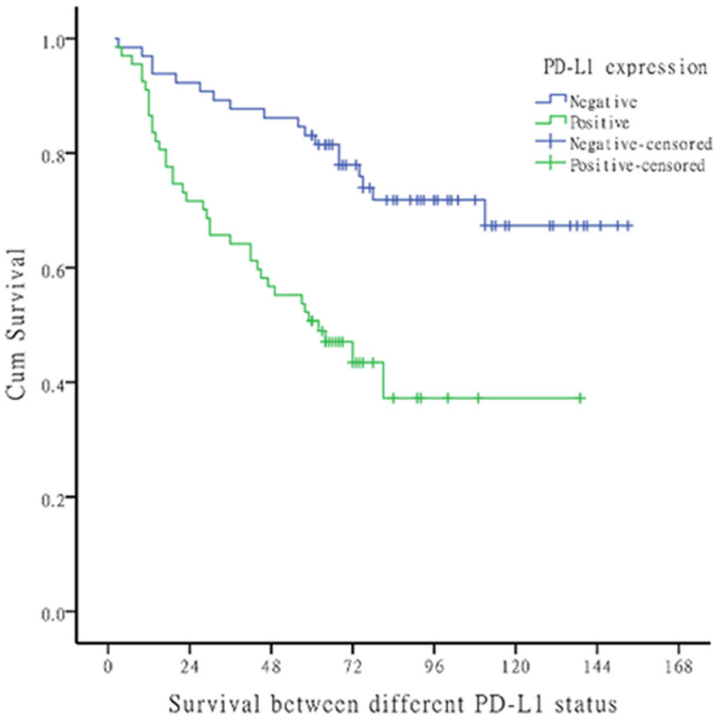

Figure 3. The survival of gastric cancer patients according to the expression of PD-L1.

significant. All tests were two-tailed. The Kaplan-Meier method was used to estimate the 5-year overall survival. For patients who remained alive, data were censored at the date of the last contact. Kaplan-Meier analysis with log-rank testing was used for univariate analysis. Variables showing a trend for association with survival ($P< 0.05$) were selected in the final multivariate Cox proportional hazards model. The Chi-squared test and t test were used to compare the relationship between PD-L1 expression and the various clinicopathological characteristics of the patients.

Ethics, consent and permissions

All patients provided written informed consent. Study approval was obtained from independent ethics committees at Cancer Center of Sun Yat-Sen University. The study was undertaken in accordance with the ethical standards of the World Medical Association Declaration of Helsinki.

Results

The characteristics of the 132 gastric adenocarcinoma patients are shown in **Table 1**. Until December 2014, there were 55 patients died from the disease.

Expression of PD-L1 in surgically resected specimens

In immunohistochemical staining of PD-L1, the protein expression in gastric adenocarcinoma patients was 50.8% (67/132) (**Figure 1** and **Table 1**).

PD-L1 expression and clinic pathological features

For the correlation with clinicopathological features, **Table 1** summarized the distribution of patients according to tumor PD-L1 (negative and positive) expression. Patients whose tumor size over 5 cm had a higher positive rate of PD-L1 expression (P=0.036). There was no statistical difference between the expression of PD-L1 and

Table 2. Multivariate analysis of overall survival in gastric cancer

Factors	Hazard ratio	*95% CI*	*P value*
Gender	0.756	0.398-1.438	0.394
Age	0.963	0.556-1.669	0.893
Stage	1.955	1.086-3.517	0.025
PD-L1	2.696	1.468-4.951	0.001
Tumor size	0.988	0.491-1.987	0.973
Histology	1.329	0.972-1.818	0.075

CI, confidence interval; PD-L1: Programmed cell death ligand 1.

age, gender, disease stage, histological grade and tumor location.

Effect of PD-L1 expression on survival

Till December 2014, 55 patients had died of cancer. The 1, 3, 5, 7-year disease specific survival rate of the entire cohort was 93.9%, 77.3%, 70.5%, 66.7% respectively. **Figure 2** showed the survival curve of the whole group of patients.

There was significantly difference between PD-L1 positive and negative patients. (5-year survival rates: 83.1% in those with PD-L1 negative patients and 50.7% in those with PD-L1 positive patients, $P<0.001$, **Figure 3**).

Univariate analysis showed that tumor stages ($P<0.001$), smaller tumor size ($P=0.003$), high tumor differentiation ($P=0.016$) and PD-L1 negative expression ($P<0.001$) were all significantly associated with longer survival.

To test the independent prognostic effect of these clinical factors, Cox's proportional hazard model was applied. The results revealed that tumor stage ($P=0.025$) and PD-L1 expression ($P=0.001$) remained independent prognostic variables for survival when age, gender, tumor size, tumor stage, PD-L1 and differentiation were all included for test (**Table 2**).

Discussion

We have demonstrated the expression of PD-L1 in surgically resected specimens of gastric cancer. The expression of PD-L1 is found in cytoplasm of cancer cells. The expression pattern of PD-L1 was consistent with previous reports, which examined their expression in human tumor tissues [13-16]. The positive rate of PD-L1 in cancer patients varied in different cancer types. **Table 3** listed some of the results using the method of immunohistochemistry to detect the expression of PD-L1 in human tumor tissues. It ranged from 23.9%-84.2%. In 2015 American Society of Clinical Oncology Gastrointestinal Cancers Symposium there was a poster about the relationship between PD-L1 expression and clinical outcomes in patients with advanced gastric cancer treated with the anti-PD-1 monoclonal antibody Pembrolizumab. They found that 40% of the advanced gastric cancer patients had PD-L1 positive tumors.

The present study also examined the relationship between the expression of PD-L1 and clinicopathological variables as well as the prognosis of gastric cancer patients. In our analyses, there was no correlation of PD-L1 expression with clinicopathologic features except for tumor size. Thompson RH et al. found that renal cell carcinoma patients with PD-L1 expression were more likely to exhibit adverse pathologic features including advanced tumor stage, tumor size over 5 cm, nuclear grade 3 or 4, and coagulative tumor necrosis [18]. However, in the disease of osteosarcoma and non-small cell lung cancer, the expression of PD-L1 had no relationship with the clinicopathologic variable [19, 20].

As for the prognostic value of PD-L1 in the malignant disease, several previous studies had demonstrated conflicting results varying from positive correlation of prognosis in non-small cell lung cancer [21] and mismatch repair (MMR)-proficient colorectal cancer [22], to observing no correlation with survival in osteosarcoma, Melanoma as well as MMR-deficient Colorectal cancer [19, 22, 23], to an inverse relationship in which high PD-L1 expression was associated with poor survival in renal cell carcinoma [18, 24]. To our knowledge, this is the first study to analyze the prognostic value of PD-L1 in gastric cancer patients. We found that PD-L1 positive was associated with poor prognosis. The multivariate analysis indicated that PD-L1 positive was an independent prognostic factor in stage II and III gastric cancer patients.

Immunotherapy has made some progress in several cancers, such as melanoma and non-small cell lung cancer [25]. Recently, immunotherapy got its indication in the lung squamous cancer. In the multicenter phase 1 trial, anti-PD-L1 antibody was given to several kinds of cancer including gastric cancer (seven pati-

Table 3. The expression of PD-L1 in different kinds of tumors

Time	Author	Country	Journal	Type of cancer	Sample size	Positive rate	Prognostic factor
2014	Shen JK et al. [19]	USA	Cancer Immunol Res	osteosarcoma	37	84.2%	No
2006	Thompson RH et al. [18]	USA	Cancer Res	Renal Cell Carcinoma	306	23.9%	Poor survival
2012	Taube JM et al. [23]	USA	Sci Transl Med	Melanoma	150	38%	No
2014	Velcheti V et al. [21]	USA	Lab Invest	non-small cell lung cancer	204	25%	Better survival
		Greek			340	36%	
2013	Droeser RA et al. [22]	Switzerland	Eur J Cancer	MMR-proficient CRC	1197	37%	Better survival
				MMR-deficient CRC	223	29%	No
2015	Kei Muro	Multicenters	ASCO GI symposium	Gastric cancer	162	40%	–
2015	Our results	China		Gastric cancer	132	49.2%	Poor survival

ents), but no clinical benefit was found in the gastric cancer patients [25]. Till now most of the target therapy agent failed in the gastric cancer except for trastuzumab and ramucirumab [26, 27]. We still have no idea whether anti PD-L1 antibody would bring survival benefit to the advanced gastric cancer patients. At least basing on our result, we found out that PD-L1 was an independent prognostic factor for stage II and III gastric cancer patients. Patients who were PD-L1 positive deserved closer follow up.

There were some limitations of our study. Firstly, we only collected patients of stage II and III. It would be a good idea to explore the patient population to stages I to IV patients and analyzed the relationship of PD-L1 expression and tumor stage. Secondly, the sample size of our study was small. Even for the limitations here, this is the first study to evaluate the prognostic value of PD-L1 in Chinese gastric adenocarcinoma patients. We found that about half of the patients had PD-L1 positive expression and PD-L1 positive indicated a poor survival.

In conclusion, The authors are not aware of any previous studies which address the clinicopathological characteristics and prognostic impact of PD-L1 with resectable gastric cancer in China. In this retrospective study conducted with 132 patients with gastric adenocarcinoma we came to the following conclusions: 1) we demonstrated the positive rate of PD-L1 in gastric cancer patients. 2) The expression of PD-L1 was associated with larger tumor size. 3) PD-L1 positive expression was associated with poor survival.

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Disclosure of conflict of interest

None.

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