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ORIGINAL ARTICLE

Expression of PD-1/PD-L1 in lung adenocarcinoma and its clinical significance*

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Abstract

Objective This study aimed to investigate PD-1/PD-L1 expression in lung adenocarcinoma and its relationship with EGFR/KRAS mutation.

Methods The expression levels of PD-1 and PD-L1 in lung adenocarcinoma were detected. Clinicopathological parameters were collected and followed up. The effects of PD-1 and PD-L1 expression on clinicopathological parameters and prognosis of patients with lung adenocarcinoma were statistically analyzed.

Results PD-L1 and PD-1 were mainly located in the membrane and cytoplasm of tumor cells. The positive expression rates of PD-1 and PD-L1 were 53% and 40%, respectively. Positive PD-1 expression had a significant effect on the incidence of KRAS mutation ($P < 0.05$), while PD-L1 expression significantly affected the incidence of EGFR mutation ($P < 0.05$). Overexpression of PD-1 and PD-L1 had a significant negative effect on disease-free survival (DFS) in patients with lung adenocarcinoma ($P<0.05$) but had no significant effect on overall survival ($P > 0.05$). EGFR gene mutation, high PD-1 expression, high PD-L1 expression, N stage, and AJCC stage were independent risk factors of DFS ($P < 0.05$).

Conclusion High PD-1/PD-L1 expression is closely related to the occurrence of lung adenocarcinoma and can be used as an independent factor to assess the prognosis of patients with lung adenocarcinoma. There were negative correlations between PD-L1 expression and EGFR mutation and between PD-1 expression and KRAS mutation.

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Key words: lung adenocarcinoma; PD-1; PD-L1; gene mutation

Lung cancer is the most common malignant tumor and the leading cause of cancer-related death worldwide. In China, lung cancer also ranks first in incidence and mortality rates [1]. Non-small-cell lung cancer (NSCLC) accounts for 80–85% of all lung cancer cases. Although the chemotherapy regimen has achieved great progress in recent decades, platinum-based chemotherapy has an effective rate of 20%–35% and provides only 8–12 months median survival [2–3]. NSCLC is a disease characterized by molecular subgroups with driver mutations. The two most important driver genes are EGFR and KRAS. About 35% of East Asian patients have tumor-associated EGFR mutations [4], and approximately 50%–65% of EGFR mutations respond to EGFR tyrosine kinase inhibitors. Cancer immunotherapy achieved an important breakthrough with the discovery of "immune checkpoints," including programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) [5]. It is unclear whether specific genomic subsets of NSCLC use the PD-1/PD-L1 pathway to achieve immune escape. The correlation between EGFR/KRAS mutation and PD-1/PD-L1 has been reported, but the results vary. Therefore, this study aimed to explore the clinical significance of PD-1/PD-L1 expression in lung cancer and its correlation with mutated EGFR and KRAS to provide additional potential targets for individualized immunotherapy of lung cancer patients.

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Materials and methods

Subjects and clinical data collection

Patients with lung adenocarcinoma admitted to the cardiothoracic surgery department of our hospital from February 2014 to December 2017 were selected as the research subjects. Inclusion criteria: (1) All patients were diagnosed with lung adenocarcinoma after operation. (2) Patients were at first diagnosis and treatment. (3) Surgical resection was performed. (4) The clinical data and follow-up data were complete. Exclusion criteria: (1) Death occurred in hospital or within 30 days after discharge. (2) The follow-up compliance was poor. Finally, 100 patients were included in the study, comprising 51 males and 49 females with average ages of 57.1 ± 14.3 and 55.2 ± 12.2 years, respectively. Clinical data including age, gender, T stage, N stage, and AJCC stage were collected in the electronic medical record system.

Immunohistochemical staining

A representative tumor region was carefully selected from a section stained with hematoxylin and eosin. After dewaxing in xylene and gradient ethanol, the antigen was heat repaired, the endogenous peroxidase was blocked by 0.3% hydrogen peroxide, and nonspecific binding was blocked by serum incubation. The sections were incubated with anti-PD-L1 antibody and anti-PD-1 antibody for 24 h. The biotin-labeled secondary antibody and horseradish peroxidase-labeled avidin were incubated with the samples and stained using the DAB method. Two doctors independently evaluated the patients' sections. The staining intensity was randomly divided into 4 grades: 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The percentage of positive cells was 0 (0%), 1 (1%–30%), 2 (31%–50%), and 3 (> 50%). The formula of positive staining is: total integral = positive percentage fraction × intensity fraction. A score of 0–2 was negative and > 2 was positive.

Follow up

All patients were followed up by outpatient visits or telephone for every 3 months in the first year and every 6 months from the second year until the patients died or the study ended. Overall survival (OS) was defined as the time from the date of surgery to death or the last follow-up, and disease-free survival (DFS) was defined as the time from the beginning of randomization to recurrence or (for any reason) death. Postoperative recurrence was diagnosed by imaging. All subjects provided informed consent for this study.

Statistical analysis

All data were analyzed by SPSS 20.0. The counting data were expressed by case number and the chi-square test. The Kaplan-Meier method and log rank test were used to analyze the survival rate. Factors showing statistically significant differences in univariate survival analysis were included in Cox regression for multivariate analysis. $P < 0.05$ was considered as statistically significant.

Results

EGFR and KRAS mutations in lung adenocarcinoma

In total, 100 patients (51 males and 49 females) with primary lung adenocarcinoma were selected. EGFR mutation was found in 60 cases (60%), and KRAS mutation in 10 cases (10%). Among cases with EGFR mutations, 19 had mutations in exon 21, 5 in exons 21/20, 3 in exons 21/19, 2 in exons 21/20/19, 2 in exons 21/18, 27 in exon 19, and 2 in exons 20/18. Among KRAS mutations, there were 4 mutations in codon 1, 1 in codon 4, 3 in codon 5, 1 in codon 6, and 1 in codon 7.

PD-1/PD-L1 immunohistochemical staining

Immunohistochemical analysis showed that the expression of PD-L1 and PD-L2 was mainly located in the cell membrane and cytoplasm of tumor cells. The positive expression rates of PD-1 and PD-L1 were 53% and 40%, respectively (Fig. 1).

Relationships between the expression of PD-1/PD-L1 and clinicopathological features

The chi-square test showed that positive PD-1 expression had a significant impact on the incidence of KRAS mutation ($P < 0.05$), and PD-L1 expression significantly influenced the incidence of EGFR mutation ($P < 0.05$). However, the expression of PD-1/PD-L1 had

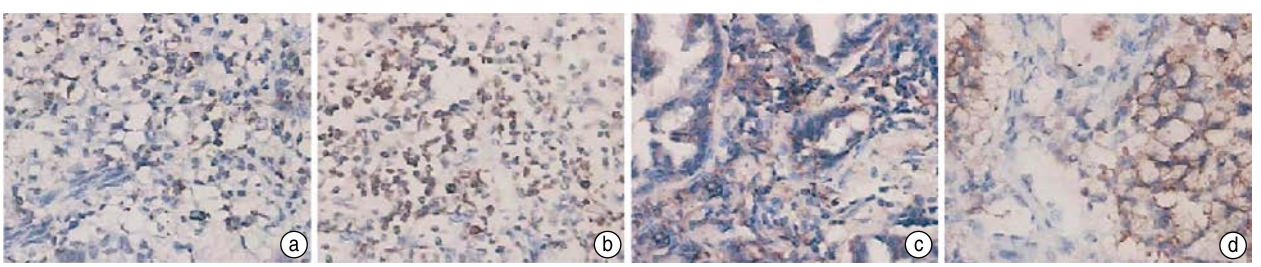

Fig. 1 Immunohistochemical staining of PD-1 / PD-L1

Table 1 Relationship between expression of PD-1 / PD-L1 and clinicopathological features

Indicators	PD-1 (–) (*n* = 47)	PD-1 (+) (*n* = 53)	χ^2	*P*	PD-1 (–) (*n* = 60)	PD-1 (+) (*n* = 40)	χ^2	*P*
Age (years)			0.117	0.732			0.168	0.682
< 60	22	23			28	17		
≥ 60	25	30			32	23		
Gender			0.662	0.416			3.528	0.060
Male	26	25			26	25		
Female	21	28			34	15		
History of smoking			0.661	0.416			0.035	0.852
No	33	41			44	30		
Yes	14	12			16	10		
EGFR mutations			1.311	0.252			6.250	0.012
No	16	24			18	22		
Yes	31	29			42	18		
KRAS mutations			4.857	0.028			0.463	0.496
No	39	51			55	35		
Yes	8	2			5	5		
PD-1 expression							0.107	0.744
Low	–	–			29	18		
High	–	–			31	22		
PD-L1 expression			0.107	0.744				
Low	29	31			–	–		
High	18	22			–	–		
T stage			0.479	0.489			1.891	0.169
T1–2	45	49			58	36		
T3–4	2	4			2	4		
N stage			0.133	0.715			1.311	0.252
N0	23	24			31	16		
> N0	24	29			29	24		
AJCC stage			1.655	0.437			0.662	0.718
I	20	22			26	16		
II	15	12			16	11		
III	12	19			28	13		

Table 2 Relationship between expression of PD-1 / PD-L1 and clinicopathological features

Indicators	β	SE	Wald	*P*	HR	95%CI
No EGFR mutations	0.867	0.325	7.138	0.008	2.553	1.262–4.503
PD-1 high expression	1.276	0.588	4.746	0.029	3.575	1.022–11.910
PD-L1 high expression	0.262	0.264	1.117	0.040	1.311	0.768–1.894
T stage	–0.594	0.352	2.935	0.084	0.548	0.274–1.092
N stage	–1.573	0.187	77.633	0.003	0.330	0.103–0.598
AJCC stage	–0.451	0.283	2.592	0.032	0.631	0.362–1.125

no significant effect on T, N, and AJCC stages ($P > 0.05$; Table 1).

Effect of PD-1/PD-L1 expression on the OS rate of patients

Kaplan-Meier survival analysis showed that overexpression of PD-1 and PD-L1 had a significant negative effect on DFS in patients with lung adenocarcinoma ($\chi^2 = 14.52$, $P < 0.05$; $\chi^2 = 7.54$, $P < 0.05$), but had no significant effect on OS ($\chi^2 = 2.74$, $P = 0.152$; $\chi^2 = 1.63$, $P = 0.405$; Fig. 2).

Cox multivariate analysis

Cox multivariate analysis showed that EGFR gene mutation, high PD-1 expression, high PD-L1 expression, N stage, and AJCC stage were independent risk factors of DFS ($P < 0.05$) (Table 2).

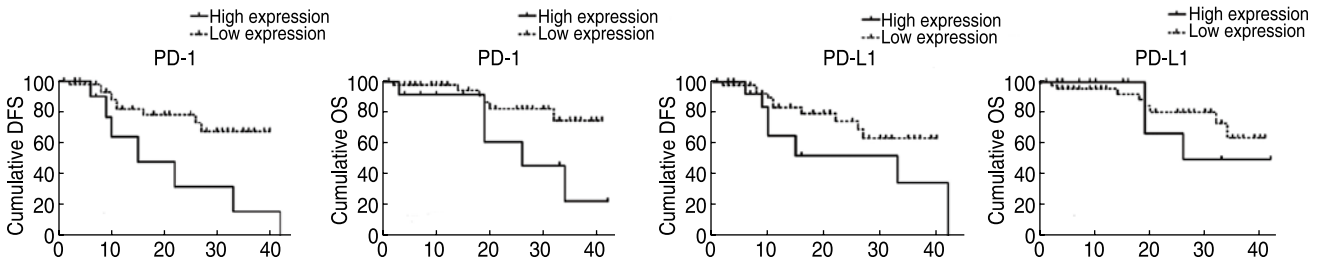

Fig. 2 Effect of PD-1 / PD-L1 expression on overall survival rate of patients

Discussion

PD-1 and its ligand, PD-L1, are immune checkpoints for cancer cells to escape destruction by T cells. PD-1 encoded by the PDCD1 gene interacts with PD-L1 to inhibit T cell activation and render immune surveillance ineffective. Accumulating evidence from experimental studies and clinical trials has shown that PD-1/PD-L1 receptor blockers exhibit good potential for application in the treatment of respiratory cancer. In a multicenter clinical trial of patients with PD-L1-positive advanced gastric cancer, the anti-PD-1 antibody pembrolizumab revealed an acceptable toxicity level and strong anti-tumor effect [6]. In another study, IFN-γ increased PD-1 expression in tumor cells through JAK signal transduction and activation of transcription pathways [7]. PD-1 and PD-L1 block immunotherapy as an effective means of treatment of various types of cancer, including lung cancer in patients with various clinical characteristics. However, currently, the specific regulatory mechanism of this new immune pathway remains unclear. Therefore, we systematically studied the expression of PD-1 and PD-L1 in lung cancer tissues to explore its relationship with the clinical parameters and survival time of lung cancer.

The frequency of EGFR mutation is highest in the East Asian population, ranging from 36.4% to 66.3% in lung adenocarcinoma [8,9]. The mutation frequency of KRAS varies from 2.3% to 9.4% in East Asia [10]. EGFR is overexpressed in about 40%–80% of NSCLC cases, and its expression level is related to the mutation of the EGFR tyrosine kinase domain. KRAS mutation is found in about 30% of lung adenocarcinoma cases but is rare in squamous cell carcinoma [11]. In this study, we found that PD-1 and PDL1 expression varied with the characteristics of patients. The expression level of PD-1 in patients without EGFR and KRAS mutations was higher than that in patients with these mutations. Current clinical studies have shown that blocking the PD-1/PD-L1 immune checkpoint can prolong the progression-free survival of NSCLC cancer patients; moreover, it is also the only new treatment option that has improved the prognosis of lung cancer patients in the past ten years. The PD-1 pathway plays an important role in inhibiting cytotoxic immune response [12]. However, not all lung cancer patients respond to the targeted therapy of these immunosuppressive checkpoints. In this sense, the clinicopathological characteristics of NSCLC patients, the changes in these inhibitory pathways, and the expected therapeutic response to these drugs are still worth further exploration. This study also found that overexpression of PD-1 and PD-L1 had a significant negative effect on DFS in patients with lung adenocarcinoma. Further Cox multivariate analysis showed that EGFR gene mutation, high PD-1 expression, high PD-L1 expression, N stage, and AJCC stage were independent risk factors for DFS. However, no correlation was found between smoking status and high PD-1/PD-L1 expression, which may be due to the relatively small sample size and sample bias.

In conclusion, this study found that the high expression of PD-1/PD-L1 is closely related to the occurrence of lung adenocarcinoma and can be used as an independent factor to assess the prognosis of patients with lung adenocarcinoma. There were negative correlations between PD-L1 expression and EGFR mutation and between PD-1 expression and KRAS mutation. These findings may provide new insights for improving the immunotherapy of PD-1/PD-L1/PD-L2 in patients with lung cancer.

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