Original Article

Simultaneous high PD-L1 and low VEGFR2 expression is associated with better overall survival in rectal cancer

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Background: The aim of the present study was to analyze the association of programmed cell death-ligand 1 (PD-L1) and vascular endothelial growth factor receptor 2 (VEGFR2) expression levels with clinicopathological characteristics and survival to provide a treatment strategy for rectal cancer.

Methods: Immunohistochemical staining of VEGFR2 and PD-L1 was carried out, and the association of PD-L1 and VEGFR2 expression levels with clinicopathological characteristics and survival were investigated in 77 pair-matched rectal cancer patients.

Results: PD-L1 and VEGFR2 expression levels in surgical tumor tissues were higher than those in paired adjacent normal tissues, respectively (both P<0.05). The results of the 5-year overall survival (OS) analysis showed that patients with low VEGFR2 expression (66.7% *vs.* 43.5%, P=0.042) and high tumor PD-L1 expression (63.9% *vs.* 26.1%, P=0.001) in tumor tissues demonstrated significantly better OS. Patients with high TNM stage had poorer OS [hazard ratio (HR): 2.093, 95% confidence interval (CI): 1.027–4.087, P=0.030]. Similar results of poorer OS could be seen in patients with low tumor PD-L1 expression (HR: 3.365, 95% CI:1.747–6.481, P=0.005), as well as patients with high tumor VEGFR2 expression (HR: 0.418, 95% CI: 0.232–0.993, P=0.048).

Conclusions: The results indicated that tumor PD-L1 and VEGFR2 expression levels were associated with OS, and the combination of tumor PD-L1 and VEGFR2 levels might be an independent prognostic factor in rectal cancer.

Keywords: Programmed cell death-ligand 1 (PD-L1); vascular endothelial growth factor receptor 2 (VEGFR2); overall survival (OS); rectal cancer

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Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of cancer-related death in women and men worldwide (1). Therefore, it is important to determine novel treatment strategy in rectal cancer. Previous studies have found that low-dose apatinib treatment increases the infiltration of lymphocytes in tumors, and that a combination of low-dose apatinib and anti-programmed cell death-ligand 1 (PD-L1) treatment could significantly impact tumor growth and metastases, prolonging survival in mouse models (2). The expression of PD-L1 and vascular endothelial growth factor receptor 2 (VEGFR2) could be of great interest for the development of rectal cancer treatment.

Positive PD-L1 expression has been associated with significantly poor prognosis in various tumors; however, the prognostic significance of PD-L1 expression in rectal cancer still remains controversial (3-5). The evaluation of PD-L1 expression is also important in the application of immunotherapy and the prediction of therapeutic effects (4). Angiogenesis is a critical process during tumor progression and metastasis. VEGF/VEGFR2 signaling is another vital molecular pathway steering tumor angiogenesis, with multiple functions in tumor microenvironment (TME), including immunosuppression (6). Using a syngeneic lung cancer mouse model, Zhao *et al.* demonstrated that low-dose apatinib could alleviate hypoxia, increase $CD8^+$ T-cell infiltration, and reduce tumor-associated macrophage recruitment (2).

There is a need for specific prognostic biomarkers for rectal cancer patients in order to reach a more effective outcome prediction strategy. In the present study, we compared tumor PD-L1 and VEGFR2 expression levels in rectal cancer. We analyzed the relationship between the expression of PD-L1 and VEGFR2, as well their potential as biomarkers for prognostic prediction in rectal cancer.

We present the following article in accordance with the REMARK reporting checklist (available at http://dx.doi.org/10.21037/tcr-20-3106).

Methods

Commercially available rectal cancer tissue microarrays contented 90 rectal cancer tissue were purchased (Hrec-Ade180Sur-03; Shanghai Outdo Biotechnology, Shanghai, China). Specimens were acquired from 90 rectal cancer patients who accepted surgery from July 2006 to August 2007. The follow-up period was 86 months. The follow-up endpoint was death. Tumors were staged according to the American Joint Committee on Cancer staging system. The following clinical pathological characteristics were collected: sex, age, TNM stage, pathological differentiation stage, and grade. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The present study was approved by the Ethics Committee of Soochow University. All enrolled patients provided signed informed consent.

The slides were dried, deparaffinized, rehydrated, antigen retrieval and endogenous peroxidases deactivation in order. The slides were incubated with VEGFR2 antibody (2479S, Cell Signaling Technology, USA) PD-L1 antibody (ab205921, Abcam, UK) at 4°C overnight. On the second day, anti-mouse/rabbit secondary antibody was added to the slices and incubated at room temperature for 30 min (Abcam, USA). A DAB substrate kit (DAB-0031, Fuzhou Maixin Biotech. Co., Ltd., Fuzhou, China) was then used for staining.

Tumor PD-L1 and VEGFR2 immunostaining was scored using the conventional Histoscore (H-score) calculation. The intensity of staining on a semiquantitative scale (0–3+) is defined as follows: 0, absent; 1+, weak; 2+, moderate; and 3+, strong membrane staining. The possible range of H-scores was from 0 to 300.

Statistical analysis

Statistical analysis was conducted via GraphPad Prism 5.0 software (GraphPad Software, San Diego, California, USA) and SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Rank sum test, χ^2-test, and Fisher's exact test were applied for group comparisons. The relationship between tumor expression levels of PD-L1, VEGFR2 and patient clinical parameters was evaluated using the Spearman's χ^2 test. Influential factors of patient survival were adjusted in the Cox model. Kaplan-Meier analysis was applied to estimate the overall survival (OS) and to calculate the hazard ratios (HRs) of the collected parameters. All tests reported a 2-sided P value with a significance level set at 0.05.

Results

Patient characteristics

In total, 90 rectal cancer samples were collected. Of these, 6 cases were missing complete clinical data, and 7 lacked

Table 1 Relationship between PD-L1 and VEGFR2 and patient characteristics (n=77)

Clinicopathological parameters	Cases (%)	PD-L1			VEGFR2		
		High (%)	Low (%)	P value	High (%)	Low (%)	P value
Age (years)				0.411			0.546
<65	38 (49)	25	13		24	14	
≥65	39 (51)	29	10		22	17	
Sex				0.230			0.525
Female	29 (38)	18	11		16	13	
Male	48 (62)	36	12		30	18	
T stage				0.257			0.553
T1–T2	8 (10)	7	1		4	4	
T3–T4	69 (90)	47	22		42	27	
pN stage				0.203			0.611
Negative	42 (55)	32	10		24	18	
Positive	35 (45)	22	13		22	13	
Distant metastasis				0.249			0.803
Negative	74 (96)	51	23		44	30	
Positive	3 (4)	3	0		2	1	
Pathological differentiation stage				0.015*			0.659
I–II	55 (71)	43	12		32	23	
III	22 (29)	11	11		14	8	
TNM stage (7th AJCC)				0.203			0.611
I–II	42 (54)	32	10		24	18	
III–IV	35 (36)	22	13		22	13	
Tumor PD–L1							
High (%)	54				33	21	
Low (%)	23				13	10	
VEGFR2				0.707			
High (%)	43	33	10				
Low (%)	34	21	13				

*, significant at P<0.05. χ^2-test was used group comparisons. Fisher's exact test was used if a variant was <5. AJCC, American Joint Committee on Cancer; PD-L1, programmed cell death-ligand 1; VEGFR2, vascular endothelial growth factor receptor 2.

enough tumor tissue for this study, resulting in 77 valid cases for the present study. The follow-up period was 86 months. The median survival time was 74 months. The clinicopathological characteristics of these patients are presented in *Table 1*. The majority of the patients were men (62%), and the mean age at diagnosis was 59.4±12.5 years (range, 31–90 years). Sixty-nine cases (90%) were T3–T4 stage. Thirty-five patients (45%) had lymph node metastases, and 3 (4%) had distant metastasis.

PD-L1 and VEGFR2 expression levels were examined using immunohistochemistry in both tumor tissues and paired adjacent normal tissues. Clinicopathological

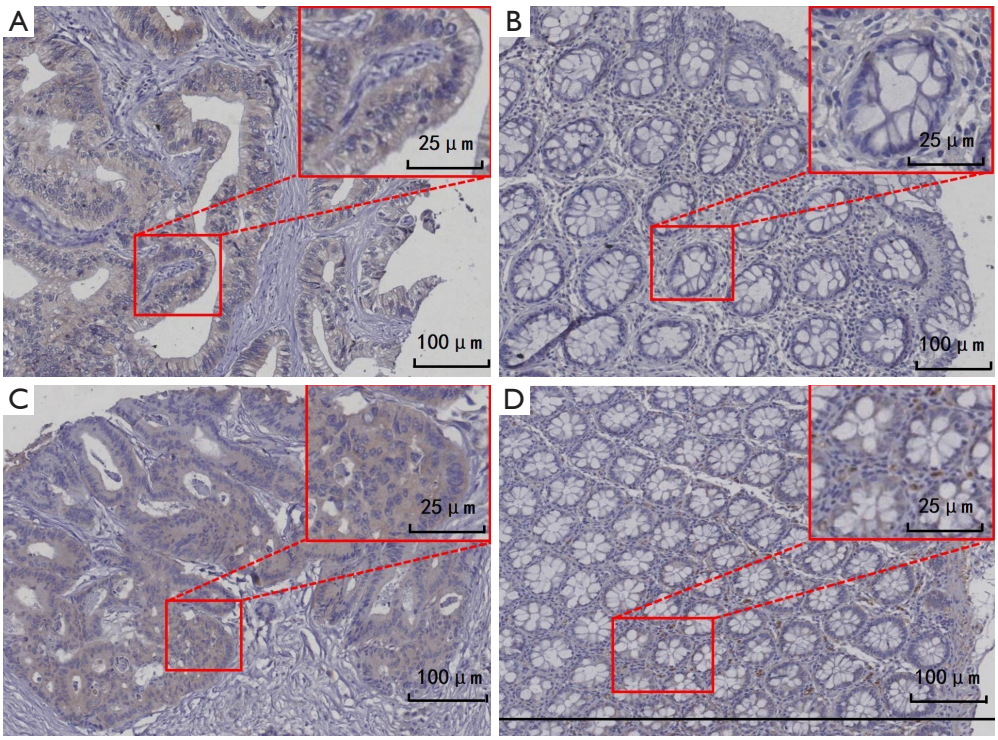

Figure 1 Programmed cell death-ligand 1 (PD-L1) and vascular endothelial growth factor receptor 2 (VEGFR2) immunohistochemistry in tumor tissue and adjust normal tissue for a patient with rectal cancer. (A) PD-L1 expression in tumor tissue. (B) PD–L1 expression in normal mucosa. (C) VEGFR2 expression in tumor tissue. (D) VEGFR2 expression in normal mucosa.

characteristics and the relationship with the expression levels of PD-L1 and VEGFR2 in surgical tumor tissues are presented in *Table 1*.

PD-L1 and VEGFR2 expression levels in tumor tissues and paired adjacent normal tissues

The expression levels of PD-L1 and VEGFR2 in surgical tumor tissues were higher than those in adjacent normal tissues, respectively (both P<0.05) (*Figure 1*). Furthermore, we analyzed the relationship between clinicopathological parameters and PD-L1, as well as VEGFR2 expression, and found that a lower pathological differentiation stage was associated with higher PD-L1 expression (P=0.015).

Tumor PD L1 expression and OS in patients

We carried out a 5-year OS analysis and found that patients with high tumor PD-L1 expression (63.9% *vs.* 26.1%, P=0.001) in tumor tissues had significantly better 5-year OS (*Table 2*). Kaplan-Meier analysis also showed that patients with a high expression of tumor PD-L1 had better OS (log-rank P=0.001) (*Figure 2A*). The findings demonstrate that high PD-L1 expression in tumor tissue indicate better clinical outcomes in rectal cancer.

Tumor VEGFR2 expression and OS in patients in the 5-year OS analysis, patients with low VEGFR2 expression (66.7% *vs.* 43.5%, P=0.042) in tumor tissues exhibited significantly better 5-year OS. Kaplan-Meier survival analysis also showed that patients with low tumor VEGFR2 level had better OS (log-rank P=0.042) (*Figure 2B*). The findings demonstrate that low VEGFR2 expression could indicate better clinical outcomes in rectal cancer patients.

Tumor PD-L1 and VEGFR2 association and OS differences in subgroups

We conducted a Spearman's rank correlation test and found no significant relationship between the expression of PD-L1 and VEGFR2 in rectal cancer (P=0.043 and P=0.77, respectively).

However, we observed survival differences between patients with different PD-L1 and VEGFR2 expression levels. Patients with high PD-L1 and low VEGFR2 levels

Table 2 Clinicopathological parameters and 5-year overall survival

Clinicopathological parameter	Cases	5-year overall survival (%)	P value
Age (years)			0.304
<65	38	60.5	
≥65	39	51.3	
Sex			0.734
Female	29	55.2	
Male	48	56.3	
T stage			0.225
T1–T2	8	87.5	
T3–T4	69	52.2	
pN stage			0.001*
Negative	42	71.4	
Positive	35	37.1	
Distant metastasis			0.006*
Negative	74	58.1	
Positive	3	0	
Pathological differentiation stage			0.203
I–II	55	60.0	
III	22	45.5	
TNM stage (7th AJCC)			0.016*
I–II	42	71.4	
III–IV	35	37.1	
Tumor PD–L1			0.001*
High	54	63.9	
Low	23	26.1	
Tumor VEGFR2			0.042*
High	43	66.7	
Low	34	43.5	
PD-L1/VEGFR2			0.001*
High/high	33	57.6	
Low/low	10	50	
High/low	21	74.6	
Low/high	13	7.7	

*, significant at P<0.05. P value was obtained from the log-rank test. Kaplan-Meier method was used for survival analysis. AJCC, American Joint Committee on Cancer; PD-L1, programmed cell death-ligand 1; VEGFR2, vascular endothelial growth factor receptor 2.

exhibited significantly better OS (P=0.001) (*Figure 2C*). This suggests that high PD-L1 and low VEGFR2 expression levels could indicate a better prognostic factor in rectal cancer. T1–2 stage also exhibited significantly better OS than T3–4 stage (P=0.016) (*Figure 2D*).

Independent risk factors for rectal cancer patients

In the univariate analysis, the OS rate of patients was associated with TNM stage, tumor PD-L1 expression and VEGFR2 expression (P<0.05). Patients with high TNM stage had poorer OS compared with that with low TNM stage [HR: 2.203, 95% confidence interval (CI): 1.139–4.261, P=0.019]. Similar risks for poorer OS could be seen in patients with low tumor PD-L1 expression (HR: 3.365, 95% CI: 1.747–6.481, P=0.005), and in those with a high tumor VEGFR2 expression (HR: 0.418, 95% CI: 0.232–0.993, P=0.048) (*Table 3*).

Subsequently, we a performed multivariate analysis, and the results were consistent with those of the univariate analysis. Patients with high TNM stage still had an increased risk for poorer OS compared with that with low TNM stage (HR:2.093, 95% CI: 1.027–4.087, P=0.030). Similar results could also be seen in patients with low tumor PD-L1 expression (HR: 4.566, 95% CI: 2.237–9.32), P=0.001), and in those with high tumor VEGFR2 expression (HR: 0.292, 95% CI: 0.133–0.642, P=0.002) (*Table 3*). These consistencies demonstrated that the combination of tumor PD-L1 and VEGFR2 levels could be an independent prognostic factor for rectal cancer patients.

Discussion

The association between PD-L1 and poor overall survival rate has been previously observed in melanoma, renal cancer, and lung cancer (7-9). However, other studies have found a correlation between tumor PD-L1 expression and improved survival outcomes in certain malignancies, such as melanomas, CRC, and non-triple-negative breast cancer (3,10-12). Previous study showed that VEGFR2 expression in tumor was higher than that in normal cells, and VEGFR-2 shows an association with invasive adenocarcinoma and poor overall survival (13). It is reported that patients with negative PD-L1 expression in tumor had worse overall and recurrence-free survivals compared to patients with positive PD-L1 expression (3). A meta-analysis indicated that a high level of PD-L1 expression might be a biomarker for a poor prognosis in CRC

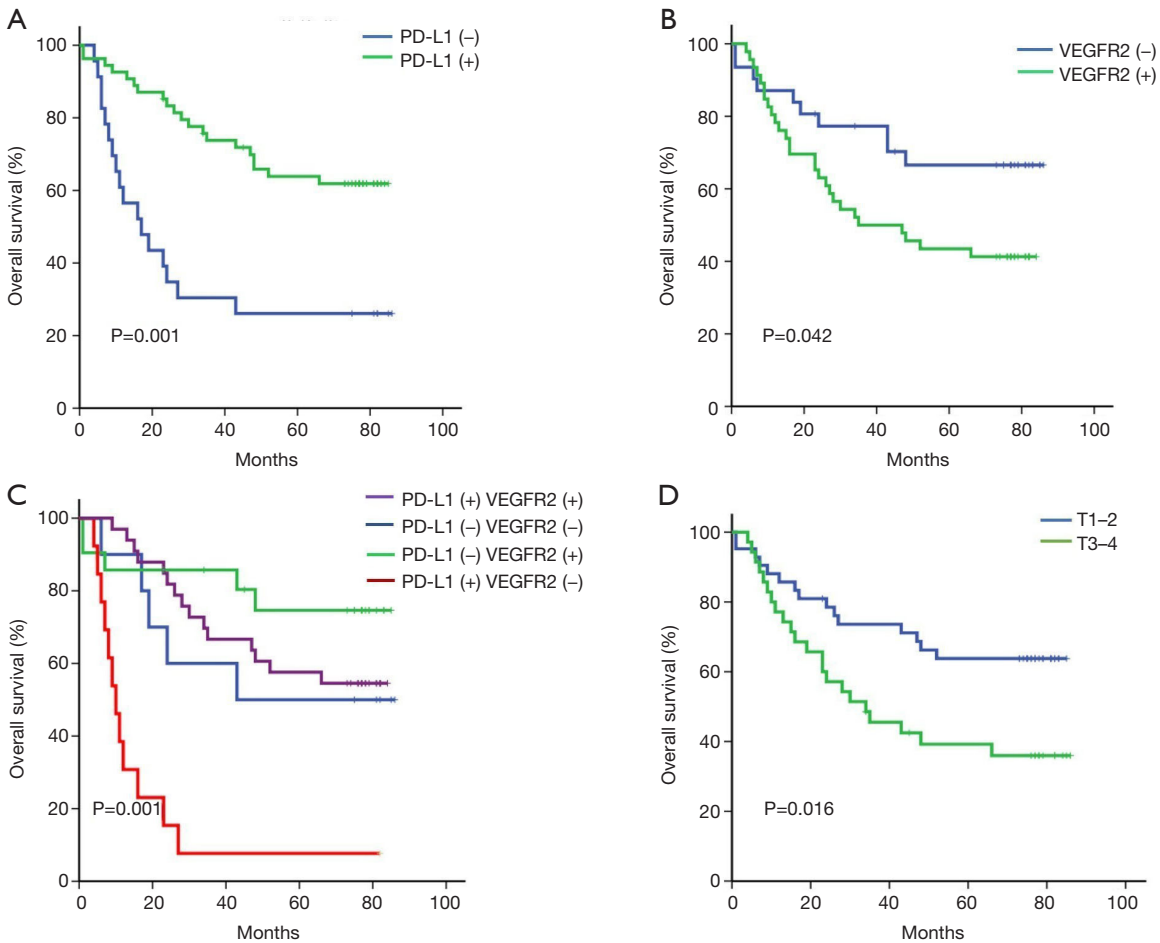

Figure 2 Kaplan-Meier curves of overall survival (OS) with tumor programmed cell death-ligand 1 (PD-L1) and vascular endothelial growth factor receptor 2 (VEGFR2) in rectal cancer patients. (A) Kaplan–Meier curves demonstrated that tumor PD-L1 expression is associated with OS in rectal cancer patients (P=0.001). (B) Kaplan–Meier curves demonstrated that tumor VEGFR2 expression is associated with OS in rectal cancer patients (P=0.042). (C) High tumor PD-L1 and low tumor VEGFR2 expressions within the tumor microenvironment are associated with improved OS in rectal cancer patients (P=0.001). (D) Kaplan-Meier curves demonstrated that tumor T stage is associated with OS in rectal cancer patients (P=0.016).

patients (4) Previous studies have indicated that patients with high PD-L1 have better OS and 5-year disease-free survival (DFS) (14). The advent of the PD-1/PD-L1-inhibiting and anti-angiogenic therapy has improved the therapeutic effect of rectal cancer.

Previous studies have indicated that adjuvant sunitinib plus anti-PD-L1 improves OS compared with either drug alone in breast cancer models (15). Therefore, in the present study, we evaluated the correlation between the expression of PD-L1 and VEGFR2 with OS and found that patients with high PD-L1 and low VEGFR2 expression had better OS compared with that low PD-L1 and high VEGFR2 expression in rectal cancer. Angiogenesis is critical for tumor growth and metastasis. VEGF/VEGFR signaling plays an important role in tumor growth, progression, metastasis, angiogenesis, and in tumor microenvironment, including immunosuppression (6,16-20). Anti-angiogenic therapy targeting VEGF/VEGFR could transform the TME into an immunological favorable state, also described as a "hot" microenvironment. Antibodies targeting VEGF/VEGFR are able to induce both anti-angiogenic and immune-supportive effects (21-23).

It has been reported that combination therapy with immunotherapy and/or anti-VEGF/VEGFR therapy

Table 3 Univariate and multivariate analyses of clinicopathological parameters for 5-year overall survival

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Sex (male *vs.* female)	1.041 (0.535–2.023)	0.906		
Age (≥65 *vs.* <65 years)	0.962 (0.505–1.834)	0.907		
T stage (T3–T4 *vs.* T1–T2)	1.522 (0.467–4.960)	0.486		
Distant metastasis (positive *vs.* negative)	1.753 (0.417–7.364)	0.444		
Pathological differentiation stage (III *vs.* I–II)	1.457 (0.730–2.906)	0.285		
TNM stage (III–IV *vs.* I–II)	2.203 (1.139–4.261)	0.019*	2.093 (1.072–4.087)	0.030*
Tumor PD-L1 expression (low *vs.* high)	3.365 (1.747–6.481)	0.005*	4.566 (2.237–9.320)	0.001*
Tumor VEGFR2 expression (low *vs.* high)	0.418 (0.232–0.993)	0.048*	0.292 (0.133–0.642)	0.002*

*P<0.05 was considered statistically significant. CI, confidence interval; HR, hazard ratio; PD-L1, programmed cell death-ligand 1; VEGFR2, vascular endothelial growth factor receptor 2.

produces favorable changes in the TME, and it has been suggested as a synergic treatment strategy in inhibiting proliferation and promoting the apoptosis of cancer cells (6,24-26). Patients with metastatic renal cell carcinoma who underwent anti-VEGF and anti-PD-L1 combination antibody therapy showed improved antigen-specific T-cell migration ability (26). This combined anti-VEGF and anti-PD-L1 antibody therapy has been trialed in patients with recurrent glioblastoma renal cell carcinoma, CRC, and ovarian cancer (27). The results indicated that combination therapy consisting of immune checkpoint inhibitor and an anti-VEGF/VEGFR antibody could further benefit rectal cancer patients (28).

In the present study, we analyzed the relationship between clinicopathological parameters PD-L1 and VEGFR2, and the OS of 77 pair-matched rectal cancer tissues. Our result showed that PD-L1 and VEGFR2 positivity were significantly higher in cancer tissue than in normal tissue. Patients with a high level of PD-L1 expression and negative pN stage had better 5-year OS. Similar to our findings, Chiang *et al.* showed that PD-L1 expression was associated with better DFS and 5-year OS in patients with locally advanced rectal cancer (14). Pyo *et al.* showed that PD-L1 expression was significantly correlated with favorable tumor behaviors and better OS (3). Patients with high tumor PD-L1 expression are most likely to benefit from immune checkpoint inhibitor treatment and improved survival outcomes in CRC (3,29). These results suggest that PD-L1 expression might be a prognostic factor for rectal cancer. High tumor PD-L1 expression induces immune microenvironment inhibition; therefore, it is reasonable to believe that checkpoint blockades might improve objective responses in PD-L1-high rectal cancer. This might help identify if those patients would benefit from immune checkpoint inhibitors.

Previous studies have shown that tumor PD-1/PD-L1 expression is significantly associated with tumor stage and prognosis (21). In present study, we analyzed the relationship between PD-L1 and various patient clinicopathological characteristics, and found that high PD-L1 was significantly associated with low pathological differentiation stage. The results of the present study demonstrated that both PD-L1 and VEGFR2 expression levels were associated with the OS of rectal cancer patients. Patients with a high level of PD-L1 expression and/or with low VEGFR2 expression were found to have better OS. Kaplan–Meier analysis showed that, compared with other groups, patients with low VEGFR2 and high PD-L1 expression had the best OS outcomes. Similar to our findings, previous studies have demonstrated that tumor PD-L1 expression is strongly associated with better OS outcomes among patients with non-triple-negative breast cancer, melanoma, and CRC (3,10-12).

Univariate and multivariate analyses showed that patients with high TNM stage exhibited an increased risk for poorer OS compared with patients with low TNM stage. Multivariate analysis models indicated that patients with high VEGFR2 and low PD-L1 expression levels had poorer OS than that with low VEGFR2 and high PD-L1 expression, indicating that tumor VEGFR2 and PD-L1 are

prognostic factors for rectal cancer. A previous study proved that for colon cancer high PD-L1 expression was collected with less aggressive tumor behavior, leading to better OS (30). These results suggest that, for rectal cancer patients, upregulated tumor PD-L1 and downregulated tumor VEGFR2 indicate good clinical outcome. The findings of the present study indicate that combinational tumor PD-L1 and VEGFR2 expression levels may provide therapeutic strategies and predict outcomes for rectal cancer patients.

It has been reported that combined anti-PD-1/PD-L1 and anti-VEGF/VEGFR2 antibodies demonstrate synergistic anti-tumor effects on some solid tumors (31). Anti-angiogenic therapy combined with PD-1/PD-L1 blockade was suggested as a potential new therapeutic approach for renal cell carcinoma patients (32). Shigeta *et al.* demonstrated that combined anti-PD-L1 and antiVEGFR2 therapy significantly inhibited primary tumor growth and improved survival in murine hepatocellular carcinoma models (33). Meder *et al.* indicated that dual anti-PD-L1/VEGF antibodies synergistically improved progression-free survival and OS in a mouse model of small cell lung cancer (30).

For rectal cancer, combining anti-VEGF/VEGFR2 antibody with immune checkpoint inhibitor treatment is potentially an efficient therapy strategy. In the future, a combination of checkpoint inhibitors and anti-angiogenic therapy should be established to improve the treatment efficacy of rectal cancer.

Conclusions

The findings of the present study highlight the impact of PD-L1 and VEGFR2 on rectal cancer by demonstrating their prognostic values in survival outcomes among rectal cancer patients. Based on tumor VEGFR2 and PD-L1 and expression levels in rectal cancer, novel prognostic and therapeutic strategies should be expected. Moreover, combined PD-L1 and VEGFR2 expression levels may be useful biomarkers to predict outcomes and establish the new treatment strategies based on the combination of immune checkpoint inhibitors and anti-angiogenesis antibodies to improve the treatment efficacy of rectal cancer.

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Footnote

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Data Sharing Statement: Available at http://dx.doi.org/10.21037/tcr-20-3106

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr-20-3106). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The present study was approved by the Ethics Committee of Soochow University. All enrolled patients provided signed informed consent.

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