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Original Article

PD-L1 Expression in Non-Small-Cell Lung Cancer Including Various Adenocarcinoma Subtypes

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Purpose: Knowledge regarding programmed death-ligand 1 (PD-L1) expression in lung cancer is limited. We aim to clarify PD-L1-positive expression in non-small-cell lung cancer (NSCLC), including adenocarcinoma subtypes.
Methods: In all, 90 NSCLC specimens containing various adenocarcinoma subtypes, in addition to squamous cell carcinoma and large-cell carcinoma were selected. PD-L1 was immunohistochemically stained by murine monoclonal antibody clone 22C3.
Results: When PD-L1-positive expression was defined by tumor proportion score (TPS) ≥1%, the positive cases were 0/11 in adenocarcinoma in situ, 0/12 in minimally invasive adenocarcinoma, 1/10 in lepidic predominant adenocarcinoma, 1/13 in papillary predominant adenocarcinoma, 8/14 in acinar predominant adenocarcinoma, 6/11 in solid predominant adenocarcinoma, 0/3 in micropapillary predominant adenocarcinoma, 0/4 in invasive mucinous adenocarcinoma, 4/9 in squamous cell carcinoma, and 2/3 in large-cell carcinoma. PD-L1 positivity was higher in males, smokers, advanced pathologic stages, positive vessel invasion, and positive lymphatic invasion. Postoperative survival analysis revealed that PD-L1-positive expression was a significantly worse prognostic factor in univariate analysis for recurrence-free survival (RFS).
Conclusion: PD-L1-positive tumors were frequent in acinar predominant adenocarcinoma and solid predominant adenocarcinoma than other adenocarcinoma subtypes. PD-L1 expression seemed to increase according to pathologic tumor progression, suggesting a worse postoperative prognosis in NSCLC patients.

Keywords: lung cancer, early adenocarcinoma, subtype, immunohistochemistry, programmed cell death 1, programmed death-ligand 1

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Introduction

Lung cancer is the leading cause of cancer deaths in most developed countries worldwide.[1)] Despite multidisciplinary therapies that have been used for patients with advanced non-small-cell lung cancer (NSCLC), the overall survival (OS) rates are still poor. Recently, several humanized monoclonal antibodies to block immune checkpoints have been developed and have proven to be useful in some selected patients with unresectable NSCLCs.[2,3)] The association between programmed cell death 1 (PD-1) and programmed death-ligand 1 (PD-L1) can target these monoclonal antibodies. Inhibition of

the PD-1/PD-L1 axis enhances antitumor immunity to prevent tumor cells from escaping from host immune responses, providing a promising strategy for effective tumor immunotherapy.[4]

Pembrolizumab, an anti-PD1 antibody has shown significant improvements in both OS and progression-free survival in first-line treatment compared with conventional chemotherapy in advanced NSCLC patients when PD-L1 positivity in the tumor cells was ≥50%.[5] Second-line treatment may be also effective when PD-L1-positive tumor cells exist ≥1%.[6] Therefore, PD-L1 expression status is critical to effectively treat by pembrolizumab in select patients. However, to date, there is limited knowledge regarding the association between PD-L1 expression and various clinicopathologic factors. Thus, we aim to clarify the PD-L1 expression and several clinicopathologic factors using resected lung cancer specimens.

Materials and Methods

The Ethics Committee of St. Marianna University School of Medicine approved this study (accession No 1461), and written informed consent was obtained from all included patients. Pathological specimens (hematoxylin-and-eosin-stained slides) of NSCLC patients who underwent surgery from 2008 to 2014 were reviewed independently by two pathologists (M.T. and M.H.), to determine the histologic type and the adenocarcinoma (Ad) subtype based on the World Health Organization (WHO) pathologic classification published in 2015.[7] Pathologists blinded from clinical information selected specimens to identify histologic types in NSCLC including various Ad subtypes. Selected cases were Ad in situ (AIS) in 11, minimally invasive Ad (MIA) in 12, lepidic predominant Ad (LPA) in 10, papillary predominant Ad (PPA) in 13, acinar predominant Ad (APA) in 14, solid predominant Ad (SPA) in 11, invasive mucinous Ad (IMA) in 4, and micropapillary predominant Ad (MPA) in 3. In addition to these 78 Ads, 9 squamous cell carcinomas (Sqs) and 3 large-cell carcinomas (Las) were selected to evaluate PD-L1 expression. Patients included 37 males and 53 females (age range: 46–81 years; mean: 66.5). The TNM stages of patients were determined according to the international staging criteria for lung cancer that were published by the International Association for the Study of Lung Cancer (IASLC) in 2009.[8] Clinical stages were c-IA in 64, c-IB in 22, c-IIA in 2, and c-IIIA in 2. Postoperative pathologic stages were p-IA in 53, p-IB in 13, p-IIA in 4, p-IIIA in 10, and undetermined in 10 due to sublobar resection without lymph node dissection. The postoperative mean follow-up period of the patients was 41 ± 21 (mean ± standard deviation [SD]) months.

Immunohistochemistry

Immunohistochemistry was performed using the PD-L1 kit (PD-L1 IHC 22C3 pharmDX; Dako, Carpinteria, CA, USA) according to the manufacturer's instructions. This antibody was selected since the Food and Drug Administration (FDA) approved this system as a companion diagnostic test to determine the applicability of treatment using pembrolizumab. In brief, serial 3-μm thick tissue sections were cut from formalin-fixed, paraffin-embedded blocks. Sections were deparaffinized in xylene and rehydrated through a graded series of ethanol concentrations. Antigen retrieval was carried out by 97°C water bath for 20 min in Envition FLEX Target Retrieval solution (Dako). Intrinsic peroxidase activity was blocked using hydrogen peroxide for 5 min. After washing the section with a Wash Buffer (Dako), primary antibodies were applied to cover the specimen. Sections were incubated at room temperature for 30 min. After three washes in the wash buffer for 5 min each, slides were incubated with anti-mouse linker antibody specific to the host species of the primary antibody, and then were incubated with a ready-to-use visualization reagent consisting of secondary antibody molecules and horseradish peroxidase molecules coupled to a dextran polymer backbone. Specimens were then counterstained with hematoxylin for 5 min and cover-slipped.

Assessment of PD-L1 expression

We obtained the final results according to the manual on "PD-L1 immunohistochemistry testing in lung cancer" reported by the IASLC.[9] PD-L1-positive tumor cells were counted by authors MH, TM, and HN. Positive tumor cells were defined as complete circumferential or partial cell membrane staining. Cytoplasmic staining was excluded from the scoring. Furthermore, tumor-associated immune cells such as macrophages were excluded from scoring. Finally, scoring was recorded as a percentage of PD-L1-positive tumor cells over the total tumor cells; tumor proportion score (TPS). Staining status was classified by TPS into three groups; <1% (negative staining), ≥1% and <49% (weakly positive), and ≥50% (highly positive). All tumors showing TPS ≥1% were considered positive expression. Representative staining is displayed in **Fig. 1A–1C**.

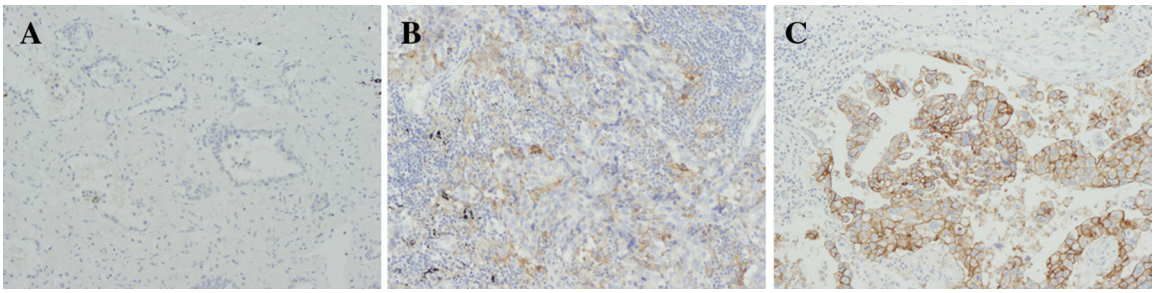

Fig. 1 Representative photograph of PD-L1 expression and TPS. (**A**) TPS <1% (negative staining) ×10, (**B**) TPS 1 to 49% (weakly positive) ×10, and (**C**) TPS ≥50% (highly positive) ×10. TPS: tumor proportion scores; PD-L1: programmed death-ligand 1

Statistical analysis

Statistical analyses for clinicopathologic characteristics by categorical variables were evaluated using the chi-squared test. OS and recurrence-free survival (RFS) were analyzed by the Kaplan–Meier method, and differences in survival rates were compared by univariate analysis using the log-rank test. Cox regression analysis was used for multivariate analysis for survival. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander to add statistical function frequently used in biostatistics.[10] A p value of <0.05 was considered statistically significant in all tests.

Results

Clinicopathological characteristics and the percentages of PD-L1-positive expression (TPS ≥1%) patients are shown in **Table 1**. PD-L1 positivity in Sq (44%) and La (67%) were larger than in Ad (21%), with a marginal significance (p = 0.064). Limited to Ad subtypes, PD-L1 positivity in APA (57%) and SPA (55%) were higher than in AIS (0%), MIA (0%), LPA (10%), PPA (8%), MPA (0%), and IMA (0%), showing significant uneven distributions (p = 0.015). Concerning other factors, PD-L1 positivity was higher in males (p = 0.001), smokers (p = 0.027), advanced pathologic stages ≥IIIA (p = 0.018), positive venous invasion (p = 0.001), and positive lymphatic invasion (p = 0.011). However, age (≥65 or <65), clinical stage (c-IIIA-IIIB or c-IA-IIB), pathologic nodal status (pN1-3 or pN0), pleural invasion (p1-3 or p0), and the status of the epidermal growth factor receptor (EGFR) mutation were not associated with PD-L1 expression.

Univariate analysis revealed that males (p = 0.040), smoking habit (p = 0.011), advanced clinical stages (p = 0.014), advanced pathologic stages (p = 0.004), pathologic nodal metastasis (p = 0.002), positive venous invasion (p = 0.010), positive pleural invasion (p <0.001), and intrapulmonary metastasis (p <0.001) were significantly worse prognostic factors for OS (**Table 2**). Significant RFS differences in univariate analysis were observed among the Ad subtypes (p = 0.002), and among histologic types in NSCLC (p = 0.018). Moreover, advanced pathologic stages (p = 0.019), pathologic nodal metastasis (p <0.001), positive venous invasion (p <0.001), positive lymphatic invasion (p <0.001), positive pleural invasion (p <0.001), intrapulmonary metastasis (p <0.001), and positive PD-L1 expression (p = 0.043) were significantly worse prognostic factors for RFS in univariate analysis. OS and RFS curves classified by PD-L1 positivity are shown in **Fig. 2A and 2B**.

Multivariate analysis revealed that pleural invasion (p = 0.045) was an independently worse prognostic factor for OS, and venous invasion (p = 0.009), pleural invasion (p = 0.029), and intrapulmonary metastasis (p = 0.003) were independently worse prognostic factors for RFS (**Table 3**).

Discussion

Many study results regarding PD-L1 protein expression in NSCLC have been reported. However, these studies occasionally showed conflicting results, and it is difficult to obtain common consensus regarding the relationship between the status of PD-L1 expression and various clinicopathologic factors. There are several plausible reasons to explain discrepancies observed in theses previous studies. Heterogeneities among the reported studies might be due to 1) anti-PD-L1 antibodies used,

Table 1 Frequencies of PD-L1-positive (≥1%) tumors in 90 patients with non-small-cell lung cancer

Characteristics	n	PD-L1 highly positive ≥50% (%)	PD-L1 positivity (%)	p
Gender				
Male	37	7 (19)	16 (43)	
Female	53	1 (2)	6 (11)	0.007*
Age (years)				
≥65	54	6 (11)	15 (28)	
<65	36	2 (6)	7 (19)	0.456
Smoking status				
Current/Former	46	6 (13)	16 (35)	
Never	44	2 (5)	6 (14)	0.027*
Histologic type				
Ad	78	7 (9)	16 (21)	
AIS	11	0	0	
MIA	12	0	0	
LPA	10	0	1 (10)	
PPA	13	0	1 (8)	
APA	14	5 (36)	8 (57)	
SPA	11	2 (18)	6 (55)	
MPA	3	0	0	
IMA	4	0	0	0.015* (Ad subtype)
Sq	9	0	4 (44)	
La	3	1 (33)	2 (67)	0.064 (Ad vs Sq vs La)
Clinical stage				
c-IA-IIB	66	8 (12)	22 (33)	
c-IIIA-IIIB	24	0	7 (29)	0.530
Pathologic stage				
p-IA-IIB	70	4 (6)	15 (21)	
p-IIIA-IIIB	10	4 (40)	6 (60)	0.018*
Not examined**	10	0	1 (10)	
Pathologic nodal status				
p-N0	67	4 (6)	15 (22)	
p-N1-3	13	4 (31)	6 (46)	0.092
Not examined**	10	0	1 (10)	
Venous invasion				
vo	75	6 (8)	13 (17)	
v1	15	2 (13)	9 (60)	0.001*
Lymphatic invasion				
ly0	72	6 (8)	13 (18)	
ly1	18	2 (11)	9 (50)	0.011*
Pleural invasion				
p0	74	5 (7)	16 (22)	
p1-3	16	3 (19)	6 (38)	0.206
Pulmonary metastasis				
pm0	88	7 (8)	21 (24)	
pm1-2	2	1 (50)	1 (50)	0.431
EGFR mutations				
Positive	37	2 (5)	7 (19)	
Negative	43	6 (14)	11 (26)	0.594
Not examined***	10	0	4 (40)	

*statistically significant; **pathologic nodal information could not be obtained in 10 cases undergoing limited resection without lymph node dissection; ***EGFR mutation was not examined in 10 cases; AIS: adenocarcinoma in situ; MIA: minimally invasive adenocarcinoma; LPA: lepidic-predominant invasive adenocarcinoma; PPA: papillary-predominant invasive adenocarcinoma; APA: acinar-predominant invasive adenocarcinoma; SPA: solid-predominant invasive adenocarcinoma; MPA: micropapillary predominant invasive adenocarcinoma; IMA: invasive mucinous adenocarcinoma; Ad: adenocarcinoma; Sq: squamous cell carcinoma; La: large-cell carcinoma; PD-L1: programmed death-ligand 1; EGFR: epidermal growth factor receptor

Table 2 Univariate analysis according to log-rank test for overall and recurrence-free survivals

Characteristics	n	5-year OS (%)	p	5-year RFS (%)	p
Gender					
Male	37	64.3		57.3	
Female	53	87.7	0.040*	67.9	0.539
Age (years)					
≥65	54	74.3		56.1	
<65	36	83.5	0.335	76.1	0.108
Smoking status					
Current/Former	46	63.7		61.3	
Never	44	91.2	0.011*	65.6	0.504
Histologic type					
Adnocarcinoma	78	80.6		63.8	
AIS	11	100		100	
MIA	12	100		100	
LPA	10	NR		NR	
PPA	13	90.9		61.5	
APA	14	70.7		43.7	
SPA	11	53.7		40.9	
MPA	3	66.7		NR	
IMA	4	NR	0.155 (adenocarcinoma subtype)	NR	0.002* (adenocarcinoma subtype)
Squamous cell carcinoma	9	66.7		77.8	
Large-cell carcinoma	3	NR	0.666 (adenocarcinoma vs squamous vs large)	NR	0.018* (adenocarcinoma vs squamous vs large)
Clinical stage					
c-IA-IIB	88	78.4		64.1	
c-IIIA-IIIB	2	NR	0.014*	NR	0.065
Pathologic stage**					
p-IA-IIB	70	79.8		65.1	
p-IIIA-IIIB	10	50.0	0.004*	23.3	0.019*
Pathologic nodal status**					
p-N0	67	81.8		68.5	
p-N1-3	13	44.0	0.002*	17.1	<0.001*
Venous invasion					
vo	75	83.7		73.8	
v1	15	51.1	0.010*	20.0	<0.001*
Lymphatic invasion					
ly0	72	82.2		76.9	
ly1	18	59.5	0.191	19.4	<0.001*
Pleural invasion					
p0	74	84.4		71.5	
p1-3	16	45.8	<0.001*	24.9	<0.001*
Pulmonary metastasis					
pm0	88	79.5		65	
pm1-2	2	NR	<0.001*	NR	<0.001*
EGFR mutations***					
Positive	37	83.4		50.6	
Negative	43	78.0	0.574	72.3	0.050
PD-L1					
Positive	22	68.2		43.6	
Negative	68	81.3	0.195	71.6	0.043*

*statistically significant; **pathologic nodal information could not be obtained in 10 cases undergoing limited resection without lymph node dissection; ***EGFR mutation was not examined in 10 cases; AIS: adenocarcinoma in situ; MIA: minimally invasive adenocarcinoma; LPA: lepidic predominant invasive adenocarcinoma; PPA: papillary predominant invasive adenocarcinoma; APA: acinar predominant invasive adenocarcinoma; SPA: solid predominant invasive adenocarcinoma; MPA: micropapillary predominant invasive adenocarcinoma; IMA: invasive mucinous adenocarcinoma; PD-L1: programmed death-ligand 1; OS: overall survival; RFS: recurrence-free survival; EGFR: epidermal growth factor receptor; NR: not reached

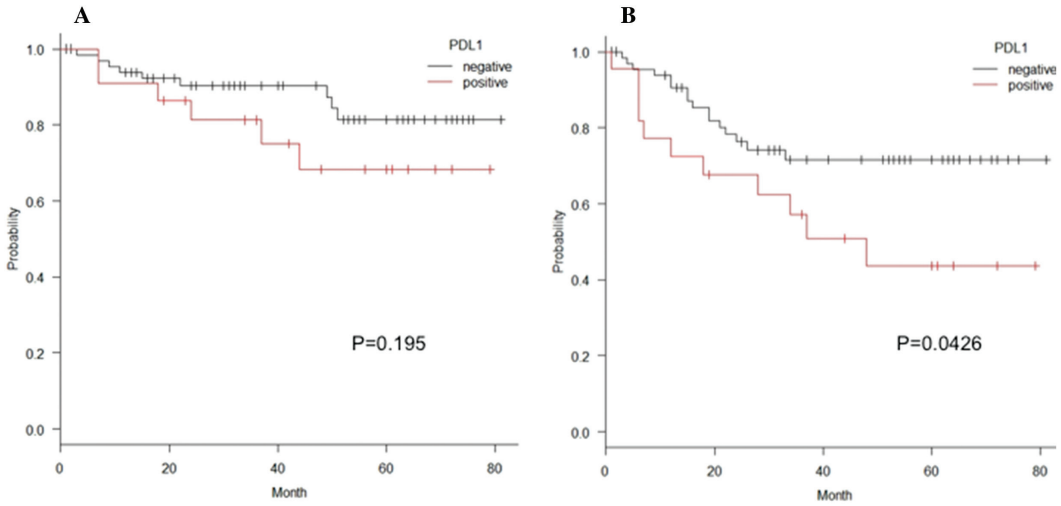

Fig. 2 Kaplan–Meier curves showing OS and RFS. (**A**) Kaplan–Meier curves showing OS. Patients with tumors with negative PD-L1 expression had slightly longer survival compared to patients with tumors of positive PD-L1 expression. There was no significant difference by log-rank test (p = 0.195). (**B**) Kaplan–Meier curves showing patients with negative PD-L1 expression had significantly longer RFS compared to patients with positive PD-L1 expression (p = 0.0426). OS: overall survival; RFS: recurrence-free survival; PD-L1: programmed death-ligand 1

2) evaluation methods of immunostained tumor cells, 3) definition of the positivity, 4) percentages of the contained histologic types such as Sq/Ad and Ad subtypes, 5) percentages of the included disease stages such as resectable/unresectable, and early stages/advanced stages, and 6) ethnicities of the enrolled patients. To minimize the technological problems of immunohistochemistry and the evaluation methods, we used companion diagnostics for pembrolizumab, murine 22C3 anti-human PD-L1 monoclonal antibody, which have been accepted by FDA to select patients suitable for pembrolizumab therapy, according to the guidelines recently published by IASLC.

Several previous studies reported associations between the percentages of PD-L1-positive tumors and histologic types although the criteria for PD-L1 positivity differed in each study. Janzic et al. assessed the resected tumors and reported that PD-L1-positive (TPS ≥5%) cases were more frequently found in Sq (52%) than in Ad (17%).[11] Scheel et al. examined specimens from patients with NSCLC and found that positive cases (TPS ≥1%) were 34% in Sq and 34% in Ad, indicating no differences between the two histologic types.[12] Lin et al. compared the PD-L1 positivity using the same criteria as Scheel et al. and showed that the positivity was higher in Sq (46%) than in Ad (27%).[12,13] Cooper et al. had reported the frequency of the high PD-L1 expression (TPS ≥50%); 8% in Sq, 12% in La, and 5% in Ad.[14] Our present study confirmed that PD-L1-positive (TPS ≥1%) lung cancers were more frequent in Sq (44%) or La (67%) than Ad (21%) with marginal significance.

In Ad subtypes, we were unable to find PD-L1-positive tumors in both AIS (n = 11) and MIA (n = 12). Since AIS and MIA are considered very early-phase Ads that usually show very slow growth, these subtypes might be in the status where PD-1/PD-L1 pathway might not function yet. Although only two tumors (9%) were positive for LPA (n = 10) and PPA (n = 13), 14 tumors (56%) were positive in APA (n = 14) and SPA (n = 11), suggesting that these subtypes frequently activated PD-1/PD-L1 pathways that lead to the suppression of anti-tumor immunity. There are only a few studies showing PD-L1 expression in Ad subtypes. Zhang et al. analyzed AIS (n = 1), MIA (n = 6), LPA (n = 8), PPA (n = 27), APA (n = 64), SPA (n = 32), MPP (n = 1), IMA (n = 3), and Enteric (n = 1), finding PD-L1-positive tumors in 0% in AIS and MIA, 46% in LPA and PPA, and 54% in APA and SPA, concluding that positive PD-L1 staining was less likely in AIS and MIA and more likely in SPA.[15] Igarashi et al. evaluated PD-L1 expression in Ad subtypes using an original scoring system (H-score), which resulted in no differences among the subtypes.[16] Our results were concordant with the study by Zhang et al.

The association between PD-L1 positivity and various clinicopathologic factors also remained unclear. Multiple meta-analyses were performed to clarify PD-L1 expression

Table 3 Multivariate analysis according to the Cox regression analysis for overall and recurrence-free survivals

Characteristics	HR (95% CI)	p
OS		
Gender (male vs female)	1.24 (0.27–5.69)	0.778
Smoking status (smoker vs nonsmoker)	3.09 (0.57–16.79)	0.190
Clinical stage (≥IIIA vs <IIIA)	1.79 (0.11–29.34)	0.682
Pathologic stage (≥IIIA vs <IIIA)**	0.71 (0.13–3.98)	0.701
Venous invasion (v1 vs vo)	3.67 (0.87–15.47)	0.077
Pleural invasion (pl1-3 vs pl0)	4.65 (1.04–20.83)	0.045*
Pulmonary metastasis (pm1-2 vs pm0)	3.91 (0.27–56.76)	0.318
RFS		
Pathologic stage (≥IIIA vs <IIIA)**	0.85 (0.28–2.61)	0.782
Venous invasion (v1 vs vo)	5.30 (1.53–18.40)	0.009*
Lymphatic invasion (ly1 vs ly0)	1.69 (0.52–5.50)	0.385
Pleural invasion (pl1-3 vs pl0)	2.72 (1.11–6.69)	0.029*
Pulmonary metastasis (pm1-2 vs pm0)	28.62 (3.15–260.40)	0.003*
PD-L1 (TPS <1% vs TPS ≥1%)	0.61 (0.22–1.71)	0.346

*statistically significant; **pathologic nodal information could not be obtained in 10 cases undergoing limited resection without lymph node dissection. OS: overall survival; RFS: recurrence-free survival; HR: hazard ratio; CI: confidence interval; TPS: tumor proportion score; PD-L1: programmed death-ligand 1

in NSCLC and associated factors. However, conflicting results were shown among the meta-analyses. Pan et al. combined 1550 NSCLC patients from nine studies and found that high PD-L1 expression was associated with poor tumor differentiation alone, and that no other factors (gender, smoking status, histologic type, invasive depth, lymph nodal metastasis, and disease stage) were associated with PD-L1 expression.[17] In contrast, Zhang et al. combined 11,444 lung cancer patients from 47 studies, showing PD-L1 expression increased in males, smokers, Sq histologic type, higher histologic grades, larger tumor sizes, positive lymph nodal metastasis, and advanced disease stages.[18] We found similar results as Zhang et al. for meta-analysis in most clinicopathologic factors; and that factors indicating tumor progression tended to increase PD-L1 expression.[18]

Regarding PD-L1 expression and EGFR mutation, there were conflicting study results. Some studies reported that PD-L1 positivity was higher in NSCLC patients carrying the EGFR mutation, and some reported that PD-L1 positivity was higher in EGFR wild-type.[19–22] Other studies reported no association between PD-L1 and EGFR.[14,23,24] Our study also showed no association.

Thus far, the reported data on the prognostic role of PD-L1 expression in NSCLCs are conflicting. Some previous studies reported that high PD-L1 expression suggested a poor prognosis in patients with lung cancer.[15,25,26] Zhang et al. reported that PD-L1 expression was an independent predictor of poor OS in Ads determined by multivariate Cox regression model adjusted for age, sex, smoking history, type of surgical resection, differentiation, TNM stage, histologic types, mutational status, and perioperative chemotherapy/radiotherapy.[15] Similarly, positive PD-L1 expression was found to be an independently worse prognostic factor for OS in other studies for non-Sqs and NSCLC.[25,26] Lin et al. reported that PD-L1 status was not associated with survival, either univariate or multivariate analysis although PD-L1 expression appeared to be lower in patients with early-stage resectable lung cancer.[13] Velcheti et al. reported conflicting results that PD-L1 expression was significantly associated with better prognosis independent of histology for NSCLC.[27] In five meta-analyses, four revealed that NSCLC patients with increased PD-L1 expression had poorer OS.[17,28–30] The other meta-analysis reported no prognostic significance of PD-L1 in NSCLC.[31] Zhang pointed out that PD-L1 expression and prognosis was dependent on ethnicity; poor in Asian populations but not in non-Asian populations.[18] In our present study, positive PD-L1 expression was a predictor of RFS in univariate, but not multivariate analysis, and was not associated with OS, showing that the prognostic value of PD-L1 was limited.

There are several limitations in this study. First, the number of enrolled patients was relatively small to obtain reliable results. We are now planning to analyze the larger number of patients in the next study. Second,

this study was not a prospective study; therefore, bias might exist in some patients with certain histologic types or Ad subtypes. As we used the archived pathologic specimens, the storage period of the paraffin-embedded tissues might affect the results of immunostaining. However, in this study, we found no statistically significant difference regarding PD-L1 positivity between the period from 2008 to 2010 (10/40, 25%) and the period from 2011 to 2014 (12/50, 24%; p = 0.9127). Third, the postoperative observation period was relatively short.

Conclusion

In conclusion, the present study confirmed that PD-L1 protein detected by the monoclonal antibody 22C3 was differentially expressed in histologic types in NSCLC, and also in subtypes for Ad. Positive expression was associated with several clinicopathologic factors such as gender, smoking status, pathologic stages, venous invasion, and lymphatic invasion. Positive PD-L1 expression was associated with a worse RFS in the only univariate analysis, indicating a limited prognostic value. Further studies including larger numbers of patients are necessary to confirm our present results.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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