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Original Article

Expression analysis of programmed cell death 1 ligand 1 (PD-L1) in patients with non-small cell lung cancer

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Abstract: We investigated 175 patients with Non-Small Cell Lung Cancer, and found that 15.43% of the patients had greater than 1% PD-L1 expression level, 16% patients had greater than 5% PD-L1 expression level, 8% of patients had greater than 50% PD-L1 expression level, while 60.57% of the patients had negative PD-L1 expression. Further, we found that the PD-L1 positive expression ratio in patients with lung adenocarcinoma was higher than that in patients with lung squamous cell carcinoma (40.60% vs 35.71%); while in elderly patients (≥60 years old) expression was higher than in non-elderly patients (43.01% vs 35.37%), and in female patients expression was higher than in male patients (46.77% vs 35.40%). Our work suggests that anti-PD-L1 treatment may be valuable for patients with partial NSCLC, especially for patients with lung adenocarcinoma, elderly patients and female patients.

Keywords: Programmed cell death 1 ligand 1, PD-L1, non-small cell lung cancer

Introduction

Eighty-five percent of patients with lung cancer have non-small cell lung cancer (NSCLC), with a limited 5-year survival of not more than 15% [1, 2]. The utility of IHC biomarkers has remained, with therapeutic options in NSCLC diagnosis and treatment [3]. It is known that programmed cell death protein-1 (PD-1) is indispensable in inhibiting immune responses when it interacts with the ligands PD-L1 and PD-L2 [4]. Tumors can evade immune surveillance by exposing PD-1/PD-L1 related signals [5]. Dysfunction of PD-L1 can cause auto-immune diseases and tumor progression [6]. Detection of PD-L1 expression in different cancers is valuable, including NSCLC [7]. There are some therapeutic drugs that are based on PD-1/PD-L1 inhibition, which show promise [8-10]. Here, we collected clinical samples from tumors in 175 NSCLC patients, by which we detected PD-L1 protein levels with immunohistochemistry. Further, we analyzed the correlation between PD-L1 protein level and different subtypes or ages or genders of NSCLC patients.

Material and methods

Tumor samples were obtained from patients who underwent surgical resection. Formalin-fixed paraffin-embedded (FFPE) tumor samples were prepared by standard procedures. PD-L1 expression level was evaluated using the 22C3 antibody concentrate. PD-L1 immuno-histochemistry (IHC) was performed with PD-L1 IHC 22C3 pharmDx kit (Dako Corp.) using a Leica BOND-III platform (Leica Biosystems), according to manufacturer recommendations. Approval for this study was obtained from the Shanghai University of Medicine & Health Sciences Ethics Committee. All patients participating in this study provided written informed consent.

SPSS 25.0 was used for data analysis. The Chi-squared test was used for statistical analysis. $P<0.05$ was considered statistically significant.

Results

We detected PD-L1 expression levels in 175 patients with Non-Small Cell Lung Cancer with immunochemistry according to standard procedures (**Figure 1**). Among them, there were 27 patients with greater than 1% PD-L1 expression level, the proportion was 15.43%; there were 28 patients had greater than 5% PD-L1 expression level, the proportion was 16%; an additional 14 patients had greater than 50% PD-L1 expression level, the proportion was 8%;

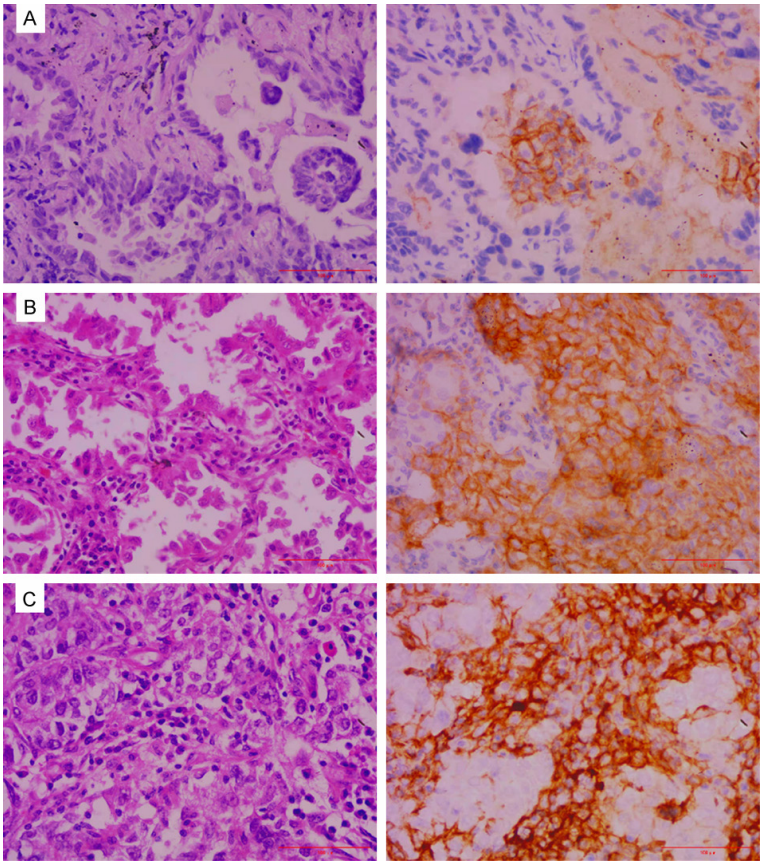

Figure 1. PD-L1 expression levels with immuno-histochemistry (IHC). Pictures were enlarged 400 times. A. H&E staining (left), PD-L1 IHC (more than 1%, right). B. H&E staining (left), PD-L1 IHC (more than 5%, right). C. H&E staining (left), PD-L1 IHC (more than 50%, right).

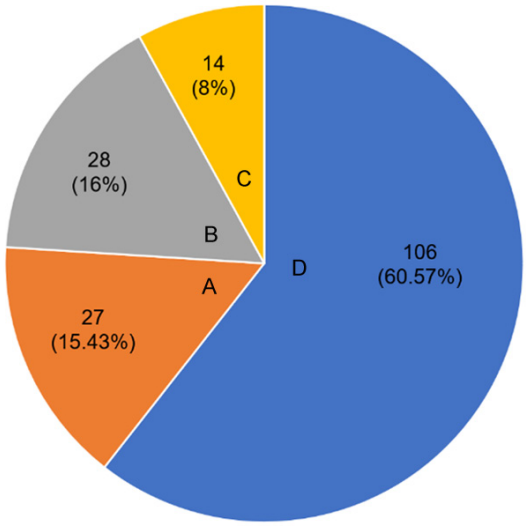

Figure 2. PD-L1 expression level in 175 NSCLC patients. A. Orange shows patients with 1% PD-L1 expression level. B. Grey shows patients with 5% PD-L1 expression level. C. Yellow shows patients with 50% PD-L1 expression level. D. Blue shows patients with negative PD-L1 expression level.

while 106 patients had negative PD-L1 expression, the ratio was 60.57% (**Figure 2**).

In our samples, 133 patients were diagnosed with lung adenocarcinoma and 42 patients were diagnosed with lung squamous cell carcinoma. We compared PD-L1 expression levels between different subtypes of NSCLC. The PD-L1 positive ratio in patients with lung adenocarcinoma was 40.60%, including 23 patients whose PD-L1 expression was more than 1%, the proportion was 17.29%; 21 patients whose PD-L1 expression was more than 5%, the proportion was 15.79%; and 10 patients whose PD-L1 expression was more than 50%, the proportion was 7.52%. Additionally, there were 79 patients whose PD-L1 expression was negative, the proportion was 59.40%. The PD-L1 positive ratio in patients with lung squamous cell carcinoma was 35.71%, including 4 patients whose PD-L1 expression was more than 1%, the proportion was 9.52%; 7 patients whose PD-L1 expression was more than 5%, the proportion was 16.67%; and 4 patients whose PD-L1 expression was more than 50%, the proportion was 9.52%. Additionally, there were 21 patients whose PD-L1 expression was negative, the proportion was 64.29% (**Figure 3**). In total, the PD-L1 positive ratio in patients with lung adenocarcinoma was higher than in patients with lung squamous cell carcinoma. However, there was no significant difference between the different subtypes groups (**Table 1**).

Among the 175 patients, there were 82 non elderly patients (20-59 years old), 46.86% of the total, including 10 patients whose PD-L1 expression level was more than 1%, the proportion was 12.20%, 14 patients whose PD-L1 expression level was more than 5%, the proportion was 17.07%, and 5 patients whose PD-L1 expression level was more than 50%, the proportion was 6.10%, and 53 patients whose

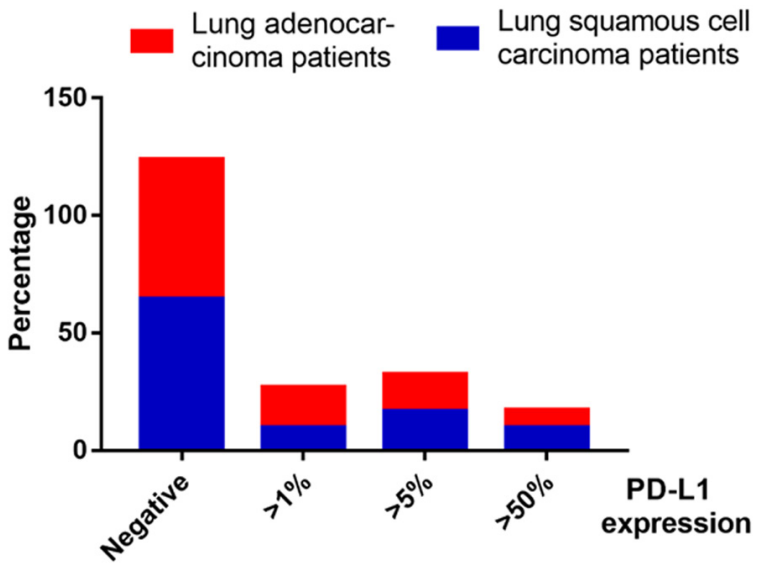

Figure 3. PD-L1 expression levels in different subtypes of NSCLC patients.

PD-L1 expression were negative, the proportion was 64.63%. There were 93 elderly patients (60-83 years old), 53.14% of the total, with 17 patients whose PD-L1 expression level was more than 1%, the proportion was 18.28%, 14 patients whose PD-L1 expression level was more than 5%, the proportion was 15.05%, 9 patients whose PD-L1 expression level was more than 50%, the proportion was 9.68%, and 53 patients whose PD-L1 expression was negative, the proportion was 56.99% (**Figure 4**). In total, the PD-L1 positive ratio in elderly patients (≥60 years old) was higher than in non-elderly patients (<60 years old). However, there was no significant difference between the age groups (**Table 1**).

Among the 175 patients, there were 113 males, 64.57% of the total, including 14 patients whose PD-L1 expression level was 1%, the proportion was 12.39%, 17 patients whose PD-L1 expression level was 5%, the proportion was 15.04%, 9 patients whose PD-L1 expression level was 50%, the proportion was 7.96%, and 73 patients whose PD-L1 expression was negative, the proportion was 64.60%. There were 62 females, 35.43% of the total, including 13 patients whose PD-L1 expression level was 1%, the proportion was 20.97%, 11 patients whose PD-L1 expression level was 5%, the proportion was 17.74%, 5 patients whose PD-L1 expression level was 50%, the proportion was 8.06%, and 33 patients whose PD-L1 expression was negative, the proportion was 53.23% (**Figure 5**). In total, the PD-L1 positive ratio in female patients was higher than that in male patients. However, there was no significant difference between the gender groups (**Table 1**).

Discussion

PD-L1 is reported to be expressed in different kinds of healthy tissues and cells as well as in some tumor cells. During tumorigenesis, binding with PD-1 helps cells escape from immune surveillance. In NSCLC, PD-L1 detection provided a relevant basis for personalized medicine. Our work showed that 39.43% of NSCLC patients had positive PD-L1 expression. Patients with different subtypes or ages or genders had different PD-L1 expression distribution. From our data, PD-L1 positive ratio in patients with lung adenocarcinoma was higher than in patients with lung squamous cell carcinoma (40.60% vs 35.71%); PD-L1 expression levels in elderly patients was higher than in non-elderly patients (43.01% vs 35.37%); PD-L1 expression levels in female patients was higher than that in male patients (46.77% vs 35.40%). This suggests better prognosis when anti PD-L1 therapies were adopted in lung adenocarcinoma, elderly and female NSCLC patients. Our study provides more clinical validation for selecting patients who will be most likely to benefit from anti PD-L1 strategeties in tumour therapies.

The dynamic changes of PD-L1 levels during tumor progression has been observed in several studies. Epidermal Growth Factor Receptor (EGFR) activation was shown to upregulate intrinsic PD-L1 expression in some tumor cells [11]. During Radio(chemo)therapy, radiation was also shown to upregulate PD-L1 expression [12].

PD-L1 negative expression in patients may be changeable and inducible in therapy. In this

Table 1. Statistical analysis for PD-L1 expression level in different groups

Item		PD-L1 expression				χ^2	*P*
		negative	1%	5%	50%		
Total		106 (60.57%)	27 (15.43%)	28 (16%)	14 (8%)		
Sub-type	Lung adenocarcinoma	79 (59.40%)	23 (17.29%)	21 (15.79%)	10 (7.52%)	1.550	0.671
	Lung squamous cell carcinoma	27 (64.29%)	4 (9.52%)	7 (16.67%)	4 (9.52%)		
Age	Non elderly people	53 (64.63%)	10 (12.20%)	14 (17.07%)	5 (6.10%)	2.275	0.517
	elderly people	53 (56.99%)	17 (18.28%)	14 (15.05%)	9 (9.68%)		
Gender	Male	73 (64.60%)	14 (12.39%)	17 (15.04%)	9 (7.96%)	2.947	0.400
	Female	33 (53.23%)	13 (20.97%)	11 (17.74%)	5 (8.06%)		

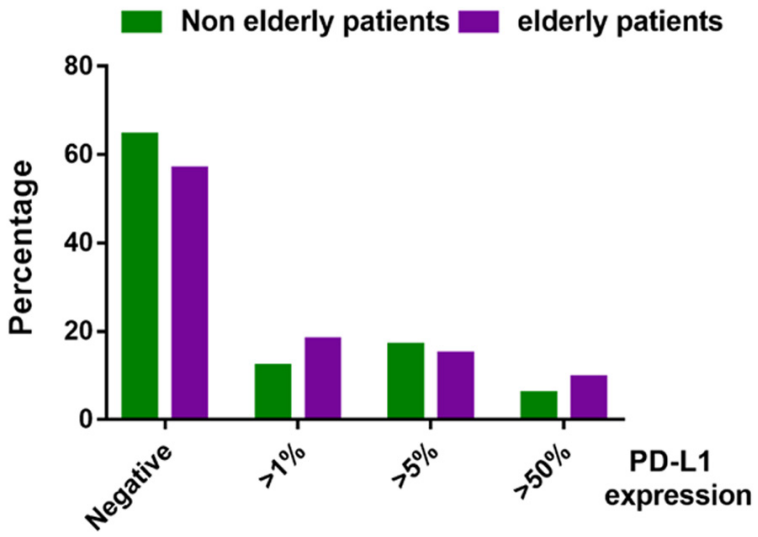

Figure 4. PD-L1 expression levels in different age groups of NSCLC patients.

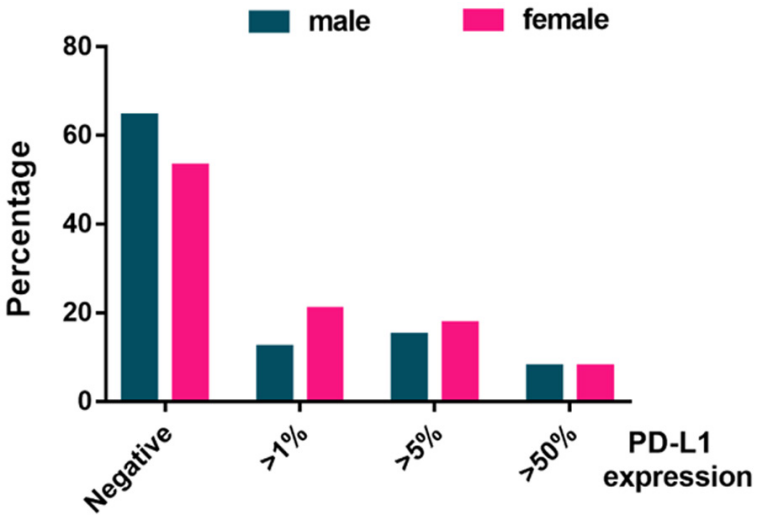

Figure 5. PD-L1 expression levels in different gender groups of NSCLC patients.

regard, checking PD-L1 expression levels is also valuable during different therapy phases for individual patients.

PD-L2 is another ligand for PD-1. The levels of PD-L2 expression were not detected because its expression is very limited to special cells, such as dendritic cells and macrophages which are related to T-cell activation. Whereas PD-L1 is expressed more commonly expressed in various tissues, including some tumors [13].

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Disclosure of conflict of interest

None.

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