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Original Article

Distribution of PD-L1 expression and its relationship with clinicopathological variables: an audit from 1071 cases of surgically resected non-small cell lung cancer

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Abstract: Background: Programmed death ligand 1 (PD-L1) was reported to predict the response of immunotherapy; however, the association between PD-L1 expression and clinicopathologic characteristics has yet to be elucidated in non-small cell lung cancer (NSCLCs). Materials and methods: We reviewed PDL1 expression investigated by immunohistochemical analysis using FFPE tissue in a total of 1071 cases of primary or metastatic NSCLC tissues analyzed between 2015-2017, and evaluated the association between PD-L1 expression and the clinicopathologic characteristics. Results: PD-L1 expression was observed in 361 (33.7%) cases with positive staining in at least 1% tumor cells and 116 (10.8%) cases had positive staining in ≥50% tumor cells. The PD-L1 positive prevalence was significantly higher in squamous cell carcinoma (SCC) than in adenocarcinoma (AD). In the AD subgroup, PD-L1 expression on tumors was higher in males and smokers, and with high histologic grade, relative high T, N, M status, advanced AJCC stage, and in ALK rearrangement patients. However, EGFR mutated patients showed relatively lower PD-L1 expression than wild type patients. Conclusion: This study revealed the unique distribution of PD-L1 expression with clinicopathologic features in East Asian NSCLCs in a single, large cohort of patients. Since immunohistochemistry of the PD-L1 protein (PD-L1 IHC) is the only clinically approved predictive biomarker for anti-PD-1/-PD-L1 therapy currently, our outcomes could help to stratify patients to ensure selection of those who would most benefit from PD-1/PD- L1 inhibitor therapy.

Keywords: PD-L1, PD-1, non-small cell lung cancer (NSCLC), immunotherapy, immune checkpoint

Introduction

Recent clinical trials have demonstrated that inhibitors of programmed death 1 (PD-1) and its primary ligand, programmed cell death ligand 1 (PD-L1) confer a survival benefit for non-small cell lung cancer (NSCLC) patients compared with conventional standard therapy [1-7]. The PD-1/PD-L1 axis is an inhibitory signaling pathway that leads to T cell exhaustion and inactivation, thus preventing autoimmune and anti-tumor responses [8]. Therefore, inhibition of such immune checkpoints has emerged as a novel and promising therapeutic option in the treatment of lung cancer. Tumor expression of PD-L1, as measured by immunohistochemistry (IHC), is reported to be a predictive marker for most patients receiving anti-PD-1/PD-L1 therapy, including NSCLC [9].

Most studies of PD-1/PD-L1 expression in NSCLC to date have been small, especially studies of Chinese populations [10-15]. Moreover, the clinicopathological features associated with PD-1/PD-L1 expression have not yet been clearly defined, with some results remaining controversial [10-15]. Here, we examined PD-L1 expression in a large cohort of 1071 patients with surgically resected NSCLC. Our primary aim was to characterize the pattern of tumor PD-L1 expression by IHC. The secondary aim was to investigate the association between PD-L1 expression and various clinicopathologic features, including age, gender, smoking, stage, histologic subtype, grade, and common driver mutations. The results may assist in stratifying the most qualified patients for immunotherapy and enrollment in clinical trials of PD-1/PD-L1 checkpoint inhibitors in NSCLC.

Materials and methods

Patients and tissue samples

A total of 1071 cases of NSCLC, which were submitted for PD-L1 expression analysis in Pathology department of West China hospital from January 2015 to June 2017, were included in this study. The specimens used for PDL1 expression analysis were from primary lung cancer in 1014 cases and metastatic lesions in 57 cases, which comprised formalin-fixed paraffin-embedded tissue from surgically resected specimens. Patients who received prior radiotherapy, chemotherapy and targeted therapy were excluded. The histologic diagnosis of these cases was made according to the 2015 WHO classification [16]. Staging was undertaken according to the 8th edition AJCC tumor, node, metastasis (TNM) classification [17]. Other clinicopathologic features including age at surgery, sex, smoking history, tumor grade, histologic subtype, EGFR and ALK mutation status, were collected from medical records. Never smokers are patients who smoked less than 100 cigarettes in their lifetime and ex-smokers quit smoking at least a year before surgery.

PDL1 immunohistochemical analysis

Immunohistochemistry was performed in 1071 patients with surgically resected NSCLC using formalin-fixed tissue sections, with a thickness of 4 μm, using standard autostaining protocols on a fully automated slide stainer (Leica Bond automated system, the United Kingdom). The primary antibody was an antihuman PD-L1 rabbit monoclonal antibody (clone SP142, dilution 1:100; ZSGB-BIO). Carcinoma cells showing membranous staining for PD-L1 were evaluated as positive cells. The proportion of PD-L1 positive cells was estimated as the percentage of total carcinoma cells. PD-L1 immunostaining results were defined as follows based on the tumor proportion score (TPS): (1) negative, when staining was absent or detected in <1% of the cells and otherwise referred as positive; (2) low expression, when membranous staining was present in 1%~49% of the cells; and (3) high expression, when membranous staining was present in ≥50% of the cells. 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.

Mutation analysis

A total of 437 cases underwent EFGR mutation analysis, carried out with Amplification Refractory Mutation System technology using the ADx EGFR Mutations Detection Kit (Amoy Diagnostics, China) which had been approved for clinical application by the State Food and Drug Administration in China. The assay was performed using a BIO-RAD CFX96 machine according to the manufacturer's instructions. A total of 1042 cases underwent ALK assessment using immunohistochemistry with the D5F3 rabbit anti-human monoclonal antibody (Ventana Medical Systems Inc), combined with an OptiView Amplification Kit and an OptiView DAB IHC Detection Kit (Ventana Medical Systems Inc). The entire staining procedure was performed on a fully automated BenchMark XT slide stainer (Ventana Medical Systems Inc). All uncertain cases subsequently underwent FISH analysis for confirmation of ALK rearrangement using an LSI ALK Dual Color Break Apart Rearrangement Probe (Abbott Molecular, USA).

Statistical analysis

Statistical analysis was performed using Graph Pad software online. Associations between PDL1 expression and various pathologic characteristics were analyzed using a chi-square test. The Fisher exact test was performed if there were five or fewer observations in a group. In all tests, two-sided *p*-values of less than 0.05 were considered significant.

Results

Clinicopathologic characteristics of patients

The characteristics of the patients included in this study are summarized in **Table 1**. The median age was 59 years (range, 23-85) and the number of male and female patients was similar (582 and 497, respectively). More than half of the patients (56.8%) were non-smokers. Most tumor specimens for IHC were from primary lung cancer (1014, 94.7%), with the remaining samples from metastatic lesions in the brain, lymph node, bone, liver, and adrenal gland (57, 5.3%). The latter group included cases with unknown pathologic T status (57, 5.3%) and N status (48, 4.5%). More than half of the patients (599, 55.9%) had stage I dis-

Table 1. Patient characteristics

Characteristics	Number of Patients (n=1071), n (%)
Age, years	
Median (range)	59.0 (23-85)
Sex	
Male	575 (53.7)
Female	496 (46.3)
Smoking status	
Never smoker	608 (56.8)
Current smoker	151 (26.8)
Ex-smoker	287 (14.1)
Unknown	25 (2.3)
Specimens resources	
Primary lung cancer	1014 (94.7)
Metastatic lesions	57 (5.3)
AJCC stage	
I	599 (55.9)
II	120 (11.2)
III	248 (23.2)
IV	104 (9.7)
T status	
Specimens from primary lesions	1014 (94.7)
T1	501 (46.8)
T2	336 (31.4)
T3	80 (7.5)
T4	97 (9.0)
Specimens from metastatic lesions	57 (5.3)
TX	57 (5.3)
N status	
Specimens from primary lesions	1014 (94.7)
N0	698 (65.2)
N1	107 (10.0)
N2	199 (18.6)
N3	10 (0.9)
Specimens from metastatic lesions	57 (5.3)
N2	3 (0.3)
N3	6 (0.5)
NX	48 (4.5)
Metastasis status	
M0	967 (90.3)
M1	104 (9.7)
Histologic type	
Adenocarcinoma	847 (79.1)
High grade	276 (32.6)
Intermediate grade	406 (47.9)
Low grade	149 (17.6)
No classification	16 (1.9)
Squamous cell carcinoma	183 (17.1)
Keratinizing	75 (41.0)
Non-keratinizing	108 (59.0)
Adenosquamous carcinoma	13 (1.2)
Neuroendocrine tumor	12 (1.1)
Large cell carcinoma	4 (0.4)
Sarcomatoid carcinomas	3 (0.3)
Lymphoepithelioma-like carcinoma	7 (0.7)
Adenoid cystic carcinoma	2 (0.2)

ease and 104 (9.7%) had stage IV metastatic disease.

The 1071 NSCLC cases comprised 847 adenocarcinomas (ADCs), 183 squamous cell carcinomas (SCCs), 13 adenosquamous carcinomas (ASCs), 12 neuroendocrine tumors (NETs; 10 large cell neuroendocrine carcinomas, 1 combined large cell neuroendocrine carcinoma, and 1 carcinoid tumor), 4 large cell carcinomas, 3 sarcomatoid carcinomas, 7 lymphoepithelioma-like carcinomas, and 2 adenoid cystic carcinomas. Among the ADCs, 276 (32.6%), 406 (47.9%), and 149 (17.6%) were poorly, moderately, and well differentiated, and 16 (1.9%) were unclassified subtypes (13 invasive mucinous ADCs, 2 fetal ADCs, and 1 enteric ADC). The SCCs consisted of 75 (41.0%) keratinizing and 108 (59.0%) non-keratinizing subtypes.

PD-L1 expression profile

Expression of PD-L1 was quantified using the tumor proportion score (TPS), which is defined as the percentage of tumor cells showing PD-L1 staining by IHC. In total, 33.7% of the 1071 tumors stained positive for PD-L1 (positivity defined as TPS≥1%) and 10.8% were classified as high expression (TPS>50%). Among the tumor subgroups, the number of cases with positive (≥1% TPS), low (1-50% TPS), and high (>50% TPS) PD-L1 expression was 221 (26.1%), 159 (18.8%), and 106 (7.3%), respectively, for ADC; and 121 (66.1%), 64 (43.2%), and 42 (22.9%), respectively, for SCC. Thus, positive PD-L1 expression (≥1% TPS) was observed in a higher proportion of SCCs than ADCs, ASCs, or NETs (**Figure 1A**), and the same trend was observed for high PD-L1 expression (>50% TPS; **Figure 1B**), although the difference was not statistically significant, possibly because of the low number of ASC and NET samples. The limited sample sizes precluded comparison of TPS for the remaining subgroups. Despite the low number of cases, we noted that the large cell, sarcomatoid, and lymphoepithelioma-like carcinomas tended to express high levels of PD-L1 (**Figure 1C** and **1D**). Representative images of PD-L1 expression in SCC and ADC samples are shown in **Figure 2**.

Correlation between PD-L1 expression and clinicopathologic features in ADC

Positive (≥1% TPS) and high (>50% TPS) PD-L1 expression in ADC was significantly associated

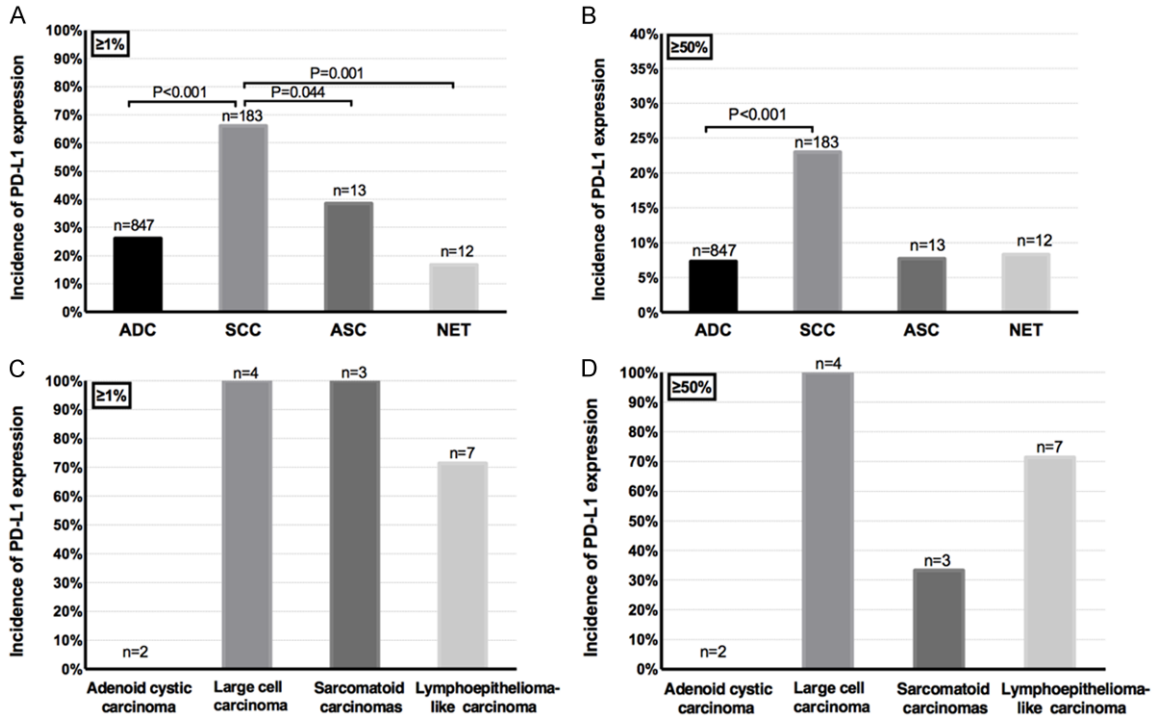

Figure 1. (A, B) Comparison of PD-L1 expression in subgroups of ADC, SCC, ASC and NET at cut-off value 1% (A) and 5% (B). (Only $P \leq 0.1$ is labeled in Figure). (C, D) PD-L1 expression in subgroups of adenoid cystic carcinoma, large cell carcinoma, sarcomatoid carcinoma, and lymphoepithelioma-like carcinoma at cut-off value 1% (C) and 5% (D).

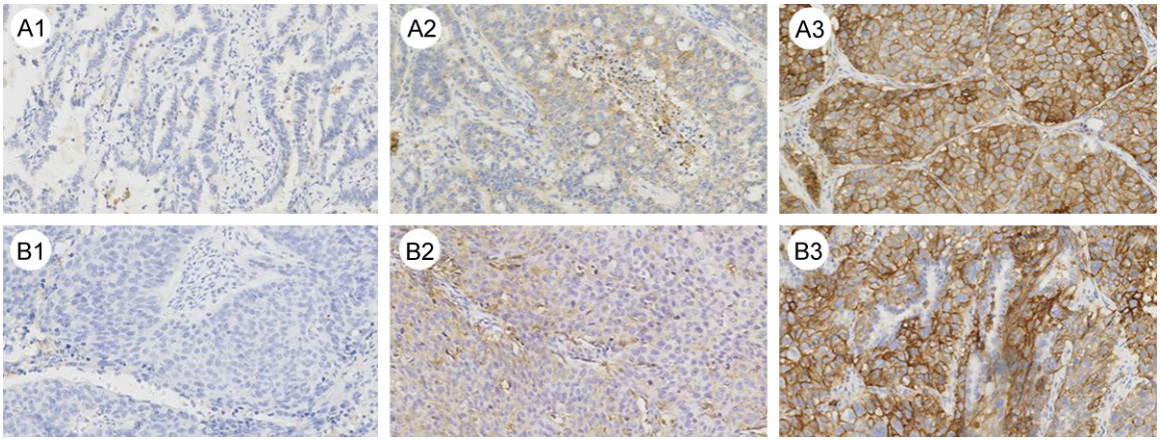

Figure 2. Representative images of PD-L1 expression in ADC (A1-A3) and SCC (B1-B3). (A1, B1: PD-L1 negative; A2, B2: low PD-L1 expression; A3, B3: high PD-L1 positive; magnification ×20).

with male gender (**Figure 3A-C**). In addition, the absolute TPS was higher for current or formers smokers compared with never smokers, whereas there was no significant difference between TPS for the former and current smokers (**Figure 3D** and **3E**). However, more current than former or never smokers had tumors expressing high PD-L1 levels (TPS>50%). Here too, there was no significant difference between the former and never smoker groups (**Figure 3F**). One possible explanation for the higher proportion and absolute level of positive PD-L1 expression among the males compared with females is that most (93.3%) of the male patients but only 13.3% of the female patients were smokers (**Figure 3J**). Among the different age groups, there were no significant differences in PD-L1 expression.

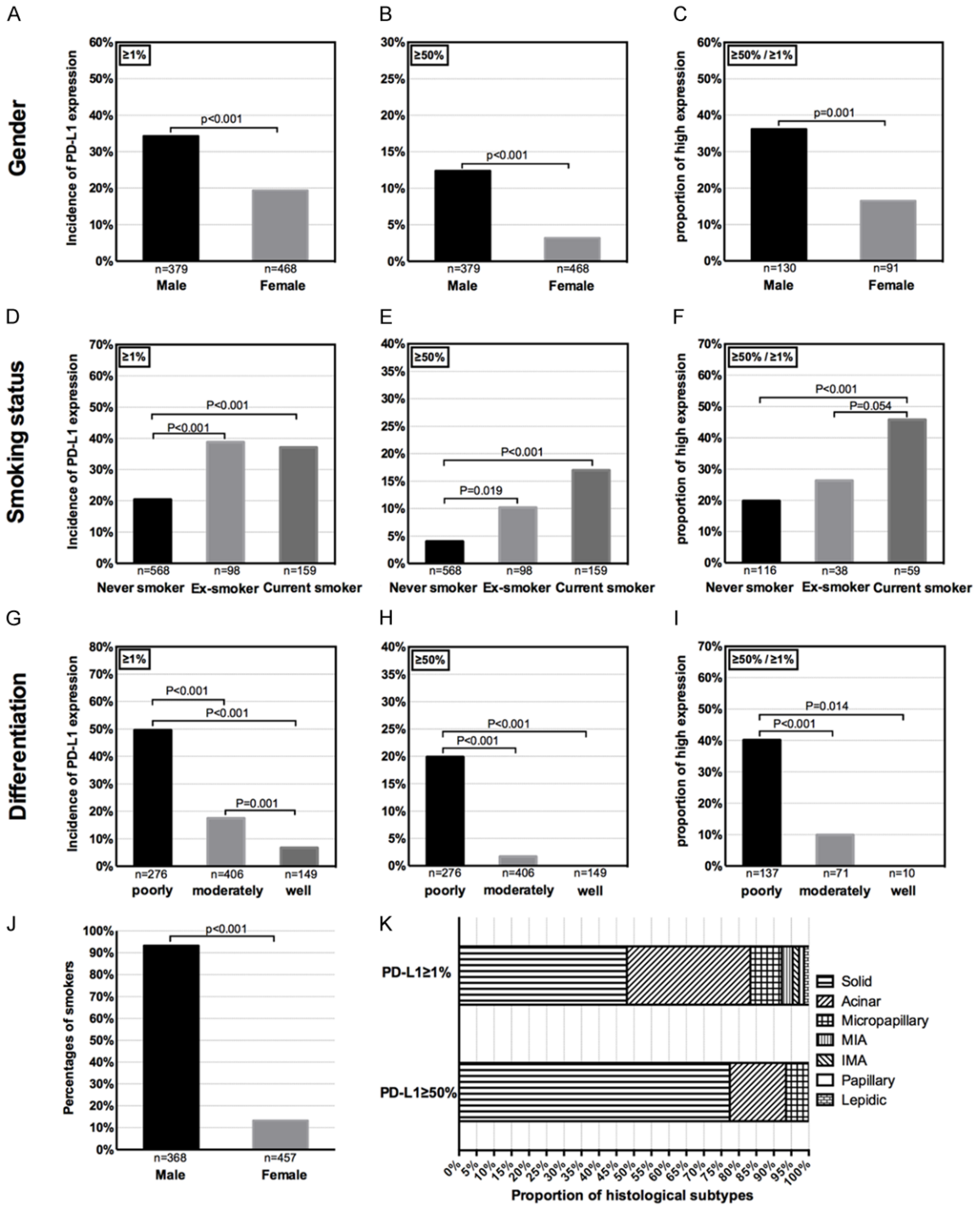

Figure 3. Association of PD-L1 expression in tumor cells with gender, smoking, and differentiation in patients with ADC according to cut-off value at 1% (A, D, G: ≥1% vs ≤1%) and 5% (B, E, H: ≥50% vs ≤50%) and the proportion of high expression cases among positive ones (C, F, I: ≥50% vs ≥1%). (J) Comparison of male and female patient smoking rates in ADC. (K) Histologic subtype components of ADC in which PD-L1 TPS≥1% and TPS≥50%. (MIA: microinvasive adenocarcinoma; IMA: invasive mucinous adenocarcinoma; Only P≤0.1 is labeled in Figure).

Positive PD-L1 expression in ADC was associated with histologic grade, with the rate being highest in poorly differentiated tumors, followed by moderately and well differentiated tumors (**Figure 3G** and **3H**). Similarly, high PD-L1 expression was significantly associated with high tumor grade (**Figure 3I**). Histological analysis of all positively stained ADCs indicated

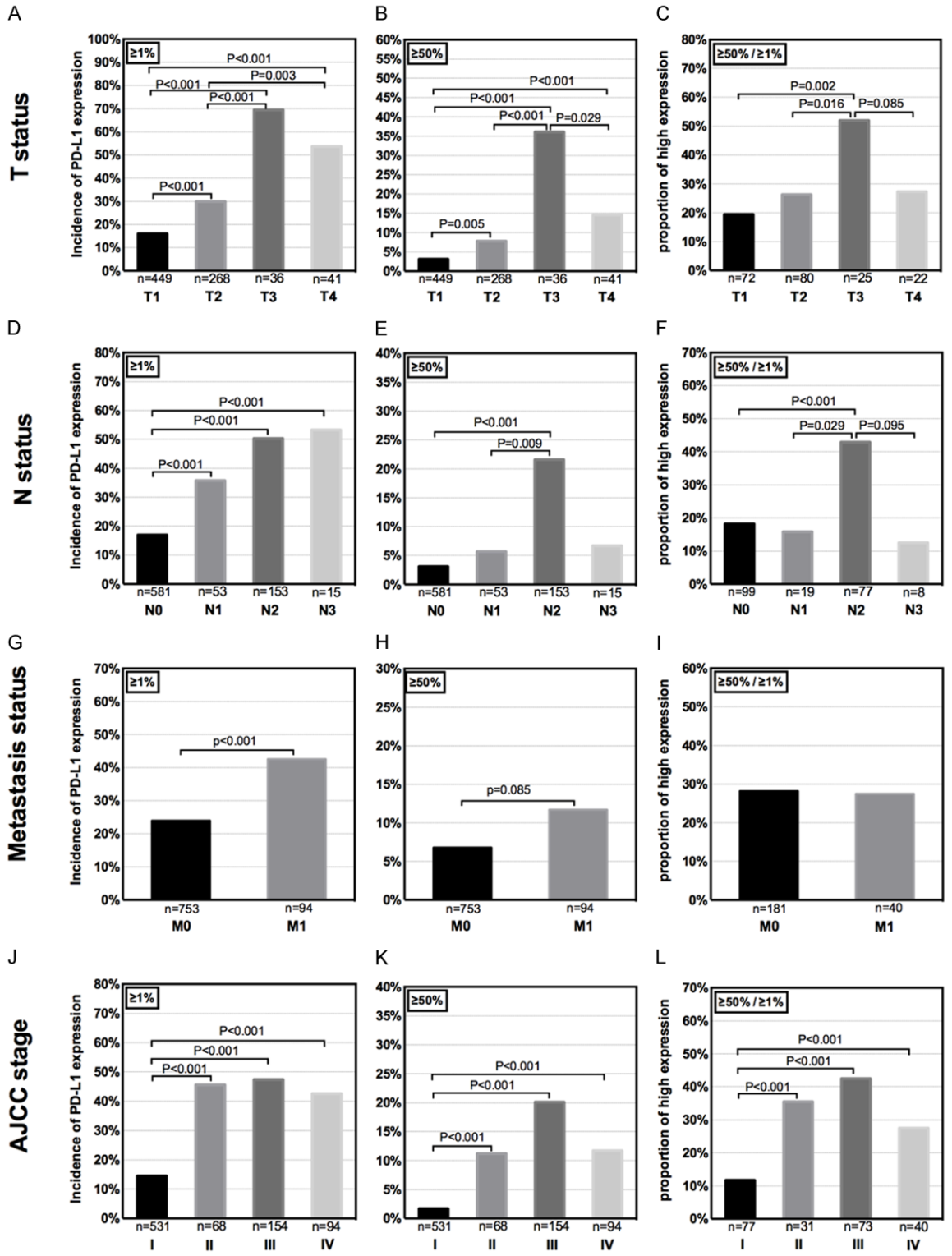

Figure 4. Association of PD-L1 expression on tumor cells with T, N, M status and AJCC stage in patients with ADC according to cut-off value at 1% (A, D, G, J: ≥1% *vs* ≤1%) and 5% (B, E, H, K: ≥50% *vs* ≤50%) and the proportion of high expression cases among positive ones (C, F, I, L: ≥50% *vs* ≥1%). (Only $P \leq 0.1$ is labeled in Figure).

that PD-L1 was mainly distributed in solid and acinar subtype growth patterns, followed by micropapillary pattern. For the high PD-L1-expressing tumors, the staining patterns were

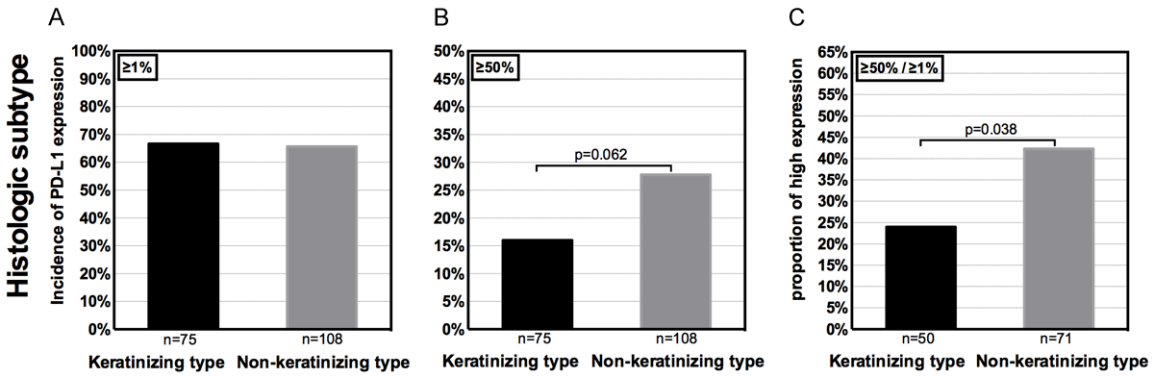

Figure 5. Association of PD-L1 expression in tumor cells with histologic subtype in patients with SCC according to cut-off value at 1% (A: ≥1% *vs* ≤1%) and 5% (B: ≥50% *vs* ≤50%) and the proportion of high expression cases among positive ones (C: ≥50% *vs* ≥1%). (Only $P \leq 0.1$ is labeled in Figure).

predominantly the solid subtype followed by acinar and micropapillary subtypes (**Figure 3K**).

We next correlated PD-L1 expression with tumor extension (T), lymph node involvement (N), and the presence of distant metastasis (M) according to the American Joint Committee on Cancer (AJCC) stages. More T3 and T4 than T1 and T2 status tumors were positive for PD-L1 (≥1%), and high expression was most commonly observed among the T3 tumors (>50%) (**Figure 4A-C**). Interestingly, significantly more patients with lymph node metastasis than without showed positive PD-L1 staining, irrespective of N status, but high PD-L1 expression was most common among patients with N2 tumors (**Figure 4D-F**). More patients with distant metastases than without had PD-L1-positive tumors, although this was significant at the ≥1% but not the >50% cut-off level (**Figure 4G** and **4H**) but there was no significant difference regarding high expression/low expression ratios of positive cases (**Figure 4I**). In the analysis of tumor stage, more advanced stage (II-IV) tumors than stage I tumors showed positive PD-L1 and high PD-L1 expression, but there were no differences among stage II, III, and IV with respect to positivity or the level of expression (**Figure 4J-L**).

Correlations between PD-L1 expression and clinicopathological features in SCC

Unlike the ADC tumors, PD-L1 expression in SCCs was not significantly related to age, gender, smoking history, T, N, M, or tumor stage. Among the SCC histologic subtypes, the rate of positive staining of non-keratinizing and keratinizing SCCs was approximately the same, whereas marginally more non-keratinizing compared with keratinizing SCCs showed high (>50%) PD-L1 expression (**Figure 5A**). Nevertheless, non-keratinizing SCCs showed a marginal tendency to be PD-L1 stained high more commonly when comparing high expression groups with low/negative groups (cut-off 50%) (**Figure 5B**). In addition, among all SCC positive cases, non-keratinizing SSCs showed a significantly higher proportion of high expression cases than keratinizing ones (**Figure 5C**).

Association between high PD-L1 expression, EGFR mutation status, and ALK translocation status

The expression of major driver oncogenes in the NSCLC patients is shown in **Table 2**. Of the 457 (42.7%) patients whose epidermal growth factor receptor (EGFR) mutation status was available, 256 (56.0%) harbored EGFR mutations. EGFR analysis was performed on 421 (49.7%) ADC patients; of these, 171 (40.6%) carried wild-type EGFR, 231 (54.9%) harbored mutations sensitive to tyrosine kinase inhibitor therapy (deletion in exon 19 and L858R), and 19 (5.4%) had other rare mutations. In contrast, all 19 (10.4%) SCC patients analyzed harbored wild-type EGFR. PD-L1 expression was significantly more common in ADC patients with wild-type than mutated EGFR when assessed at both the ≥1% cut-off value (35.1% vs 22.8%, P=0.006) and >50% cut-off value (12.9% vs 3.2%, P<0.001). Also, patients of wild type EGFR status had a greater proportion of high expression cases (36.7% vs 14.0%, P=0.005) (**Table 2**).

Table 2. Patient driver oncogene status

Major driver oncogenes	n (%)	PD-L1 expression				*P*
		≥1%	*P*	≥50%	*P*	
EGFR status	457	137 (30.0)		37(8.1)		
Adenocarcinoma	421 (92.1)		0.006*		<0.001*	0.005*
Wild type	171 (40.6)	60 (35.1)		22 (12.9)		
L858R	117 (27.8)	22 (18.8)		2 (1.7)		
Exon 19 deletion	114 (27.1)	29 (25.4)		5 (4.4)		
Others†	19 (4.5)	6 (31.6)		1 (5.3)		
Squamous cell carcinoma	19 (4.2)					
Wild type	19 (9.5)	13 (68.4)		5 (26.3)		
Adenosquamous carcinoma	8 (1.8)					
Wild type	3 (1.5)	1 (33.3)		0		
L858R	2 (40.0)	1 (50.0)		0		
Exon 19 deletion	2 (40.0)	1 (50.0)		0		
T790M+L858R	1 (20.0)	0		0		
Lymphoepithelioma-like carcinoma	2 (0.4)					
Wild type	1 (50.0)	1 (50.0)		1 (50.0)		
Exon 19 deletion	1 (50.0)	0		0		
Other tumors	7 (1.5)					
Wild type	7 (100)	3 (42.9)		1 (14.3)		
ALK status	1034	346 (33.5)		115 (11.1)		
Adenocarcinoma	825 (77.8)		0.038*		0.505	0.222
Wild type	788 (95.5)	199 (25.3)		59 (7.5)		
Rearrangement	37 (4.5)	15 (40.5)		3(8.1)		
Squamous cell carcinoma	172 (16.6)					
Wild type	170 (98.8)	113 (66.5)		41 (24.1)		
Rearrangement	2 (1.2)	1 (50.0)		0		
Adenosquamous carcinoma	13 (1.3)					
Wild type	11 (84.6)	3 (27.3)		1 (50.0)		
Rearrangement	2 (15.4)	2 (100)		0		
Neuroendocrine tumor	11 (1.1)			0		
Wild type	10 (90.9)	2 (20.0)		1 (10.0)		
Rearrangement	1 (9.1)	0		0		
Other tumors	13 (1.3)					
Wild type	13 (100)	11 (84.6)		10 (76.9)		

*Significant *P* values. †Insertion in exon 20 (n=6), G719X (n=5), L861Q (n=4), L858R+T790M (n=1), 19Del+T790M (n=2), G719X+T790M (n=1).

Of the 1034 patients whose ALK mutation status was available, only 42 (4.1%) harbored an ALK translocation. The 1034 patients included 825 (97.4%) with ADC, of whom 37 (4.5%) were positive for the ALK translocation, and 172 (94%) with SCC, among whom only 2 (1.1%) were positive. PD-L1 expression was significantly more common among the ALK translocation-positive compared with the -negative group at the ≥1% cut-off (40.5% *vs* 25.3%, *P*=0.038) but not the >50% cut-off. There was also no significant relationship between ALK translocation status and the ratio of low (≥1%-50%) and high (>50%) PD-L1-expressing tumors (**Table 2**). No further analyses were performed because of limited sample sizes.

Discussion

Immunotherapy for lung cancer is evolving quickly and has the potential to revolutionize cancer treatment [18, 19]. Previous clinical tri-

als have shown impressive overall response rates to antibodies to PD-1 and PD-L1 in patients with NSCLC [1-7]. Currently, PD-L1 protein detection by IHC is the only clinically approved predictive biomarker for anti-PD-1/-PD-L1 therapy [9]. However, the prevalence of PD-L1 expression in patients with different clinicopathologic parameters has not been fully defined. Further clinical studies are needed to assess the predictive value of tumor PD-L1 expression in patients with defined clinicopathologic features to select those most suitable for PD-1/PD-L1 checkpoint blockade therapy.

Here, we retrospectively assessed tumor PD-L1 expression and its correlation with clinicopathologic variables in a large cohort of patients with surgically resected NSCLC. Overall, PD-L1 expression (≥1% TPS) was detected by IHC in 33.7% of patients and strong expression (>50% TPS) was detected in 10.8%, which is similar to a previous IHC analysis using clone SP142 anti-PD-L1 antibody [15]. PD-L1 expression was much greater in SCC compared with ADC tumors, suggesting that East Asian SCC patients may be more suitable for PD-1/PD-L1 immunotherapy. In general, PD-L1 expression in ADC was associated with male gender, smoking history, wild-type EGFR, high histological grade, high AJCC stage, large tumor size, lymph node metastasis, and distant metastasis. However, we found no association between PD-L1 expression and age.

We found that PD-L1 expression was markedly higher in ADC specimens from smokers compared with never smokers, but there was no significant difference between former and current smokers. In addition, more current smokers expressed high levels of PD-L1 compared with former and never smokers. These observations could be explained by the pro-inflammatory effects of smoking on the immune system [20]. Alternatively, smoking-induced carcinomas typically carry a high mutational burden and express neoantigens that can trigger antitumor immune responses [21]. Consequently, aberrant activation of PD-L1 expression on tumor cells might counteract the host immune response. Importantly, a high tumor mutational burden has been shown to be associated with smoking and better efficacy of PD-1 inhibitors [22]. Other studies also reported that smoking history was related to better responses to immunotherapy [23, 24], which may suggest the inflammatory processes and immune response statuses of smokers and that effects might be more rigorous in current smokers. In addition, it could to some extent explain why high PD-L1 expression was more prevalent among SCC than ADC patients, since more SCC patients were smokers (86.7% vs 31.2%, respectively).

In our study, PD-L1 expression was positively associated with male gender, which could be a consequence of the higher incidence of cigarette smoking among males. Alternatively, it might reflect the influence of hormones, as suggested by the finding that a high PD-L1 immunoscore negatively correlates with hormone receptor expression in breast cancer [25], which estrogen and progesterone had less effect. Poorly differentiated ADC showed much higher PD-L1 expression than did moderately or well differentiated tumors, and there was also a high concordance between high grade tumors and the solid-predominant subtype, which is in line with previous work [10]. One earlier study showed that the density of tumor-associated inflammatory cells was higher in ADCs with solid tumor histology compared with other histologicsubtypes [26], and these cells might stimulate tumor PD-L1 expression. We also found positive staining mainly distributed in tumor areas with acinar subtype, which has not previously been reported. In general, solid-predominant ADCs carry no targetable (e.g., EGFR) mutations [27], suggesting that patients with this subtype ADC might be good candidates for immunotherapy. Tumor-mediated suppression of host immunity has been associated with tumor burden in several models [28], which could, at least to some extent, explain our finding that PD-L1 expression is associated with high T, N, and M status and with tumor stage. Thus, PD-L1 is a potential marker of disease progression. Consistent with this, PD-L1 expression has been shown to be significantly associated with radiologic and pathologic invasive tumors and high maximum standardized uptake value by ^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography [29].

Unexpectedly, we saw no significant association between PD-L1 expression and any clinical variables in the SCC group, except that the non-keratinizing subtype SCCs expressed marginally higher PD-L1 expression. Further work will be

needed to understand the factors associated with PD-L1 expression in SCC.

There is increasing evidence that oncogenic EGFR activation drives immune evasion; for example, high PD-L1 levels in bronchial epithelial cells in vitro are associated with expression of mutant EGFR [30]. In contrast, however, we found lower PD-L1 expression in NSCLC tumors carrying mutant EGFR compared with those carrying wild-type EGFR. There are many possible explanations for these disparate findings, including the complexity of signaling pathways in vivo. Other studies have reported that mutant EGFR is associated with low response rates to PD-1 pathway blockade, and combination immunotherapy and EGFR-targeted therapy increases the risk of side effects [31-34]. Based on the results of our study, we suggest that anti-PD-L1 therapy may provide better outcomes for lung ADC patients expressing wild-type compared with mutant EGFR. Interestingly, we found that wild-type EGFR was also associated with smoking history, male gender, and less tumor differentiation. In contrast, PD-L1 expression was positively associated with ALK rearrangement. It is possible that ALK-mediated activation of the STAT3 pathway might contribute to PD-L1 expression in these tumors [33].

To date, clinical trials of PD-1/PD-L1-targeted therapies have employed a number of IHC assays using various cut-off values to define positive PD-L1 expression. A TPS score of >50% predicted significantly prolonged survival in patients with NSCLC treated with pembrolizumab compared with cytotoxic chemotherapy [3, 6]. The United States Food and Drug Administration has approved pembrolizumab for treatment of patients with NSCLC as follows: (a) first-line treatment for patients with metastatic NSCLC, tumor PD-L1 TPS of >50%, and no genomic EGFR or ALK tumor aberrations, or (b) treatment of patients with metastatic NSCLC, tumor PD-L1 TPS of >1%, and disease progression on or after platinum-containing chemotherapy [34]. Another anti-PD-L1 antibody, nivolumab, showed better clinical outcomes than cytotoxic chemotherapy in patients with SCC, regardless of PD-L1 expression [2]. However, nivolumab efficacy was highest in patients with non-SCC NSCLC and tumor PD-L1 TPS of ≥1% [1]. Nivolumab has also been approved in the United States for the treatment of metastatic NSCLC patients with disease progression on or after platinum-containing chemotherapy [35]. Based on these findings, we selected TPS≥1% and >50% as the cut-off values to assess correlations between PD-L1 expression and clinicopathologic features in the present study.

The limitations of this study include its retrospective design and the primary antibody used for PD-L1 IHC. There is currently no standardization of IHC antibodies or cut-off values to define positive PD-L1 expression in tumors. However, a strength of our study was the selection of surgically resected NSCLC specimens to assess PD-L1 expression, given that they are superior to biopsy samples often used in the diagnosis of advanced NSCLC.

In conclusion, our data from this large cohort of NSCLC patients confirm the correlation between PD-L1 expression and certain clinicopathological parameters in East Asian patients. To our knowledge, this is the most comprehensive analysis of the largest number of surgically resected samples of NSCLC in China. Although the molecular mechanisms underlying the relationships between PD-L1 expression and the clinicopathologic parameters are still unclear, further studies will undoubtedly advance our understanding. The outcomes of our study may help to stratify patients to ensure selection of those who would most benefit from PD-1/PD-L1 inhibitor therapy.

Disclosure of conflict of interest

None.

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