Int J Clin Exp Pathol 2017;10(3):2701-2711
www.ijcep.com /ISSN:1936-2625/IJCEP0049124

Original Article

PD-L1 is correlated with p53 expression in patients with lung adenocarcinoma

Cheng Xu, Hongjin Hua, Tianyu Chen, Weiming Zhang, Guoxin Song, Zhihong Zhang

Department of Pathology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, P. R. China

Received January 18, 2017; Accepted February 22, 2017; Epub March 1, 2017; Published March 15, 2017

Abstract: PD-L1 plays a key role in immune evasion of tumor cells, and individual study found that PD-L1 status was regulated by p53 gene. Whether PD-L1 expression is related to p53 status or prognosis in Chinese patients with lung adenocarcinoma has not been clearly clarified. In our study, immunohistochemical analysis of PD-L1 and p53 expression was performed in 229 surgically resected lung adenocarcinomas by using tissue microarray technology. PD-L1 positivity in tumor cells was found in 20.5% of the samples and was associated with female (P=0.007), nodal metastasis (P=0.005), solid predominant type (P<0.001), poor differentiation (P<0.001). PD-L1 positivity in tumor-infiltrating immune cells was found in 23.6% of the samples and was more frequent in male patients (P=0.049), solid predominant tumors (P<0.001) and poor differentiation (P<0.001), and there was a significant correlation between PD-L1 expression in tumor cells and immune cells (P<0.001). p53 aberrant expression was significantly associated with PD-L1 positivity in tumor cells (P<0.001) and tumor-infiltrating immune cells (P=0.001). PD-L1 positive patients with lung adenocarcinoma showed poor recurrence-free survival (P<0.001) and overall survival (P<0.001) on univariate analysis but it was not independent prognostic factor. In conclusion, PD-L1 expression in tumor cells was significantly associated with PD-L1 expression in tumor-infiltrating immune cells and P53 status.

Keywords: Lung adenocarcinoma, PD-L1, p53, tissue microarray, prognosis

Introduction

Lung cancer accounts for more than 1.8 million newly diagnosed cancer cases and 1.6 million cancer-related deaths (19.4% of the total) worldwide every year, while non-small cell lung cancer (NSCLC) accounts for about 85% of all lung cancer cases [1]. In the past few years, targeted therapy has achieved some therapeutic effects against driver mutation (such as EGFR, ALK), but the five-year survival rate was still less than 15% of all patients due to the increasing drug resistance [2]. Emerging immunotherapy has become the new direction for the treatment of NSCLC, of which programmed death-1 (PD-1) and its ligand PD-L1 play a significant role in tumor immune evasion and have become effective therapeutic targets.

PD-1 is a member of the extended CD28/CTLA-4 immunoglobulin family. It is encoded by the PDCD1 gene located in chromosome 2 and its main ligand PD-L1 (also named B7-H1, a number of B7 family) is encoded by Cd274 located in chromosome 9 [3]. PD-L1 is expressed on resting T cells, B cells, dendritic cells, macrophage, vascular endothelial cells and pancreatic islet cells [3]. It is expressed in various types of cancers, especially in NSCLC, melanoma, renal cell carcinoma, gastric cancer, and so on [2]. PD-1/PD-L1 pathway regulates immune suppression by multiple mechanisms, such as inducing apoptosis of activated T cells, facilitating T cell anergy and exhaustion, enhancing the function of regulatory T cells, inhibiting the proliferation of T cells [2]. In brief, the binding of PD-L1 to PD-1 will transfer inhibitory signals to suppress T cell functions so that the tumor cells will protect themselves from immune destruction [4]. However, the study on PD-L1 in lung adenocarcinoma was restricted in tumor cells, while individual study [5] including ours found that PD-L1 also expressed in immune cells and it was also worth studying.

TP53 is the most frequently studied molecular mutation in human cancer and p53 protein expression has been found in more than 50% of human tumors. It is a tumor suppressor gene which plays an important role in cell cycle, DNA repair, cellular senescence [6, 7]. We hypothesized that PD-L1 may be correlated with P53 aberrant expression and predict a poor prognosis. To test this hypothesis, we undertook

the current study to use immunohistochemical staining for PD-L1 and p53 protein to evaluate its prognostic significance by tissue microarrays in a large cohort of patients with lung adenocarcinoma.

Materials and methods

Patients and samples

A total of 229 samples from patients with lung adenocarcinoma who had undergone initial surgery at the First Affiliated Hospital of Nanjing Medical University between 2012 and 2013 were included in this study. None had received chemotherapy before surgery or had distant metastasis at the time of diagnosis. Clinical data and follow-up information was updated through June 2016 by reviewing medical records and telephone survey. This research protocol was approved by the local Ethical Committee. All patients provided a written informed consent statement, and our study follows the principles of the Declaration of Helsinki.

Histologic review and tissue microarrays

Samples were classified as lepidic, acinar, papillary, micropapillary, solid, or invasive mucinous adenocarcinoma according to the 2015 World Health Organization classification [8]. T staging was performed in accordance with the standards of the American Joint Committee on Cancer, 7th Edition.

Sections were reviewed by a pathologist who selected representative areas of the tumors from which to acquire cores for microarray analysis. Tissue microarray blocks were constructed by taking core samples from morphologically representative areas of formalin-fixed paraffin-embedded (FFPE) tumor tissues and assembling them on a recipient paraffin block by Manual Tissue Arrayer (Beecher Instruments, America). For each sample, two or three cores of 1.5 mm were selected in consideration of the heterogeneity of the tumors. Sample tracking was based on coordinate positions for each tissue spot in the tissue microarray block. The spots were transferred onto tissue microarray slides for staining. This sample tracking system was linked to a data base that contained clinicopathologic and survival data on the patients who provided the samples, thereby allowing rapid links between histologic data and clinical features. The array was read according to the given tissue microarray map; each core was scored individually, and the results were presented as the mean of all cores. Samples in which no tumor was found or no cores were available were excluded from the final data analysis [9].

Immunohistochemistry for PD-L1 and p53

Rabbit anti-PD-L1 monoclonal antibody (Clone SP142, ZSGB-BIO, Beijng, China) and mouse anti-p53 monoclonal antibody (Clone DO-7, MXB, Fuzhou, China) showed the appropriate sensitivity based on the previous study [5, 10] and were used as the primary antibody in this study. Briefly, TMA blocks were sectioned at a thickness of 4 μm before deparaffinized and rehydrated, and antigen retrieval was done by high pressure method. After incubation with the primary antibody at 4°C for one night, the slides were then washed by phosphate-buffered saline (PBS) and incubated with secondary antibodies at room temperature for 17 min followed by incubation with 3, 3'-diaminobenzidine (DAB). Sections were counterstained with hematoxylin.

Two authors who were blinded to the clinical data assessed the immunostaining independently, and discrepancies were resolved by reviewing the corresponding sections and by discussion. PD-L1 expression in tumor cells was considered 'positive' if greater than or equal to 5% of tumor cells showed membranous staining, otherwise negative [5, 11-13]. PD-L1 expression in tumor-infiltrating immune cells was considered positive if greater than or equal to 1% of immune cells showed membranous or cytoplasm staining, as references described [14]. p53 expression was defined as 'aberrant expression' if tumor cells showed either nuclear expression in greater than 50% or complete absence of staining and as 'wild type expression' if tumor cells showed no aberrant expression (1-50% staining), as references described [5, 15, 16].

EGFR mutation analysis

Hematoxylin and eosin (H&E)-stained slides were reviewed and tumor regions were manually microdissected from consecutive formalin-fixed, paraffin-embedded tissue sections. After deparaffinization, specimens were subjected to mutation analysis of exons 18, 19, 20 and 21 of the epidermal growth factor receptor (EGFR) gene. ADx-ARMS kit (Amoy Diag-

Table 1. Correlation between clinicopathological features and expression of PD-L1 in tumor cells and tumor-infiltrating immune cells

	PD-L1 expression in tumor cells			PD-L1 expression in tumor-infiltrating immune cells		
	Positive, n (%)	Negative, n (%)	*P* value	Positive, n (%)	Negative, n (%)	*P* value
Gender						
Male	13 (12.6)	90 (87.4)	0.007	36 (28.6)	90 (71.4)	0.049
Female	34 (27.0)	92 (73.0)		18 (17.5)	85 (82.5)	
Age						
≤60	23 (20.7)	88 (79.3)	0.943	25 (22.5)	86 (77.5)	0.757
>60	24 (20.3)	94 (79.7)		29 (24.6)	89 (75.4)	
Smoking status						
Never smokers	30 (17.6)	140 (82.4)	0.067	36 (21.2)	134 (78.8)	0.146
Former or current smokers	17 (28.8)	42 (71.2)		18 (30.5)	41 (69.5)	
Size						
≤3 cm	23 (15.2)	128 (84.8)	0.006	33 (21.9)	118 (78.1)	0.392
>3 cm	24 (30.8)	54 (69.2)		21 (26.9)	57 (73.1)	
Nodal metastasis						
No	24 (15.4)	132 (84.6)	0.005	35 (22.4)	121 (77.6)	0.551
Yes	23 (31.5)	50 (68.5)		19 (26.0)	54 (74.0)	
Vascular invasion						
No	41 (19.2)	173 (80.8)	0.053	49 (22.9)	165 (77.1)	0.357
Yes	6 (40.0)	9 (60.0)		5 (33.3)	10 (66.7)	
T status						
I	15 (15.8)	80 (84.2)	0.460	24 (25.3)	71 (74.7)	0.283
II	28 (24.1)	88 (75.9)		26 (22.4)	90 (77.6)	
III	4 (23.5)	13 (76.5)		3 (17.6)	14 (82.3)	
IV	0 (0)	1 (100)		1 (100)	0 (0)	
Histologic subtype						
Lepidic	2 (7.4)	25 (92.6)	<0.001	9 (33.3)	18 (66.7)	<0.001
Acinar	10 (9.9)	91 (91.0)		14 (13.9)	87 (86.1)	
Mucinous	0 (0)	15 (100)		0 (0)	15 (100)	
Papillary	1 (5)	19 (95.0)		0 (0)	20 (100)	
Micropapillary	3 (27.3)	8 (72.7)		2 (18.2)	9 (81.8)	
Solid	31 (56.4)	24 (43.6)		29 (52.7)	26 (47.3)	
Solid predominant type						
Negative	16 (9.2)	158 (90.8)	<0.001	25 (14.4)	149 (85.6)	<0.001
Positive	31 (56.4)	24 (43.6)		29 (52.7)	26 (47.3)	
Pathologic differentiation						
Well/moderate	8 (5.8)	131 (94.2)	<0.001	19 (15.8)	120 (86.3)	<0.001
Poor	39 (43.3)	51 (56.7)		35 (38.9)	55 (61.1)	
EGFR						
Wild type	19 (25.3)	56 (74.7)	0.100	21 (28.0)	54 (72.0)	0.109
Mutant	12 (14.8)	69 (85.2)		14 (17.3)	67 (82.7)	
p53 status						
Aberrant expression	39 (36.1)	69 (63.9)	<0.001	18 (14.9)	103 (85.1)	0.001
Wild type expression	8 (6.6)	113 (93.4)		36 (33.3)	72 (66.7)	
PD-L1 expression in tumor-infiltrating immune cells						
Negative	21 (12.0)	154 (88.1)	<0.001			
Positive	26 (48.1)	28 (51.9)				

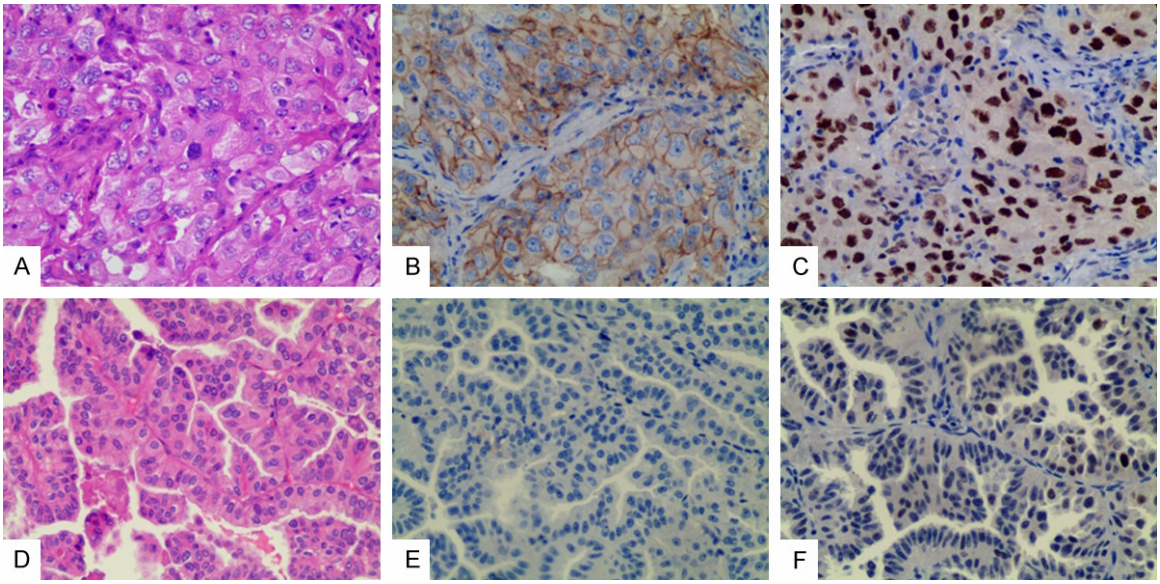


Figure 1. Images for PD-L1 and p53 immunohistochemistry in tumor cells. A. Microscopic examination manifests the histology are solid predominant, which consists of solid nests of large epithelial cells with abundant cytoplasm and prominent nucleoli (H&E stain, Original magnification ×200). B. The tumor cells show markedly diffuse membranous staining of PD-L1. (Original magnification ×200). C. The tumor cells show nucleus staining of p53 in more than 50% of tumor cells which means p53 aberrant expression. (Original magnification ×200). D. Microscopic examination manifests the histology are papillary predominant, which consists of malignant cuboidal to columnar tumor cells growing on the surface of fibrovascular cores. (H&E stain, Original magnification ×200). E. Immunohistochemical staining is negative for PD-L1 in membrane. (Original magnification ×200). F. Immunohistochemical staining is occasionally positive for p53 in nucleus which means p53 wild type. (Original magnification ×200).

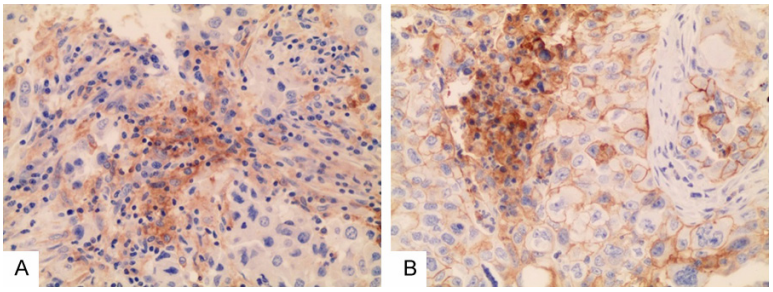


Figure 2. Images for PD-L1 immunohistochemistry in tumor-infiltrating immune cells. A, B. Tumor-infiltrating immune cells show membranous or cytoplasm staining of PD-L1 (Original magnification ×200).

nostics, Xiamen, China) method were used for the EGFR mutation testing as described previously [17], following the kit manual.

Statistical analyses

All statistical analyses were conducted by IBM SPSS 22. Relationships between clinicopathologic parameters were evaluated by the Pearson's Chi-square or Fisher's exact test. Independent predictive factors were examined by multivariate logistic regression analysis. Recurrence-free survival and overall survival were evaluated by the Kaplan-Meier method using the log-rank test. The Cox proportional hazards model was applied for multivariate survival analysis. All statistical significance was set as $P< 0.05$.

Result

Patient characteristics, histological and genetic subtype

A total of 229 lung adenocarcinoma samples were collected from 126 females and 103 males aged 36-83 years (median =60 years). This population included 170 non-smokers (74.2%) and 59 former or current smokers (25.8%). Tumor size ranged from 1.1 to 9 cm (median =2.8 cm). Histologically, 27 cases (11.8%) were classified as lepidic predominant type, 101 (44.1%) as acinar predominant, 15 (6.6%) as mucinous subtype, 20 (8.7%) as papillary predominant, 11 (4.8%) as micropapillary predominant, 55 (24.2%) as solid predominant subtype (**Table 1**). 156 patients underwent EGFR mutation testing, including 75 wild type (48.1%), 81 mutant types (51.9%, in-

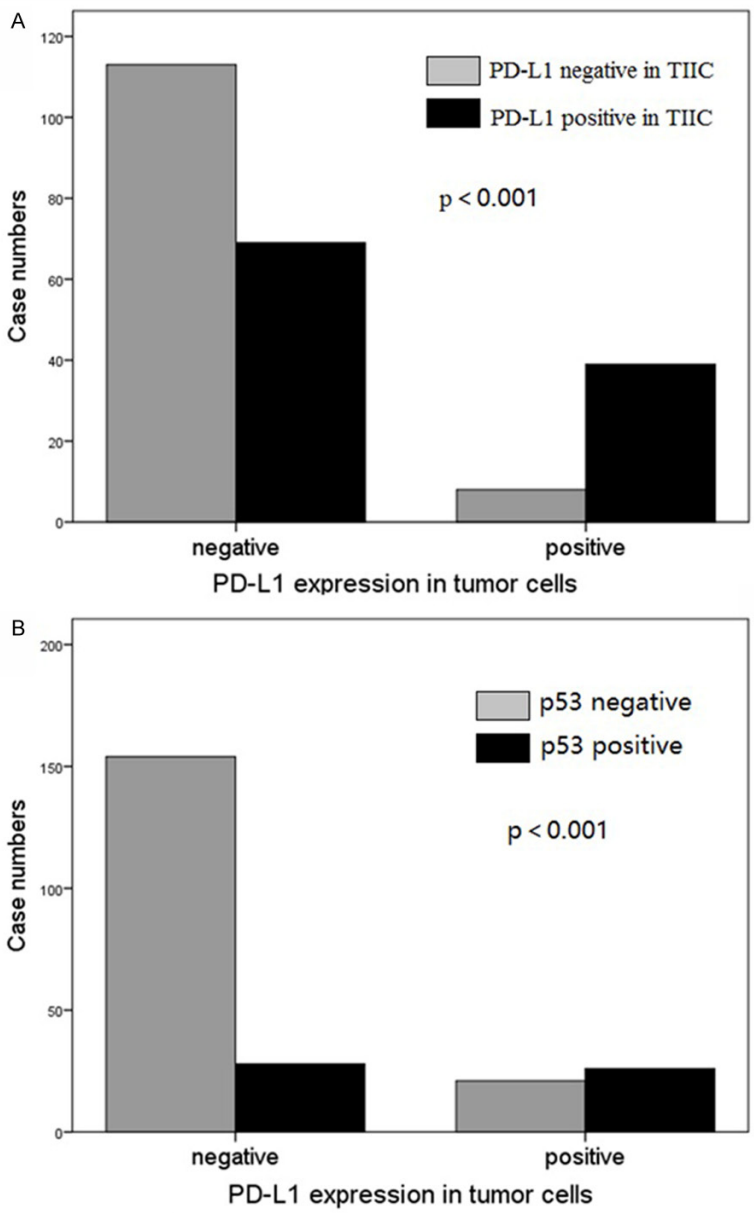


Figure 3. The relationships between PD-L1 positivity tumor cells and other parameters. A. PD-L1 positivity in tumor cells were significantly correlated with PD-L1 positivity in tumor-infiltrating immune cells (P<0.001). B. PD-L1 positivity in tumor cells were significantly correlated with aberrant p53 expression (P<0.001) (TIIC, tumor-infiltrating immune cells).

cluding 36 exon 19 mutations, 45 exon 21 mutation (**Table 1**).

Cliniopathologic and genetic features of PD-L1-positive adenocarcinoma

PD-L1 positivity was found in 47 of 229 cases of tumor cells (20.5%) (**Figure 1**). PD-L1 positivity of tumor cells was more frequent in female patients (12.6% in male vs 27% in female, P=0.007), larger tumors (15.2% in ≤3 cm tumors vs 30.8% in >3 cm tumors, P=0.006), node metastasis (15.4% in No vs 31.5% in N1/N2, P=0.005), solid predominant tumors (9.2% in other types vs 56.4% in solid predominant type, P<0.001), poor differentiation (5.8% in well/moderate differentiation vs 43.3% in poor differentiation, P<0.001). There were no significant correlations between PD-L1 positivity and age, smoking history, T status, vascular invasion or EGFR mutation status. Detailed clinicopathologic characteristics are shown in **Table 1**.

PD-L1 positivity in tumor-infiltrating immune cells was found in 54 of 229 cases (23.6%) (**Figure 2**). PD-L1 positivity was more frequent in male patients (28.6% in male vs 17.5% in female, P=0.049), solid predominant tumors (14.4% in other types vs 52.7% in solid predominant type, P<0.001), poor differentiation (15.8% in well/moderate differentiation vs 38.9% in poor differentiation, P<0.001) (**Table 1**). 11.4% (26/229) of samples were positive both in tumor cells and immune cells, and there was a significant correlation between PD-L1 expression in tumor cells and immune cells (P<0.001) (**Figure 3A**).

p53 aberrant expression was observed in 108 cases (47.2%), diffuse positive (≥50%) in 82 cases and complete absence of staining in 26 cases (**Figure 1**). p53 aberrant expression was associated with PD-L1 positivity in tumor cells (P<0.001) (**Figure 3B**) and PD-L1 positivity in immune cells (P=0.001), node metastasis (P=0.003), solid predominant tumors (P<0.001), poor differentiation (P<0.001) (Supplementary Table 1).

Table 2. Logistic regression analysis of the association of clinicopathological variables with PD-L1 expression in tumor cells

Risk factor	Odds ratio	95% confidence interval	*P* value
P53	4.173	1.593-10.932	0.004
PD-L1 expression in tumor-infiltrating immune cells	3.178	1.271-7.948	0.013
Pathologic differentiation	3.440	1.180-10.024	0.024
Gender	0.367	0.147-0.920	0.033

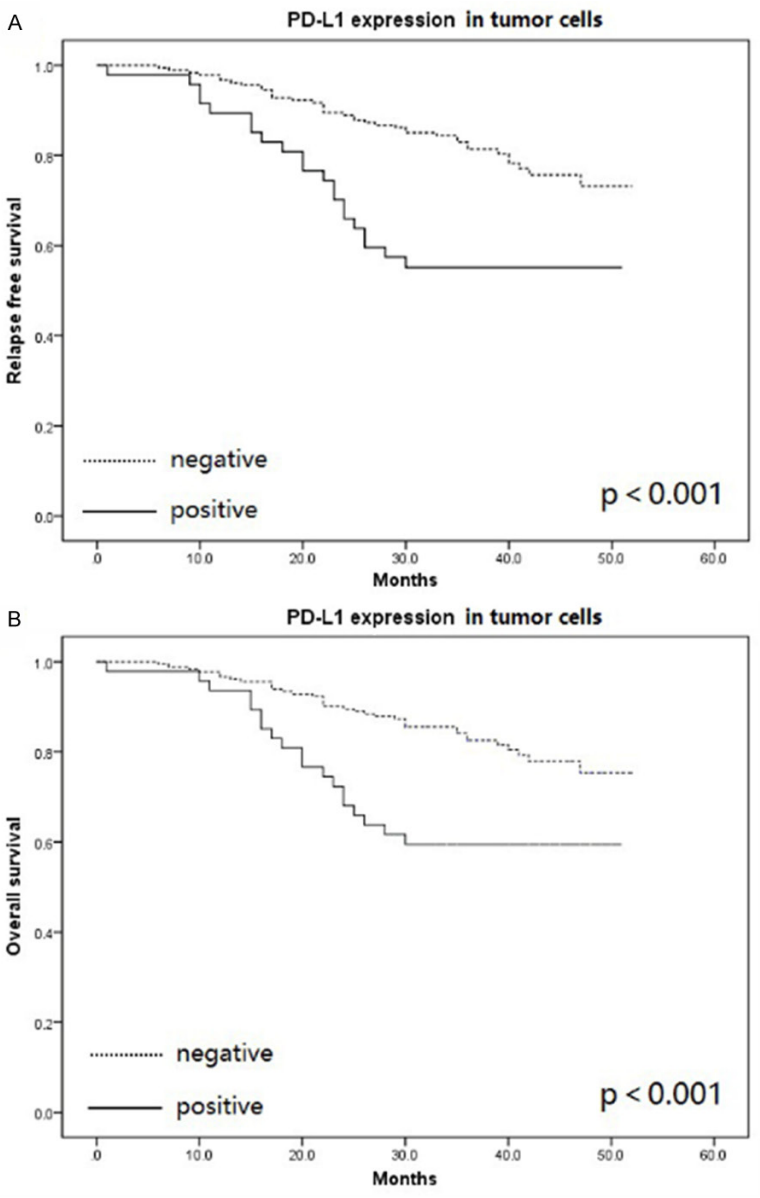


Figure 4. Survival curves among lung adenocarcinoma patients according to PD-L1 expression in tumor cells. A. Kaplan-Meier plots with the log-rank test for disease-free survival according to PD-L1 expression in tumor cells. B. Kaplan-Meier plots with the log-rank test for overall survival according to PD-L1 expression in tumor cells.

Logistic regression analysis including gender, tumor size, node metastasis, pathologic differentiation, solid predominant type, p53 status and PD-L1 expression in tumor-infiltrating immune cells revealed that p53 status (P=0.004), PD-L1 expression in tumor-infiltrating immune cells (P=0.013), poor differentiation (P=0.024) and female (P=0.033) were independently parameters affecting PD-L1 expression in lung adenocarcinomas (**Table 2**). Logistic regression analysis including gender, pathologic differentiation, solid predominant type, p53 status and PD-L1 expression in tumor cells revealed that PD-L1 expression in tumor cells (P=0.005) and solid predominant type (P=0.006) were independently parameters affecting PD-L1 expression in lung adenocarcinomas (**Table 2**).

Prognostic significance of PD-L1 expression

The median follow-up time of this cohort was 35.6 months (95% CI: 34.1-37.0 months). In univariate analysis, PD-L1 positivity in tumor cells was significantly correlated with poorer relapse free survival (RFS) (P<0.001) and overall survival (OS) (P=0.002) (**Figure 4**), while PD-L1 positivity in immune cells did not show prognostic significance (Supplementary Figure 1). P53 aberrant expression (P=0.003 for RFS; P=0.004 for OS) (**Figure 5A**, **5B**), EGFR wild type (P=0.032 for RFS; P=0.044 for OS) (**Figure 5C**, **5D**), node metastasis (P<0.001 for

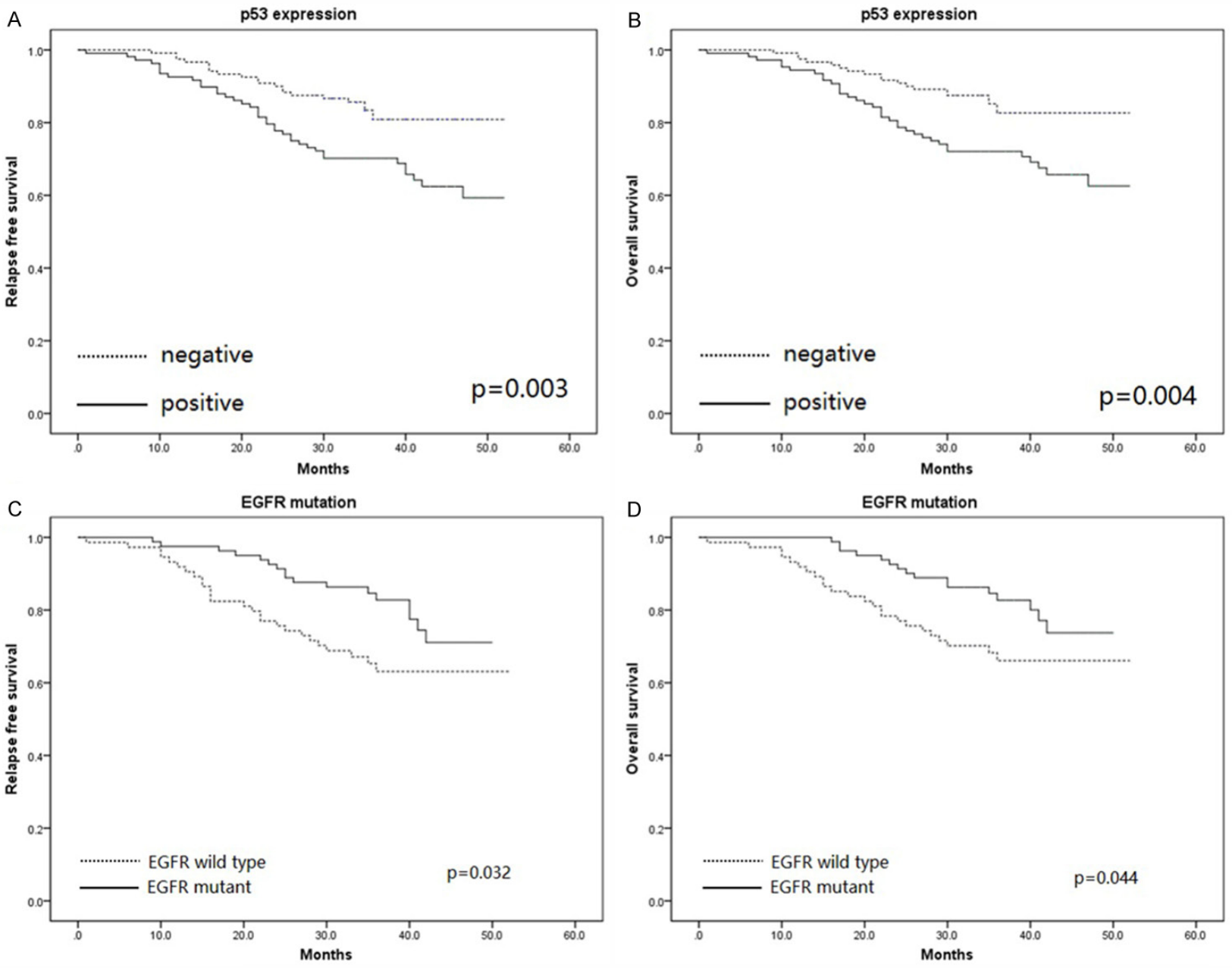

A
p53 expression
Relapse free survival
negative
positive
p=0.003
Months
B
p53 expression
Overall survival
negative
positive
p=0.004
Months
C
EGFR mutation
Relapse free survival
EGFR wild type
EGFR mutant
p=0.032
Months
D
EGFR mutation
Overall survival
EGFR wild type
EGFR mutant
p=0.044
Months

Figure 5. Survival curves among lung adenocarcinoma patients according to p53 status and EGFR mutation status. Kaplan-Meier plots with the log-rank test for disease-free survival (A) and overall survival (B) according to p53 status. Kaplan-Meier plots with the log-rank test for disease-free survival (C) and overall survival (D) according to EGFR mutation status.

RFS; P<0.001 for OS), tumor size (>3 cm) (P<0.005 for RFS; P=0.034 for OS), solid predominant tumors (P<0.001 for RFS; P<0.001 for OS) and poor pathologic differentiation (P<0.001 for RFS; P<0.001 for OS) were also significantly associated with poorer survival. Multivariate analysis including PD-L1 expression in tumor cells, p53 status, EGFR mutation status, node metastasis, tumor size, solid predominant tumors and pathologic differentiation revealed that solid predominant tumors (P= 0.041) were independent prognostic factors of RFS, but PD-L1 expression and p53 status were not (Supplementary Table 2).

Discussion

We examined the expression of PD-L1 and P53 in lung adenocarcinoma in this paper and found that PD-L1 expression in tumor cells was significantly associated with PD-L1 expression in tumor-infiltrating immune cells and P53 status. Some studies demonstrated that PD-L1 was expressed in 18.6%-59% of tumor cells in lung adenocarcinoma [5, 11, 13, 18-20], while that was 20.5% in our study. The discrepancy between the present and previous studies might be attributed to several reasons, which were shown as follows: firstly, the baseline characteristics of lung cancer among these studies were heterogeneous; secondly, the standardized antibody or diagnostic kit for immunotherapy has not been established. we chose SP142 on the base of a study which compared the performance of 6 monoclonal antibodies (SP142, E1L3N, et al.) and all 6 antibodies had high levels of concordance [21].

There were controversial results concerning the relationship between PD-L1 positivity in tumor cells and clinicopathological features. It was found in our study that PD-L1 positive was markedly associated with larger tumors, node metastasis, solid predominant tumors, and poor differentiation, all of which would admittedly predict poor development of tumors, as previously described [11, 13, 18]. Most studies demonstrated that PD-L1 expression signified shorter relapse-free survival and overall survival [13, 18], which was consistent with our results with large sample size and reliable results. However, it was not shown in our study that PD-L1 positive was an independent prognostic factor. Individual study showed that PD-L1 expression had no relationship with the survival, thus, more studies with larger sample size in different people were still necessary.

The relationship between PD-L1 expression and genetic mutation is worthy discussed. K. Azuma et al. found that the presence of EGFR mutations were significantly associated with increased PD-L1 expression [22], while some studies including ours' demonstrated that there were no statistical correlation between them [11]. Further studies are necessary for the drug combination of PD-1 inhibitors and EGFR-tyrosine kinase inhibitor (EGFR-TKI).

Few studies have been performed in PD-L1 expression in tumor-infiltrating immune cells. We demonstrated that PD-L1 positivity in tumor-infiltrating immune cells was more frequent in male patients, solid predominant tumors and poor differentiation, and showed a significant correlation with that in tumor cells but it had no relationship with RFS or OS, which was consistent with Cha YJ's results [5]. However, Akihito K et al. found that Neither PD-L1 expression on tumor cells nor that on tumor-infiltrating immune cells was an independent prognostic factor in gastric cancer patients [23]. Thus the study on PD-L1 in lung adenocarcinoma could be extended to immune cells, such as tumor-infiltrating lymphocytes, rather than being restricted in tumor cells.

The published data about p53 expression in lung carcinoma were limited. As was found in some studies, the expression of p53 in lung carcinoma was only associated with women, which was of no prognostic significance [24, 25], while our study found that patients with aberrant expression of p53 showed poor prognosis and were significantly associated with node metastasis, solid predominant tumors, and poor differentiation. Our study demonstrated that aberrant p53 expression was significantly independent factor affecting PD-L1 positivity in tumor cells. Some studies had sh-

own that p53 interacted with the immune response by regulating the inflammatory cytokines, toll-like receptors, and IFN signaling [26-28]. However, whether p53 was involved in tumor immune evasion was poorly understood. It was discovered in a comprehensive study that p53 regulated PD-L1 status via miR-34, which directly bound to the PD-L1 3' untranslated region in models of NSCLC, and further research showed that PD-L1 was lost or expressed at reduced levels in cells that expressed wild-type p53, suggesting that induction of p53 promoted the downregulation of PD-L1 relative to the controls [29]. It provided molecular support for our results. Previous study also showed that PD-L1 expression was related to p53 status in hepatocellular carcinoma and acute myeloid leukemia [30, 31]. All these studies including ours' linked tumor immune evasion to other tumor suppressor pathways previously described for p53 [32, 33].

The current understanding is that PD-L1 expression could be induced mainly by adoptive immune resistance (secondary to development of multiple passenger mutations) and innate immune resistance (secondary to oncogenic signaling). Since p53 were independently parameters affecting PD-L1 expression in lung adenocarcinomas, it is thought that TP53 is mutated as part of passenger mutations, and p53 status could be a new biomarker for immune resistance.

PD-1 inhibitors, such as Pembrolizumab and Nivolumab, had been verified to be effective in some types of advanced lung carcinoma [34, 35], but the effective rate was less than 50% and 20% in patients with adenocarcinoma [35] and squamous carcinoma [34], respectively. However, the therapy was very expensive, so it was necessary to search for the best marker for predicting responses to immune checkpoint inhibitors in the further study. Interestingly, there is some evidence that tumor expression of PD-L1 is associated with tumor response to PD-1/PD-L1 pathway inhibition suggesting PD-L1 expression may be a predictive marker of response to treatment [36]. More studies about PD-L1 are needed before they could be used in the clinic.

A final note about our study was that we only demonstrated the correlation between PD-L1 and p53 protein, but whether we could use p53 as a surrogate marker of TP53 gene remained a question? We could speculate the potential correlation between PD-L1 expression and TP53 gene mutation by virtue of the previous studies on the ovarian and lung cancers which indicated the veracity and the feasibility of the method [10, 15, 24, 37]. However, an immunohistochemical study demonstrated only 70% concordance between over-expression of p53 protein and mutation of p53 gene in lung carcinoma [37]. Consequently, further studies about the relationship between PD-L1 and TP53 gene mutation were necessary. In addition, we evaluated PD-L1 expression by tissue microarray, the cores of which might not be representative of the entire tissue because of the heterogeneity of the tumor cells, so we chose two or three cores which showed different histological subtypes or pathological differentiation to make up for the deficiency.

Conclusion

We demonstrate that PD-L1 expression in tumor cells is significantly associated with aberrant p53 expression, PD-L1 expression in tumor-infiltrating immune cells, poor pathological differentiation and female, PD-L1 expression in lung adenocarcinoma patients predicted poor prognosis but it was not independent prognostic factor. All these closely related factors should be considered when analyzing the clinical outcomes of patients treated with anti-PD1/PD-L1 immune checkpoint inhibitors.

Acknowledgements

This work was supported by the National Scientific Foundation of Jiangsu Provincial of China (BK20151582), and the Fund of the priority Academic Program Development of Jiangsu Higher Education Institution (JX1023-1801).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhihong Zhang, Department of Pathology, The First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, P. R. China. Tel: 86-25-68217512; Fax: 86-25-83724440; E-mail: zhangzhih2001@aliyun.com

References

[1] Gridelli C, Rossi A, Carbone DP, Guarize J, Karachaliou N, Mok T, Petrella F, Spaggiari L,

Rosell R. Non-small-cell lung cancer. Nat Rev Dis Primers 2015; 1: 15009.
[2] He J, Hu Y, Hu M, Li B. Development of PD-1/PD-L1 pathway in tumor immune microenvironment and treatment for non-small cell lung cancer. Sci Rep 2015; 5: 13110.
[3] Carreno BM, Collins M. The B7 family of ligands and its receptors: new pathways for costimulation and inhibition of immune responses. Annu Rev Immunol 2002; 20: 29-53.
[4] Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008; 26: 677-704.
[5] Cha YJ, Kim HR, Lee CY, Cho BC, Shim HS. Clinicopathological and prognostic significance of programmed cell death ligand-1 expression in lung adenocarcinoma and its relationship with p53 status. Lung Cancer 2016; 97: 73-80.
[6] Mir R, Masroor M, Javid J, Ahamad I, Farooq S, Yadav P, Zuberi M, Lone M, Ray PC, Saxena A. Clinical implications of cytosine deletion of exon 5 of P53 gene in non small cell lung cancer patients. South Asian J Cancer 2016; 5: 33-6.
[7] Ryan KM, Phillips AC, Vousden KH. Regulation and function of the p53 tumor suppressor protein. Curr Opin Cell Biol 2001; 13: 332-7.
[8] Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. Introduction to the 2015 World Health Organization classification of tumors of the lung, pleura, thymus, and heart. J Thorac Oncol 2015; 10: 1240-2.
[9] Rosen DG, Yang G, Deavers MT, Malpica A, Kavanagh JJ, Mills GB, Liu J. Cyclin E expression is correlated with tumor progression and predicts a poor prognosis in patients with ovarian carcinoma. Cancer 2006; 106: 1925-32.
[10] López-Guerrero JA, Bolufer-Gilabert P, Vera-Sempere FJ, Marugán de la Concha I, Barragán-González E. C-erbB-2 expression and its relationship with ploidy, p53 abnormalities and epidermal growth factor receptor content in human non-small cell lung cancer. Clin Chim Acta 1999; 285: 105-20.
[11] Yang CY, Lin MW, Chang YL, Wu CT, Yang PC. Programmed cell death-ligand 1 expression in surgically resected stage I pulmonary adenocarcinoma and its correlation with driver mutations and clinical outcomes. Eur J Cancer 2014; 50: 1361-9.
[12] Topalian SL, Hodi FS, Brahmer JR. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. [J]. N Engl J Med 2012, 366: 2443.
[13] Koh J, Go H, Keam B, Kim MY, Nam SJ, Kim TM, Lee SH, Min HS, Kim YT, Kim DW, Jeon YK, Chung DH. Clinicopathologic analysis of programmed cell death-1 and programmed cell death-ligand 1 and 2 expressions in pulmonary adenocarcinoma: comparison with histology and driver oncogenic alteration status. Mod Pathol 2015; 28: 1154-66.
[14] Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, Mcdermott DF, Powderly JD, Gettinger SN. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014; 515: 563-7.
[15] Yemelyanova A, Vang R, Kshirsagar M, Lu D, Marks MA, Shih Ie M, Kurman RJ. Immunohistochemical staining patterns of p53 can serve as a surrogate marker for TP53 mutations in ovarian carcinoma: an immunohistochemical and nucleotide sequencing analysis. Mod Pathol 2011; 24: 1248-53.
[16] Shim HS, Kenudson M, Zheng Z, Liebers M, Cha YJ, Hoang HQ, Onozato M, Phi LL, Heist RS, Iafrate AJ. Unique genetic and survival characteristics of invasive mucinous adenocarcinoma of the lung. J Thorac Oncol 2015; 10: 1156-62.
[17] Ma XM, Cao YZ, Ji WL, Zhao F, Fang XZ. [Analysis of epidermal growth factor receptor mutations and its clinico-pathologic characteristics of the primary lung adenocarcinoma in Xinjiang Uighur Autonomous Region]. Beijing Da Xue Xue Bao 2016; 48: 663-6.
[18] Zhang Y, Wang L, Li Y, Pan Y, Wang R, Hu H, Li H, Luo X, Ye T, Sun Y, Chen H. Protein expression of programmed death 1 ligand 1 and ligand 2 independently predict poor prognosis in surgically resected lung adenocarcinoma. Onco Targets Ther 2014; 7: 567-73.
[19] Cooper WA, Tran T, Vilain RE, Madore J, Selinger CI, Kohonen-Corish M, Yip P, Yu B, O'Toole SA, McCaughan BC, Yearley JH, Horvath LG, Kao S, Boyer M, Scolyer RA. PD-L1 expression is a favorable prognostic factor in early stage non-small cell carcinoma. Lung Cancer 2015; 89: 181-8.
[20] Lin C, Chen X, Li M, Liu J, Qi X, Yang W, Zhang H, Cai Z, Dai Y, Ouyang X. Programmed death-ligand 1 expression predicts tyrosine kinase inhibitor response and better prognosis in a cohort of patients with epidermal growth factor receptor mutation-positive lung adenocarcinoma. Clin Lung Cancer 2015; 16: e25-35.
[21] Gaule P, Smithy JW, Toki M, Rehman J, Patell-Socha F, Cougot D, Collin P, Morrill P, Neumeister V, Rimm DL. A quantitative comparison of antibodies to programmed cell death 1 ligand 1. JAMA Oncol 2016; [Epub ahead of print].
[22] Azuma K, Ota K, Kawahara A, Hattori S, Iwama E, Harada T, Matsumoto K, Takayama K, Takamori S, Kage M, Hoshino T, Nakanishi Y, Okamoto I. Association of PD-L1 overexpression with activating EGFR mutations in surgically resected nonsmall-cell lung cancer. Ann Oncol 2014; 25: 1935-40.

[23] Kawazoe A, Kuwata T, Kuboki Y, Shitara K, Nagatsuma AK, Aizawa M, Yoshino T, Doi T, Ohtsu A, Ochiai A. Clinicopathological features of programmed death ligand 1 expression with tumor-infiltrating lymphocyte, mismatch repair, and Epstein-Barr virus status in a large cohort of gastric cancer patients. Gastric Cancer 2016; [Epub ahead of print].

[24] Guinee GD Jr, Travis WD, Trivers GE, De Benedetti VM, Cawley H, Welsh JA, Bennett WP, Jett J, Colby TV, Tazelaar H, et al. Gender comparisons in human lung cancer: analysis of p53 mutations, anti-p53 serum antibodies and C-erbB-2 expression. Carcinogenesis 1995; 16: 993-1002.

[25] Geradts J, Fong KM, Zimmerman PV, Maynard R, Minna JD. Correlation of abnormal RB, p16ink4a, and p53 expression with 3p loss of heterozygosity, other genetic abnormalities, and clinical features in 103 primary non-small cell lung cancers. Clin Cancer Res 1999; 5: 791-800.

[26] Porta C, Hadj-Slimane R, Nejmeddine M, Pampin M, Tovey MG, Espert L, Alvarez S, Chelbi-Alix MK. Interferons alpha and gamma induce p53-dependent and p53-independent apoptosis, respectively. Oncogene 2005; 24: 605-15.

[27] Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, Cordon-Cardo C, Lowe SW. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature 2007; 445: 656-60.

[28] Menendez D, Shatz M, Azzam K, Garantziotis S, Fessler MB, Resnick MA. The toll-like receptor gene family is integrated into human DNA damage and p53 networks. PLoS Genet 2011; 7: e1001360.

[29] Cortez MA, Ivan C, Valdecanas D, Wang X, Peltier HJ, Ye Y, Araujo L, Carbone DP, Shilo K, Giri DK, Kelnar K, Martin D, Komaki R, Gomez DR, Krishnan S, Calin GA, Bader AG, Welsh JW. PDL1 regulation by p53 via miR-34. J Natl Cancer Inst 2015; 108.

[30] Kan G, Dong W. The expression of PD-L1 APE1 and P53 in hepatocellular carcinoma and its relationship to clinical pathology. Eur Rev Med Pharmacol Sci 2015; 19: 9.

[31] Xi W, Li J, Ke D, Fang L, Min L, Ouyang Y, Wei J, Xi C, Weng Y, He T, Zhang H. Tumor suppressor miR-34a targets PD-L1 and functions as a potential immunotherapeutic target in acute myeloid leukemia. Cell Signal 2015; 27: 443-52.

[32] He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, Ridzon D, Jackson AL, Linsley PS, Chen C, Lowe SW, Cleary MA, Hannon GJ. A microRNA component of the p53 tumour suppressor network. Nature 2007; 447: 1130-4.

[33] Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, Feldmann G, Yamakuchi M, Ferlito M, Lowenstein CJ, Arking DE, Beer MA, Maitra A, Mendell JT. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell 2007; 26: 745-52.

[34] Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, Waterhouse D, Ready N, Gainor J, Aren Frontera O, Havel L, Steins M, Garassino MC, Aerts JG, Domine M, Paz-Ares L, Reck M, Baudelet C, Harbison CT, Lestini B, Spigel DR. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 2015; 373: 123-35.

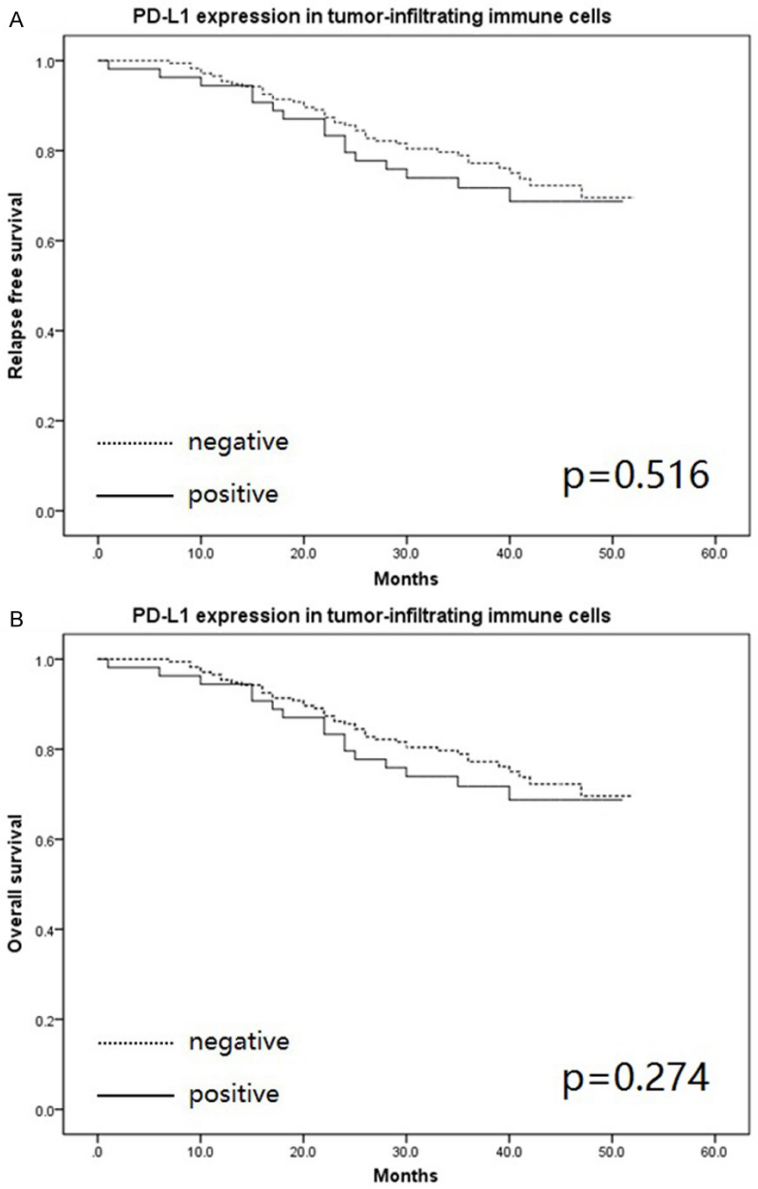
[35] Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, Patnaik A, Aggarwal C, Gubens M, Horn L, Carcereny E, Ahn MJ, Felip E, Lee JS, Hellmann MD, Hamid O, Goldman JW, Soria JC, Dolled-Filhart M, Rutledge RZ, Zhang J, Lunceford JK, Rangwala R, Lubiniecki GM, Roach C, Emancipator K, Gandhi L; KEYNOTE-001 Investigators. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015; 372: 2018-28.

[36] Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366: 2443.

[37] Brattström D, Bergqvist M, Lamberg K, Kraaz W, Scheibenflug L, Gustafsson G, Inganas M, Wagenius G, Brodin O. Complete sequence of p53 gene in 20 patients with lung cancer: comparison with chemosensitivity and immunohistochemistry. Med Oncol 1998; 15: 255-61.

Supplementary Table 1. Correlation between clinicopathological features and expression of p53 in lung adenocarcinomas

	P53 aberrant expression, n (%)	P53 wild type expression, n (%)	*P* value
Gender			
Male	62 (49.2)	64 (50.8)	0.493
Female	46 (44.7)	57 (55.3)	
Age			
≤60	53 (47.7)	58 (52.3)	0.863
>60	55 (46.6)	63 (53.4)	
Smoking status			
Never smokers	79 (46.5)	91 (53.5)	0.722
Former or current smokers	29 (49.2)	30 (50.8)	
Size			
≤3 cm	68 (45.0)	83 (55.0)	0.369
>3 cm	40 (51.3)	38 (48.7)	
Nodal metastasis			
No	63 (40.4)	93 (59.6)	0.003
Yes	45 (61.6)	28 (38.4)	
Vascular invasion			
No	101 (47.2)	113 (52.8)	0.968
Yes	7 (46.7)	8 (53.3)	
T status			
I	42 (44.2)	53 (55.8)	0.551
II	59 (50.9)	57 (49.1)	
III	7 (41.2)	10 (58.8)	
IV	0 (0)	1 (100)	
Histologic subtype			
Lepidic	9 (33.3)	18 (66.7)	<0.001
Acinar	48 (47.5)	53 (52.5)	
Mucinous	3 (20.0)	12 (80.0)	
Papillary	3 (15.0)	17 (85.0)	
Micropapillary	3 (27.3)	8 (72.7)	
Solid	42 (76.4)	13 (23.6)	
Solid predominant type			
Negative	66 (37.9)	108 (62.1)	<0.001
Positive	42 (76.4)	13 (23.6)	
Pathologic differentiation			
Well/moderate	49 (35.3)	90 (64.7)	<0.001
Poor	59 (65.6)	31 (34.4)	
EGFR			
Wild type	42 (56.0)	33 (44.0)	0.149
Mutant	36 (44.4)	45 (55.6)	
PD-L1 positivity in tumor cells			
Negative	69 (37.9)	113 (62.1)	<0.001
Positive	39 (83.0)	8 (17.0)	
PD-L1 positivity in tumor-infiltrating immune cells			
Negative	72 (41.1)	103 (58.9)	0.001
Positive	36 (66.7)	18 (33.3)	



Supplementary Figure 1. Survival curves among lung adenocarcinoma patients according to PD-L1 expression in tumor-infiltrating immune cells. Kaplan-Meier plots with the log-rank test for disease-free survival (A) and overall survival (B) according to PD-L1 expression in tumor-infiltrating immune cells.

Supplementary Table 2. Multivariate analysis of prognostic factors of survival

Parameters	Relapse-free survival		Overall survival	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Node metastasis	0.687-2.564	0.400	0.737-2.872	0.279
Pathologic differentiation	0.827-4.338	0.131	0.861-4.644	0.107
Solid predominant type	1.037-5.405	0.041	0.673-3.711	0.293
EGFR mutation status	0.371-1.333	0.281	0.366-1.372	0.307
PD-L1 positive	0.379-1.875	0.675	0.493-2.589	0.772
Tumor size	0.802-2.829	0.203	0.717-2.652	0.336
P53 status	0.507-1.936	0.979	0.546-2.220	0.789

Abbreviations: HR, Hazard ratio; CI, Confidence interval.