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Overexpression of Programmed Cell Death-Ligand 1 in Small-Cell Lung Cancer and Survival Analysis

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Abstract

Purpose: Programmed cell death-ligand-1 (PD-L1) has identified overexpression in many solid carcinomas. However, the expression in small cell lung cancer (SCLC) remains unclear and the association between the PD-L1 expression and prognosis is not well investigated.

Methods: The expression of PD-L1 was evaluated in 136 specimens of SCLC by immunohistochemistry in Huai'an First People's Hospital. PD-L1 expression was defined as tumors staining in over 5% of tumor cells. Survival analysis was evaluated using the Kaplan-Meier method. Multivariate regression was performed with the Cox proportional hazards model.

Results: One hundred and thirty-six patients were enrolled in present study,including 78 of extensive and 58 with limited stage. PD-L1 expression was detected in 54.4% of all the patients (53.8% in extensive and 55.2% in limited stage). Patients with PD-L1 positive expression showed better overall survival (OS) than PD-L1 negative patients regardless of extensive or limited small cell lung cancer (P value were 0.002 and 0.016, respectively). One hundred and twenty-one patients were with recurrence or metastasis. Median progression free survival of first-line chemotherapy in PD-L1 positive patients was 5.30 and 3.50 months in PD-L1 negative patients (P=0.030). PD-L1 remained as significant prognostic factor for better survival with multivariate analyses (HR=0.76; P=0.041).

Conclusion: Our results that PD-L1 is overexpressed in 54.4% of SCLC patients. Expression of PD-L1 is correlated with a favorable PFS and OS in SCLC.

Keywords: Programmed cell death-ligand-1; Small cell lung cancer; Prognosis

Introduction

Small cell lung cancer (SCLC) is one of the main subtypes of lung cancer that accounts for approximately 15-20% of all lung cancer [1,2]. More than 60%-70% of SCLC patients were with metastasis at diagnosis. Despite a high rate of response to initial chemotherapy, most of patients may suffer progression [3,4]. New therapeutic method has not emerged currently and with median survival time under one year in extensive SCLC.

Programmed cell death (PD-1/PD-L1) pathway plays an important role to limit the activity of T lymphocyte in tissues at the time of an inflammatory response to infection [5,6]. PD-L1 is broadly expressed in human carcinomas [7,8]. Several preclinical or clinical trials have demonstrated that inhibition of this pathway in solid carcinomas with anti-PD-1 or PD-L1 antibodies exerts a promising effect [9,10]. However, the studies of PD-L1 expression in SCLC have remained unknown. We examined PD-L1 expression in SCLC and investigated its association with clinicopathologic characteristics and prognostic value.

Materials and Methods

Study population

Totally, 136 patients with primary SCLC diagnosed at Huai'an First People's Hospital from 2009 to 2015 were enrolled in present study. The inclusion criteria were as follows: (1) pathologically proven primary SCLC, and without combined SCLC (2) all patients were staged according to the Veterans' Administration Lung Study Group staging system [11], (3) disease progression was confirmed using brain MRI, chest/abdomen computed tomography (CT), and ultrasound examination, (4) none of the patients had received chemotherapy or radiotherapy treatment at the time of diagnosis, and (5) Eastern Cooperative Oncology Group performance status (ECOG) of 0 to 2. The study protocol was approved by the Institutional Review Board of Huai'an First People's Hospital and was conducted in accordance with the Helsinki Declaration of 1964 (revised 2008). All of the participants gave informed consent before taking part in the present study.

Immunohistochemical analysis of PD-L1 expression

Immunohistochemical (IHC) staining of PD-L1 expression was performed on 4-6 μm thick formalin-fiated, paraffi-embedded tissue. The concentration of rabbit primary antibody that reacts to PD-L1 The concentration of rabbit primary antibody that reacts to PD-L1 (Proteintech Group Inc., Chicago, IL, USA, Catalog number: 66248-1-

Ig) was 1:100 in Dako antibody diluent; slides were incubated with this antibody overnight at 4°C. Then, the slides were incubated with Ventana Omni Mapanti-rabbit secondary antibody for 60 min. AVentana Chromo MapKit was used for antibody detection, and then the slides were counterstained with hematoxylin. Next, the slides were dehydrated and cover slipped as per normal laboratory protocol. All slides were examined by two pathologists independently;

PD-L1 criteria were according to intensity and extent of staining: (1) negative, when staining was absent or detected in <5% of the cells; and (2) positive, when membranous staining was present in ≥ 5% of the cells.

Follow-up

All patients were evaluated progression-free survival (PFS) and overall survival (OS), and none were lost to follow-up. The median follow-up period was 13.5 months (5.6-23); Last follow-up day was Dec 31, 2016.

Statistical analysis

The Chi-squared test was used to evaluate the relationships between clinical characteristics and PD-L1 expression. Survival curves were calculated using the Kaplane-Meier method from the start of confirmed pathology to date of death or last follow-up. The PFS encompassed the time from the start of treatment to documented progression or last follow-up time. Cox regression model was used for multivariate analysis. Statistical analysis was used with the SPSS 18 software (Chicago, IL, US). P<0.05 were considered statistically significant.

Variable	Number
Gender	
Male	108
Female	28
Age	
Range	31-78
Median	58
<65	105
≥ 65	31
Smoking status	
Never	31
Former/current	105
Performance status	
0-1	112
2	24
Serum NSE level	
Normal	45
Abnormal	91
Serum LDH level	
Normal	52
Abnormal	84
Recurrence or metastasis	
Yes	121
No	15
PD-L1 expression	
Positive	74
Negative	62
Stage at diagnosis	
Limited	58
Extensive	78

Table 1: Demographic characteristics of the study population.

Results

Patient characteristics

The clinical and pathological characteristics of the 136 patients are listed in Table 1. Of all patients enrolled, there were 108 males and 28 females with a median age of 58 years (range, 31-78 years). The performance status (PS) was 0-1 in 112 patients (82.4%) and PS 2 accounted for 17.6%. Seventy-eight was in extensive stage and fifty-eight with limited stage on presentation. One hundred and five patients had a history of smoking. One hundred and twenty-one were with recurrence or metastasis and received first-line chemotherapy. Platinum-based first-line chemotherapy was applied in all of the 121 patients. Seventy-six patients received EP (etoposide+cisplatin), 40 with EC (etoposide+carboplatin) regimen and five with other regimens.

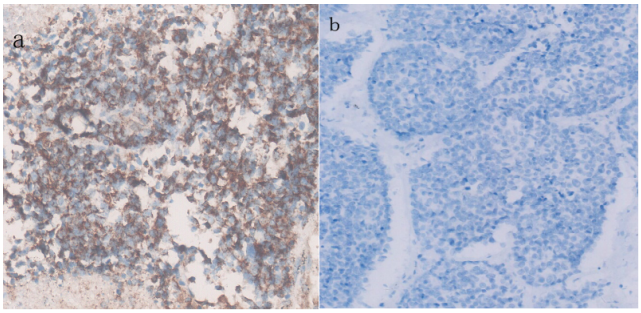

Figure 1: (a) Positive PD-L1 immunohistochemical staining in small cell lung cancer (b) Negative PD-L1 immunohistochemical staining in small cell lung cancer (40X).

PD-L1 expression and its correlation with patient characteristics

Seventy-four (54.4%) patients had PD-L1 staining positive. Table 2 showed the correlation between patient characteristics and PD-L1 expression. No significant correlation existed between PD-L1

expression and gender (P=0.45), age (P=0.548), smoking history (P=0.72), PS (P=0.11), serum NSE level (P=0.58), serum LDH level (P=0.55) and stage (P=0.88).

Survival analysis

Totally, 121 patients were with recurrence or metastasis. The median overall survivals were 18 and 10.6 months in limited and extensive small cell lung cancer, respectively. Patients with PD-L1 positive expression showed better OS than PD-L1 negative patients (15.40 *vs.* 11.0 months, P=0.001; regardless of limited (18.0 *vs.* 12.3 months, P=0.013) or extensive stage (12.4 *vs.* 8.7 months, P=0.002) (Figures 1a and 1b). The median PFS for first-line chemotherapy for the 121 patients was 4.8 months. The PD-L1-positive group showed longer PFS than the PD-L1-negative group (5.40 *vs.* 3.50 months, P=0.030; Figure 2).

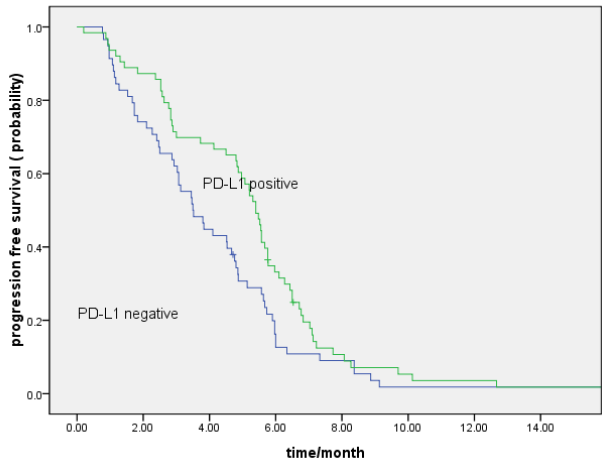

Figure 2: Kaplan-Meier progression free survival curves or extensive small cell lung cancer (p=0.030).

Univariate analysis demonstrated that PD-L1 expression (P=0.001), good PS (P<0.001), and limited stage (P<0.001) were associated with a favorable OS (Table 3). Multivariate analysis was performed to detect factors that associated with OS. Good PS, PD-L1 expression positive and limited stage were significant favorable predictive factors for OS (Table 4).

Discussion

We demonstrated that PD-L1 was overexpression in 54.4% of SCLC, and that PD-L1 expression was associated with favorable survival regardless of extensive and limited stage patients. Progression free survival of first-line chemotherapy in PD-L1 positive patients was longer than those in PD-L1 negative patients.

Variable	PD-L1 positive (n=74)	PD-L1 negative (n=62)	P
Gender			
Male	57	51	0.45
Female	47	11	
Age			
<65	58	47	0.72
≥ 65	16	15	
Smoking status			
Never	17	14	0.96
Former/current	57	48	
Performance status			
0-1	58	54	0.11
2	16	8	
Serum NSE level			
Normal	26	19	0.58
Abnormal	48	43	
Serum LDH level			
Normal	30	22	0.55
Abnormal	44	40	
Stage			
Limited	32	26	0.88
Extensive	42	36	

Table 2: Comparison clinical characteristics between the PD-L1 positive and PD-L1 negative patients.

Variable	First-line PFS (n=121)	P	Median overall survival (n=136)	P
Gender				
Male	4.67	0.133	12.7	0.17
Female	5.67		16.5	
Age				
<65	4.8	0.212	15.3	0.512
≥ 65	3.07		12.6	
Smoking status				
Never	5.4	0.322	16.5	0.089
Former/current	4.5		12.9	
Performance status				
0-1	5.07	<0.001	17.7	<0.001
2	2.83		8.6	
Serum NSE level				
Normal	5.25	0.212	15.5	0.087
Abnormal	4.43		10.7	
Serum LDH level				

Normal	5.43	0.043	14.5	0.196
Abnormal	4.11		11.3	
Stage at diagnosis				
Limited	4.97	0.95	18	<0.001
Extensive	4.56		10.6	
PD-L1 expression				
Positive	5.4	0.03	15.4	0.001
Negative	3.5		11	

Table 3: Univariate analysis of patients' first-line progression-free survival and overall survival according to the clinicopathologic characteristics.

An association of PD-L1 expression with clinicopathologic factors has been reported in several studies [12-14]. The differentiation status was identified to be correlated with PD-L1 expression in a meta-analysis revolving the NSCLC [15]. Some driver genes such as EGFR, KRAS have been showed with higher PD-L1 expression in NSCLC in several studies [16,17], while, other reports observed that there was no significant correlation between PD-L1 expression and driver genes [18]. For driver gene is not widely detection in SCLC, the relationship between driver gene of SCLC and PD-L1 expression is not known currently.

Variable	OS		
	HR	95% CI	p
Stage (Limited *vs.* extensive)	0.45	0.27-0.89	0.007
Performance status (2 *vs.* 0-1)	2.77	1.17-3.98	0.019
PD-L1 expression (yes *vs.* no)	0.76	0.56-0.97	0.041

Table 4: Multivariate survival analysis for overall survival.

Ishii et al. demonstrated that expression of PD-L1 was significantly correlated with a limited stage but not extensive stage disease [19]. However, only 41 patients with limited stage were included in Ishii et al. study. The limited number of patients may influence the results of this report. No clinical factors were found to be associated with PD-L1 expression in current cohort, regardless of limited or extensive stage.

Previous reports have demonstrated that expression of PD-L1 is associated with prognosis in many solid carcinomas. However, the results were conflicting [20,21]. Studies previously have reported that PD-L1 expression was associated with poor survival in patients with several solid carcinomas [20,21]. In contrast, PD-L1 is correlated with a favorable survival in other carcinomas [13,15]. Ishii et al. showed that patients with expression of PD-L1 had significantly better prognosis than those with negative expression in limited SCLC [19]. In current study, the results demonstrated that patients with PD-L1 expression have a better PFS and OS in SCLC, including in limited and extensive stage. To our knowledge, a significant association between PD-L1 expression and better PFS and OS has not been specifically reported previously in extensive SCLC.

There were some limitations in our study. One major limitation was its retrospective nature and with relatively small number patients. Secondly, different antibodies are used in different anti-PD-1 or PD-L1 drugs in clinical trials currently; the choice of antibody and threshold for positivity might be influence the results of different studies. Only one antibody and 5% as threshold were used in our study, different antibodies of PD-L1 needs to be validate in the same sample in future studies.

In summary, the present study is the report with largest number patients' evaluated PD-L1 levels in SCLC. Our results suggest association between the presences of PD-L1 with overall survival. However, for the limitation of our study with retrospective nature, the value of PD-L1 expression in SCLC as a candidate prognosis factor needs to be validated in the future.

Conflict of Interest

The authors declare that they have no competing interests.

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