

ORIGINAL ARTICLE

Circulating PD-L1 in NSCLC patients and the correlation between the level of PD-L1 expression and the clinical characteristics

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Background

Some research has shown that activation of the programmed cell death-1/programmed cell death-1 ligand (PD-1/PD-L1) signal pathway can result in the formation of an immunosuppressive tumor microenvironment, which causes tumor cells to escape organism immune surveillance and killing, while blocking the PD-1/PD-L1 signal pathway can reverse the tumor immune microenvironment and enhance the endogenous antitumor immune effect.¹

The PD-1 receptor is a member of the immunoglobulin B7-CD28 family, and can be expressed on activated CD4+T,

Abstract

Background: The programmed cell death-1/programmed cell death-1 ligand (PD-1/PD-L1) pathway plays a crucial role in tumor evasion. This study evaluated the association between circulating PD-L1 expression and clinical characteristics in patients with advanced non-small cell lung cancer (NSCLC).

Methods: A total of 109 advanced NSCLC and 65 healthy patients from the Beijing Cancer Hospital were enrolled in the study. Circulating PD-L1 expression was tested by enzyme-linked immunosorbent assay. The associations between the level of PD-L1 expression and clinicopathologic features and prognosis were statistically analyzed.

Results: The expression of PD-L1 in advanced NSCLC patients was significantly upregulated compared with the healthy control ($P < 0.001$). The expression of PD-L1 was significantly correlated with abdominal organ metastasis ($P = 0.004$). A high PD-L1 expression had a worse prognosis than a low expression in patients (18.7 vs. 26.8 month, $P < 0.001$).

Conclusions: PD-L1 was elevated in advanced NSCLC patients and may play an important role in tumor immune evasion and patient prognosis.

CD8+T, B, natural killer T, mononuclear, and dendritic cells.² PD-1 includes two ligands: PD-L1 and PD-L2. PD-L1 is the main ligand of PD-1, and has high expression in many malignant tumors, including NSCLC, melanoma, glioma, and renal cell, prostatic, and breast cancers. PD-1/PD-L1 signal pathway activation can cause a tumor T cell immune effect in the local microenvironment, which reduces to mediate tumor immune escape and promote tumor growth, which may include the following aspects: inducing T cell tolerance, inducing T cell apoptosis, inducing T cell exhaustion, enhancing Treg cell function, inhibiting T cell proliferation, inducible co-stimulatory molecule, and PD-1 disbalance.

Programmed cell death-1/programmed cell death-1 ligand signal pathway inhibitors have great potential for the treatment of malignant tumors. Phase I clinical trial results of the anti-PD-L1 antibody BMS-936559 have shown lasting tumor retrogression in NSCLC, melanoma, renal cell, and cervical cancers, with an overall response rate of 6–17%.³ Another clinical trial of the anti-PD-L1 antibody MPDL3280A reported that it has good tolerance and safety in advanced systematically treated NSCLC, with a total effective rate of 23% and a 24-week progression-free survival ratio of 45%.⁴

At present, the feasibility of PD-L1 expression level as a prognosis index has not been confirmed. Retrospective analysis has shown that over expression of PD-L1 in NSCLC cells indicates high invasiveness and poor prognosis, and similar conclusions have been reported in liver, colorectal, and other cancers.^{5–8} A study by Yang *et al.* reported that pulmonary adenocarcinoma patients with a high expression of PD-L1 had longer recurrence-free survival.⁹ Research by Velcheti *et al.* showed that patients with the PD-L1 protein or ribonucleic acid overexpression had longer total survival, which was not correlated with age, staging or tissue type.¹⁰ There have been reports on immunohistochemical detection of the expression of immunity-correlated molecules; however, the results have been disputed. There are fewer reports on the detection of soluble PD-L1 in the peripheral blood of lung cancer patients. This study investigates the expression of PD-L1 in the peripheral blood of advanced NSCLC patients, and analyzes its correlation with clinicopathologic characteristics, evaluating whether it could be a forecasting and prognostic factor.

Materials and method

Patients and collection of specimens

Peripheral blood specimens and complete medical history data of 109 patients with advanced NSCLC admitted to the Department of Thoracic Oncology II, Beijing Cancer Hospital from January 2012 to March 2014, and 65 healthy controls, were collected. Cases were identified as advanced NSCLC by pathological histology. Cases without complete clinical data or patients not capable of providing specimens were excluded. Tumor node metastasis staging of lung cancer was performed according to Union for International Cancer Control guidelines. Complete clinicopathological data were followed up and recorded, including age, gender, and histology. Each patient provided informed consent before enrollment. The ethics committee of the Peking University Cancer Hospital approved the study.

Peripheral blood specimens were collected before treatment. The serum was obtained by centrifugation (at 4000 g for 10 minutes), aliquoted, and stored at -80°C until analysis. The ethics committee of the Peking University Cancer

Hospital approved blood collection and analyses. Clinicians analyzed all cases.

Enzyme-linked immunosorbent assay determination

Programmed cell death-1/programmed cell death-1 ligand expression in the serum specimens of 109 patients with advanced NSCLC and blood serum specimens of 65 healthy controls was determined using an enzyme-linked immunosorbent assay kit (Beijing Keyingmei Science and Technology Ltd., Beijing, China; PD-L1 antibody Article Number: ab156361). Briefly, 96-well plates were incubated with standards at different concentrations, and serum samples were incubated for two hours at 37°C . After covering biotinylated antibodies and several aspiration/wash processes, horseradish peroxidase-conjugated streptavidin was prepared at 37°C for 15–25 minutes, and protected from light. The liquid turned brown after the addition of substrate solution. Enzymatic reactions were developed and the absorbance was immediately measured at 450 nm (A450) using a Bio-Rad Model 680 Microplate Reader (BioRad Laboratories Inc., Tokyo, Japan). A standard curve diagram was drawn according to each determination, and protein levels were calculated. The PD-L1 values in blood serum specimens of healthy controls were determined by the same method.

Statistical methods

SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The relationships among variables were investigated by logistic regression analysis; overall survival (OS) and other factors were analyzed by the Kaplan–Meier method. A two-sided test was adopted, and $P < 0.05$ indicated that the difference had statistical significance.

Results

The mean PD-L1 of the peripheral blood serum in advanced NSCLC patients and healthy controls were $0.723 \pm 0.081\text{ng/ml}$ and $0.565 \pm 0.048\text{ng/ml}$, respectively. There was statistical difference in PD-L1 expression levels between the two groups ($P < 0.001$). A cut-off value of 0.636ng/ml was distinguished in patients for correlation and survival analysis. The area under the curve value was 0.956 (95% confidence interval [CI]: 0.927–0.985) (Fig 1).

This study included 109 patients with advanced NSCLC with a median age of 57 years (range: 15–76). Sixty-five patients (59.6%) were male, and 44 (40.4%) were female. The demographic characteristics are shown in Table 1.

After 27.6 months of median follow-up (6.5–38.3 months; to January 2015), 39 of 109 (35.8%) patients had died. The loss ratio of follow-up was 0.0%.

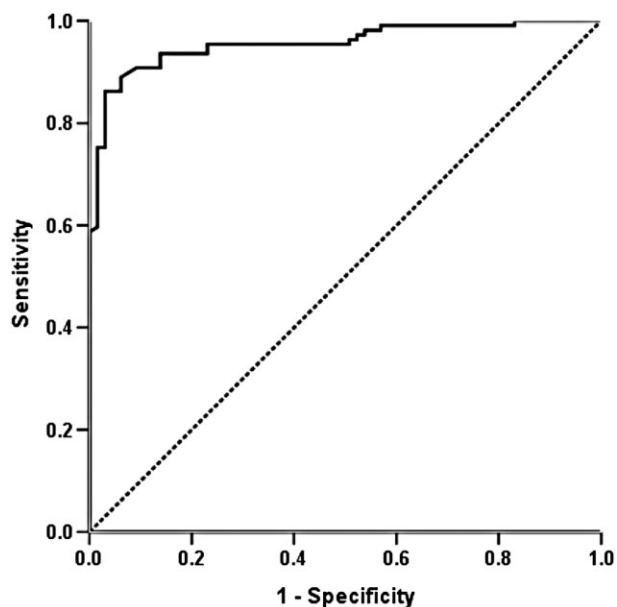


Figure 1 Receiver operating characteristic curve for patients with advanced non-small cell lung cancer.

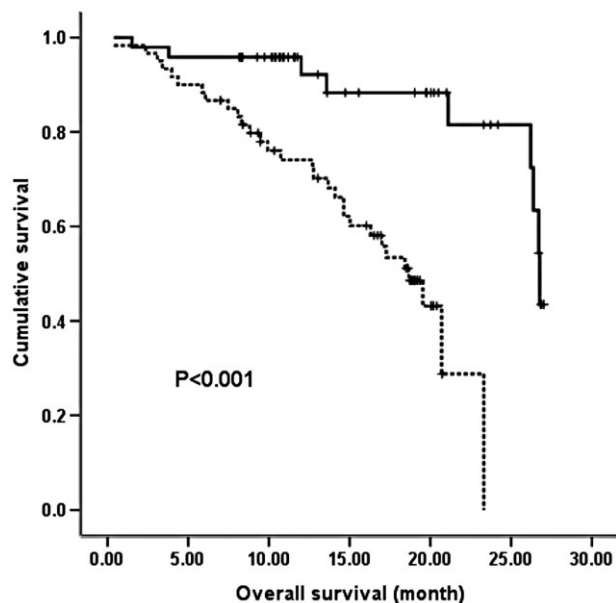


Figure 2 Kaplan–Meier overall survival curves in patients with low and high expression. —, PD-L1 low expression; ·····, PD-L1 high expression; +, censored.

The cut-off value was best distinguished in patients with low and high expression. There were 48 cases in the low (44.0%) and 61 in the high expression group (56.0%). The median OS of the low and high expression groups were 26.8 (95% CI: 26.2–27.4 months) and 18.7 months (95% CI: 15.9–21.5 months). OS in patients in the low expression group was longer than in the high ($P < 0.001$, Fig 2).

Table 1 Patients characteristics

Variable	Patients
Patients (%)	109 (100.0)
Gender	
Male (%)	65 (59.6)
Female (%)	44 (40.4)
Mean age (range)	57 (15–76)
Histology	
Adenocarcinoma (%)	57 (52.3)
Squamous (%)	52 (47.7)
Differentiation	
Poor (%)	91 (83.5)
Moderate (%)	17 (15.6)
Well (%)	1 (0.9)
Metastatic organ	
≥3 (%)	45 (41.3)
<3 (%)	64 (58.7)
Smoking history	
Never (%)	17 (15.6)
Former (%)	58 (53.2)
Current (%)	20 (18.3)
Unknown (%)	14 (12.8)

The tumor tissues of 73 patients were detected as having the epidermal growth factor receptor (EGFR) gene, and the mutation rate was 32.9% (24/73). In 24 EGFR mutation-positive patients, the median OS of the high and low expression groups were 17.3 (95% CI: 11.5–23.1 months) and 25.4 months (95% CI: 22.3–28.5 months), respectively, indicating that there was a trend in the difference between the two groups ($P = 0.058$), but no statistical significance.

As shown in Table 2, PD-L1 expression appeared to be significantly associated with abdominal organ metastasis ($P = 0.004$, Table 2). No significant association was observed

Table 2 Associations between the expression level of PD-L1 and clinical-pathological features

Variable	P
Gender	0.763
Age	0.188
Histology	0.330
Differentiation	0.581
Smoking history	0.962
Brain metastasis	0.385
Liver metastasis	0.167
Abdominal organ metastasis	0.004*
Bone metastasis	0.097
Number of metastatic organs	0.373
Effect of chemotherapy	0.660

*The difference had statistical significance. PD-L1, programmed cell death-1 ligand.

between serum PD-L1 level and other clinicopathological variables.

Discussion

Lung cancer is the leading cause of cancer-related mortality worldwide, and although target therapy has seen rapid development in recent years, many patients without gene mutations cannot benefit from it. Compared with a chemotherapy regimen, molecular target therapy can prolong the progression-free survival of NSCLC patients; however, the total survival time is not prolonged.¹¹ Standard therapy has reached a bottleneck because of severe adverse reactions, high target drug price, and other economic factors. Immunotherapy provides a new treatment direction for NSCLC patients.

The clinical significance of immune checkpoint molecules PD-1 and PD-L1 in NSCLC has been widely researched. Different from the cell membrane expression mode, the peripheral blood soluble B7 family molecule has been found.¹² Some research has shown that a high expression level of PD-L1 in advanced gastric cancer patients was correlated with tumor differentiation degree ($P = 0.026$), and serum PD-L1 level can be used as a latent prognostic factor in advanced gastric cancer patients.¹³ Our study indicated that the average PD-L1 level in the peripheral blood serum of advanced NSCLC and healthy controls were 0.723 ± 0.081 ng/ml and 0.565 ± 0.048 ng/ml, respectively, with a significant difference in PD-L1 expression level between the two groups ($P < 0.001$). The study indicated that the PD-L1 level in peripheral blood of advanced NSCLC patients was higher than in healthy controls.

The current study showed median OS in peripheral blood in the low and high expression groups of 26.8 (95% CI: 26.2–27.4 months) and 18.7 months (95% CI: 15.9–21.5 months), respectively. This indicated that the OS of patients in the low expression group was longer than in the high ($P < 0.001$), and that patients with a low PD-L1 expression level may have a longer survival time.

A recent study assessed PD-1 and PD-L1 expression in NSCLC patients by immunohistochemistry.¹⁴ PD-1 positive was significantly associated with current smoking status ($P = 0.02$) and the presence of Kirsten rat sarcoma mutation ($P = 0.006$), whereas PD-L1 positive was significantly associated with adenocarcinoma histology ($P = 0.005$) and the presence of EGFR mutations ($P = 0.001$). In our study, the circulating PD-L1 expression appeared to be significantly associated with abdominal organ metastasis ($P = 0.004$). No significant association was observed between the serum PD-L1 level and other clinicopathological variables. The small sample sizes in this study may have had an influencing factor. The human body is a microenvironment full of immune activities; tumor cell and peripheral blood soluble PD-L1 expression are influ-

enced by test reagents, test methods, tumor sample quality, tumor types, tumor heterogeneity and other factors, which may be related to infiltrative immunocyte or expressions of other immunosuppressant molecules. Therefore, further research, in which the same individual's peripheral blood and tumor tissue paired specimens can be considered, is required.

Some studies report a correlation between a high expression of PD-L1/EGFR mutation in NSCLC patients.^{14,15} Another study revealed that PD-L1 expression on the cell membrane can depend upon the EGFR signal pathway.¹⁶ Our study indicated that in 24 EGFR mutation-positive patients, the OS of the high expression in peripheral blood group was shorter than in the low expression group, and there was a survival difference trend.

Conclusion

Our study observed the circulating level of PD-L1 expression in advanced NSCLC patients. PD-L1 was elevated in advanced NSCLC patients and may play an important role in tumor immune evasion and patient prognosis. An investigation of the molecular mechanisms of PD-L1 expression in EGFR mutation positive NSCLC patients using larger sample sizes is necessary to confirm our results.

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