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Original Article

PD-L1 expression in colon cancer and its relationship with clinical prognosis

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Abstract: Objectives: PD-L1 is closely associated with tumorigenesis and development. However, expression of PD-L1 protein in colon cancer and its significance in clinical prognosis are yet to be fully clarified. This study examined the relationship between PD-L1 expression with the clinicopathological features and prognosis of colon cancer. Methods: This study collected cases of primary colon cancer that had not undergone preoperative chemotherapy and had complete clinical data. Eighty specimens each were obtained from cancer tissues, paracancer tissues, and normal tissues. Immunohistochemical assays were performed to detect PD-L1 expression. The relationship between PD-L1 expression and clinicopathologic features was compared. This was combined with follow-up data, to analyze the relationship between positive or negative PD-L1 expression and prognosis. Results: Among 80 tumor specimens, 22 cases (27.5%) showed high PD-L1 expression, 24 cases (30.0%) showed moderate expression, and 34 cases (42.5%) showed weak or no PD-L1 staining. High expression of PD-L1 in paracancer and normal tissues were 9 (11.3%) and 5 (6.3%) cases, respectively. PD-L1 expression was also positively correlated with TNM stage (P=0.009), lymph node metastasis (P=0.000), distant metastasis (P=0.014). There were no significant differences in different age, gender, histologic grade, and tumor size groups (P>0.05). Regression analysis revealed that poorer tumor differentiation, later TNM stages, presence of lymph node metastasis, and positive PD-L1 expression were factors that influenced prognosis. Multivariate analysis indicated that late TNM stage and positive PD-L1 expression were independent risk factors that influenced prognosis. Conclusion: PD-L1 expression is significantly elevated in colon cancer tissues, and is closely associated with lymph node metastasis, prognosis, and survival.

Keywords: PD-L1, colon cancer, prognosis

Introduction

Colorectal cancer is the third most common cancer and the fourth leading cause of cancer death worldwide, and its incidence is increasing each year [1]. In China, the incidence and mortality rates of colon cancer are also increasing [2]. The first-line treatment for colon cancer involves comprehensive treatment with surgery and adjuvant chemotherapy, however recurrence and metastasis are major causes of treatment failure [3]. Although some recent progress has been made in both diagnosis and treatment, colorectal cancer continues to have a huge impact on human lives and health [3]. Therefore, new targeted therapeutic interventions are greatly needed.

With increasing research on the effects of the tumor immune microenvironment on tumorigenesis and development, cancer immunotherapy has now become the fourth type of cancer treatment. From various cancer vaccines to adoptive T cell therapy, and to current immune checkpoint blockers, these treatment methods have continued to achieve significant success [4]. They have revolutionized cancer treatment and represent a major breakthrough in current methods of treatment. Programmed cell death protein 1 (PD-1) has been confirmed to be an inhibitory receptor that is expressed on the surface of T lymphocytes [5]. PD-1 has two ligands, namely PD-L1 and PD-L2. PD-L1 is a transmembrane molecule belonging to the B7 family [5]. It acts via the PD-1/PD-L1 transduction pathway to inhibit the differentiation and proliferation of T cells, and to block signal transduction and the secretion of various cytokines, thereby causing the invasion and metastasis of cancer cells [6]. The human PD-L1 gene is known as *CD279*, and is located on chromosome 2q37.35. It has a relative

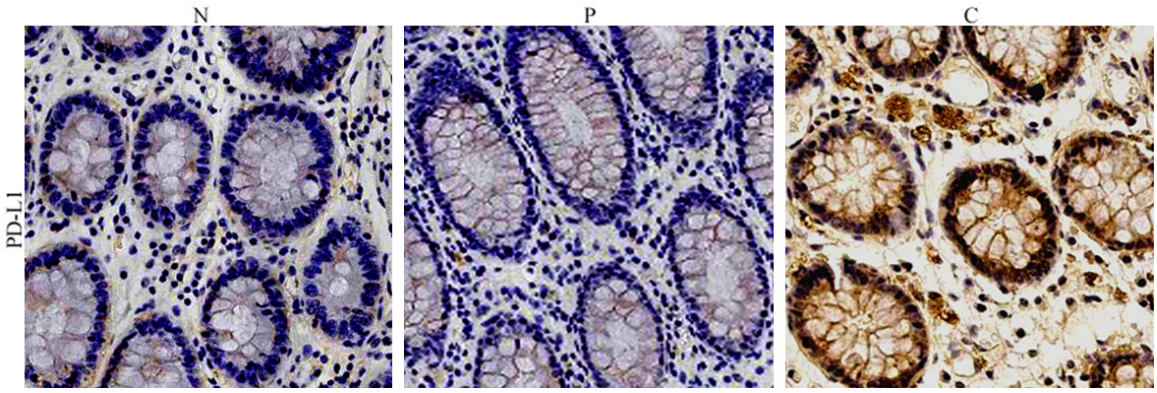

Figure 1. Expression of PD-L1 in cancer tissues, paracancer tissues, and normal tissues.

Table 1. Association between PD-L1 protein expression and clinicopathologic factors in colonic cancers

Characteristics	Frequency (80)	PD-L1 ≥2 46 (%)	PD-L1 1 24 (%)	PD-L1 0 10 (%)	χ^2	*P*
Age (years)					1.666	0.197
>60	38	19 (50.0)	12 (31.6)	7 (18.4)		
≤60	42	27 (64.2)	12 (28.6)	3 (7.1)		
Gender					0.205	0.651
Female	40	22 (55.0)	13 (32.5)	5 (12.5)		
Male	40	24 (60.0)	11 (27.5)	5 (12.5)		
Differentiation					1.361	0.506
I	10	5 (50.0)	4 (40.0)	1 (10.0)		
II	46	29 (63.0)	15(32.6)	2 (4.3)		
III	24	12 (50.0)	5 (20.1)	7 (29.1)		
Tumor size					1.228	0.541
≤2 cm	24	12 (50.0)	10 (41.7)	2 (8.3)		
2~5 cm	48	30 (65.2)	12 (25.0)	6 (12.5)		
>5 cm	8	4 (50.0)	2 (25.0)	2 (25.0)		
Nodal metastasis					20.925	0.000
Negative	25	5 (20.0)	14 (56.0)	6 (24.0)		
Positive	55	41 (74.5)	10 (18.2)	4 (7.3)		
Distant metastasis					6.016	0.014
Absent	70	37 (52.8)	23 (32.9)	10 (14.3)		
Present	10	9 (90.0)	1 (10.0)	0 (0.0)		
T Stage					7.769	0.009
I/II	26	10 (7.1)	9 (34.6)	7 (26.9)		
III	44	29 (27.3)	13 (29.5)	2 (4.5)		
IV	10	7 (70.0)	2 (20.0)	1 (10.0)		

molecular mass of 55 kDa, and is composed of extracellular, transmembrane, and intracellular domains [6]. In addition to its expression on the surfaces of natural killer cells and macrophages, PD-L1 expression is also upregulated in lung cancer [7], breast cancer [8], pancreatic cancer [9], hepatocellular carcinoma [10], melanoma [11], and other tissues. There is currently a lack of systematic research on colon cancer in China. Therefore, this study collected specimens of radical colectomy from 80 patients who were treated at our hospital, and measured the expression of PD-L1 in colon cancer tissue using immunohistochemistry. Our objective was to explore the relationship between PD-L1 expression and clinicopathological features, and the effects of PD-L1 on cancer prognosis, in the hopes that PD-L1 could become a new marker and target in the treatment of colon cancer.

Materials and methods

Tissue specimens

A total of 80 fresh colorectal cancer tissues and paired adjacent non-tumor tissues were obtained from patients who had undergone surgical resection of colorectal cancer between 2013 and 2017 at the Second Affiliated Hospital of Xi'an Jiaotong University, China. All of the tissue samples were washed with sterile

phosphate-buffered saline before being fixed in 10% formaldehyde solution, followed by paraffin embedding, sectioning, and pathologic examination. The colorectal cancer diagnosis was confirmed by an experienced pathologist. No patients had been treated with radiotherapy or chemotherapy before surgery. This study was approved by the Ethics Committee of Xi'an Jiaotong University and informed consent was obtained from each patient involved in the study.

Immunohistochemistry

PD-L1 protein was detected immuno-histochemically using a standardized streptavidin-peroxidase (SP) method. Tissue sections (4 μm) were incubated overnight with primary antibody at a proper concentration. The next day, the slides were incubated for 30 min with biotinylated goat anti-rabbit IgG, followed by incubation with peroxidase-conjugated streptavidin for 20 min at room temperature. Color was developed using 0.02% 3, 3'-diaminobenzidine (DAB) in 50 mM Tris-HCl buffer (pH 7.6) for 5-7 min. Finally, the sections were counterstained with hematoxylin, rinsed with water, dehydrated, cleared and cover slipped. Negative controls for immunostaining replaced the primary antibody with nonimmune goat or rabbit serum. The antibodies against PD-L1 and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Stain scoring

DAKO Hercept Test scoring system was used. PD-L1 positive staining defines as yellow or brown granules in cytoplasm. The staining strength and number of stained cells per 100 was determined under a microscope (Olympus Optical, Tokyo, Japan) in five visual felds, at a ×400 magnification. The staining strength was scored as 0 (no staining), 1 (light yellow), 2 (brown), 3 (deep brown). The number of stained cells per 100 was scored as 1 (≤10%), 2 (10%~50%), 3 (≥50%). High PD-L1 expression was defined when the product of staining strength score multiplied and number of stained cells per 100 score was no less than 3.

Statistical analysis and patient outcome

Relationships between PD-L1 expression and clinicopathological features were analyzed by the χ^2 or two-sided Fisher's exact test, as appropriate. Survival rates were calculated by the Kaplan-Meier method, and differences were examined by the log-rank test. Factors found to be significant were then selected for a stepwise Cox's multivariate proportional hazard model to determine their prognostic values. $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed using SPSS 13.0 statistical software (SPSS, Chicago, IL, USA).

Result

Expression of PD-L1 in colon cancer

To determine the expression of PD-L1 in colon cancer, immunohistochemical assays were performed to evaluate the tumor specimens, paracancer tissues, and normal tissues of patients with colon cancer. Our results indicated that PD-L1 is localized in the cell membrane and cytoplasm (**Figure 1**). Among the 80 tumor specimens, 22 cases (27.5%) showed high PD-L1 expression, 24 cases (30.0%) showed moderate expression, and 34 cases (42.5%) showed weak or no PD-L1 staining. High expression of PD-L1 in paracancer and normal tissues were 9 (11.3%) and 5 (6.3%) cases, respectively.

Relationship between PD-L1 expression and clinicopathological features

The relationship between PD-L1 expression and clinicopathological features can be summarized as follows (**Table 1**): The expression level of PD-L1 in Stage I-II cases was significantly lower than that in Stage III and Stage IV ($P<0.05$). PD-L1 expression was also positively correlated with TNM stage ($P=0.009$), lymph node metastasis ($P=0.000$), and distant metastasis ($P=0.014$). There were no significant differences in different age, gender, histologic grade, and tumor size groups ($P>0.05$).

Relationship between PD-L1 and the prognosis for colon cancer

To determine the relationship between PD-L1 and prognosis of colon cancer, we analyzed PD-L1 expression and cumulative survival rates of the patients (**Figure 2**). Specimens with weak or no PD-L1 staining were defined as negative and those with moderate or strong staining were defined as positive. The 1-year cumulative survival rate of patients negative for

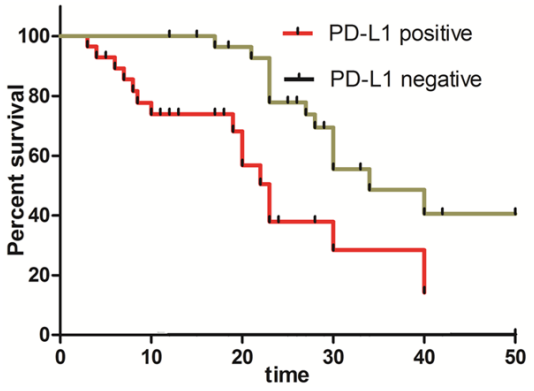

Figure 2. PD-L1 expression in colon cancer and patients' post-operative survival.

PD-L1 (n=34) was 100.0%, whereas in contrast, that of patients positive for PD-L1 (n=46) was 78.6%. Cox survival analysis indicated that poorer tumor differentiation, later TNM stages, presence of lymph node metastasis, and positive PD-L1 expression were independent risk factors influencing prognosis and survival. Kaplan-Meier analysis indicated that positive PD-L1 expression in colon cancer was associated with patients' post-operative survival (P<0.05).

Discussion

Immune tolerance is a major barrier in cancer immunotherapy, and exists in a variety of human tumors. Within the tumor microenvironment, tumor cells rely on different mechanisms to protect themselves and escape the body's immune responses [12]. PD-L1 is an important molecule in the tumor microenvironment, and its upregulation is one of the key mechanisms of tumor immune evasion. PD-L1 is an apoptosis-related protein that plays a crucial role in the immune response. After binding of T lymphocytes to their receptor PD-1, PD-L1 can induce transmission of inhibitory signals leading to T cell apoptosis, thus regulating immune tolerance of peripheral T cells [12]. Therefore, it plays a key role in the body's anti-tumor immune responses. The disruption of the PD-1/PD-L1 pathway and tumor immune evasion is an immunotherapy target that is of general concern in current cancer research. Using drugs to block the PD-1/PD-L1 pathway will enhance T cell function, and lead to tumor cell lysis, thus opening a new avenue for cancer treatment [12]. Recent studies have found that PD-L1 is widely expressed in many types of human tumor tissue [12]. However, a clear consensus has not been reached on the relationship of PD-L1 with the clinicopathological features and prognosis of patients with colon cancer. Our study results revealed that PD-L1 expression is significantly elevated in tumor tissues. Its expression was not significantly correlated with age, gender, and tumor size, but was associated with tumor TNM stage and lymph node metastasis. This study further found that high PD-L1 expression in tumor tissue is an independent factor of poor prognosis in patients with colon cancer. Currently, the value of PD-L1 expression in tumors with respect to clinicopathological features and prognosis is still controversial. A meta-analysis showed that among 1,157 patients with non-small cell lung cancer (NSCLC), those with positive PD-L1 expression had shorter overall survival, and was related to poor tumor differentiation [13]. In addition, another meta-analysis performed by Pan et al. on this issue revealed that PD-L1 expression was related to poor tumor differentiation, and could be an indicator of poor prognosis [14]. The results of this study are consistent with the findings above. This study has provided preliminary evidence of the possible role of PD-L1 in colon cancer. Therefore, PD-L1 could be a key target in the treatment of colon cancer.

In normal immune systems, PD-L1 plays an extremely important role in maintaining the balance between protective immunity and immune tolerance in the normal body. However, in the tumor microenvironment, PD-L1 expression is upregulated via various mechanisms, leading to the abnormal activation of the PD-L1/PD-1 signaling pathway. This, in turn, will inhibit the proliferation and differentiation of T cells through different mechanisms, and induces T cell apoptosis, thereby mediating tumor immune evasion. Cho et al. [15] showed that PD-L1 positive tumor tissues were related to low-density tumor-infiltrating lymphocytes (TILs). Furthermore, Konishi et al. [16] also showed that in the tumor tissues of NSCLC patients, the number of TILs in the PD-L1 positive group was significantly lower than the negative group. In addition, PD-L1 not only exerts its effect as a ligand of PD-1, but can also act as a receptor to transmit negative signals, thereby blocking Fas-FasL-mediated apoptosis in tumor cells, and resisting cytotoxic T lymphocyte-mediated cell lysis. The mechanisms by which PD-L1 achieves immune eva-

sion are by no means limited to the above. It can also induce the joint action of multiple mechanisms, including T cell anergy and exhaustion, loss of function in tumor-associated antigen-presenting cells, aggregation of regulatory T cells, etc. Although these mechanisms have been clarified in *in vitro* studies, investigations on their effects *in vivo* and their relevant molecular mechanisms are still unclear. The specific mechanisms of the PD-1/PD-L1 pathway and whether it consists of multiple receptors are yet to be confirmed. Therefore, the future preparation of PD-L1 monoclonal or polyclonal antibodies to block this pathway could be a new direction in the treatment of colon cancer.

There are two hypotheses regarding the primary mechanisms underlying the participation of PD-1/PD-L1 in tumor immune evasion: Inherent mechanisms: whereby changes in the genome or transcriptome of tumor cells themselves cause high PD-L1 expression. The most common of these is the abnormal activation of Akt, STAT3, and other signaling pathways inherent in the tumors, which lead to high PD-L1 expression, thereby inhibiting the activation of cytotoxic T cells. For example, several types of tumors present with *PTEN* gene deletion, which often leads to the over-activation of the PI3K/AKT signaling pathway, thus increasing downstream PD-L1 expression [17, 18]. Adaptive mechanisms involve the protective mechanisms of tumor cells against the eliminating action of immune cells in the immune microenvironment. The IFN-γ secreted by a series of cells participating in tumor elimination (i.e. CD4+ Th1 cells and activated T cells) may induce high PD-L1 expression in tumor cells [19].

Studies have shown that PD-1/PD-L1 not only participates in the regulation of tumor immunity, but also plays a role in tumorigenesis, growth, and metastasis. High PD-L1 expression in skin accelerated inflammation-associated carcinogenesis in a methylcholanthrene-induced model of squamous cell carcinoma, and induced epithelial-mesenchymal transition (EMT) features in the tumor [20]. This indicates that PD-L1 facilitated tumorigenesis and EMT. Recently, Kleffel et al. [21] detected PD-1 expression on the surface of melanoma cells. They found that endogenous PD-1/PD-L1 binding was possible in these melanoma cells without participation of immune cells, and that the mTOR signaling pathway was activated to promote their own proliferation. At present, many anti-PD-1 or anti-PD-L1 drugs have been used in clinical trials. A key feature of these treatments is good patient tolerance. The maximum tolerated dose could not be found in the majority of these drugs in phase I monotherapy clinical trials. Currently, two anti-PD-1 monoclonal antibodies have received marketing approval by the United States Food and Drug Administration, and good clinical activity has been observed in melanoma and NSCLC patients [22].

In summary, the PD-1/PD-L1 signaling pathway has been widely acknowledged and emphasized in research on cancer immunotherapy, and has provided us with new molecular targets. At this stage, our study has shown that PD-L1 expression is upregulated in colon cancer, and is closely related to late TNM stage, lymph node metastasis, and poor prognosis. Therefore, PD-L1 could be a new biological marker for invasion and metastasis in colon cancer. In the next step of our investigations, we will focus on whether PD-L1 can be a key target for immunotherapy in colon cancer, and its possible mechanisms.

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Disclosure of conflict of interest

None.

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