Research in Oncology
2024; Vol. 20, No. 2: 24-32
DOI: 10.21608/resoncol.2024.213776.1196

Original Article

PD-L1 Expression in Colorectal Cancer and Its Relation to Microsatellite Instability and Cytotoxic Tumor-Infiltrating Lymphocytes

Suzan M. Talaat [1] ✉, Hany William [2], Nouran M. Roby [2], Eman M. S. Keshk [1]

[1] Pathology Department, Ahmed Maher Teaching Hospital, Cairo, Egypt; [2] Clinical Oncology Department, Ahmed Maher Teaching Hospital, Cairo, Egypt

Abstract

Background: Colorectal cancer (CRC) is the third most common cancer worldwide. Tumor cell PD-L1 expression has been shown to enable immune evasion by suppressing the immune system's active T-cell-mediated response.
Aim: To investigate the expression of PD-L1 in CRC and its correlation with microsatellite instability (MSI) and cytotoxic tumor-infiltrating lymphocytes.
Methods: The pathological specimens of 49 cases of CRC were studied for PD-L1 expression and its correlation with different clinicopathological parameters, including MSI and cytotoxic CD8+ve tumor-infiltrating lymphocytes.
Results: High PD-L1 expression in the microenvironment was significantly higher in low-grade tumors compared to high-grade tumors (50% vs. 12%, respectively; p = 0.008). Additionally, high PD-L1 expression in the microenvironment was significantly associated with a lack of mucinous change (p = 0.019), low T stage (p = 0.001), and non-infiltrative (pushing) tumor borders (p = 0.037). High PD-L1 expression in cancer cells was more prevalent in low T stage tumors and those with a high peritumoral CD8+ lymphocyte count. All cases of high PD-L1 expression in cancer cells also showed high PD-L1 expression in the microenvironment (p < 0.001). There was a significant relationship between intratumoral CD8+ lymphocyte count and MSI (p = 0.026), with all MSI-low cases showing a high intratumoral CD8+ lymphocyte count. There was no significant relationship between PD-L1 expression and MSI.
Conclusions: PD-L1 expression in the microenvironment was significantly correlated with tumor grade, mucinous change, type of tumor border, and T stage. This suggests a prognostic role for PD-L1 expression in colon cancer.

Keywords: Colorectal cancer, Cytotoxic tumor-infiltrating lymphocytes, Microenvironment, Microsatellite instability, PD-L1
Corresponding author: Suzan M. Talaat, MD; Pathology Department, Ahmed Maher Teaching Hospital, Cairo, Egypt; Email: suzabella0000@gmail.com
Received: 1-July-2023, **Accepted:** 13-May-2024, **Published online:** 28-June-2024

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and is characterized by specific genetic changes, an expression signature, and a therapy response [1]. It is caused by genetic alterations of tumor cells and affected by tumor-host interactions [2]. One of these interactions is the histological reaction of tumor infiltrating lymphocytes (TILs) that constitutes a favourable prognostic factor in CRC, acting as gatekeepers in preventing tumor dissemination [3].

The immune system is distinguished by the presence of inhibitory mechanisms that stop excessive lymphocyte activation [4]. Programmed cell death receptor 1 (PD-1; CD279) is typically expressed by activated lymphocytes [5]. Its engagement by specific ligands, including PD ligand 1 (PD-L1; B7-H1; CD274) and PD ligand 2 (PD-L2; B7-DC; CD273), causes down-regulation of lymphocyte proliferation and cytokine production resulting in lymphocyte exhaustion and induction of immunological tolerance [6-9].

PD-L1 is expressed by T and B cells, macrophages and dendritic cells and is up-regulated through

activation by interferons [8, 9]. It is also expressed by other cell types including endothelial, pancreatic and muscle cells [5]. In contrast, PD-L2 expression is much more restricted and is detected in activated dendritic cells and macrophages [9].

By suppressing the active T-cell-mediated immune response, tumor cells that express PD-L1 can evade the immune system [10, 11]. As a result, the production of PD ligands on tumor cells inhibits CD8+ T-cells' cytotoxicity [12].

Several tumor cells have been identified to express PD-L1 and, to a lesser extent, PD-L2, including glioblastoma [13], renal cell carcinomas [14], squamous cell carcinoma of the head and neck [15], esophageal [16] and non-small cell lung cancers [17].

Because immunological checkpoints are one of the pathways for immune evasion and tumor formation, blockage of these molecules with monoclonal antibodies may restore host immune response and, consequently, stop tumor growth and lead to tumor regression. For this reason, immunotherapy employing immune checkpoint inhibitors is a rapidly growing technique for the treatment of some human malignancies [18, 19].

One of the predictive markers for the response to checkpoint inhibitors is the immunohistochemical expression of PD-L1. Inhibitors targeting PD-L1, or PD-1 protein can improve clinical outcomes in several cancer types such as malignant melanoma, non-small cell lung cancer, renal cell carcinoma, and bladder cancer. Although initial studies suggested no role for immunotherapy in CRC [20], Le et al showed that 40% of patients with CRC-mismatch repair deficiency (dMMR), treated with pembrolizumab (anti-PD-1 monoclonal antibody) responded to therapy vs 0% of patients with CRC-mismatch repair proficiency (pMMR) [21].

Although chromosomal instability is the predominant mechanism for CRC development, 12-15% of cases contain dMMR, which causes microsatellite instability (MSI) [22, 23]. Increased mutations caused by this deficiency result in the production of abnormal proteins that act as neo-antigens and trigger an immunological response in TILs. Compared to other forms of CRCs, MSI CRC exhibits a T immune infiltrate that is linked to the overexpression of T cell checkpoints such PD-L1. It also has a better prognosis [24, 25].

The aim of this study was to explore the expression of PD-L1 in colorectal cancer and its relationship with MSI and cytotoxic tumor-infiltrating lymphocytes.

Methods

The pathological specimens of forty-nine cases of CRC were retrieved from the pathology department, Ahmed Maher teaching hospital, Egypt during the period from January to December 2020. Demographic and clinical data of the patients were collected from the hospital files.

Immunohistochemistry

Five µm thick sections were cut from formalin-fixed paraffin embedded tissue blocks and stained with haematoxylin and eosin for histopathological examination. Six extra sections were cut from each paraffin block and stained with mismatch repair proteins (MLH1, PMS2, MSH2 and MSH6), CD8 and PD-L1.

Immunohistochemical staining was performed using immunostainer (Shandon Sequenza) using the labelled streptavidin biotin method with the following reagents: Diva Decloaker, pretreatment antigen retrieval (Biocare Medical, catalogue number: DV2004 LX, MX), hydrogen peroxide block (Lab Vision, USA, catalogue number: TA-060-HP), ultravision large volume detection system (Lab Vision, USA, catalogue number: TP-060- HL) including Ultra V block, biotinylated goat anti - polyvalent plus (link) and streptavidin peroxidase plus (label), and DAB plus substrate system (Lab Vision, USA, catalogue number: TA-060-HDX) including DAB plus chromogen and DAB plus substrate.

The primary antibodies were PMS-2: a mouse polyclonal antibody (Biocare Medical, catalogue number: PM 344 AA), MLH-1: a mouse monoclonal antibody (Biocare Medical, catalogue number: PM 220 AA), MSH-6: a mouse monoclonal antibody (Biocare Medical, catalogue number: PM 265 AA), MSH-2: a mouse monoclonal antibody (Biocare Medical, catalogue number: PM 219 AA), and PD-L1: 22C3 pharmDx (Code SK006).

For mismatch repair antibodies, non-neoplastic colonic mucosa, stromal cells, infiltrating lymphocytes or the centres of lymphoid follicles, were used as internal positive controls. Sections of the same tissue were used following the same procedure, but the phosphate buffered saline (PBS) was used instead of the primary antibody being used as internal negative controls.

Cases were categorized into positive (nuclear staining within tumor cells) and negative (complete absence of nuclear staining within tumor cells with concurrent internal positive controls) [26]. Then cases

were interpreted as microsatellite stable (MSS) when all the four antibodies show positive nuclear staining of tumor cells, as microsatellite instable low (MSI-L) when one antibody shows negative nuclear staining of tumor cells, and as microsatellite instable high (MSI-H) when two antibodies or more show negative nuclear staining of tumor cells [27].

For PD-L1, the kit set had positive and negative control slides. PD-L1 stained tissues were scored as low or high when <5% or ⩾5% of the cells are positive, respectively [28].

For CD8, intratumoral CD8-positive T cell density, defined as CD8 cells that infiltrated into cancer nests, were scored low or high when their mean number was <50 or ⩾50, respectively. Peritumoral CD8-positive T cell density, defined as CD8 cells that were distributed along the invasive margin of cancer, were scored low or high when the mean number was <200 or ⩾200, respectively. CD8-positive T cells were counted twice in a microscopic field at a magnification of x200 [28].

Statistical methods

Categorical data were described using frequencies and percentages, while quantitative data were presented as means and standard deviations. The Chi-square test or Fisher's exact test, as appropriate, was used to compare categorical data. A p-value of < 0.05 was considered statistically significant. Data analysis was conducted using IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp).

Results

The age of patients ranged from 27 to 87 years, with a mean of 55.3 years (standard deviation: 13.55). Twenty-seven cases (55.1%) were men, while 22 (44%) were women. Tumours were located in the left colon in 27 (55.1%) cases, the right colon in 20 (40.8%) and the transverse colon in only two (4.1%). The pathological characteristics are illustrated in Table 1.

Figures 1, 2, and 3 illustrate various patterns of PD-L1 expression in cancer cells and the surrounding microenvironment, as well as the infiltration of CD8+ lymphocytes both within and around the tumor. Table 2 illustrates the correlation between PD-L1 expression in cancer cells and the microenvironment and the pathological parameters of the studied cases.

Table 1: Pathological characteristics of 49 colorectal carcinoma cases

Characteristic	*n* (%)
Tumor type	
Adenocarcinoma	38 (77.6)
Mucinous adenocarcinoma	6 (12.2)
Signet ring carcinoma	5 (10.2)
Tumor grade	
Low	32 (65.3)
High	17 (34.7)
T stage	
T1	1 (2)
T2	5 (10.2)
T3	24 (49)
T4	19 (38.8)
N stage	
N0	20 (40.8)
N1	17 (34.7)
N2	12 (24.5)
Lymphovascular invasion	
Present	24 (49)
Absent	25 (51)
Tumor borders	
Infiltrative	31 (63.3)
Pushing	18 (36.7)
Tumor necrosis	
Present	22 (44.9)
Absent	27 (55.1)
Microsatellite stability	
Microsatellite stable	27 (55.1)
Microsatellite instable - Low	4 (8.2)
Microsatellite instable - High	18 (36.7)
PD-L1 in cancer cells	
Low	39 (79.6)
High	10 (20.4)
PD-L1 in microenvironment	
Low	31 (63.3)
High	18 (36.7)
CD8 +ve intratumoral lymphocytes	
Low	30 (61.2)
High	19 (38.8)
CD8 +ve peritumoral lymphocytes	
Low	43 (87.7)
High	6 (12.2)

There was a significant correlation between PD-L1 expression in the microenvironment and tumor grade. Low-grade tumors were more likely than high-grade tumors to show high PD-L1 expression in the microenvironment (50% vs. 12%, respectively; $p = 0.008$) (Figures 1 and 2). All 31 cases of low PD-L1 expression in microenvironment were of high T stage while all low T stage cases were associated with high PD-L1 expression in microenvironment ($p = 0.001$) (Figure1). Similarly, high PD-L1 expression

in microenvironment was significantly associated with adenocarcinoma type (Table 2).

PD-L1 expression in microenvironment was significantly correlated with type of tumor borders (p = 0.037) as most of low expression cases (74.2%) had infiltrative borders while cases with pushing borders showed more high expression. High PD-L1 expression in cancer cells was more prevalent in tumors with lower T stages (50%) compared to those with higher T stages (16%) (Figure 2, Table 2).

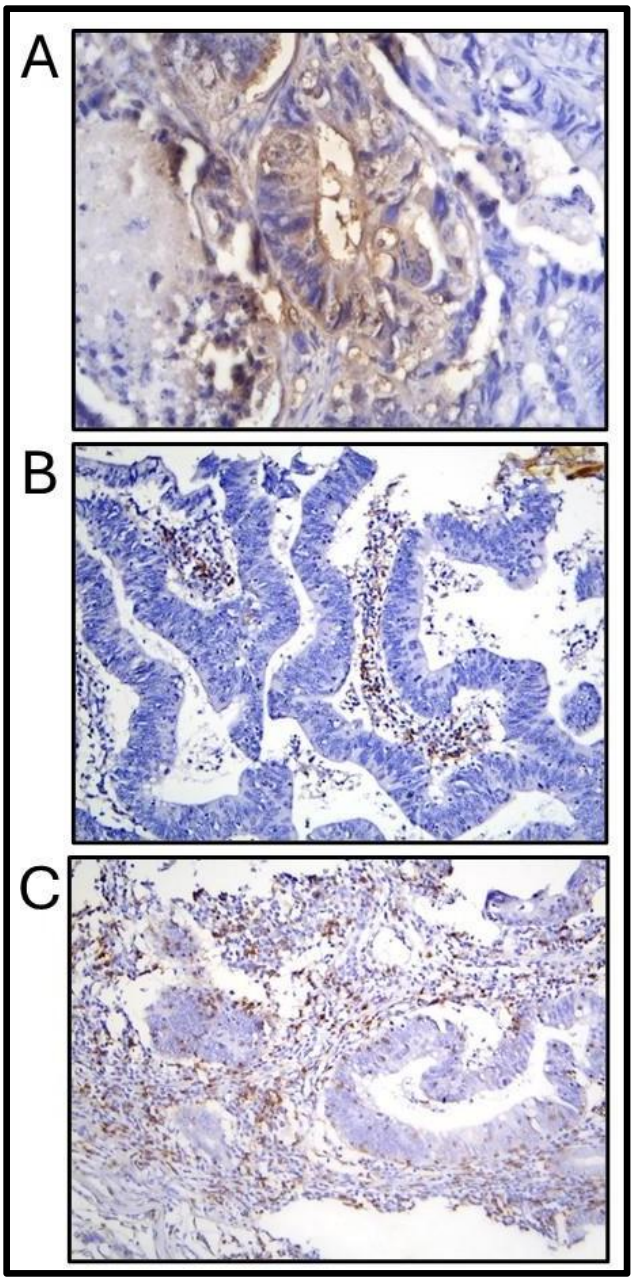

Figure 1: Low grade colon adenocarcinoma, MSS, T2, with **A)** High PD-L1 expression in cancer cells (x400), **B)** High PD-L1 expression in the surrounding microenvironment (x200), and **C)** Intratumor and peritumor CD8+ve lymphocytes (x200)

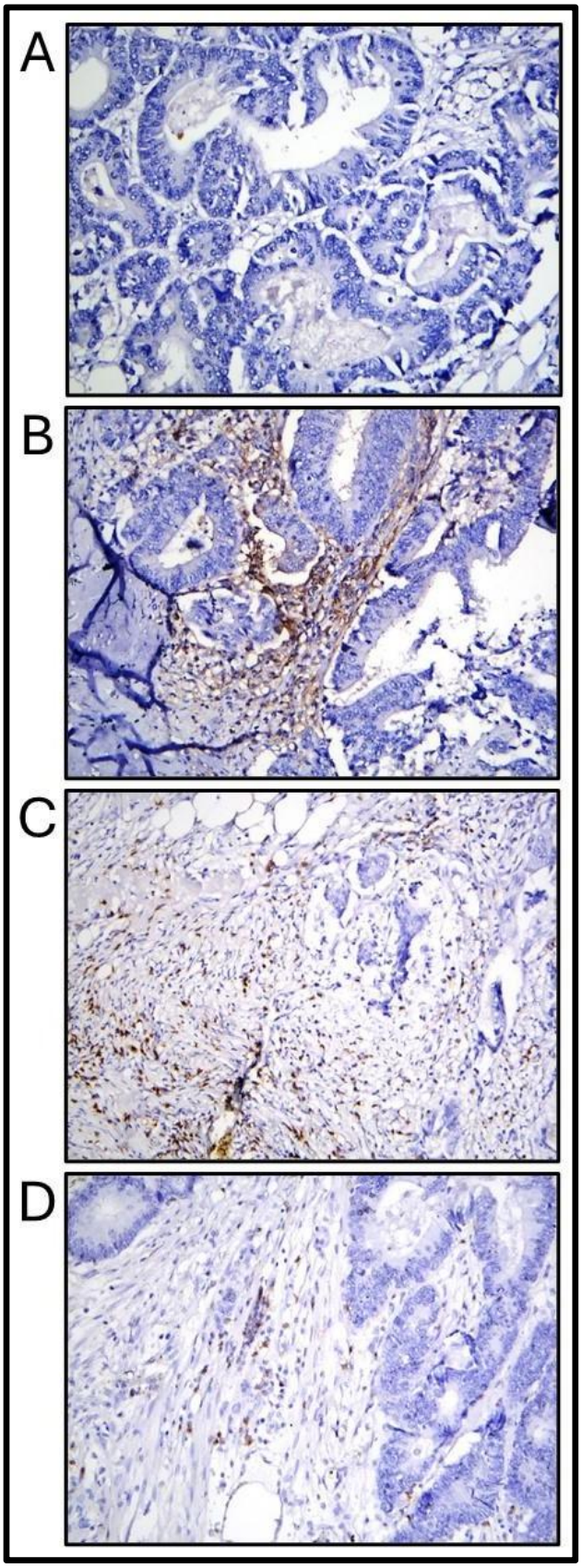

Figure 2: Low grade colon adenocarcinoma, MSS, T4, with **A)** Low PD-L1 expression in cancer cells (x200), **B)** High PD-L1 expression in surrounding microenvironment (x200), **C)** High peritumor CD8+ve lymphocytes (x200), and **D)** Low intratumor CD8+ve lymphocytes (x200)

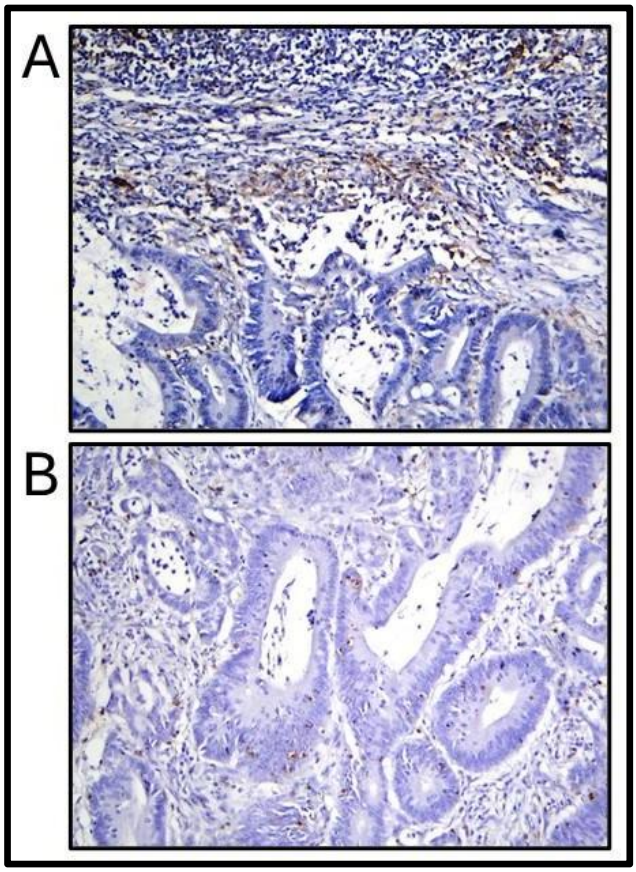

Figure 3: Low grade colon adenocarcinoma with mucinous differentiation, MSI-H, T2, with **A)** Low PD-L1 expression in cancer cells and high expression in surrounding microenvironment (x200), and **B)** Low intratumor and peritumor CD8+ve lymphocytes (x200)

A significant correlation was found between PD-L1 expression in microenvironment and PD-L1 expression in cancer cells ($p < 0.001$) as all cases of high PD-L1 expression in cancer cells showed also high PD-L1 expression in microenvironment and 79.5% of low PD-L1 expression in cancer cells showed also low PD-L1 expression in microenvironment (Figure 1).

High PD-L1 expression in cancer cells was more prevalent in tumors with CD8 peritumoral lymphocytes count as 92.3% of cases of low PD-L1 expression in cancer cells showed low CD8 peritumoral lymphocytes count (Figure 3). No significant correlation was found between PD-L1 expression in cancer cells and CD8 intratumoral lymphocytes count. Also, no significant correlation was found between PD-L1 expression in surrounding microenvironment and intratumoral or peritumoral CD8 lymphocytes.

A significant correlation was found between intratumoral CD8 lymphocytes count and microsatellite stability status ($p = 0.026$) as all MSI-L cases showed high intratumoral CD8 lymphocytes count while 72.2% of MSI-H cases showed low count and 63% of MSS showed also low count. No significant correlation was found between PD-L1 expression in cancer cells or surrounding microenvironment or peritumoral CD8 lymphocytes and microsatellite stability status.

Discussion

In our study, the majority of cases exhibited low PD-L1 expression in cancer cells (79.6%) and in the surrounding microenvironment (63.3%). Similarly, Huang et al. and Koganemaru et al. reported low PD-L1 expression in cancer cells (56% and 91.9%, respectively) [2, 28]. Additionally, Koganemaru et al. found low PD-L1 expression in the microenvironment (84.7%) [28].

Rosenbaum et al. found positive PD-L1 expression in only 9% of cases [29]. Masugi et al. observed positive PD-L1 expression in cancer cells in 89% of cases and in the microenvironment in 5% of cases[30]. In contrast, Valentini et al. found positive PD-L1 expression in cancer cells in 25% of cases and in the microenvironment in 78% of cases [31]. The findings of Masugi et al. were particularly distinctive as they examined PD-L1 expression levels in 823 cases of CRC. They assessed tumor expression levels in both the cytoplasm (intensity ranging from 0 to 3) and the membrane (absent [0] or present [1], if distinct membrane staining above the cytoplasmic staining level was observed), then used the sum of both scores in each case for further analyses [30].

In this study, PD-L1 expression in the microenvironment was significantly correlated with tumor grade, mucinous change, type of tumor borders, and T stage. High PD-L1 expression in the microenvironment was associated with favorable parameters such as low tumor grade, low T stage, pushing borders, and absence of mucinous change. These findings differed from those of most studies. However, Droeser et al. found that high PD-L1 expression was associated with early T stage, absence of lymph node metastasis, lower tumor grade, absence of vascular invasion, and MMMR proficiency [32]. Lee et al. reported that PD-L1 expression in TILs was linked to low TNM stage, absence of distant metastasis, and absence of lymphovascular and perineural invasion. In contrast, PD-L1 expression in cancer cells was correlated with adverse parameters such as high MSI, poor differentiation, high TNM stage, presence

Table 2: Correlation between PD-L1 expression in cancer cells and the microenvironment with the pathological parameters of colorectal cancer

Parameter	PD-L1 in cancer cells		*p* value	PD-L1 in microenvironment		*p* value
	High, *n* (%)	Low, *n* (%)		High, *n* (%)	Low, *n* (%)	
Tumor type						
Adenocarcinoma	9 (24.3)	28 (75.7)	0.232	17 (46)	20 (54.1)	0.019
Mucinous / Signet ring adenocarcinoma	1 (8.3)	11 (91.7)		1 (8.3)	11 (91.7)	
Tumor grade						
Low	8 (25)	24 (75)	0.274	16 (50)	16 (50)	0.008
High	2 (11.8)	15 (88.2)		2 (11.8)	15 (88.2)	
T stage						
Low (T1 / T2)	3 (50)	3 (50)	0.055	6 (100)	0	0.001
High (T3 / T4)	7 (16.3)	36 (83.7)		12 (27.9)	31 (72.1)	
N stage						
N0	4 (20)	16 (80)	0.888	9 (45)	11 (55)	0.518
N1	3 (17.6)	14 (82.4)		6 (35.3)	11 (64.7)	
N2	3 (25)	9 (75)		3 (25)	9 (75)	
Lymphovascular invasion						
Present	5 (20)	20 (80)	0.942	9 (36)	16 (64)	0.913
Absent	5 (20.8)	19 (79.2)		9 (37.5)	15 (62.5)	
Tumor border						
Infiltrative	4 (12.9)	27 (87.1)	0.087	8 (25.8)	23 (74.2)	0.037
Pushing	6 (33.3)	12 (66.7)		10 (55.6)	8 (44.4)	
Tumor necrosis						
Present	5 (22.7)	17 (77.3)	0.716	9 (40.9)	13 (59.1)	0.584
Absent	5 (18.5)	22 (81.5)		9 (33.3)	18 (66.7)	
Microsatellite stability						
Microsatellite stable	6 (22.2)	21 (77.8)	0.877	11 (40.7)	16 (59.3)	0.774
Microsatellite instable - Low	1 (25)	3 (75)		1 (25)	3 (75)	
Microsatellite instable - High	3 (16.7)	15 (83.3)		6 (33.3)	12 (66.7)	
CD8 +ve intratumoral lymphocytes						
Low	4 (13.3)	26 (86.7)	0.123	8 (26.7)	22 (73.3)	0.066
High	6 (31.6)	13 (68.4)		10 (52.6)	9 (47.4)	
CD8 +ve peritumoral lymphocytes						
Low	7 (16.3)	36 (83.7)	0.055	15 (34.9)	28 (65.1)	0.472
High	3 (50)	3 (50)		3 (50)	3 (50)	
PD-L1 in cancer cells						
Low	---	---	----	8 (20.5)	31 (79.5)	<0.001
High	---	---		10 (100)	0	

of metastasis, and lymphatic and perineural invasion [33]. Koganemaru et al. found that high PD-L1 expression in tumor-infiltrating mononuclear cells (TIMCs) was associated with a good prognosis [28].

We also found that PD-L1 expression in cancer cells had a significant correlation only with tumor T stage, with most low-expression cases corresponding to high T stage. There was no significant correlation with other pathological parameters. These results differ from those of other studies. Koganemaru et al. found that high PD-L1 expression in tumor cells was associated with more extensive lymph node metastasis and advanced TNM stage [28]. Li et al. reported that PD-L1 expression was linked to poor differentiation and right-sided colon location [34]. Shan et al. observed that PD-L1 expression in stages I-II cases was significantly lower than in stages III-IV, and it was significantly correlated with TNM stage, lymph node involvement, and distant metastasis [35]. Lee et al. found that high PD-L1 expression was correlated with medullary morphology, right-sided location, younger age, high TILs, and a high peritumoral score [33]. Huang et al. discovered a correlation between PD-

L1 expression and perineural invasion and tumor differentiation [2]. Additionally, Enkhbat et al. found that PD-L1 expression was associated with poor prognosis and significantly correlated with age, lymphatic invasion, and tumor location [36]. Jung et al. demonstrated that PD-L1 expression was associated with MSI-high status, mucinous features, poor cell differentiation, and right-sided tumor location [1].

In the current study, there was a significant correlation between PD-L1 expression in cancer cells and the count of CD8 peritumoral lymphocytes, but no significant correlation between PD-L1 expression in cancer cells and CD8 intratumoral lymphocytes count. Huang et al. detected a direct correlation between PD-L1 expression in tumor cells and CD8+ TILs [2]. Koganemaru et al. also found that tumors with high PD-L1 expression on TIMCs were significantly associated with increased intra CD8-positive T cells [28]. Both Jung et al. and Rosenbaum et al. revealed that PD-L1 positive tumors had a higher number of CD8 positive TILs [1, 29]. In contrast, Masugi et al. reported that PD-L1 expression was not significantly associated with CD3+, CD8+, or CD45RO+ cell density [30]. Additionally, Cho et al. found that PD-L1 positive tumors were related to low-density TILs [37]. These differences are likely due to the use of different methods to score CD8 T cells.

A significant correlation was also found between PD-L1 expression in the microenvironment and its expression in cancer cells, as all cases with high PD-L1 expression in cancer cells also showed high PD-L1 expression in the microenvironment. Similarly, Valentini et al. found that all specimens with PD-L1 positive neoplastic cells also showed PD-L1 expression in infiltrating immune cells [31]. In contrast, Koganemaru et al. reported that no patient had high PD-L1 expression in both tumor cells and the tumor microenvironment [28].

A significant correlation was found between intratumoral CD8 lymphocyte count and microsatellite stability status. All MSI-L cases showed a high intratumoral CD8 lymphocyte count, while unexpectedly, 72.2% of MSI-H cases showed a low count. Additionally, 63% of MSS cases showed a low count, which is expected, as in MSS patients, non-immunogenic tumors develop and are recognized by the immune system as self. Consequently, the host is unable to generate an immune response, resulting in minimal lymphocyte recruitment in these tumors. In contrast, Drescher et al. and Phillips et al. revealed that the percentage of CD8 T cells in the colon cancer infiltrates of MSI-H patients was elevated compared to that observed in the tumors of MSS individuals [38, 39]. Numerous studies have emphasized that MSI-H CRC harbors an increased number of TILs compared to MSS carcinomas and that intraepithelial T-lymphocytes are a characteristic of MSI-H tumors. However, there is wide variability in the methodology used to count TILs. Moreover, our study is limited by the small number of cases.

We did not find a significant correlation between PD-L1 expression in cancer cells or the surrounding microenvironment and peritumoral CD8 lymphocytes or MSI status. However, Rosenbaum et al. reported that PD-L1 positive tumors were more likely to exhibit MSI [29]. Similarly, Valentini et al. found that PD-L1 expression in cancer cells was significantly associated with MSI [31]. In contrast, Masugi et al. found a significant inverse association between PD-L1 expression and MSI status[30].

Our study has several limitations. It was a retrospective, single-institution study with a small sample size. Additionally, PD-L1 expression was evaluated using only one antibody (22C3 clone).

Conclusion

The prognostic value of PD-L1 expression in colon cancer is highly debated. While some studies have demonstrated a better outcome, others have shown a worse outcome. Therefore, further studies are required to clarify the prognostic role of PD-L1 expression.

Acknowledgments
Not applicable.

Authors' contribution
Conception: SMT; Design: HW, NMR & EMSK; Data Collection: All authors; Data Analysis and Interpretation: SMT, NMR & EMSK; Drafting the manuscript: SMT; Revising the manuscript: HW & NMR; Approval of the final version of the manuscript: All authors; Agreement to be accountable for all aspects of the work: All authors;

Conflict of interest
The authors declare that they have no conflict of interest to disclose.

Data availability
Data is available from the corresponding author upon request.

Ethical considerations
This study was conducted in accordance with the Declaration of Helsinki.

Funding
Not applicable.

Study registration
Not applicable.

References

1. Jung DH, Park HJ, Jang HH, Kim SH, Jung Y, Lee WS. Clinical impact of PD-L1 expression for survival in curatively resected colon cancer. Cancer Invest. 2020; 38(7): 406-414.
2. Huang CY, Chiang SF, Ke TW, et al. Clinical significance of programmed death 1 ligand-1 (CD274/PD-L1) and intra-tumoral CD8+ T-cell infiltration in stage II-III colorectal cancer. Sci Rep. 2018; 8(1): 15658.
3. Kwak Y, Koh J, Kim DW, Kang SB, Kim WH, Lee HS. Immunoscore encompassing CD3+ and CD8+ T cell densities in distant metastasis is a robust prognostic marker for advanced colorectal cancer. Oncotarget. 2016; 7(49): 81778-81790.
4. Khoury SJ, Sayegh MH. The roles of the new negative T cell costimulatory pathways in regulating autoimmunity. Immunity. 2004; 20(5): 529-538.
5. Okazaki T, Honjo T. The PD-1-PD-L pathway in immunological tolerance. Trends Immunol. 2006; 27(4): 195-201.
6. Curiel TJ, Wei S, Dong H, et al. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. Nat Med. 2003; 9: 562–567.
7. Latchman Y, Wood CR, Chernova T, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol. 2001; 2(3): 261-268.
8. Keir ME, Liang SC, Guleria I, et al. Tissue expression of PD-L1 mediates peripheral T cell tolerance. J Exp Med. 2006; 203(4): 883-895.
9. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008; 26: 677-704.
10. Ostrand-Rosenberg S, Horn LA, Haile ST. The programmed death-1 immune-suppressive pathway: barrier to antitumor immunity. J Immunol. 2014; 193(8): 3835-3841.
11. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell. 2015; 160(1-2): 48-61.
12. Hirano F, Kaneko K, Tamura H, et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res. 2005; 65(3): 1089-1096.
13. Wintterle S, Schreiner B, Mitsdoerffer M, et al. Expression of the B7-related molecule B7-H1 by glioma cells: a potential mechanism of immune paralysis. Cancer Res. 2003; 63(21): 7462-7467.
14. Thompson RH, Gillett MD, Cheville JC, et al. Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci U S A. 2004; 101(49): 17174-17179.
15. Strome SE, Dong H, Tamura H, et al. B7-H1 blockade augments adoptive T-cell immunotherapy for squamous cell carcinoma. Cancer Res. 2003; 63(19): 6501-6505.
16. Ohigashi Y, Sho M, Yamada Y, et al. Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res. 2005; 11(8): 2947-2953.
17. Konishi J, Yamazaki K, Azuma M, Kinoshita I, Dosaka-Akita H, Nishimura M. B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clin Cancer Res. 2004; 10(15): 5094-5100.
18. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012; 12(4): 252-264.
19. Lynch D, Murphy A. The emerging role of immunotherapy in colorectal cancer. Ann Transl Med. 2016; 4(16): 305.
20. Bupathi M, Wu C. Biomarkers for immune therapy in colorectal cancer: mismatch-repair deficiency and others. J Gastrointest Oncol. 2016; 7(5): 713-720.
21. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015; 372(26): 2509-2520.
22. Williams DS, Bird MJ, Jorissen RN, et al. Nonsense mediated decay resistant mutations are a source of expressed mutant proteins in colon cancer cell lines with microsatellite instability. PLoS One. 2010; 5(12): e16012.
23. Mlecnik B, Bindea G, Angell HK, et al. Integrative analyses of colorectal cancer show immunoscore is a stronger predictor of patient survival than microsatellite instability. Immunity. 2016; 44(3): 698-711.
24. Llosa NJ, Cruise M, Tam A, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov. 2015; 5(1): 43-51.
25. Dudley JC, Lin MT, Le DT, Eshleman JR. Microsatellite instability as a biomarker for PD-1 blockade. Clin Cancer Res. 2016; 22(4): 813-820.
26. Yuan L, Chi Y, Chen W, et al. Immunohistochemistry and microsatellite instability analysis in molecular subtyping of colorectal carcinoma based on mismatch repair competency. Int J Clin Exp Med. 2015; 8(11): 20988-21000.
27. Rustgi AK. The genetics of hereditary colon cancer. Genes Dev. 2016; 21(20): 2525-2538.
28. Koganemaru S, Inoshita N, Miura Y, et al. Prognostic value of programmed death-ligand 1 expression in patients with stage III colorectal cancer. Cancer Sci. 2017; 108(5): 853-858.
29. Rosenbaum MW, Bledsoe JR, Morales-Oyarvide V, Huynh TG, Mino-Kenudson M. PD-L1 expression in colorectal cancer is associated with microsatellite instability, BRAF mutation, medullary morphology and cytotoxic tumor-infiltrating lymphocytes. Mod Pathol. 2016; 29(9): 1104-1112.
30. Masugi Y, Nishihara R, Yang J, et al. Tumour CD274 (PD-L1) expression and T cells in colorectal cancer. Gut. 2017; 66(8): 1463-1473.

31. Valentini AM, Di Pinto F, Cariola F, et al. PD-L1 expression in colorectal cancer defines three subsets of tumor immune microenvironments. Oncotarget. 2018; 9(9): 8584-8596.
32. Droeser RA, Hirt C, Viehl CT, et al. Clinical impact of programmed cell death ligand 1 expression in colorectal cancer. Eur J Cancer. 2013; 49(9): 2233-2242.
33. Lee KS, Kim BH, Oh HK, et al. Programmed cell death ligand-1 protein expression and CD274/PD-L1 gene amplification in colorectal cancer: Implications for prognosis. Cancer Sci. 2018; 109(9): 2957-2969.
34. Li Y, He M, Zhou Y, et al. The prognostic and clinicopathological roles of PD-L1 expression in colorectal cancer: A systematic review and meta-analysis. Front Pharmacol. 2019; 10:139.
35. Shan T, Chen S, Wu T, Yang Y, Li S, Chen X. PD-L1 expression in colon cancer and its relationship with clinical prognosis. Int J Clin Exp Pathol. 2019; 12(5): 1764-1769.
36. Enkhbat T, Nishi M, Takasu C, et al. Programmed cell death ligand 1 expression is an independent prognostic factor in colorectal cancer. Anticancer Res. 2018; 38(6):3367-3373.
37. Cho YA, Yoon HJ, Lee JI, Hong SP, Hong SD. Relationship between the expressions of PD-L1 and tumor-infiltrating lymphocytes in oral squamous cell carcinoma. Oral Oncol. 2011; 47(12): 1148-1153.
38. Drescher KM, Sharma P, Watson P, Gatalica Z, Thibodeau SN, Lynch HT. Lymphocyte recruitment into the tumor site is altered in patients with MSI-H colon cancer. Fam Cancer. 2009; 8(3):231-239.
39. Phillips SM, Banerjea A, Feakins R, Li SR, Bustin SA, Dorudi S. Tumour-infiltrating lymphocytes in colorectal cancer with microsatellite instability are activated and cytotoxic. Br J Surg. 2004; 91(4): 469-475.