Original Article

Programmed cell death-ligand 1 expression and its prognostic significance in completely resected primary small cell carcinoma of esophagus

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Background: Programmed cell death-ligand-1 (PD-L1) has been highly up-regulated in various malignancies. The present study was intended to explore the frequency of PD-L1 expression and understand its role as a prognostic factor for overall survival in primary small cell carcinoma of esophagus (SCCE).

Methods: Immunohistochemistry was employed for evaluating the expression of PD-L1 in 78 specimens of SCCE at Zhejiang Cancer Hospital. Tumors with staining in over 5% of tumor cells were scored as positive for PD-L1 expression. Survival outcome was evaluated by the Kaplan-Meier method and multivariate regression was performed with a Cox proportional hazard model.

Results: PD-L1 expression was detected in 53.8% (42/78) of all patients and it was not correlated with gender (P=0.34), age (P=0.39), stage (P=0.87), smoking history (P=0.34), lymph node metastasis (P=0.56), tumor length (P=0.49) or histology (P=0.13). Their median disease-free and overall survivals were 13.0 and 27.5 months, respectively. The median disease-free survivals in PD-L1 positive and negative SCCE patients were 19.0 and 8.8 months, respectively (P=0.001). The median overall survival was longer in PD-L1 expression positive patients than that in negative counterparts (34.0 *vs.* 21.0 months, P=0.019). Multivariate analyses indicated PD-L1 was a significant prognostic factor for more favorable survival (HR=0.85; P=0.041).

Conclusions: Over half of SCCE patients expressed PD-L1 and its positive expression was associated with more favorable survival.

Keywords: Programmed cell death-ligand-1 (PD-L1); primary small cell carcinoma of esophagus (SCCE); prognosis

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Introduction

Esophageal carcinoma is one of the most common carcinomas in China (1). The most common histological subtype is squamous cell carcinoma occurring in approximately 90–95% of all patients (2). As a rare and aggressive subtype, primary small cell carcinoma of esophagus (SCCE) accounted for around 0.8% to 3.1% of all esophageal malignancies (3-6).

For rarity of this tumor, treatment protocols have not been standardized and the prognosis has remained generally disappointing (7-10). Surgical resection, radiotherapy and chemotherapy have been used alone and in combination for SCCE. However, due to few studies reported (3-10), the management of SCCE is ill-defined and the role of current treatment has remained controversial for limited-disease SCCE.

Blockade of immune checkpoints with monoclonal antibodies has recently attempted for various solid carcinomas (11-14). The patients with PD-L1 expression benefited from anti-PD-L1 antibody treatment (15,16). PD-L1 expression has been regarded as a feasible biomarker for immunotherapy. Furthermore, it has been correlated with prognosis for various carcinomas. However, the prognostic significance of PD-L1 expression is largely unknown in SCCE.

Here retrospective reviews were conducted for the clinical data of patients with limited-disease SCCE. We analyzed the association between clinicopathologic characteristics and PD-L1 expression to further elucidate its prognostic significance.

Methods

Patient recruiting

A total of 78 eligible patients underwent complete resection of ESCC at Zhejiang Cancer Hospital between January 2004 and January 2014. Their postoperative diagnoses were confirmed pathologically as SCCE by two independent pathologists (i.e., Profs. H Zhu & B Chen). The tumor-node-metastasis (TNM) stages were assessed by the classification scheme of 2009 American Joint Committee on Cancer (AJCC) (6). SCCE was diagnosed by the 2004 World Health Organization (WHO)'s histological criteria. The inclusion criteria consisted of: (I) pathologically proven primary SCCE; (II) no preoperative radiotherapy or chemotherapy; (III) complete resection.

Immunohistochemistry of PD-L1 expression

Immunohistochemical (IHC) staining of PD-L1 expression was performed on 4 μm thick FFPE tissues. PD-L1 (Proteintech Group Inc., Chicago, IL, USA) was used for IHC. UltraVision Quanto Detection System HRP DAB (Thermo Fisher Scientific, Fremont, CA, USA) was employed for detecting PD-L1 according to the manufacturer's instructions. Semiquantitative H score (maximum value of 300 corresponding to 100% of tumor cells positive for PD-L1 with an overall staining intensity score of 3) was defined as multiplying the percentage of stained cells by an intensity score (0, absent; 1,weak; 2, moderate; and 3, strong). A 5% proportion of membranous staining of tumor cells which were defined as H-score ≥5 have been used as cutoff for PD-L1 positivity. Two pathologists independently assessed the expression of PD-L1 status. Semiquantitative H score were recorded as the average score.

Statistical analyses

Chi-squared test was performed for evaluating the relationship between clinical characteristics and PD-L1 expression. Survival curves were plotted by the Kaplane-Meier method from the start of definite pathology to the date of mortality or last follow-up. Multivariate analysis was conducted with a Cox regression model. Statistical analyses were performed with SPSS 18 software (Chicago, IL, US). The last follow-up time was January 31, 2015. $P<0.05$ was considered statistically significant.

Results

Patient profiles

SCCE was diagnosed in 2.1% (78/3,714) of all completely resected esophageal malignancies. There were 60 males and 18 females with a median age of 58 years. Fifty-two of them had a history of smoking and 26 were non-smokers. According to the 2009 AJCC staging scheme, the stages were I (n=23, 29.5%), II (n=37, 47.4%) and III (n=18, 23.1%) respectively. Pure SCCE accounted for 83.3% (n=65) while the remainder belonged to SCCE mixed with squamous cell carcinoma (n=10) or adenocarcinoma (n=3). Their clinical characteristics are summarized in *Table 1*.

Treatment

Complete resections were performed via such operative approaches as Ivor-Lewis (n=37), left thoracotomy (n=24) and cervico-thoracoabdominal procedure (n=17). Thirty-two of them received postoperative radiotherapy at a dose range of 45 to 60 Gy. Twelve patients received chemotherapy and seventeen with chemoradiotherapy. The remainder 17 patients received no adjuvant treatment.

Correlation of PD-L1 expression and clinicopathological parameters

Among 78 tumors, the status of PD-L1 expression was positive in 42 (n=42) and negative in 36 patients (*Figure 1*). No association existed between PD-L1 expression and gender (P=0.34), age (P=0.39), stage (P=0.87), smoking history (P=0.34), lymph node metastasis (P=0.56),

Table 1 Clinical characteristics of present study and comparison between the PD-L1 positive and PD-L1 negative patients

Variable	Total (n=78)	PD-L1 positive (n=42)	PD-L1 negative (n=36)	P
Gender				0.71
Male	60	33	27	
Female	18	9	9	
Age				0.39
<60	43	25	18	
≥60	35	17	18	
Smoking status				0.34
Never	26	16	10	
Former/current	52	26	26	
Stage				0.87
I + II	60	32	28	
III A	18	10	8	
Lymph node metastasis				0.56
Yes	22	13	9	
No	56	29	27	
Treatment after surgery				0.93
Yes	61	33	28	
No	17	9	8	

PD-L1, programmed cell death-ligand-1.

tumor length (P=0.49) or histology (P=0.13). The clinicopathological characteristics are summarized in *Table 1*.

Survival analyses

No postoperative mortality was reported. Sixty-seven patients were recurrent (n=15) or metastastic (n=52) and 41 died from tumor progression. Their median values of disease-free survival (DFS) and overall survival (OS) were 13.0 and 27.5 months respectively. The results of univariate analyses for clinicopathologic factors are listed in *Table 2*. PD-L1 expression was a favorable factor for DFS and OS (*Figures 2,3*). Earlier stage and postoperative interventions also predicted a better prognosis. No differences existed in DFS and OS among gender, age, smoking history, lymph node metastasis, tumor length or histology.

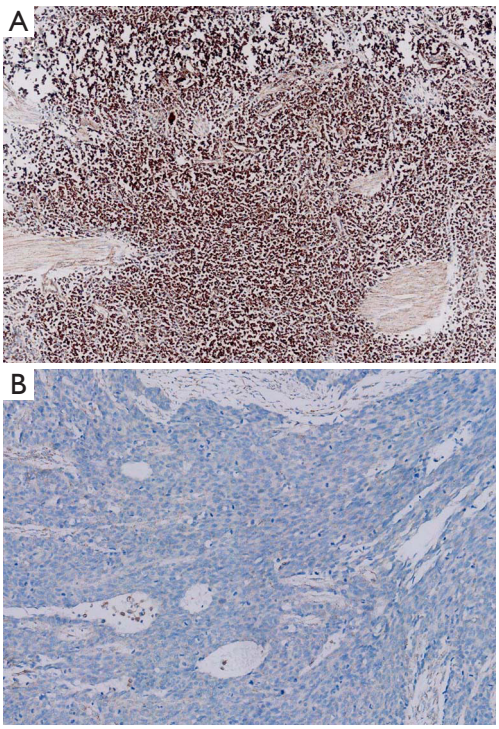

Figure 1 PD-L1 immunohistochemical staining in SCCE (A) positive (B) negative. (A) Positive PD-L1 immunohistochemical staining in SCCE (×40); (B) negative PD-L1 immunohistochemical staining in SCCE (×40).

A multivariate Cox's regression model was constructed with the variables of postoperative treatment, stage and PD-L1 expression. These three parameters were independent prognostic factors for OS (*Table 3*).

Discussion

Our results have shown that more than half of SCCE patients were positive for PD-L1 expression and PD-L1 expression was correlated with a more favorable prognosis. To our knowledge, this is the first study of detecting the frequency of PD-L1 expression and assessing its prognostic significance in SCCE.

As a regulator of cellular immune responses, PD-L1 is widely expressed in various solid tumors, including non-small cell lung cancer, breast, renal and head & neck cancers (11-16). Several recent clinical trials have demonstrated encouraging efficacies for several carcinomas using human antibodies against PD-1/PD-L1 signaling pathway. PD-L1 expression had achieved better clinical efficacies for some anti-PD-1/PD-L1 drugs. PD-L1 has been identified

Table 2 Univariate analysis of all the study population

Variable	Median DFS	P	Median OS	P
Gender		0.32		0.23
Male	11.5		24.5	
Female	14.3		30.2	
Age		0.21		0.42
<60	15.0		29.0	
≥60	11.2		25.5	
Smoking status		0.11		0.37
Never	15.3		30.7	
Former/current	11.4		25.5	
Stage		<0.001		<0.001
I+II	22.5		36.0	
IIIA	8.9		21.5	
Treatment after surgery		0.012		0.037
Yes	16.7		32.1	
No	10.6		24.5	
PD-L1 expression		0.001		0.019
Positive	19.0		34.0	
Negative	8.5		21.0	

PD-L1, programmed cell death-ligand-1; OS, overall survival; DFS, disease-free survival

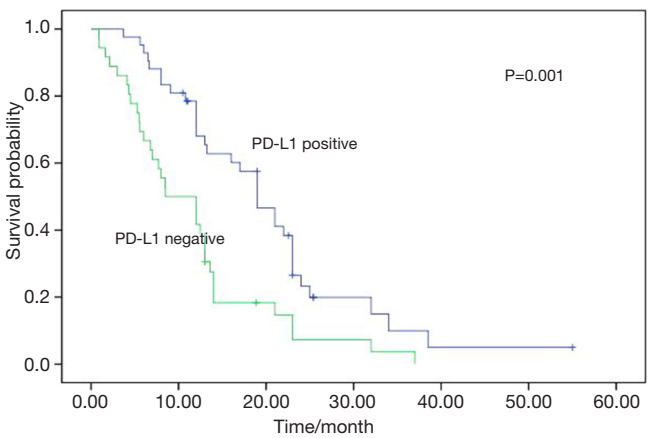

Figure 2 Disease free survival curves for SCCE patients with positive and negative expressions of PD-L1 (P=0.001). PD-L1, programmed cell death-ligand-1; SCCE, small cell carcinoma of esophagus.

as an effective target for immunotherapy (15-16). The expression of PD-L1 varied greatly for various solid

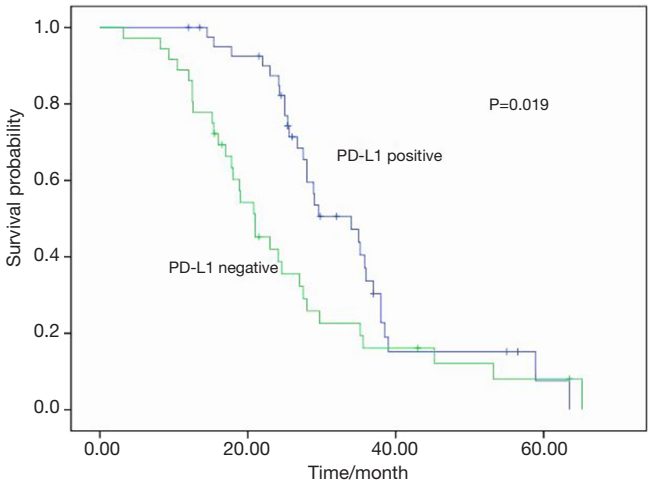

Figure 3 Overall survival curves for SCCE patients with positive and negative expressions of PD-L1 (P=0.019).

Table 3 Multivariate overall survival analysis

Variable	OS		
	HR	95% CI	P
Stage (I+II *vs*. IIIA)	0.37	0.12–0.78	0.017
Treatment after surgery(yes *vs*. no)	0.76	0.45–0.98	0.045
PD-L1 expression(yes *vs*. no)	0.85	0.57–0.96	0.041

OS, overall survival; PD-L1, programmed cell death-ligand-1.

tumors. Its frequency was shown to be correlated with such clinicopathological parameters as smoking history and histological subtypes for various carcinomas (17-20). Due to its rarity, no study has detected the frequency of PD-L1 in SCCE. The present study revealed that 53.8% patients were positive for PD-L1 expression and no correlation existed between PD-L1 expression and clinical characteristics. One reason was probably due to a small sample size. For the higher aggressive characteristics and poor prognosis in SCCE, treatment with anti-PD-1/PD-L1 drugs may be a promising therapy.

There is controversial for PD-L1 as a prognosis marker in solid carcinomas (17-24). PD-L1 expression might achieve superior OS for some solid tumors while it was a poor prognosis factor for some carcinomas (17-24). PD-L1 expression was identified as a poor survival factor in most of previous studies regarding to esophageal squamous cell carcinomas and gastrointestinal tract cancer, different from

previous studies, it was found here that PD-L1 expression-positive SCCE patients had a significantly better prognosis than their counterparts. The reason is currently unclear. More aggressive characteristics in SCCE may explain the different survival results between SCCE and other subtype of esophageal carcinomas. One previous study identified PD-L1 expression as a favorable prognostic factor for small cell lung cancer (24). Its finding was consistent with our results. Our result demonstrated that the role of PD-L1 expression may be different between small cell lung cancer and other carcinomas.

The present study had some inherent limitations. Firstly, one major shortfall was its retrospective nature. Hence, the results must be further prospectively validated by new population-based studies. Secondly, a relatively small number of enrolled patients might compromise the results. Thirdly, immunohistochemistry was used in present study to detect the PD-L1 expression, which may results in false positivity, and there is a need of another method, such as qRT-PCR, to confirm the results of PD-L1 expression based on immunohistochemistry. However, as the first study of detecting the frequency of PD-L1 expression and its prognosis significance for SCCE, our results has some clinical relevance.

Conclusions

In summary, this is the first report of quantifying the protein level of PD-L1 in SCCE. It was shown that half of SCCE patients were positive for PD-L1 expression and PD-L1 expression was correlated with a more favorable prognosis. PD-L1 expression may be a potential molecular marker for selecting ESCC patients for immunotherapy.

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Footnote

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2016.07.13). 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 study protocol was approved by Zhejiang Cancer institutional Ethics Committee (IRB-2015-69) and individual consent for this retrospective analysis was waived.

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