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 Map anti-rabbit secondary antibody for 60 min. Avantana 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 Kaplan-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.

Table 1: Demographic characteristics of the study population.

| 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           |        |

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).

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                    |    |

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 |             |   | 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                           |    |

Figure 2: Kaplan-Meier progression free survival curves or extensive small cell lung cancer (p=0.030).
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 validated 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 presents 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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### Table 3: Univariate analysis of patients' first-line progression-free survival and overall survival according to the clinicopathologic characteristics.

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

| Stage at diagnosis | Abnormal | Normal |
|--------------------|----------|--------|
| Limited            | 4.97     | 18     | <0.001 |
| Positive           | 5.4      | 15.4   | 0.001  |
| Negative           | 3.5      | 11     |        |

### Table 4: Multivariate survival analysis for overall survival.

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