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Induction of Patient-Derived Xenograft Formation and Clinical Significance of Programmed Cell Death Ligand 1 (PD-L1) in Lung Cancer Patients

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Background: The immune checkpoint of programmed cell death ligand 1 (PD-L1) commonly expressed in solid cancers, and the blockade of this molecule show promising results in advanced cancers, including lung cancer. The relevance of PD-L1 to patient-derived xenograft (PDX) formation and clinicopathological characteristics in early stage lung cancer have not been fully elucidated.

Material/Methods: Cell counting kit-8 and flow cytometry were carried out to examine proliferation and apoptosis in PC9 and H520 cells transfected with siRNAs. Nod-scid mice were used to establish PDX. Immunohistochemistry was done to investigate PD-L1 expression in tumor tissues.

Results: PD-L1 was detected in lung cancer cell lines and 45.45% of primary tumor tissues from a cohort of 209 lung cancer patients. Cell growth was restrained and apoptosis was induced when PD-L1 was inhibited in PC9 and H520 cells. In addition, we successfully established 16 PDX models from tissues from 43 cases of primary lung cancer. Higher PD-L1 expression rates (75%) was observed in primary tumors with PDX formation compared to protein expression rate (44.44%) in tumors without PDX formation. Consistently, a 1.9-fold increase of PDX formation frequency was identified in the PD-L1 positive tumors than in the PD-L1 negative tumors. Moreover, PD-L1 was found to be related to smoking, histological type, and pathological stage. Importantly, PD-L1 over-expression was associated with shorter overall survival (OS) of lung cancer patients.

Conclusions: This study suggests that overexpression of PD-L1 could induce PDX formation and is related to poor outcome for the lung cancer patients.

MeSH Keywords: Antigens, CD274 • Cell Proliferation • Lung Neoplasms

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Background

Lung cancer is the most common cause of death among malignant cancers worldwide [1]. Lung cancer consists of approximately 15% small cell lung cancer (SCLC) and 85% non-small cell lung cancer (NSCLC) which includes adenocarcinoma (Ad), squamous carcinoma (SCC) and larger cell lung cancer (LCLC). Although conventional treatments like surgical resection, chemotherapy, and radiotherapy have been developed in the past decades, there has not been a significant improvement in overall survival (OS) for lung cancer patients. Therefore, we need to understand the molecular basis of the disease and novel therapies for improving patient outcomes. Patient-derived xenografts (PDX), in which surgically resected tumors are directly inoculated into immune-compromised mice, can reproduce the biological characteristics of human cancers. It has been identified that approximately 30–40% of lung cancer tissues could successfully form PDX with more than two passages [2]. In addition, the frequency of PDX formation was associated with recurrence of patients in early stage lung cancer [3].

Recently, the immunosuppressive molecule of programmed cell death ligand-1 (PD-L1) has been found to be commonly express in tumor tissues of several solid carcinomas, including melanoma, breast, ovary, esophagus, and lung cancers [4–10]. PD-L1 on the antigen-presenting cells has been shown to negatively regulate immune response through binding to its receptor PD-1 on T cells [11,12]. The effect of PD-L1 on tumor cells demonstrates that blockade of PD-L1 could inhibit tumor growth of metastatic renal cells, melanoma, and lung cancers *in vitro* and *in vivo* [13]. PD-L1 expression has been found to be related to prognosis for several cancer types including lung cancer [14–16].

PD-L1 has previously been detected by immunohistochemistry (IHC) in the formalin-fixed paraffin-embedded (FFPE) tissue samples that could predict clinical response to immune therapy of targeted PD-1/PD-L1 [17,18]. However, data on the role of PD-L1 in tumor formation and the mechanism of progression for lung cancer is limited. In our study, we explored how PD-L1 influenced PDX formation and the clinical significance of this protein for early stage lung cancer patients. Knockdown of PD-L1 inhibited cell growth and induced apoptosis in lung cancer cell lines PC9 and H520. It was further verified that higher expression frequency of PD-L1 was observed in tumors with PDX formation than in tumors without PDX formation. In addition, higher PDX formation frequency was identified in PD-L1 positive tumors than in PD-L1 negative tumors. In a cohort of 209 lung cancer patients, PD-L1 expression was related to smoking, histological type, stage and poor outcomes. Our data indicated that PD-L1 played an important role in PDX formation capacity and could be a poor prognostic factor in early stage lung cancer.

Material and Methods

Cell lines and transient transfection

Human lung cancer cell lines PC-9 and H520 (American Type Culture Collection, Manassas, VA, USA) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 g/mL streptomycin (Invitrogen, Grand Island, NY, USA) in a humidified atmosphere of 5% CO_2 at 37°C incubator. After cells were seeded in six-well plates overnight, PD-L1 siRNA or scramble siRNA (Suzhou GenePharma Co., China) were transfected into cells with X-tremeGENE siRNA Transfection Reagent according to the manufacture's instruction (Roche, USA).

Quantitative real-time-PCR (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Then 2 μg of total RNA was used to synthesize cDNA using the Oligo dT primer. Quantitative real-time PCR (qRT-PCR) reactions were performed using Light-Cycler®480 Real-Time PCR System (Roche, USA) and the reagent LightCycler®480 SYBR Green I Master (Roche, USA). The cycling condition was as follows: five minutes at 95°C, followed by 45 cycles of 10 second at 95°C, 30 second at 60°C, and 20 second at 72°C. The $2^{-\Delta\Delta Ct}$ method (where $\Delta Ct=Ct_{target}-Ct_{control}$) was used to analyze comparative gene expression with normalization to the internal reference level of GAPDH. The sequences of the used primers were as following: PD-L1: forward primer-GACCTATATGTGGTAGAGTATGGTAGC, reverse primer-TTCAGCTGTATGGTTTTCCTCAGGATC; GAPDH: forward primer-GACCCCTTCATTGACCTCAAC, reverse primer-CTTCTCCATGGTGGTGAAGA.

Cell proliferation assay

Cell growth was tested using a cell counting kit-8 assay. PC-9 and H520 cells (2×10^3) were cultivated in 96-well plates for 0, 24, 48, and 72 hours. At each time, FLUO star OPTIMA (BMG LAB-TECH, Offenburg, Germany) was added for one hour. Then the number of viable cells was determined by measuring absorbance at 450 nm, according to the manufacturer's instructions.

Annexin V assay by flow cytometry

After transfection with PD-L1 siRNA or scramble siRNA in A549 and H520 cells for 48 hours, the cells were trypsinized and suspended in binding buffer including propidium iodide (PI) and Annexin V (FITC Annexin V Apoptosis Detection Kit 1, BD Biosciences) for 30 minutes at 4°C in the dark. The cells were resuspended in PBS through a 70-μm nylon mesh followed by evaluation with flow cytometer (BD Biosciences) within 30 minutes.

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PDX establishment and clinical samples of lung cancer

Forty-three cases of fresh tumor tissues were obtained from lung cancer patients who underwent resection at Peking University Cancer Hospital. Four- to six-week old nonobese diabetic/severe combined immunodeficient (Nod-scid) mice were purchased from Vitalriver (Beijing, China) and maintained in specific pathogen-free conditions. After the tumors were removed, the fresh tissue was immediately maintained in 1640 medium with 2% fetal bovine serum (FBS). The tumor tissue was then cut into multiple pieces and inoculated subcutaneously into mice, as previously described [19,20]. Xenografted mice with tumors with more than two passages were considered successful for PDX establishment. Study protocols involving mice were approved by the Animal Care and Use Committee at Peking University Cancer Hospital.

In addition, we collected the tumor tissue from 209 lung cancer patients who underwent radically surgical resection between July 2006 and January 2013 at Peking University Cancer Hospital. Clinicopathological characteristics collected included age, sex, smoking status, histological type, and TNM stage. All patients were followed up every three to six months until February 2016. Among these lung cancer patients, 120 out of 145 cases with pathological stage II-IIIa received adjuvant chemotherapy, and chemotherapy combined with radiation were conducted for N2 positive disease. In addition, 11 out of 42 stage Ib patients with risk factor like venous invasion and pleural invasion also underwent adjuvant chemotherapy. The present study was approved by the Ethics Committee of Peking University Hospital and written informed consent was received from all the patients according to the tenets of Declaration of Helsinki.

Hematoxylin-eosin staining (H&E) and immunohistochemistry

For H&E, paraffin-embedded tumor tissues were cut into 4-mm sections and the sections were then placed on the positively charged microscope slides. The slides were rehydrated in xylene and graded ethanol solution. Tumor tissues were added with hematoxylin for five minutes followed by washing in water. After differentiating in 1% acid alcohol for 30 seconds, eosin was used to stain the slide for 10 minutes. Then the tissues were dehydrated through low concentration of alcohol to absolute alcohol and two changes of xylene followed by mounting with xylene based mounting medium.

The immunohistochemistry assays were performed as described previously [21]. Briefly, after 4-mm paraffin-embedded slides were deparaffinized, endogenous peroxidase activity was blocked by 3% H_2O_2 in PBS for 30 minutes. The slides were then incubated with primary antibody of PD-L1 (1: 500 dilution; Abcam, UK) at 4°C overnight. The HRP-conjugated secondary antibody was used to incubate the slides (Sigma-Aldrich, Poole, Dorset, UK). Sections were counterstained with hematoxylin and mounted with a cover glass.

Two independent pathologists analyzed the morphology and immunostained sections. PD-L1 staining was graded based on the intensity of staining, including membrane/cytoplasm alone and the combination of membrane and cytoplasm location (1, weak; 2, moderate; 3, strong) and the percentage of stained cells (0, for <5%; 1, for 5–25%; 2, for 26–50%; and 3, >50%.) according to the method reported in our previous study [21]. When combining these two parameters, 0–1 and >1 were ranked negative and positive expression, respectively.

Statistical analysis

Data are demonstrated as mean ±SEM and the experiments were independently performed in triplicate. χ^2 test and Fisher's exact test were used to analyze the associations between the PD-L1 expression and clinical variables. Kaplan-Meier and Cox model were used to assess survival rates. A value of $p<0.05$ was considered as significant difference and all the statistical analyses were done using SPSS 17.0 statistical software.

Results

Suppression of PD-L1 decreased cell growth and increased apoptosis in lung cancer cells

First, we investigated the capacity for proliferation and apoptosis in the lung cancer cell lines PC9 and H520 transfected with PD-L1 siRNA or scramble siRNA. Suppression of PD-L1 was validated in tumor cells transfected with PD-L1 siRNA compared to control cells transfected with scramble siRNA by qRT-PCR (Figure 1A). Slower growth was demonstrated in PD-L1-siRNA transfected PC9 and H520 cells than the corresponding control cells ($p<0.01$, Figure 1B, 1C). Next, we detected apoptosis for PC9 and H520 cells with siRNAs transfection by Annexin V assay. The data revealed higher percentage of apoptotic cells in PD-L1-siRNA transfected cells compared to scramble-siRNA transfected cells ($p=0.05$ for PC9 and $p=0.02$ for A549, Figure 1D). These results indicated that PD-L1 could increase proliferation and decrease apoptosis in lung cancer cells.

PD-L1 promoted PDX formation

Based on the inductive effect of PD-L1 on proliferation in lung cancer cell lines, we further detected whether this molecule influenced primary tumor growth in mice models. We collected primary tumor tissues from 43 cases of lung cancer patients to inject into Nod-scid mice for establishing PDX. The

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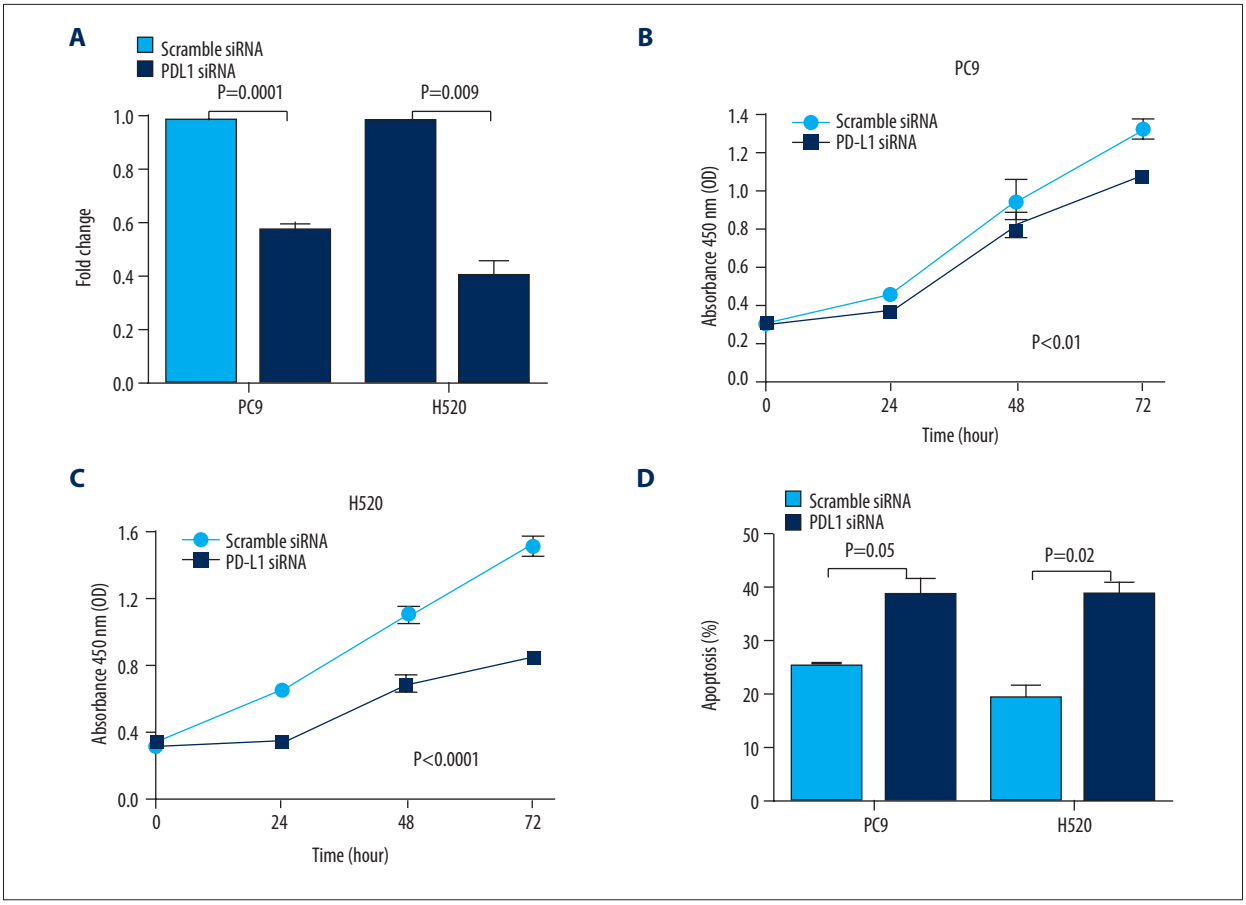

Figure 1. Proliferative and apoptotic abilities of lung cancer cells PC9 and H520 transfected with siRNAs. (**A**) The suppression of PD-L1 was evaluated in cells with PD-L1-siRNA by qRT-PCR. (**B, C**) Growth curves show that proliferation was inhibited in PC9 and H520 cells with knockdown of PD-L1. (**D**) siRNA-PD-L1 induced apoptosis of lung cancer cells by Annexin V assay.

clinical information for these patients is listed in Table 1. The median age was 63 years old (range from 29 to 82 years) with 60.47% male. There were 31 cases of Ad, nine cases of SCC, one case of LCLC, and two cases of SCLC for these stage I to IIIa patients. Among the 43 cases of tumors from lung cancer patients, we successfully established 16 PDX models. The histology of primary and PDX tissues were validated by H&E staining (Figure 2A). Higher frequency of PDX formation was observed in lung cancer patients with older age (>63 years, 43.5%), male (46.2%), and SCC types (66.7%) compared to that in patients age <63 years (30%), female (23.5%), and Ad type (32.3%), respectively (Table 1).

We further asked whether PD-L1 expression could influence the ability of PDX formation for these lung cancers. PD-L1 was stained on the membrane or both the membrane and cytoplasm of primary tumor tissues, and formed PDX tissues by immunohistochemistry (Figure 2B). Among these 43 cases of primary tumors, 24 of the tumors were detected with PD-L1 expression. Higher expression rate of PD-L1 was identified in PDX formed tumors (75%) than in tumors without PDX formation (44.4%) (Figure 2C left panel). Compared to the 26.7% of PDX formation in tumors which did not have detected PD-L1 expression, 50% of lung cancers with PD-L1 expression formed PDX with a 1.9-fold increase (Figure 2C right panel). Our data suggested that the lung cancers with PD-L1 expression greatly induced PDX formation.

Clinicopathologic significance of PD-L1 in lung cancer patients

Next, we assessed the clinical significance of PD-L1 expression in lung cancer patients. We detected PD-L1 status in tumor tissues from a cohort of 209 patients by immunohistochemistry. Based on the evaluation of PD-L1 expression by pathologists, 95 cases (45.5%) were identified as positive expression and 114 cases (54.5% as negative expression. Among patients with positive expression of PD-L1, there were 59 cases with both membrane and cytoplasm staining, 35 cases with cytoplasm expression and one case with the membrane expression. We then divided PD-L1 expression into negative and positive groups and analyzed the relationship of clinical significance

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Table 1. Clinical features of 43 lung cancer patients with PDX establishment.

Variable	No.		PDX establishment			
			Unformation (n=27)		Formation (n=16)	
Age (median 63, range 29–82)						
<63	20	(46.5%)	14	(70%)	6	(30%)
≥63	23	(53.5%)	13	(56.5%)	10	(43.5%)
Sex						
Male	26	(60.5%)	14	(53.9%)	12	(46.1%)
Female	17	(39.5%)	13	(76.5%)	4	(23.5%)
Histology						
Ad	31	(72.1%)	21	(67.7%)	10	(32.3%)
SCC	9	(20.9%)	3	(33.3%)	6	(66.7%)
LCLC	1	(2.3%)	1	(100%)	0	(0%)
SLCL	2	(4.7%)	2	(100%)	0	(0%)
Venous invasion						
Negative	33	(76.7%)	20	(60.6%)	13	(39.4%)
Positive	10	(23.3%)	7	(70%)	3	(30%)
Stage						
I	23	(53.6%)	15	(65.2%)	8	(34.8%)
II	10	(23.2%)	6	(60%)	4	(40%)
III	10	(23.2%)	6	(60%)	4	(40%)

Ad – adenocarcinoma; SCC – squamous cell carcinoma; LCLC – large cell lung cancer; SCLC – small cell lung cancer.

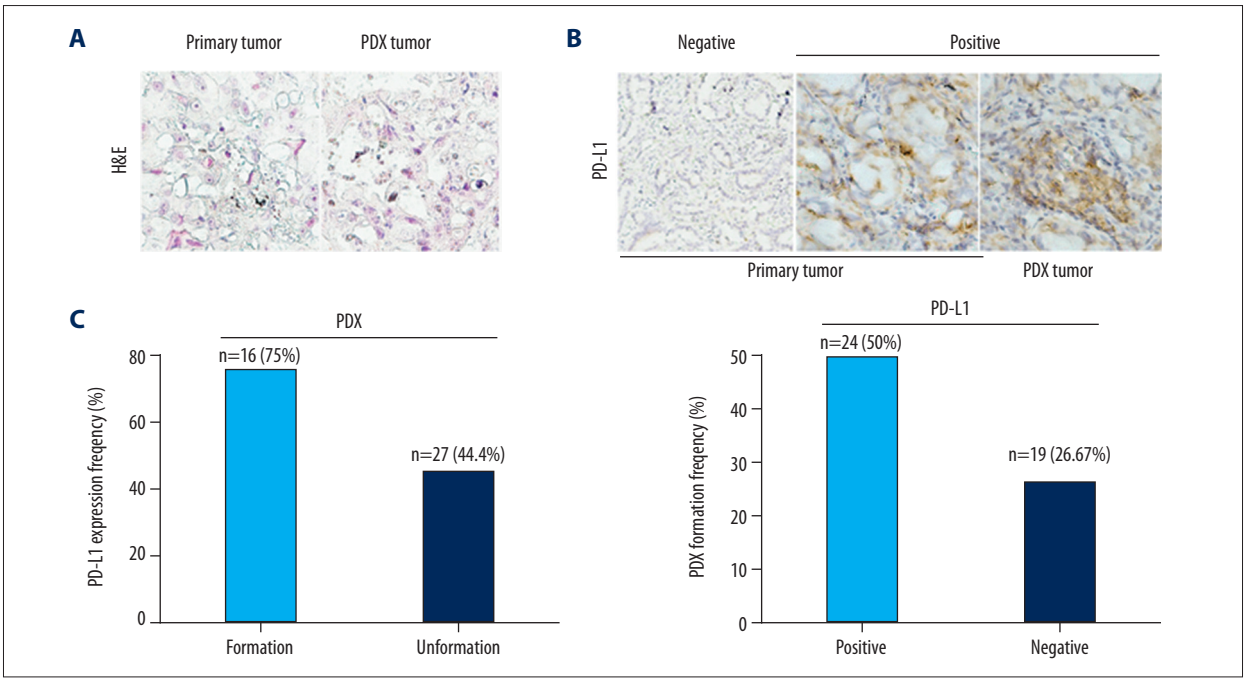

Figure 2. Correlation between PD-L1 expression and PDX formation of lung cancer. (**A**) Representative images of H&E staining in primary and PDX tumors. (**B**) Representative pictures of positive/negative PD-L1 expression in primary tumors and PD-L1 staining in PDX tumors. (**C**) Higher frequency of PD-L1 expression was shown in PDX formed tumors than in unformed PDX tumors (left panel). Increased rate of PDX formation was seen in PD-L1 positive tumors compared to PD-L1 negative tumors (right panel).

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Table 2. Clinicopathological variables and PD-L1 expression in the lung cancer patients with resected surgery (n=209).

Variable	Case no.	PD-L1 expression		*P* value
		Negative	Positive	
Gender				*p*=0.094
Male	158 (75.6%)	81 (51.3%)	77 (48.7%)	
Female	51 (24.4%)	33 (64.7%)	18 (35.3%)	
Age (median 58)				*p*=0.064
≤60	80 (38.3%)	37 (46.2%)	43 (53.8%)	
>60	129 (61.7%)	77 (59.7%)	52 (40.3%)	
Smoking history				***p*=0.041**
Yes	147 (75.6%)	74 (50.3%)	73 (49.7%)	
No	62 (24.4%)	40 (64.5%)	22 (35.5%)	
Venous invasion				*p*=0.175
Negative	167 (79.9%)	95 (56.9%)	72 (43.1%)	
Positive	42 (20.1%)	19 (45.2%)	23 (54.8%)	
Histology type				***p*=0.010**
Ad	98 (46.9%)	64 (65.3%)	34 (34.7%)	
SCC	102 (48.8%)	47 (46.1%)	55 (53.9%)	
LCC	9 (4.3%)	3 (33.3%)	6 (66.7%)	
Differentiation				*p*=0.350
Poor	99 (47.4%)	49 (49.5%)	50 (50.5%)	
Moderate	97 (46.4%)	58 (59.8%)	39 (40.2%)	
Well	13 (6.2%)	7 (53.8%)	6 (46.2%)	
TNM stage				***p*=0.045**
I	64 (30.6%)	41 (64.1%)	23 (35.9%)	
II/IIIa	145 (69.4%)	73 (50.3%)	72 (49.7%)	

P-value was calculated using Pearson's χ^2 test. Bold values are significant (P<0.05). Ad – adenocarcinoma; SCC – squamous cell carcinoma; LCLC – large cell lung cancer.

for these lung cancer patients. Clinicopathologic characteristics of the studied cohort are summarized in Table 2; these patients were diagnosed as stage I–IIIa lung cancer disease with median age of 58 years old. Our data showed that there was no significant correlation between PD-L1 expression and gender, age, venous invasion, and differentiation of these patients (Table 2). Higher PD-L1 expression frequencies was shown in patient smokers, SCC and LCLC types, and stage II/III compared to those in patient nonsmokers, Ad type, and stage I, respectively with statistically significant difference (Table 2).

PD-L1 expression was associated with poor prognosis

We analyzed the correlation between PD-L1 expression and disease free progression (DFS) and overall survival (OS) for these 209 lung cancer patients. Our results demonstrated that relatively higher PD-L1 expression rate was seen in patients with shorter DFS than in patients with longer DFS (Figure 3A, *p*=0.03). Importantly, PD-L1 expression correlated with poor OS in this cohort, with statistically significant difference (Figure 3B, *p*=0.007). Moreover, both univariate and multivariate proportional hazard Cox analyses suggested that PD-L1 (HR, 1.726; 95% CI, 1.157–2.575; *p*=0.007), TNM stage (HR, 2.286; 95% CI, 1.415–3.692; p=0.001), and tumor size (HR, 1.670; 95% CI, 1.108–2.516; *p*=0.014) are strong and independent predictors of prognosis for lung cancer patients (Table 3).

Discussion

Lung cancer is an aggressive disease with a five-year survival rate ranging from 6% to 18% [22]. Most lung cancers are diagnosed at an advanced stage with bad outcomes compared to early stage disease. Thus, effective screening modalities for detecting early stage lung cancer are needed. It has been known that computed tomography (CT) screening improved

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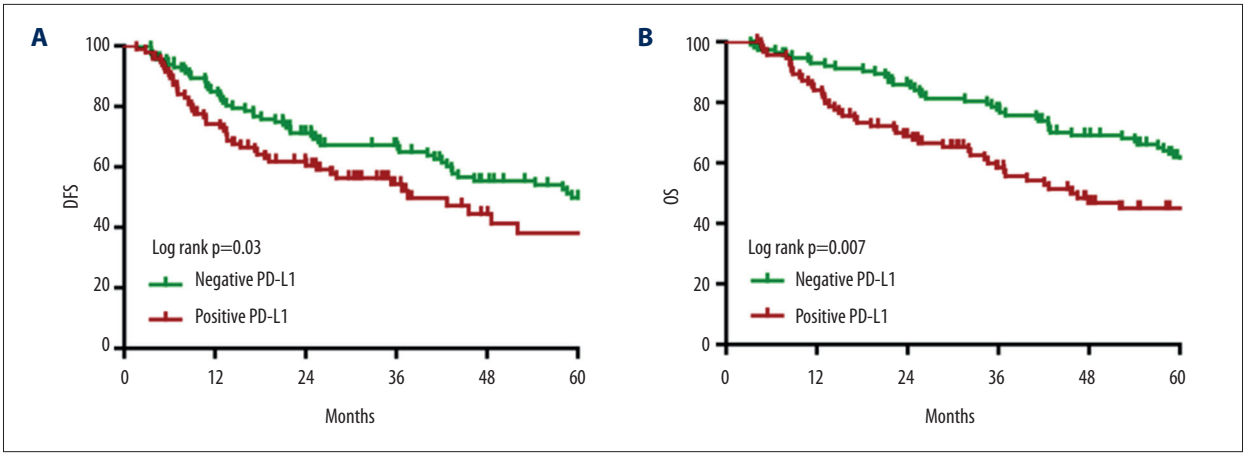

Figure 3. Association between PD-L1 expression and DFS and OS in 209 cases of lung cancer patients. (**A**) Higher expression rate of PD-L1 was observed in patients with shorter DFS (p=0.03). (**B**) Kaplan-Meier survival curve demonstrates that PD-L1 positive expression was associated with poor OS (p=0.007).

Table 3. Univariate and multivariate analysis of overall survival in 209 of the patients.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Gender	0.937 (0.595–1.47)	0.776		
Age	0.707 (0.482–1.070)	0.104		
Smoking history	1.236 (0.811–1.916)	0.313		
Venous invasion	0.821 (0.491–1.330)	0.413		
History type		0.110		
Differentiation		0.075		
TNM stage	0.463 (0.337–0.755)	**0.001**	2.286 (1.415–3.692)	**0.001**
PD-L1	0.589 (0.375–0.847)	**0.0027**	1.726 (1.157–2.575)	**0.007**

P-value was calculated using Pearson's χ^2 test. Bold values are significant (P<0.05). HR – hazard ratio (log-rank); CI – confidence interval.

long-term survival by identifying the increased number of early stage lung cancer patients [23]. However, the CT scan with associated radiation can induce long-term cancer risk as well [24]. Therefore, we attempted to find a new predictor or prognostic factor for lung cancer patients from a different perspective.

Among the suppressive molecules, PD-L1 combined with PD-1 has been proven to contribute to tumor cells' immune-escape and also has been associated with cancer patient outcomes [25,26]. However, there is little known about the role of PD-L1 in tumor formation and clinical significance of early stage lung cancer.

PD-L1 binding to PD-1 on activated T cells can induce T cell apoptosis [25,27] that might be the mechanism driving cancer cells to overexpress PD-L1 in response to immune attacks [28].

In our current study, we focused on the role of PD-L1 in lung cancer cells. We tested how PD-L1 influenced the ability of proliferation and apoptosis in lung cancer cell lines PC9 and H520. Cell growth was decreased and apoptosis was increased when PD-L1 was knocked down in these cells. Apoptosis has been identified to play an import role in malignant properties like drug response in lung cancer [29,30]. It is well known that the MAPK pathway is involved in proliferation of cancer cells. In melanoma cells, MEK inhibitor or ERK1/2 siRNAs function to reduce PD-L1 expression [31]. MAPK signal pathway has been found to result in increased expression of PD-L1 by oncogenic activation of EGFR in lung cancer [32]. PD-L1 promotion of proliferation of lung cancer cells might be through MAPK signal pathway and associated with apoptosis.

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We identified how PD-L1 influenced tumor growth *in vivo*. It is worth mentioning that the PDX models, which are the most valuable tools for preclinical evaluation of novel therapeutic strategies for cancer, have closely related genetic features with the primary tumors of our study patients. For lung cancer, approximately 30–40% of PDX formation was observed [2]. In our current study, 37.2% (16 out of 43 cases) of PDX formations from lung cancer tissues was obtained. It has also been reported that PDX formation is associated with disease recurrence in patients with early stage lung cancer [3]. Higher frequencies of PDX formation were observed in patients with older age, male sex, and SCC type compared with patients with younger age, female sex and Ad type. We identified higher PD-L1 expression rates in the PDX formed tumors than in the PDX unformed tumors. A 1.9-fold increase of frequency of PDX formation was found in the tumors with PD-L1 expression compared to the tumors without this protein expression. These results indicated that the lung cancer PDX model could provide a preclinical model to investigate mechanisms of responsiveness and even resistance to drugs of PD-L1 inhibitors.

We looked at the correlation between PD-L1 expression and clinicopathological characteristics. The primary tumor tissues from 209 cases of lung cancer patients were evaluated by immunohistochemistry for detection of PD-L1. Among the 95 cases of positive PD-L1 expression, we observed 35 tumors with membrane location of this protein, one tumor with cytoplasm expression, and 59 tumors with both membrane and cytoplasm staining. Since there were a limited number for each group for the different locations, we divided PD-L1 expression into two groups of positive and negative expression. Previous studies found higher frequency of PD-L1 expression in smoker compared to non-smoker lung cancer patients [33,34]. Consistently, more smoker lung cancer patients showed PD-L1 expression compared with nonsmoker patients. In summary, PD-L1 could be induced by smoking. It has been reported that higher expression of PD-L1 was observed in stage IV lung cancer patients compared to stage I–III patients [33]. Among the early stage lung cancer patients in our current study, the patients with stage II/IIIa showed higher expression rates compared to patients with stage I disease. In addition, PD-L1 expression varied among different types of histology for Ad, SCC, and LCLC with significant differences.

PD-L1 overexpression has been correlated with poor prognosis in melanoma [4,26], cervical [35], esophageal [14,36], gastric [16,37], and colorectal cancers [38]. In a meta-analysis of different cohorts of lung cancer patients, there was no significant association between PD-L1 expression and five-year OS [39]. Velcheti et al. reported PD-L1 expression was related to longer survival in both Greek University (291 cases) and Yale University (153 cases) and mRNA analyses (288 cases from Greek University, and 112 cases from Yale University) [40]. In contrast, the study by Mu et al. showed that high expression of PD-L1 might be a poor prognostic factor in lung cancer patients from the Soochow University in the south of China (n=109) [26]. In addition, Yang et al. demonstrated that patients with positive PD-L1 expression had longer recurrence free disease than those with negative PD-L1 expression, but the OS between the two groups had no significant difference (n=163, from Taiwan University) [41]. In our study, we found positive PD-L1 expression was associated with poor outcome for DFS and OS in 209 lung cancer patients at Peking University Cancer Hospital of northern China. All in all, the relationship between PD-L1 expression and prognosis varied between different cohorts. More studies of lung cancer patient populations are needed.

Conclusions

PD-L1 induced PDX formation of lung cancer tissue and positive expression of this protein could predict poor prognosis for lung cancer patients.

References:

1. Siegel RL, Miller KD, Jemal A: Cancer statistics, 2016. Cancer J Clin, 2016; 66: 7–30
2. Moro M, Bertolini G, Tortoreto M et al: Patient-derived xenografts of non small cell lung cancer: Resurgence of an old model for investigation of modern concepts of tailored therapy and cancer stem cells. J Biomed Biotechnol, 2012; 2012: 568567
3. John T, Kohler D, Pintilie M et al: The ability to form primary tumor xenografts is predictive of increased risk of disease recurrence in early-stage non-small cell lung cancer. Clin Cancer Res, 2011; 17: 134–41
4. Taube JM, Anders RA, Young GD et al: Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med, 2012; 4: 127ra137
5. Bai ZP, Deng XS: [Effect of oleum fructus brucease injection via bronchial arterial infusion in treating advanced lung cancer]. Zhongguo Zhong Xi Yi Jie He Za Zhi, 2010; 30: 838–40
6. Hsu YL, Wu CY, Hung JY et al: Galectin-1 promotes lung cancer tumor metastasis by potentiating integrin alpha6beta4 and Notch1/Jagged2 signaling pathway. Carcinogenesis, 2013; 34: 1370–81
7. Muto S, Owada Y, Inoue T et al: Clinical significance of expanded Foxp3(+) Helios(–) regulatory T cells in patients with non-small cell lung cancer. Int J Oncol, 2015; 47: 2082–90
8. Srivastava MK, Zhu L, Harris-White M et al: Targeting myeloid-derived suppressor cells augments antitumor activity against lung cancer. Immunotargets Ther, 2012; 2012: 7–12
9. Haroon M, Bond U, Phelan M, Regan M: How are we doing in reviewing those new patients who need to be seen as early as possible? An audit of 264 consecutive new patients seen over 6 months in a university hospital in Ireland. J Rheumatol, 2009; 36: 454–55; author reply 455
10. Hao J, Shi FD, Abdelwahab M et al: Nicotinic receptor beta2 determines NK cell-dependent metastasis in a murine model of metastatic lung cancer. PLoS One, 2013; 8: e57495

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

11. Dong H, Strome SE, Salomao DR et al: Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. Nat Med, 2002; 8: 793–800
12. Freeman GJ, Long AJ, Iwai Y et al: Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med, 2000; 192: 1027–34
13. Sznol M, Chen L: Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. Clin Cancer Res, 2013; 19: 1021–34
14. Ohigashi Y, Sho M, Yamada Y et al: Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res, 2005; 11: 2947–53
15. Thompson RH, Kuntz SM, Leibovich BC et al: Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res, 2006; 66: 3381–85
16. Wu C, Zhu Y, Jiang J et al: Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. Acta Histochem, 2006; 108: 19–24
17. Brahmer JR, Tykodi SS, Chow LQ et al: Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med, 2012; 366: 2455–65
18. Tamura H, Ishibashi M, Yamashita T et al: Marrow stromal cells induce B7-H1 expression on myeloma cells, generating aggressive characteristics in multiple myeloma. Leukemia, 2013; 27: 464–72
19. Cutz JC, Guan J, Bayani J et al: Establishment in severe combined immunodeficiency mice of subrenal capsule xenografts and transplantable tumor lines from a variety of primary human lung cancers: Potential models for studying tumor progression-related changes. Clin Cancer Res, 2006; 12: 4043–54
20. Merk J, Rolff J, Becker M et al: Patient-derived xenografts of non-small-cell lung cancer: A pre-clinical model to evaluate adjuvant chemotherapy? Eur J Cardiothorac Surg, 2009; 36: 454–59
21. Zhang L, Zhang J, Ma Y et al: Testicular orphan receptor 4 (TR4) is a marker for metastasis and poor prognosis in non-small cell lung cancer that drives the EMT phenotype. Lung Cancer, 2015; 89: 320–28
22. Youlden DR, Cramb SM, Baade PD: The international epidemiology of lung cancer: Geographical distribution and secular trends. J Thorac Oncol, 2008; 3: 819–31
23. Church TR, Black WC, Aberle DR et al: Results of initial low-dose computed tomographic screening for lung cancer. N Engl J Med, 2013; 368: 1980–91
24. Puri S, Hu R, Quazi RR et al: Physicians' and midlevel providers' awareness of lifetime radiation-attributable cancer risk associated with commonly performed CT studies: relationship to practice behavior. Am J Roentgenol, 2012; 199: 1328–36
25. Butte MJ, Keir ME, Phamduy TB et al: Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity, 2007; 27: 111–22
26. Mu CY, Huang JA, Chen Y et al: High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. Med Oncol, 2011; 28: 682–88
27. Francisco LM, Salinas VH, Brown KE et al: PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med, 2009; 206: 3015–29
28. 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
29. Zhang J, Yang Y, Lei L, Tian M: Rhizoma paridis saponins induces cell cycle arrest and apoptosis in non-small cell lung carcinoma A549 cells. Med Sci Monit, 2015; 21: 2535–41
30. Zhu X, Jiang H, Li J et al: Anticancer effects of paris saponins by apoptosis and PI3K/AKT pathway in gefitinib-resistant non-small cell lung cancer. Med Sci Monit, 2016; 22: 1435–41
31. Jiang X, Zhou J, Giobbie-Hurder A et al: The activation of MAPK in melanoma cells resistant to BRAF inhibition promotes PD-L1 expression that is reversible by MEK and PI3K inhibition. Clin Cancer Res, 2013; 19: 598–609
32. Chen N, Fang W, Zhan J et al: Upregulation of PD-L1 by EGFR activation mediates the immune escape in EGFR-driven NSCLC: Implication for optional immune targeted therapy for NSCLC patients with EGFR mutation. J Thorac Oncol, 2015; 10: 910–23
33. Calles A, Liao X, Sholl LM et al: Expression of PD-1 and its ligands, PD-L1 and PD-L2, in smokers and never smokers with KRAS-mutant lung cancer. J Thorac Oncol, 2015; 10: 1726–35
34. Koh J, Go H, Keam B et al: 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
35. Karim R, Jordanova ES, Piersma SJ et al: Tumor-expressed B7-H1 and B7-DC in relation to PD-1+ T-cell infiltration and survival of patients with cervical carcinoma. Clin Cancer Res, 2009; 15: 6341–47
36. Chen L, Deng H, Lu M et al: B7-H1 expression associates with tumor invasion and predicts patient's survival in human esophageal cancer. Int J Clin Exp Pathol, 2014; 7: 6015–23
37. Geng Y, Wang H, Lu C et al: Expression of costimulatory molecules B7-H1, B7-H4 and Foxp3+ Tregs in gastric cancer and its clinical significance. Int J Clin Oncol, 2015; 20: 273–81
38. Shi SJ, Wang LJ, Wang GD et al: B7-H1 expression is associated with poor prognosis in colorectal carcinoma and regulates the proliferation and invasion of HCT116 colorectal cancer cells. PLoS One, 2013; 8: e76012
39. Wu P, Wu D, Li L et al: PD-L1 and survival in solid tumors: A meta-analysis. PLoS One, 2015; 10: e0131403
40. Velcheti V, Schalper KA, Carvajal DE et al: Programmed death ligand-1 expression in non-small cell lung cancer. Lab Invest, 2014; 94: 107–16
41. Yang CY, Lin MW, Chang YL et al: 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: 1361–69

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