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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

Prognostic Impact of Programmed Death-Ligand 2 Expression in Primary Lung Adenocarcinoma Patients

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ABSTRACT

Background. Programmed death-ligand 1 (PD-L1) expression on lung cancer cells is a prognostic marker and a predictive biomarker for response to immunotherapy. However, previous clinical trials have suggested that other programmed cell death 1 ligands, including programmed death-ligand 2 (PD-L2), might have clinical impacts. This study aimed to analyze the prognostic significance of PD-L2 expression in lung adenocarcinoma patients.

Methods. The study included 433 patients who underwent surgical resection for lung adenocarcinoma between 2003 and 2012 at Kyushu University Hospital. Both PD-L1 and PD-L2 expression were evaluated by immunohistochemistry. The cutoff value for PD-L2 positivity was set at 1% according to a time-dependent receiver operating characteristic curve for 5-year survival.

Results. Of the 433 patients, 306 (70.7%) were positive for PD-L2. No significant association between PD-L1 and PD-L2 expression was observed ($P = 0.094$). The multivariate analysis showed that the independent predictors of PD-L2 positivity were never-smoker status ($P = 0.002$), poor differentiation grade ($P = 0.008$), and advanced stage ($P = 0.048$). The PD-L2-positive patients had significantly shorter disease-free survival (DFS) ($P = 0.018$) and overall survival (OS) ($P = 0.016$). Both PD-L1 and PD-L2 positivity were independent predictors of OS ($P < 0.001$ and $P = 0.027$, respectively). In the subgroup analysis of the PD-L1-negative patients, PD-L2 positivity was significantly associated with shorter DFS ($P = 0.018$).

Conclusions. The study demonstrated that the clinical characteristics of patients with PD-L1 expression may differ from those of patients with PD-L2 expression, and that both might contribute to poor prognoses. The potential role of PD-L2 expression as a predictive biomarker for response to immunotherapy should be investigated in future studies.

Lung cancer is one of the most fatal malignancies worldwide, and non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases.[1,2] There have been dramatic pharmacotherapy developments for NSCLC patients. In addition to chemotherapy, molecular-targeted drugs, such as epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase tyrosine kinase inhibitors, have been adopted in the clinic.

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Recently, immunotherapy targeting programmed cell death 1 (PD-1) or programmed death-ligand 1 (PD-L1) has gathered great attention as a novel pharmacologic treatment. The CheckMate-017, CheckMate-057 and KEYNOTE-024 clinical trials found that immunotherapy was associated with significantly longer progression-free survival and overall survival (OS) than standard conventional chemotherapy for NSCLC patients.[3–5]

Since immunotherapy became one of the standard pharmacologic therapies for NSCLC patients, predictive biomarkers for responses to anti-PD-1 drugs have been investigated. According to previous reports, PD-L1 expression on tumor cells is a promising predictive biomarker for the response to immunotherapy.[6–8] However, the CheckMate-057 trial showed that the OS benefits of anti-PD-1 drugs were equivalent to those of conventional chemotherapy for the patients who were negative for PD-L1 expression on tumor cells.[3] Thus, other PD-1 ligands, including programmed death-ligand 2 (PD-L2), also may have clinical roles.

The PD-L2 protein is mainly expressed by dendritic cells, macrophages, and tumor cells and downregulates the effector functions of T cells via the PD-1/PD-L2 axis in the tumor microenvironment.[9,10] The clinical significance of PD-L2 expression has not been well investigated, but a few reports have described the prognostic marker of PD-L2 expression.[11–14] Additionally, whether PD-L2 expression is significantly associated with poor prognoses remains controversial.[11–14] Therefore, in this translational study, we analyzed the postoperative prognostic significance of PD-L2 expression in lung adenocarcinoma patients. We also investigated associations of PD-L2 and PD-L1 expression with clinicopathologic factors, and finally, we studied the clinical significance of PD-L2 expression in lung adenocarcinoma.

MATERIALS AND METHODS

Patients and Samples

The study enrolled 690 consecutive patients with a diagnosis of NSCLC between January 2003 and December 2012 in the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan. The inclusion criteria for this study specified patients who had pathologic stage 1, 2, or 3 NSCLC and no history of cancer, pathologic lung adenocarcinoma diagnoses confirmed by re-review, and R0 resection and systematic ipsilateral mediastinal lymphadenectomy. With regard to preoperative staging methodology, all the patients underwent positron emission tomography-computed tomography (PET-CT), brain magnetic resonance imaging (MRI), and contrast-enhanced computed tomography.

The data of 433 patients were retrospectively analyzed in this study. The investigated clinical characteristics included sex, age, smoking history, tumor differentiation, pathologic stage, pleural or lymphovascular invasion, histologic subtype, surgical procedure, and EGFR mutation status. The criteria for intentional sublobar resection specified a total tumor size of 2 cm or smaller and a consolidation/tumor ratio of 0.25 or less.[15]

Compromised sublobar resections were performed when patients may not have tolerated lobar resection due to decreased pulmonary function or comorbid disease. Sections stained with Victoria blue-van Gieson were investigated for the presence of vascular and pleural invasion. Lymphovascular invasion was reported when tumor cells or emboli were identified in lymphatic or blood vessel lumens.

The EGFR mutation status of tumors was determined using the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp method (Mitsubishi Chemical Medience, Tokyo, Japan). The 7th edition of the American Joint Committee on Cancer staging system for lung cancer was used to determine pathologic stages.

Routine examinations after surgery were performed as follows. Chest radiography was conducted at 3-month intervals for the first 3 years and at 6-month intervals after surgery. Computed tomography was performed twice a year for the first 3 years and then annually thereafter. The methods for patient follow-up evaluation included both telephone calls and outpatient clinical checkups. Patients with local recurrence received surgery or radiotherapy, and surgery was determined by the patients' pulmonary function, performance status, or both. Clinical information and follow-up data were collected using medical records. Our local institutional review board approved this study (Kyushu University, IRB no. 29-261).

Immunohistochemical Analysis of PD-L1 and PD-L2 Expression in Tumor Specimens

Immunohistochemical analyses were performed using commercial antibodies against PD-L1 (SP142, 1:100; Spring Bioscience, Ventana, Tucson, AZ, USA) and PD-L2 (176611, 1:200; R&D Systems, Inc., Minneapolis, MN, USA). Immunohistochemistry for PD-L1 was performed as previously described.[8] For PD-L2 immunohistochemistry, 4-μm-thick sections were prepared and mounted on glass slides using B Bond-III autostainer (Leica Microsystems, Newcastle, UK). Briefly, mouse monoclonal anti-human PD-L2 antibody was incubated with the sections for 30 min after a 5-min proteinase K treatment (Agilent/Dako, Santa Clara, CA, USA). We then used the automated Refine

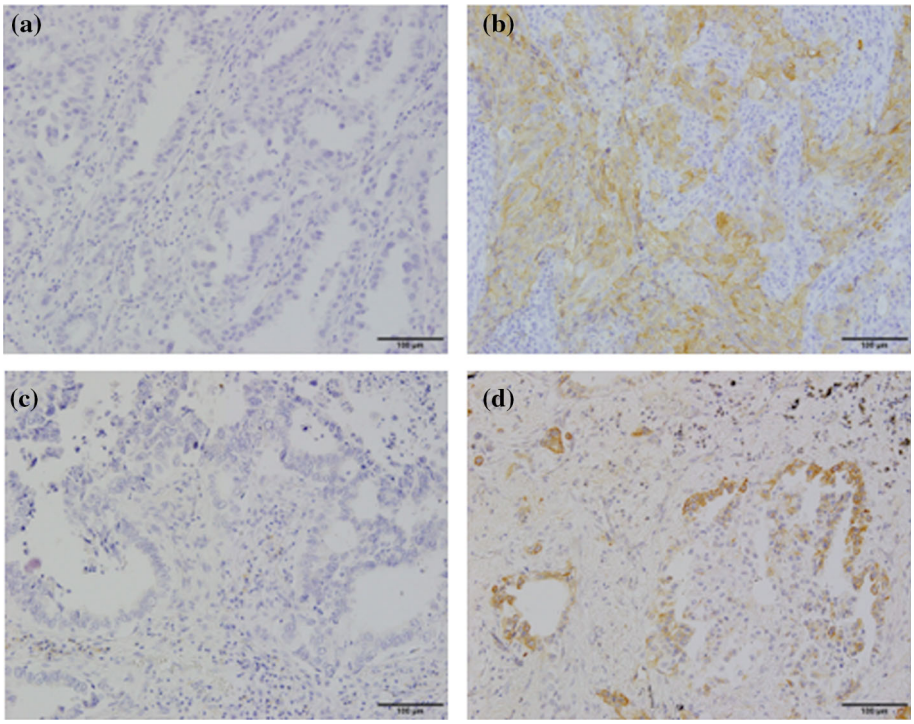

FIG. 1 Immunohistochemical staining of programmed death-ligand 1 (PD-L1) and programmed death-ligand 2 (PD-L2) in primary lung adenocarcinoma samples. **a** Negative staining for PD-L1. **b** Representative image of a PD-L1-positive case. The tumor membrane is stained. **c** Negative staining for PD-L2. **d** Representative image of a PD-L2-positive case. Both the tumor membrane and cytoplasm are stained. Carcinoma cells showing positive membrane staining for PD-L2 were classified as PD-L2-positive given its function as a ligand. Scale bar: 100 μm

polymer detection system (Leica Microsystems) with horseradish peroxidase-polymer as the secondary antibody and 3,3′ diaminobenzidine as the chromogen. Slides were visualized using diaminobenzidine.

Carcinoma cells showing positive membrane staining for PD-L1 and PD-L2 were classified respectively as PD-L1-positive and PD-L2-positive. The proportion of PD-L1- and PD-L2-positive carcinoma cells was estimated from the percentage of total carcinoma cells in whole sections. All immunohistochemical images were independently evaluated by three investigators (S.T., K.T., and T.J.), who were blinded to outcomes. For cases in which determinations differed among the three observers, slides were reviewed by all three investigators together to achieve consensus, which was used for further analyses.

We set the cutoff value for PD-L1 positivity at 1% as previously reported.[8] We set the cutoff value for PD-L2 positivity at 1% according to a time-dependent receiver operating characteristic curve for 5-year survival using a five-grade system as follows: 0 (0%), 1 (1–4%), 2 (5–10%), 3 (11–49%), 4 (≥ 50%) (Fig. S1). Additionally, a 5% cutoff value for PD-L2 was used according to the previous report by Koh et al.[13]

Statistical Analysis

Associations between PD-L2 expression and clinical factors were analyzed using Fisher's exact two-sided test. Predictors for PD-L2 positivity were investigated using uni- and multivariate logistic analyses. In the multivariate logistic analysis, the backward elimination method was used. In brief, the model was run with all the variables, and one variable with the highest *P* value was excluded. The model was run again with the other variables, and one variable with the highest *P* value was excluded repeatedly to retain only those with a *P* value lower than 0.05. Cox proportional hazard regression models were used to calculate hazard ratios for positive risk factors with a similar backward elimination method. The Kaplan–Meier method with the log-rank test was used to estimate survival probabilities. All *P* values lower than 0.05 were considered statistically significant. All analyses were performed using JMP software v13 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Association Between PD-L2 Expression and Clinicopathologic Characteristics in Primary Lung Adenocarcinoma Patients

The patient characteristics are shown in Table S1. Immunohistochemical staining for PD-L1 and PD-L2 was evaluated by detecting membrane staining in carcinoma cells (Fig. 1). Associations between PD-L2 expression and patient characteristics are summarized in Table 1. Using the cutoff value of 5%, 244 patients (56.4%) were found to be positive for PD-L2, whereas 306 (70.7%) were found to be positive using a 1% cutoff value. The findings showed

TABLE 1 Association between programmed death-ligand 2 (PD-L2) expression and the clinicopathologic factors of lung adenocarcinoma patients (n = 433)

Factors	n	1% Cutoff			5% Cutoff		
		PD-L2 n (%)		P Value	PD-L2 n (%)		P Value
		Negative	Positive		Negative	Positive	
Age (years)							
< 70	231	66 (52.0)	165 (53.9)	0.751	96 (50.8)	135 (55.3)	0.382
≥ 70	202	61 (48.0)	141 (46.1)		93 (49.2)	109 (44.7)	
Sex							
Men	215	72 (56.7)	143 (46.7)	0.073	101 (53.4)	114 (46.7)	0.176
Women	218	55 (43.3)	163 (53.3)		88 (46.6)	130 (53.3)	
Smoking							
Never	226	54 (42.5)	172 (56.2)	0.011	86 (45.5)	140 (57.4)	0.016
Ever	207	73 (57.5)	134 (43.8)		103 (54.5)	104 (42.6)	
Grade							
G1	208	74 (58.3)	134 (43.8)	0.008	105 (55.6)	103 (42.2)	0.007
≥ G2	225	53 (41.7)	172 (56.2)		84 (44.4)	141 (57.8)	
Stage							
1	319	105 (82.7)	214 (69.9)	0.006	146 (77.3)	173 (70.9)	0.153
≥ 2	114	22 (17.3)	92 (30.1)		43 (22.7)	71 (29.1)	
Pl							
Absent	336	105 (82.7)	231 (75.5)	0.128	153 (81.0)	183 (75.0)	0.163
Present	97	22 (17.3)	75 (24.5)		36 (19.0)	61 (25.0)	
Ly							
Absent	371	117 (92.1)	254 (83.0)	0.015	168 (88.9)	203 (83.2)	0.099
Present	62	10 (7.9)	52 (17.0)		21 (11.1)	41 (16.8)	
V							
Absent	312	99 (78.0)	213 (69.6)	0.099	142 (75.1)	170 (69.7)	0.235
Present	121	28 (22.0)	93 (30.4)		47 (24.9)	74 (30.3)	
Histologic subtype							
Micropapillary/solid	27	11 (8.7)	16 (5.2)	0.193	15 (7.9)	12 (4.9)	0.231
Others	406	116 (91.3)	290 (94.8)		174 (92.1)	232 (95.1)	
EGFR[a]							
Wild-type	129	46 (53.5)	83 (51.6)	0.791	63 (50.0)	66 (54.6)	0.525
Mutant	118	40 (46.5)	78 (48.4)		63 (50.0)	55 (45.4)	
PD-L1							
Negative	287	92 (72.4)	195 (63.7)	0.094	130 (68.8)	157 (64.3)	0.357
Positive	146	35 (27.6)	111 (36.3)		59 (31.2)	87 (35.7)	

Pl pleural invasion, *Ly* lymphatic invasion, *V* vascular invasion, *EGFR* epidermal growth factor receptor, *PD-L1* programmed death-ligand 1

[a]Cases for which data were available

PD-L2 expression to be significantly higher in nonsmokers than in smokers and in the patients with poorer differentiation grades at both cutoff values. Additionally, PD-L2 expression was significantly higher in advanced-stage tumors and those with lymphatic invasion using the 1% cutoff value. Multivariate logistic analyses for PD-L2 expression showed that the independent predictors of PD-L2 positivity were never-smoker status (P = 0.002), poor differentiation grade (P = 0.008), and advanced stage (P = 0.048) (Table 2).

Univariate Analyses of Disease-Free Survival (DFS) and OS According to PD-L2 Expression in Primary Lung Adenocarcinoma Patients

Associations of PD-L2 expression with DFS and OS were analyzed at both PD-L2 cutoff values. As shown in

TABLE 2 Uni- and multivariate logistic analyses for programmed death-ligand 2 (PD-L2) expression in patients with lung adenocarcinoma (n = 433)

Factors	OR (95% CI)	P Value
Univariate logistic analysis		
Age (< 70/≥ 70 years)	1.08 (0.71–1.64)	0.711
Sex (woman/man)	1.49 (0.98–2.26)	0.060
Smoking status (never/ever)	1.74 (1.14–2.64)	0.010
Grade (≥ G2/G1)	1.79 (1.18–2.72)	0.006
Stage (≥ 2/1)	2.05 (1.22–3.45)	0.007
Pl (present/absent)	1.55 (0.91–2.63)	0.104
Ly (present/absent)	2.40 (1.18–4.88)	0.016
V (present/absent)	1.54 (0.95–2.51)	0.079
Histologic subtype (others/micropapillary) or solid)	1.72 (0.77–3.81)	0.183
EGFR[a] (mutant/wild-type)	1.08 (0.64–1.83)	0.772
PD-L1 (positive/negative)	1.50 (0.95–2.36)	0.082
Multivariate logistic analysis		
Smoking status (never/ever)	2.04 (1.32–3.17)	0.002
Grade (≥ G2/G1)	1.86 (1.18–2.93)	0.008
Stage (≥ 2/1)	1.74 (1.01–3.00)	0.048

Cutoff value of 1%

OR odds ratio, *CI* confidence interval, *Pl* pleural invasion, *Ly* lymphatic invasion, *V* vascular invasion, *EGFR* epidermal growth factor receptor, *PD-L1* programmed death-ligand 1

[a]Cases for which data were available

Fig. 2a and b, at the 1% cutoff value, the PD-L2-positive patients had significantly shorter DFS (P = 0.018, log-rank test) and OS (P = 0.016, log-rank test). However, the difference in DFS and OS was not statistically significant between the PD-L2-positive and -negative patients at the 5% cutoff value (respectively P = 0.307 and P = 0.088, log-rank test; Fig. 2c.

In the subgroup analysis of the PD-L1-negative patients (using the 1% cutoff value), the PD-L2-positive patients (using the 1% cutoff value) were significantly associated with a shorter DFS than the PD-L2-negative patients (P = 0.018, log-rank test; Fig. S2a). However, the difference in OS was not significant between the PD-L2-positive and -negative patients using the 1% cutoff value (P = 0.142, log-rank test; Fig. S2b). In the subgroup analysis of the PD-L1-positive patients (using the 1% cutoff value), differences in DFS and OS were not significant between the PD-L2-positive and -negative patients (using the 1% cutoff value) (respectively P = 0.488 and P = 0.062, log-rank test; Fig. S2c and d).

Additionally, we conducted a subgroup analysis limited to the patients with N1, N2 disease who were indicated for adjuvant chemotherapy. The difference in DFS and OS was not significant between the PD-L2-positive and -negative patients at the 1% cutoff value (respectively P = 0.376 and P = 0.291, log-rank test; data not shown).

Multivariate Analysis of DFS and OS According to PD-L2 Expression in Primary Lung Adenocarcinoma Patients

Using the results obtained in the univariate analyses, we adopted the 1% cutoff value for further survival analyses. Multivariate analysis with Cox proportional hazard models showed that age of 70 years or older, poor differentiation grade, advanced T and N stages, lymphatic invasion, and PD-L1 positivity were independent predictors of shorter DFS (Table 3), whereas PD-L2 positivity, age of 70 years or older, male sex, advanced T and N stages, lymphatic invasion, and PD-L1 positivity were independent predictors of shorter OS (PD-L2-positive vs PD-L2-negative: hazard ratio [HR], 1.88; 95% confidence interval [CI], 1.07–3.53; P = 0.027; Table 3).

Subgroup Univariate Analysis of DFS and OS According to Expression of Both PD-L1 and PD-L2 in Primary Lung Adenocarcinoma Patients

Subgroup univariate analyses of DFS and OS according to PD-L1 and PD-L2 status were performed. Expression of both PD-L1 and PD-L2 was significantly associated with postoperative DFS and OS ($P < 0.001$, log-rank test, for both; Fig. S3a and b). The patients positive for both PD-L1 and PD-L2 showed significantly shorter DFS than those negative for both PD-L1 and PD-L2 (HR, 3.50; 95% CI, 1.99–6.59; $P < 0.001$; Fig. S3a). The patients positive for

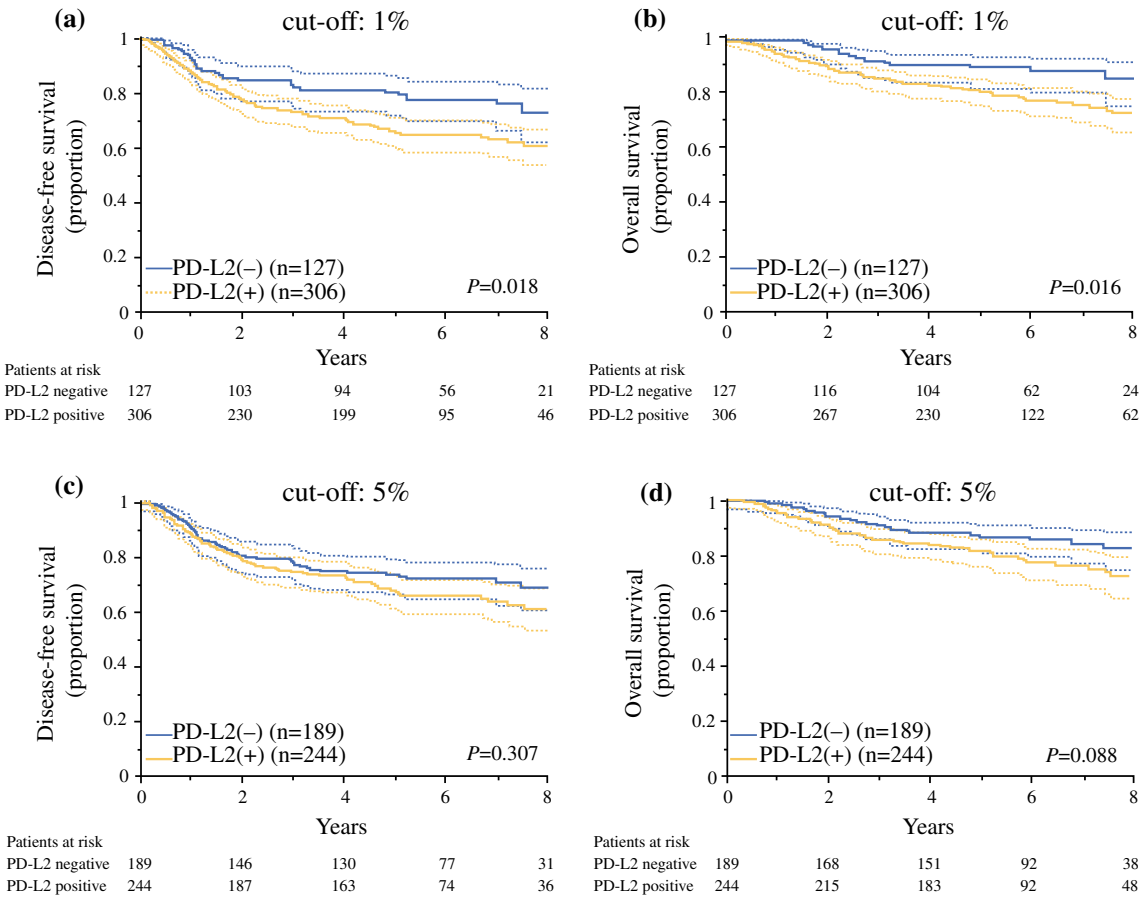

FIG. 2 Kaplan–Meier survival curves of all primary lung adenocarcinoma patients according to programmed death-ligand 2 (PD-L2) expression. **a** Disease-free survival and **b** overall survival among all patients according to PD-L2 expression status using the 1% cutoff value. **c** Disease-free survival and **d** overall survival among all patients according to PD-L2 expression status using the 5% cutoff value

both PD-L1 and PD-L2 showed significantly shorter OS than those negative for both PD-L1 and PD-L2 (HR, 4.98; 95% CI, 2.37–12.18; $P < 0.001$; Fig. S3b). Finally, the patients with either PD-L1-positivity or PD-L2-positivity had significantly shorter DFS and OS than the patients negative for both PD-L1 and PD-L2 ($P < 0.001$, log-rank test, for both; Fig. S4a and b).

DISCUSSION

This study demonstrated that PD-L1 and PD-L2 were variably expressed in lung adenocarcinoma. Our previous study showed that higher PD-L1 expression was observed in male patients, smokers, patients without EGFR mutations, patients with poorly differentiated tumors, and patients with more advanced-stage disease.[8] Conversely, this study demonstrated that PD-L2 expression was significantly higher in nonsmokers, patients with poorly differentiated tumors, and patients with more advanced-stage disease.

The patient characteristics associated with PD-L2 positivity were similar to those of PD-L1 in terms of tumor cell differentiation and stage. This finding suggests that both PD-L1 and PD-L2 expression may be related to the intrinsic malignancy of cancer cells and tumor progression. In contrast to PD-L1 expression, PD-L2 was significantly associated with never-smokers, which also was found by previous studies.[13,14,16]

The discrepant expression of PD-L1 and PD-L2 may result from the differential inflammatory state of the tumor. Upregulation of PD-L1 is mainly induced by Th1 cytokine, including interferon-gamma (IFN-γ), which is upregulated by cigarette smoking.[17] Expression of PD-L2 also is induced by IFN-γ,[18–20] but only weakly.[21] Gene transcription of PD-L2 is regulated by interleukin-4R/signal transducer and activator of transcription 6 signaling, which is related to Th2 cytokine.[21] Another possible explanation is that tumors express less PD-L1 due to low-level exposure to IFN-γ, and PD-L2 would compensate for a lack of PD-L1 expression, allowing tumors to escape the antitumor immune response.

TABLE 3 Uni- and multivariate analyses of disease-free and overall survival in lung adenocarcinoma patients (n = 433)

Factors	n	Disease-free survival		Overall survival	
		Univariate analysis HR(95% CI) *P* Value	Multivariate analysis HR(95% CI) *P* Value	Univariate analysis HR(95% CI) *P* Value	Multivariate analysis HR(95% CI) *P* Value
Age (years)					
≥ 70	202	1.43 (1.02–2.00)	1.43 (1.02–2.03)	2.59 (1.65–4.16)	3.03 (1.91–4.93)
< 70	231	0.039	0.040	< 0.001	< 0.001
Sex					
Men	215	1.91 (1.36–2.72)	1.59 (1.12–2.27)	2.35 (1.50–3.79)	2.25 (1.42–3.67)
Women	218	< 0.001	0.010	< 0.001	0.001
Smoking					
Ever	207	1.51 (1.08–2.12)		1.76 (1.14–2.77)	
Never	226	0.017		0.011	
Grade					
≥ G2	225	4.11 (2.78–6.24)	1.82 (1.12–2.99)	4.05 (2.43–7.14)	
G1	208	< 0.001	0.015	< 0.001	
T factor					
≥ 3	33	3.11 (1.85–4.93)		2.50 (1.25–4.52)	
≤ 2	400	< 0.001		0.012	
N factor					
≥ 2	45	5.15 (3.47–7.47)	2.09 (1.35–3.20)	4.68 (2.85–7.45)	2.56 (1.49–4.29)
≤ 1	388	< 0.001	0.001	< 0.001	0.001
Pl					
Present	97	3.52 (2.48–4.95)	1.74 (1.19–2.55)	3.65 (2.34–5.66)	2.07 (1.30–3.29)
Absent	336	< 0.001	0.015	< 0.001	0.002
Ly					
Present	62	5.06 (3.51–7.20)	2.99 (1.92–4.57)	4.28 (2.68–6.70)	2.88 (1.67–4.85)
Absent	371	< 0.001	< 0.001	< 0.001	< 0.001
V					
Present	121	3.21 (2.28–4.50)		3.56 (2.30–5.54)	
Absent	312	< 0.001		< 0.001	
Histologic subtype					
Micropapillary/solid	27	2.14 (1.20–3.55)		1.20 (0.46–2.53)	
Others	406	0.012		0.682	
Surgical procedure					
≥ Lobectomy	333	1.67 (1.08–2.71)		1.77 (0.99–3.43)	
Sublobar	100	0.020		0.053	
EGFR[a]					
Wild-type	129	1.67 (1.05–2.73)		2.17 (1.15–4.32)	
Mutant	118	0.032		0.016	
PD-L1					
Positive	146	2.04 (1.45–2.86)	1.67 (1.14–2.44)	2.76 (1.78–4.31)	2.76 (1.76–4.34)
Negative	287	< 0.001	0.008	< 0.001	< 0.001
PD-L2[b]					
Positive	306	1.63 (1.10–2.50)		2.01 (1.16–3.72)	1.88 (1.07–3.53)
Negative	127	0.015		0.011	0.027

HR hazard ratio, *CI* confidence interval, *Pl* pleural invasion, *Ly* lymphatic invasion, *V* vascular invasion, *EGFR* epidermal growth factor receptor, *PD-L1* programmed death-ligand 1, *PD-L2* programmed death-ligand 2

[a]Cases for which data were available

[b]Cutoff value of 1%

Our results showing that patients with either PD-L1-positive or PD-L2-positive expression had significantly shorter DFS and OS than those negative for both factors (Fig. S4a and b) supports a complementary role for PD-L2 to PD-L1 in activating PD-1 to exhaust anticancer activity of cytotoxic T lymphocytes. With regard to EGFR mutation status, unlike PD-L1, PD-L2 expression was not associated with EGFR mutation status, which also is in line with previous reports.[13,14] Thus, the patient characteristics associated with PD-L2 positivity were different from those of PD-L1 in terms of smoking history and EGFR status.

The significance of PD-L2 as a prognostic marker and the relationship with clinicopathologic features has been reported previously.[11–14,16] These reports are summarized in Table S2. In previous studies, PD-L2 immunohistochemistry was evaluated based not only on the proportion of membranous staining but also on the intensity and/or cytoplasmic staining in tumor cells. In this study, we focused only on the proportion of PD-L2-positive tumor cells because PD-L2 expression downregulates the effector functions of T cells via interactions between PD-1 on T cells and PD-L2 expression on the tumor cell membrane.[9,10]

To the best of our knowledge, this is the first study to show the clinical impact that PD-L2 expression only on tumor cells has on the postoperative outcome. This study also was the first to demonstrate a significant influence of PD-L2 expression on DFS. Other studies have used tissue microarrays or biopsy specimens and have analyzed only one part of the tumor.[12,13,16] Some studies have used whole-section specimens but involved much smaller samples than our study, which investigated 433 patients.[11,14] Thus, we believe our findings provide important value because of the large sample size with whole-section specimens.

In the subgroup analysis limited to the PD-L-negative patients, the PD-L2-positive patients were significantly associated with shorter DFS than the PD-L2-negative patients. Additionally, PD-L1 expression was not an independent predictor of PD-L2 positivity, and PD-L1 and PD-L2 positivity both were independent predictors of OS. Notably, PD-L2 positivity combined with PD-L1 positivity was a strong prognostic marker. According to a previous report, co-occurring PD-L1 and PD-L2 positivity was an independent predictor of poor OS (HR, 2.54; 95% CI, 1.35–4.79),[14] which was consistent with our results.

Recently, potential therapeutic agents targeting PD-L2 have been investigated. A previous study showed that blocking PD-L2 selectively activated CD8 T cells and suppressed cancer metastasis using murine models of metastatic pancreatic ductal adenocarcinoma.[22] Additionally, an ongoing clinical trial is evaluating the efficacy of peptide vaccination against PD-L2 in follicular lymphoma patients (ClinicalTrials.gov Identifier: NCT 03381768). Finally, a recent study showed that the intrinsic and extrinsic pathways of PD-L2 expression regulated PD-L2 positivity in NSCLC.[23] Thus, future studies will clarify the immunotherapeutic effect of anti-PD-L2 agents in lung adenocarcinoma patients.

This study had some limitations. First, it was a single-institution retrospective study. Future prospective studies investigating the clinicopathologic characteristics and prognostic significance of PD-L2-positive patients will be important to draw the definitive conclusions. Second, we examined PD-L1 and PD-L2 expression using only the clones SP142 and 176611, respectively. Both PD-L1 and PD-L2 expression should be reevaluated using other antibodies and/or cutoff values in future studies.[24,25]

In conclusion, this study demonstrated that the clinical characteristics of patients with PD-L1 and PD-L2 expression may be different, and that both may independently contribute to poor prognoses for lung adenocarcinoma patients.

DISCLOSURE All authors have declare that they have no conflicts of interest.

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