Prognostic value of programmed death-ligand 1 expression in patients with esophageal squamous cell carcinoma

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esophageal cancer, PD-L1, immunochemistry, prognosis

Abstract

Background

To investigate the expression of PD-L1(programmed death-ligand 1)in patients with esophageal squamous cell carcinoma (ESCC) and its clinical significance.

Methods

The tissue expression of PD-L1 protein in 139 cases of ESCC and 50 adjacent non-malignant epithelial tissues (> 5 cm from the tumor resection margins) were identified by immunohistochemical staining. Subsequently, the relationship between expression and the observed clinical characteristics was analyzed.

Results

The positive expression rate of PD-L1 protein was increased in tumor tissues compared to that of adjacent noncancerous mucosa tissues (40.3% vs. 22.0%, $P < 0.05$). The findings also indicated that PD-L1 protein expression had no significant correlation with age, gender, tumor location, differentiation and lymph node (N) status ($P > 0.05$). The 91 months follow-up Kaplan-Meier survival analysis showed that patients in positively expressed PD-L1 group experienced a lower survival rate compared to their negatively expressed PD-L1 counterparts (32.1% vs. 48.2%, $P < 0.05$). The COX regression analysis results suggested that PD-L1 represented an independent prognosis factor for ESCC.

Conclusions

The findings indicated that PD-L1 plays an important role in the progression of ESCC and might represent a potential therapeutic and prognostic target for ESCC patients.

Background

Esophageal carcinoma is one of the deadliest most aggressive gastrointestinal malignancies, ranking sixth among cancer-related deaths [1]. It is one of the leading causes of cancer-related deaths in China, with 90% of cases being esophageal squamous cell carcinoma (ESCC) [2, 3]. Despite breakthroughs in esophageal carcinoma treatment methods such as chemotherapy, radiotherapy, targeted therapy, and immunotherapy, the incidences of local recurrence and distant metastasis remain higher, with an overall five-year survival rate of only about 30% [1, 4]. Therefore, new clinically-oriented prognostic parameters and effective treatment strategies are urgently needed.

Programmed death ligand 1 (PDCD1, PD-1) belongs to the CD28 family of immunoglobulin superfamily and chiefly expressed on the surface of T lymphocytes, B lymphocytes, and dendritic cells. Programmed death receptor ligand 1 (PDCD1LG1, PD-L1) is the prime ligand of PD-1 and is expressed on the surface of activated T lymphocytes, B lymphocytes, macrophages, dendritic cells, fibroblasts, and some other neoplasm cells [5]. The PD-L1 signaling pathway is closely relevant to the immune escape of tumor cells [6, 7], and a combination of PD-1 and PD-L1 can induce apoptosis or failure of activated T lymphocytes [8]. Researches have found that the expression of PD-L1 is related to poor prognosis in patients with a variety of malignant tumors, including those with non-small-cell lung cancer, colorectal cancer, renal cell cancer, gastric cancer and breast cancer [9–13]. Studies have also shown that immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway represent promising therapeutic agents in the treatment of various human tumors [6]. To date, several previous studies have pointed out variations in PD-L1 expression rates in individuals with ESCC. However, the relationship between those variations and patient prognosis remains controversial. Therefore, this paper aims to study PD-L1 expression levels in pathological tissues of ESCC patients and assess their connection with observed clinicopathological features and prognosis, hoping to provide further clues for future treatment of esophageal carcinoma.

Methods

Patients

We retrospectively reviewed medical records of 139 patients who underwent curative esophagectomy with R0 resection for histologically verified stage tumor (T) 3 ESCC between January 2012 and December 2016 at the First Affiliated Hospital of Bengbu Medical College. Patients with a history of neoadjuvant treatment before the procedure, distant metastasis, or immune system diseases were excluded from this study. The postoperative treatment is based on the patient's surgical pathology and is comprised of chemotherapy and radiotherapy according to the guidelines proposed by the oncology team of our hospital. All tissue specimens involved in this study were obtained from the pathological tissue bank of our hospital.

All subject’s clinicopathological data, including age, gender, tumor location, tumor differentiation, and

lymph node metastasis, were recorded. The original histological diagnosis was based on criteria established by the World Health Organization's guidelines [14], and all tumors were pathologically staged according to the American Joint Committee on Cancer (AJCC, 2002) TNM staging system. The postoperative follow-ups included clinical examinations and laboratory test results and were spaced as follows: every 3–6 months during the first two years after the surgical procedure then annually afterward or until patient death. The median follow-up time was 29.5 months, with a range of 2.0 to 91 months. The last follow-up was performed on December 31, 2019.

Tissue Microarray

Formalin-fixed paraffin-embedded (FFPE) tumor tissue samples and corresponding adjacent non-malignant epithelial samples were independently stained by hematoxylin and eosin (H&E) and double-checked by two pathologists. A tissue microarray of all 139 cases and 50 control specimens was constructed by paraffin tissue blocks. A 2 mm in diameter region was removed from every specimen's FFPE tissue blocks. Series of 4-μm-thick sections were cut and transferred to slides for immunohistochemistry according to the manufacturer's instructions.

Immunohistochemistry staining

Immunohistochemistry (IHC) staining was performed using the rabbit anti-PD-L1 monoclonal antibody (1:200, ab205921, Sa Yingsi biotechnology co. LTD, Hefei, China) to evaluate PD-L1 tissue expression. The tissue microarray was deparaffinized and rehydrated. Then the sections underwent neutralization of endogenous peroxidase and finally blockage with goat blocking serum. The samples were later incubated overnight with the PD-L1 antibody followed with polyclonal peroxidase-conjugated anti-rabbit IgG (Maixin biotechnology development Co, Fuzhou, China) for 20 min. Finally, the slides were counterstained with hematoxylin.

Immunostaining Evaluation

The immunohistochemistry results were examined under low power (× 100) to identify regions containing positively stained tumor cells. The dyeing intensity and proportions of tumor cells showing high and low staining in each selected field were determined at high magnification for sections with positive staining. The immunoreactivity scoring pattern (ISP) used in the study was a combination of the intensity category and the proportion category [15]. The intensity category was graded as follows:

0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The proportion category was graded as follows: 0 (no or less than 1%), 1 (1%-30%), 2 (31–60%) and 3 (61%-100%). The final score was obtained by multiplying the intensity category grade with the proportion category grade and ranged from 0 to 9. In our study, the expression of PD-L1 in tumor tissues was classified as either negative (ISP□3) or positive (ISP ≥ 3). Tumor samples were reviewed and blindly scored by two pathologists. Conflicting cases were discussed until consensus was achieved.

Statistical analysis

Patients were assigned to two groups according to their PD-L1 status. The clinicopathological factors and PD-L1 expressions were assessed using the $\chi 2$ or Fisher's exact test for categorical variables. Significant correlations between clinical data and PD-L1 positivity were assessed by logistic regression analysis. The Kaplan–Meier survival method was used to evaluate disease-free survival (DFS) and overall survival (OS). The prognostic relevance of each variable was compared using a log-rank test. Risk factors related to ESCC patient's prognosis were assessed using Cox's proportional hazard model. A $p < 0.05$ was considered statistically significant for all statistical analyses. All analyses were performed on SPSS 26.0 (IBM Corporation, New York, USA) software. GraphPad Prism 8 (San Diego, USA) software was used for graphic creation.

Results

PD-L1 expression

IHC data from TMA slides shown that, compared to adjacent non-malignant epithelial tissues, PD-L1 was over-expressed in tumor cell membranes or cytoplasm (or both) in surgically excised tumor tissues (Fig. 1), although it was also distributed in the cytoplasm. Figure 2 shown representative images of PD-L1 antibody negatively stained TMA sections (Fig. 2A-C; ISP = 0, 1, 2, respectively) and positive staining (Fig. 2D-F; ISP ≥ 3, respectively). Among the 139 esophageal cancer tissues, 56 (40.3%) were PD-L1-positive, and 83 (59.7%) were negatively. The positive expression rate of PD-L1 protein was increased in cancer tissue when compared with that in adjacent noncancerous mucosa tissues (40.3 vs. 22.0%, $P < 0.05$).

Association Between Clinicopathological Factors And PD-L1 Protein

The clinicopathological factors associated with ESCC and their relationship with PD-L1 protein

expression are shown in Table 1. The diagnostic median age was 65 years, with a range from 40 to 78 years. Of the 139 patients, 78 (56.1%) were younger than 65 years old, and 110 were males (79.1%). In this cohort, 6 (4.3%) tumors were highly differentiated, 95 (68.3%) were moderately differentiated, and 38(27.4%) were poorly differentiated. Additionally, the results indicated lymphatic metastasis in 80 patients (57.6%), while 59 (42.4%) of the remaining subjects did not show signs of lymphatic metastasis. The analysis results did not indicate a significant correlation between PD-L1 expression and age, gender, tumor location, differentiation, or lymph node (N) stage.

Table 1
Association of PD-L1 expression with clinicopathological factors in 139 ESCC patients

Category	Cases (number, %)	PD-L1 expression in tumor cells		P value*
		- (n=83)	+ (n=56)	
Age (years) ≤ 65 > 65	78(56.1) 61(43.9)	45 38	33 23	0.583
Gender Male Female	110(79.1) 29(20.9)	64 19	46 10	0.474
Tumor location Upper Middle Lower	2(1.4) 81(58.3) 56(40.3)	2 48 33	0 33 23	0.353
Differentiation Well Moderate Poor	6(4.3) 95(68.3) 38(27.4)	3 58 22	3 37 16	0.838
N stage# N0 N+	59(42.4) 80(57.6)	36 47	23 33	0.788
#According to the 8th IUCC/AJCC staging system, location of the primary cancer site was defined by the position of the upper (proximal) edge of the tumor in the esophagus, and the remaining missing patients were ominous				
*The statistical method was chi-square test.				

Prognostic Value Of PD-L1 Protein Expression

The univariate analysis was used to assess risk factors related to the OS and DFS. As shown in Table 2, tumor differentiation, lymph node metastasis, and PD-L1 expression were associated with both OS and DFS in ESCC patients (all $P < 0.05$). The multivariate analysis using the Cox regression model indicated that PD-L1 expression represented a significant independent prognostic factor for ESCC patients ($P < 0.05$). Moreover, it showed that tumor differentiation and N status also had evalues in such cases (all $P < 0.05$) (Table 3).

Table 2

Univariate analysis of risk factors for prognosis of 139 ESCC patients

	DFS			OS		
	P value	HR	95%CI	P value	HR	95%CI
Age (≤ 60, > 60 years)	0.185	1.019	0.991–1.047	0.426	1.012	0.983–1.041
Gender (female, male)	0.584	1.150	0.697–1.898	0.518	1.189	0.703–2.010
Tumor location (up/middle, low)	0.765	1.063	0.713–1.584	0.885	1.031	0.681–1.561
Differentiation (well/moderate, poor)	0.010*	1.686	1.132–2.511	0.011*	1.719	1.134–2.608
Lymph node metastasis (negative, positive)	< 0.001*	2.555	1.609–4.059	< 0.001*	3.010	1.836–4.934
PD-L1 expression (negative, positive)	0.024*	1.630	1.067–2.488	0.021*	1.672	1.080–2.593

*P less than 0.05 is significant.

Table 3

Multivariate Cox regression analysis of clinicopathological factors for risk prediction in 139 ESCC patients

Factor	Risk	95% CI	P value
Differentiation	1.576	1.028–2.417	0.037*
Lymph node metastasis	2.812	1.713–4.614	< 0.001*
PD-L1 expression	1.670	1.077–2.589	0.022*

*P < 0.05 was considered significant.

Additional results have indicated a significant correlation between PD-L1 expression and a higher ESCC recurrence rate making it a significant predictor of shorter survival (Table 2 and Fig. 3). PD-L1 positive patients had significantly worse DFS and OS compared to their negative counterparts (P = 0.020, P = 0.018, respectively) (Fig. 3A and Fig. 3B). Moreover, we divided 139 patients into two different groups according to their lymph node status. PD-L1 expression was associated with poorer DFS and OS in patients without lymph node metastasis (P = 0.016, P = 0.026, respectively) (Figs. 4A, 4B). However, no association between PD-L1 expression and DFS or OS in patients with lymph node metastasis was found (P = 0.275, P = 0.197, respectively) (Fig. 4C, 4D).

Discussion

Although many studies have investigated the relationship between ESCC patient's prognosis, clinicopathological characteristics, and PD-L1 expression, the query yielded controversial results. This study examined 139 well-documented ESCC specimens in order to investigate the possible role and clinical significance of PD-L1 expression in locally resectable patients with advanced ESCC. We

discovered that there was no association between tumor cells with higher PD-L1 expressions and the clinicopathological features of ESCC patients. The results also showed that higher PD-L1 expressions represented a significant independent prognostic factor for those patients.

Positive PD-L1 expression is usually assessed through the percentage and number of TCs with PD-L1 expression [16–19]. Previous studies have indicated a positive PD-L1 expression rate ranging between 18.4% and 79.7%. ISP was assessed in this study, considering that the positive PD-L1 expression rate might not effectively reflect the intensity of PD-L1 expression. ISP ≥ 3 was viewed as a positive PD-L1 expression. Consequently, the positive PD-L1 expression rate in this study was 40.3%. The difference in the positive PD-L1 expression rate between our study and prior ones can be attributed to the difference in detection methods, reagents, sample size, and evaluation standards.

Previous studies performed by Ohigashi et al. and Ito et al. revealed an association between tumor stage, lymph node status and PD-L1 expression [18, 20]. However, some other studies have suggested that none of the clinicopathological features were associated with PD-L1 expression [17, 21–24]. Our results did not find any association between PD-L1 expression and clinicopathological factors such as age, gender, tumor location, differentiation, and stage of lymph node metastasis.

PD-L1 expression has also been explored as a prognostic predictor for ESCC. Previous reports on the connection between PD-L1 expression and patient’s clinical outcome have yielded controversial results [20, 21, 23, 25–30], with the major studies showing poor prognosis [20, 23, 26–28, 30] and some studies reported opposite results [21, 25, 29]. For the above studies, researchers have used immunohistochemistry to detect PD-L1 expression, and have come to different conclusions. The discrepancies might be related to variations in detection methods, reagent sensitivities, and evaluation criteria. In this study, we also found that high PD-L1 expression is an independent risk factor for poor outcome in patients with resectable locally advanced ESCC.

Additionally, we found that the expression of PD-L1 was significantly associated with poor clinical outcome in patients with earlier stage ESCC (without lymph node metastasis), while there was a lack of associated between PD-L1 expression and DFS or OS in advanced patients(with lymph node metastasis) was seen. This indicated that the prognostic value of PD-L1 expression was limited and

not useless in every stages of ESCC. The associations between PD-L1 expression and poor outcome have also been observed in several human cancers, including non-small cell lung cancer (NSCLC) [31], colorectal cancer [32], breast cancer [33], and melanoma [34]. The mechanism by which PD-L1 over-expression leads to a poor prognosis in ESCC patients remains unclear. It may be reasonable to assume that PD-L1 expression in those patients is reflective of the immunosuppressive effect. In the early stages of cancer, the antitumor response is reduced due to immunosuppressive effects of the PD-1 / PD-L1 pathway, thus leading to worse survival. However, in the later stages of cancer, the effect of lymph node metastasis on the patient's prognosis is far greater than that of PD-L1 expression. Therefore, partially explaining the inconsistencies seen in studies assessing the prognostic role of PD-L1 over-expression in ESCC patients. When addressing conflicting results in survival analyses in the future, researchers should remember to take into consideration the patient's lymph node status.

The limitations of this study are as follows: firstly, this is a retrospective single centered study, therefore prone to selection bias. Secondly, the study sample is relatively small, making it less reflective. Thirdly, all patients included in this study were patients with stage T3. The correlation between different TNM staging and PD-L1 expression was not analyzed, which might affect the final results. Fourthly, we only examined the expression of PD-L1 in TMA sections. Considering that those sections only capture a small volume of tissue and that tumors may heterogeneously express PD-L1, this might lead to an over or underestimation of the true expression levels of PD-L1 protein.

Conclusions

This study findings showed that ESCC tissues had a significantly increased (40.3%) positive PD-L1 protein expression rate and that its expression was not associated with present clinicopathological factors. Additionally, PD-L1 protein expression was determined to be an independent prognosis factor for patients with locally resected advanced ESCC. However, this association was not consistent in patients with different lymph node status. The results further predicted poor prognosis for positive PD-L1 expression patients without lymph node metastasis as compared to those with lymph node metastasis. This revealed that the prognostic value of PD-L1 expression is closely linked with the

patient’s lymph node status. This study showed that PD-L1 expression is a critical predictor of prognosis for ESCC patients. Therefore, it may be of tremendous help for future patient-oriented therapeutic options. Further large-scale, multi-centered clinical trials are needed to verify our discoveries.

Abbreviations

ESCC (esophageal squamous cell carcinoma); PD-L1 (programmed death ligand 1); ISP (immunoreactivity scoring pattern); TCs (tumor cells); OS (overall survival); DFS (disease-free survival); CI (confidence interval); HR (hazard ratio); TMA (tissue microarray); NSCLC (non-small cell lung cancer); FFPE (formalin-fixed paraffin-embedded); H&E (hematoxylin and eosin).

Declarations

Ethics approval and consent to participate

All experimental protocols were approved by the Ethics Committee of the First Affiliated Hospital of Bengbu Medical Collegeand and performed in accordance with the relevant guidelines and regulations. Written informed consent was obtained from all patients.

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Availability of data and material

Not Applicable.

Authors’ contributions

ZQ and LF contributed equally to the work. ZQ and LF prepared most of the figures and tables and drafted the manuscript. CP, LHW and YHM conceived the idea for the project. ZCM and ZL provided the statistical data. LDJ provided critical review of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figures

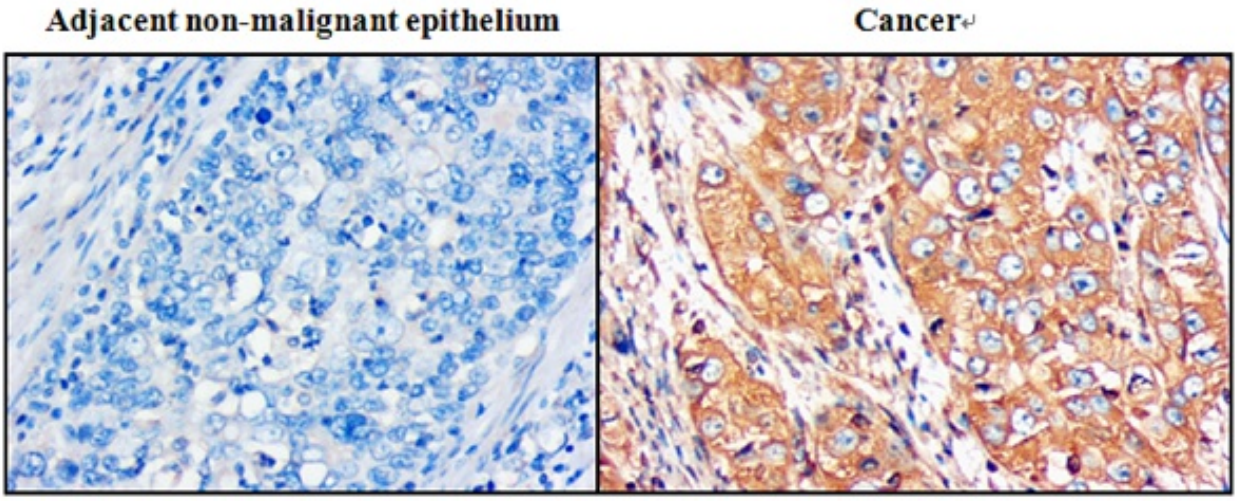
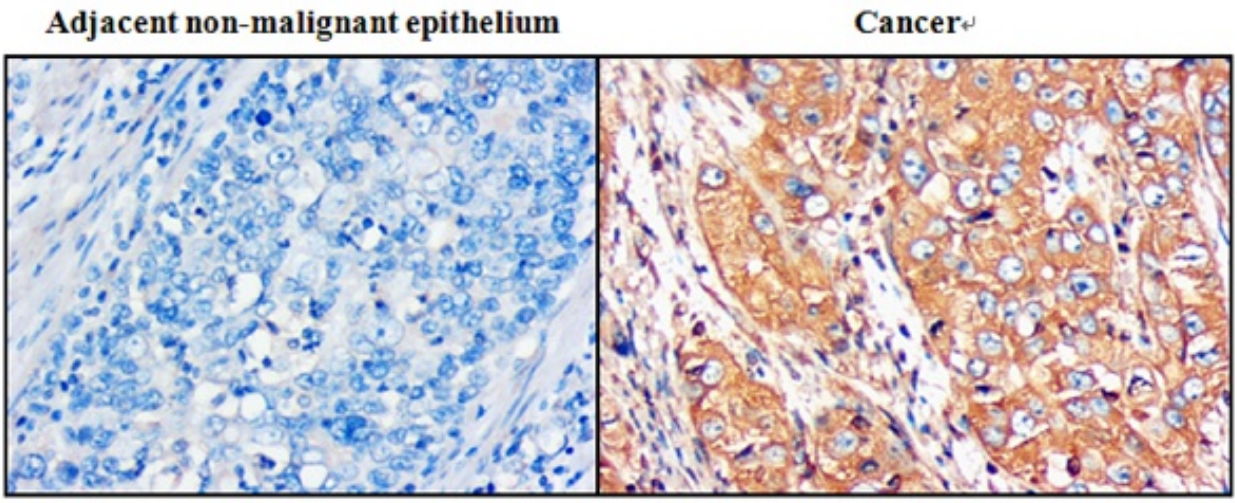


Figure 1

PD-L1 levels in ESCC tissues. Representative IHC staining images of esophageal cancer and adjacent non-malignant epithelial tissues on TMA blocks.



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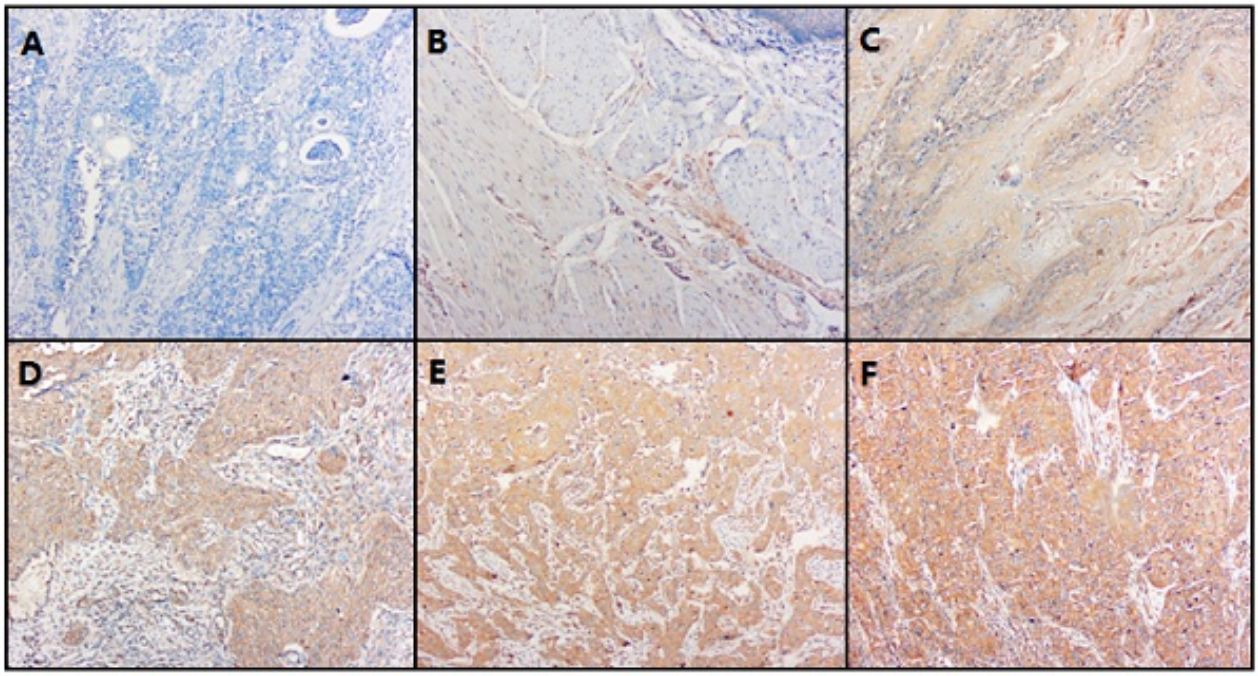
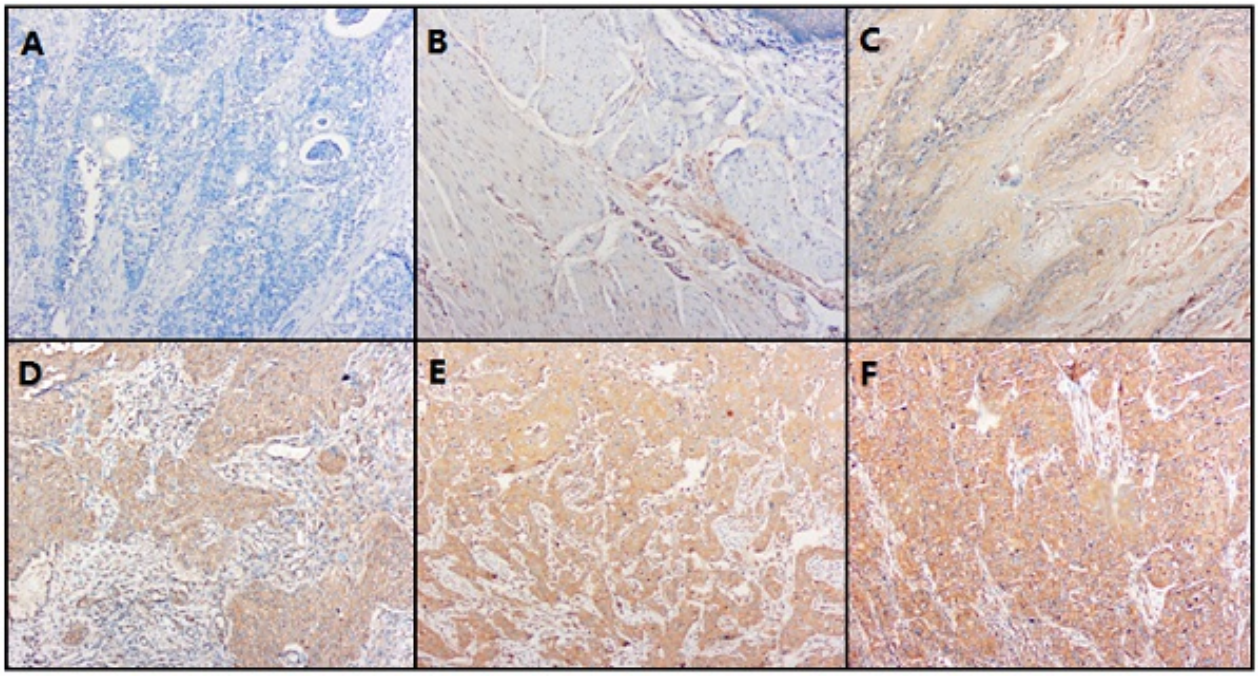


Figure 2

Representative photomicrographs of ESCC TMA sections. Figure 2A-C shows representative photomicrographs stained with programmed death-ligand 1 (PD-L1) with ISP scores = 0, 1, 2, respectively. Figure 2D-F shows representative photomicrographs stained with PD-L1with ISP scores ≥3.



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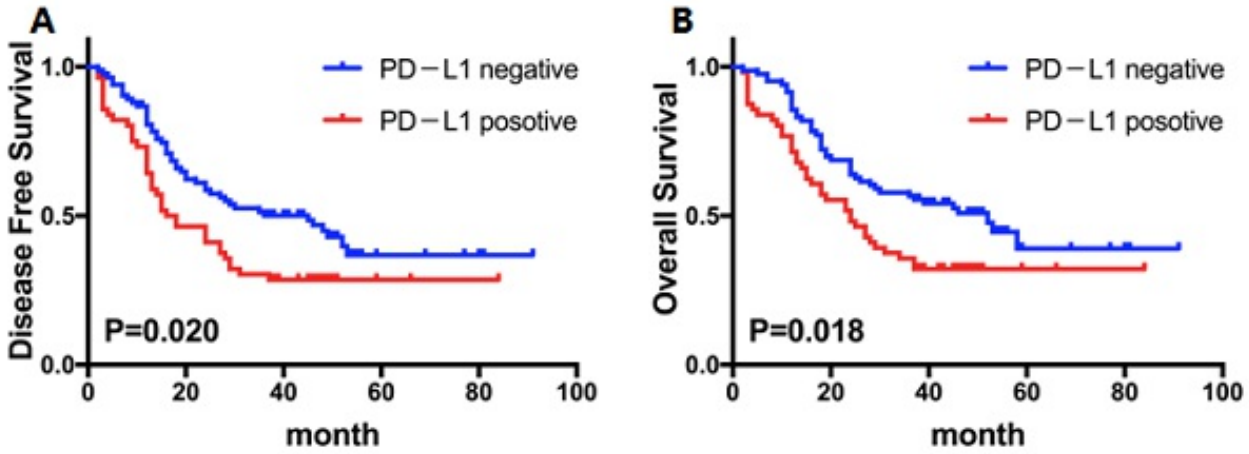


Figure 3

Kaplan-Meier curves showing ESCC patient’s survival and PD-L1 expression. (A) Disease-free survival (DFS), and (B) Overall survival (OS).

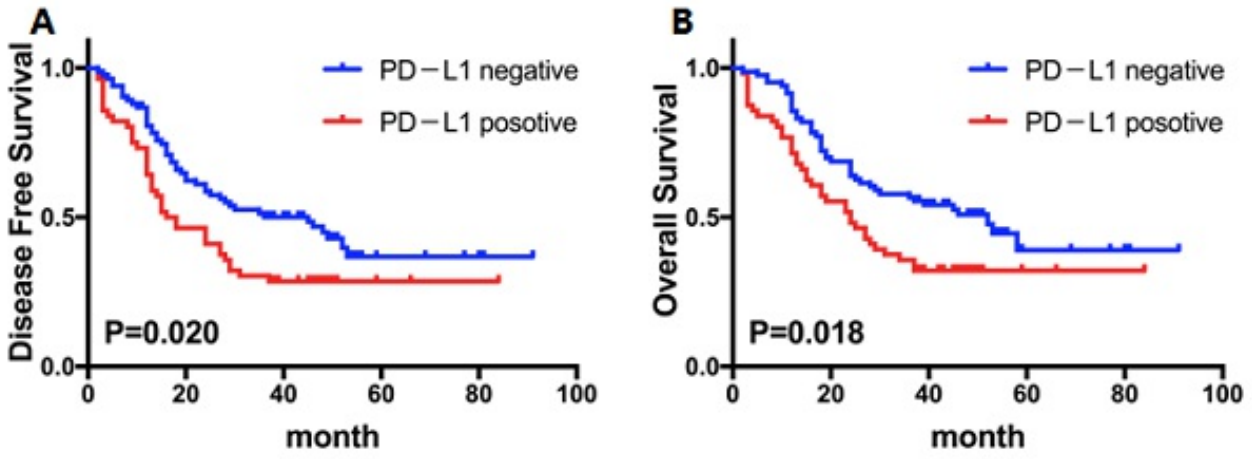


Figure 3

Kaplan-Meier curves showing ESCC patient’s survival and PD-L1 expression. (A) Disease-free survival (DFS), and (B) Overall survival (OS).

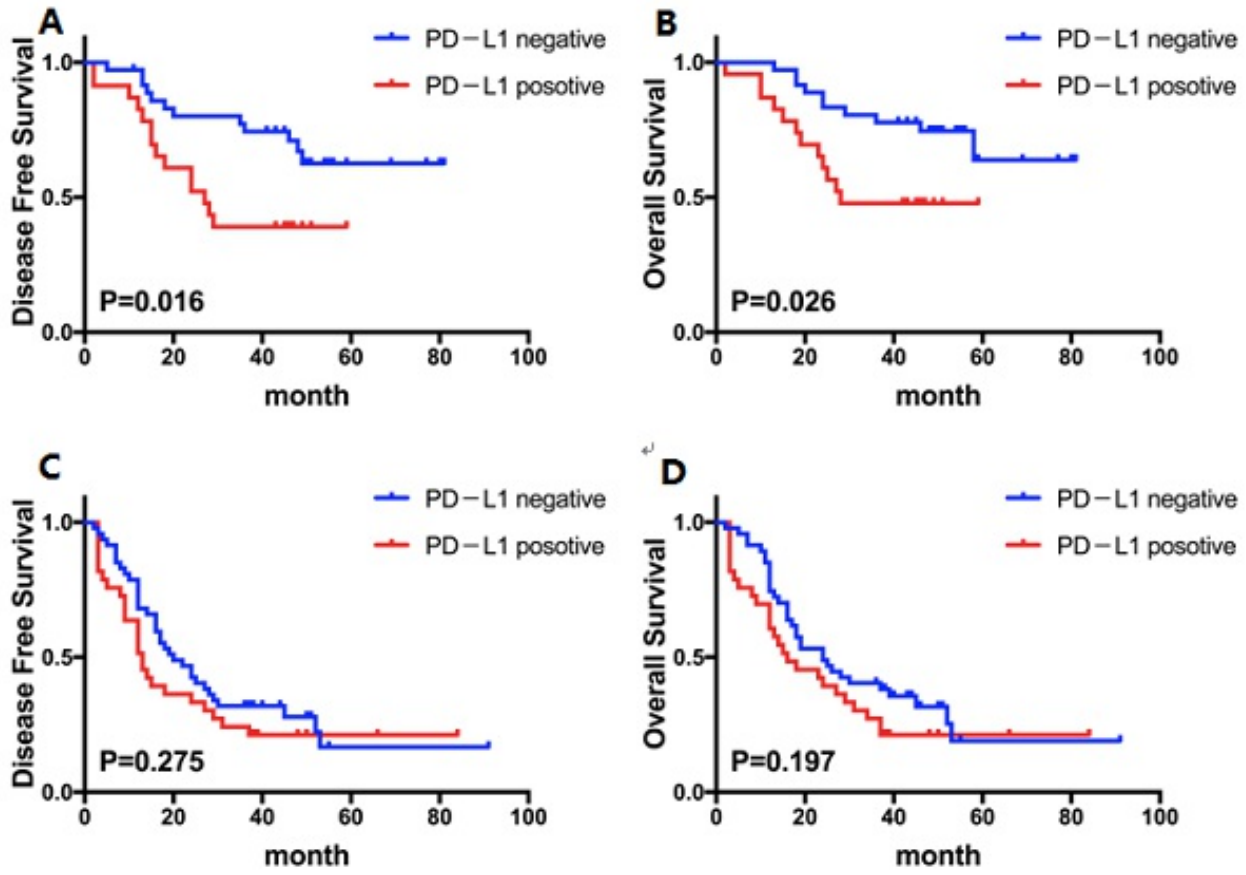
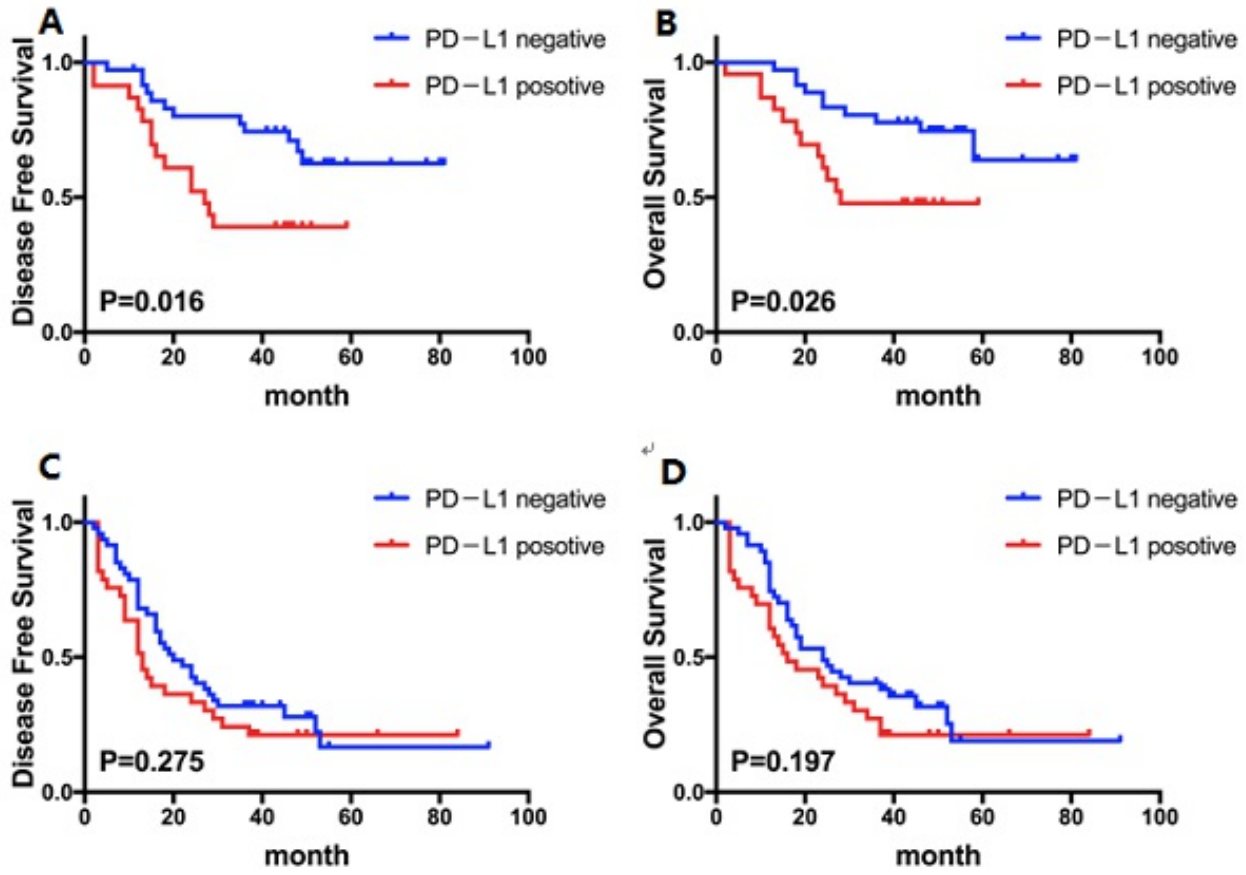


Figure 4

ESCC survival analysis based on lymph node status. PD-L1 expression was associated with worse (A) DFS (P=0.016) and (B) OS (P=0.026) in patients without lymph node metastasis. However, no clear association between PD-L1 expression and (C) DFS (P=0.275) or (D) OS (P=0.197) was seen in patients with lymph node metastasis.



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