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Association Between PD-L1 Expression and Metabolic Activity on ^{18}F-FDG PET/CT in Patients with Small-sized Lung Cancer

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Abstract. *Aim: We evaluated the metabolic characteristics of small-sized lung cancer using ^{18}F-fluorodeoxyglucose positron-emission tomography/computed tomography (^{18}F-FDG PET/CT) with regard to programmed cell death ligand 1 (PD-L1) expression. Materials and Methods: PD-L1 expression was evaluated by immunohistochemistry with the antibody clone SP142 in 263 patients with surgically resected primary small-sized lung cancer. Specimens with <5% tumor membrane staining were considered negative. We examined the association between the frequency of PD-L1 expression and the maximum standardized uptake value (SUVmax) in preoperative ^{18}F-FDG PET/CT. Results: Among patients with non-small cell lung cancer (NSCLC), the SUVmax was significantly higher in those with PD-L1 expression than in those without (p<0.0001). However, there was no correlation between SUVmax and PD-L1 expression in patients with neuroendocrine tumors (p=0.9638). Multivariate analysis revealed that smoking and a high SUVmax were independent predictors of PD-L1 expression. Conclusion: PD-L1 expression was related to high glucose metabolism in small-sized NSCLC.*

Immune checkpoint inhibition that targets programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) has recently been shown to improve the prognosis of multiple cancer types (1, 2). In recent phase II/III studies, PD-1/PD-L1 inhibitors such as nivolumab, pembrolizumab, and atezolizumab exhibited a survival benefit compared to conventional standard therapy in patients with non-small cell lung cancer (NSCLC) (3-8), and PD-1 inhibitors have become standard treatment for NSCLC. Because PD-L1 protein expression in tumor cells is expected to serve as a predictive marker for efficacy of PD-1/PD-L1 inhibitors, it is important to elucidate the clinical significance of PD-L1 protein expression in patients with NSCLC.

^{18}F-Fluorodeoxyglucose positron-emission tomography/computed tomography (^{18}F-FDG PET/CT) is an essential imaging tool in the diagnosis and staging of lung cancer (9, 10). We recently evaluated the metabolic characteristics of lung cancer using ^{18}F-FDG PET/CT with regard to PD-L1 protein expression (unpublished observation). In our analysis of 548 patients with NSCLC, the maximum standardized uptake value (SUVmax) was significantly higher in patients with than in those without PD-L1 protein expression, and multivariate analysis revealed that smoking, the presence of pleural invasion, and a high SUVmax were independent predictors of PD-L1 protein expression. Glucose metabolism in cancer tissues as measured by ^{18}F-FDG PET/CT is a significant biomarker for characterization of lung cancer. Previous studies have revealed correlations between FDG uptake and biological features of cancer such as proliferation, histological type, tumor differentiation, and hypoxia (11-16). These findings indicate that PD-L1-positive NSCLC has a high glucose metabolism and that PD-L1 protein expression in patients with NSCLC may be associated with a poor prognosis. In our above-mentioned study, however, we did not exclude patients with small tumors in order to avoid the bias of a partial volume effect (17). Therefore, whether these results would be the same in an analysis only of patients whose chest computed tomographic (CT) findings reveal a primary tumor size of <2 cm requires examination.

In this translational study, we examined PD-L1 protein expression in primary small-sized (≤2 cm) lung cancer among patients who had undergone complete surgical resection and investigated the association between PD-L1 protein expression and the SUVmax by preoperative ^{18}F-FDG PET/CT.

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Key Words: FDG, PD-L1, PET/CT, small-sized lung cancer, SUVmax.

Materials and Methods

Patients and samples. We retrospectively examined patients with primary small-sized (≤2 cm) lung cancer who underwent complete surgical resection at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University. Among them, we selected patients who underwent chest CT and ^{18}F-FDG PET/CT before surgery. The following patients were included in this study: 220 patients with adenocarcinoma (ADC) and 30 with squamous cell carcinoma (SCC) until December 2015, and one patient with large cell carcinoma (LCC), seven with small cell lung carcinoma (SCLC), and five with large cell neuroendocrine carcinoma (LCNEC) until June 2016. In total, 263 paraffin-embedded specimens were retrieved from the registry of the Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University. Patients with a history of SCC of the head and neck or esophagus were excluded from this study because of the possibility of metastatic SCC. Patients who had received neoadjuvant therapy were also excluded because of inconsistency in the expression of PD-L1 on tumor cells before and after neoadjuvant chemotherapy (18). Clinicopathological features including age at surgery, sex, smoking status, pathological tumor-node-metastasis stage (seventh edition of the Lung Cancer Staging System) (19), pleural or lymphovascular invasion, and SUVmax were examined. Clinical information and follow-up data were obtained from the patients' medical records. We obtained informed consent from each patient, and this study was approved by our Institutional Review Board (Kyushu University, IRB no. 28-100).

Chest CT. Chest CT scans were performed during an inspiratory breath-hold with the patient in the supine position using various multidetector row scanners: Aquilion 4 (Toshiba, Tokyo, Japan), Aquilion 64 (Toshiba), Aquilion ONE (Toshiba), Aquilion ONE Vision (Toshiba), SOMATOM Plus 4 Volume Zoom (Siemens Medical Solutions, Erlangen, Germany), Brilliance CT (Philips Healthcare, Amsterdam, the Netherlands), and Brilliance iCT (Philips Healthcare). The imaging parameters for thin-section CT were as follows: tube voltage, 120 kVp; tube current, 100-500 mA; scan field of view, 320-360 mm; and slice thickness, 2 mm. Real exposure control (Toshiba) or automatic exposure control (Siemens Medical Solutions and Philips Healthcare) was used in each study. All CT data sets were transferred to a Picture Archiving and Communication System, which was accessible for the workstation (Volume Analyzer SYNAPSE VINCENT; Fujifilm, Tokyo, Japan) with a specialized application for the lungs.

^{18}F-FDG PET/CT. For each patient, 185 MBq of FDG was intravenously administered after fasting for at least 4 h. Scans were conducted from the middle of the thigh to the top of the skull 60 min after FDG administration. ^{18}F-FDG PET/CT images were obtained by an integrated PET/CT scanner (Discovery STE; GE Medical Systems, Milwaukee, WI, USA; or Biograph mCT; Siemens Medical Solutions). All emission scans were performed in three-dimensional mode, and the acquisition time per bed position was 3 min for the Discovery STE and 2 min for the Biograph mCT. We reconstructed the PET images using the ordered-subset expectation–maximization method (VUE Point Plus) with two full iterations of 28 subsets for the Discovery STE and iterative True-X algorithm and time-of-flight (ultra-high-definition PET) with two full iterations of 21 subsets. The True-X algorithm incorporates an additional specific correction for the point-spread function. The full-width at half-maximum values of the Discovery STE and Biograph mCT were 5.2 and 4.4 mm, respectively. A low-dose 16-slice CT scan (tube voltage, 120 kV; effective tube current, 30-250 mA; Discovery STE) and a low-dose 32-slice CT scan (tube voltage, 120 kV; use of angular and longitudinal dose modulation; CARE Dose 4D®, Biograph mCT) were performed from the vertex to the proximal thigh for attenuation correction and for determining the precise anatomic location of the lesions before acquisition of PET images. The CT scans were reconstructed by filtered back projection into 512×512 pixel images with a slice thickness of 5 mm to match the PET scan. FDG uptake in lesions was evaluated using the SUVmax, which was calculated by the dedicated workstation of each scanner.

Immunohistochemical analysis. Immunohistochemistry was performed in 263 surgically resected primary small-sized (≤2 cm) lung cancer specimens using formalin-fixed and paraffin-embedded tumor tissue sections according to our previously described PD-L1 immunohistochemistry protocol (20-23).

The primary antibody was a rabbit monoclonal antibody to human PD-L1 (clone SP142, dilution 1:100; Spring Bioscience, Ventana, Tucson, AZ, USA). Carcinoma cells showing membranous staining for PD-L1 were evaluated as positive cells. The proportion of PD-L1-positive cells was independently estimated as a percentage of the total carcinoma cells in whole sections by three investigators (K.T., K.K., and G.T.). If the independent assessments did not agree, the slides were reviewed by all three investigators together to achieve consensus. Judgment obtained by consensus were adopted as the final result. Cases with <5% tumor membrane staining were considered negative in this study. Sections from human placentas were used as positive controls.

Statistical analysis. Univariate and multivariate analyses of the relationship between PD-L1 protein expression and other patient characteristics were performed by logistic regression analysis with the backward elimination method. The cut-off SUVmax was determined by receiver operating characteristic curve analyses. We examined the association between the frequency of PD-L1 protein expression and SUVmax by preoperative ^{18}F-FDG PET/CT using Student's *t*-test. Correlations between the proportion of PD-L1-positive carcinoma cells and the SUVmax on preoperative ^{18}F-FDG PET/CT were assessed using Spearman's correlation coefficient test. All statistical analyses were performed by JMP Statistical Discovery Software (v11.0; SAS Institute, Cary, NC, USA). A *p*-value of less than 0.05 was considered statistically significant.

Results

Association between PD-L1 protein expression and SUVmax in patients with small-sized lung cancer. Table I shows the clinicopathological characteristics of all patients in the present study. A total of 263 patients with primary small-sized lung cancer who underwent complete surgical resection were included in the present study. One hundred and forty-eight (56.3%) patients were male, and 155 (58.9%) were smokers. The median age of the whole study population was 68 years (range=36-89 years).

Table I. *Clinicopathological characteristics of all patients.*

Factor	Value
Age (years)	68 (36-89)
Gender	
Male	148
Female	115
Smoking status	
Never-smoker	108
Smoker	155
Tumor size (mm)	15 (4-20)
T	
T1	221
T2	40
T3	2
T4	0
N	
N0	234
N1	12
N2	17
N3	0
Stage	
IA	211
IB	22
IIA	10
IIB	2
IIIA	18
IIIB	0
IV	0
Pleural invasion	
Absent	225
Present	38
Lymphatic invasion	
Absent	250
Present	13
Vascular invasion	
Absent	224
Present	39
Histology	
Adenocarcinoma	220
Squamous cell carcinoma	30
Large cell carcinoma	1
Small cell carcinoma	7
Large cell neuroendocrine carcinoma	5

Data are presented as median (range) for continuous factors or number of patients.

Representative images of thin-section CT, ^{18}F-FDG PET/CT, and immunohistochemistry staining for PD-L1 in patients with and without PD-L1 protein expression are shown in Figure 1. Table II shows a summary of the frequency of PD-L1 protein expression and the SUVmax on ^{18}F-FDG PET/CT. The SUVmax was significantly higher in patients with than in those without PD-L1 protein expression in the analyses of cancer overall (p<0.0001), ADC/SCC/LCC (p<0.0001), ADC (p<0.0001), and SCC (p=0.0137) (Table II and Figure 2). In contrast, there was no correlation between the SUVmax and PD-L1 protein expression in neuroendocrine tumors (SCLC/LCNEC) (p=0.9638) (Figure 2). Spearman's correlation coefficient test also showed a positive correlation between the proportion of PD-L1-positive carcinoma cells and the SUVmax in the analyses of cancer overall (Spearman's rho=0.4700, p<0.0001), ADC/SCC/LCC (Spearman's rho=0.4892, p<0.0001), ADC (Spearman's rho=0.4376, p<0.0001), and SCC (Spearman's rho=0.3865, p<0.0349) (Figure 3).

Univariate and multivariate analyses of the relationship between PD-L1 protein expression and other patient characteristics. We examined the association between PD-L1 protein expression and other patient characteristics. The cut-off SUVmax obtained by receiver operating characteristic curve analysis was 3.20, 3.20, 3.20, and 8.83 for cancer overall, ADC/SCC/LCC, ADC, and SCC, respectively (Figure 4). Multivariate analysis revealed that the SUVmax on ^{18}F-FDG PET/CT was a predictor of PD-L1 protein expression in patients with small-sized lung cancer, especially NSCLC (Table III). Additionally, smoking was a predictor of PD-L1 protein expression. Table IV shows the frequency of PD-L1 protein expression according to the patients' smoking history and SUVmax on ^{18}F-FDG PET/CT. The frequency of PD-L1 protein expression was very high in patients with a smoking history (or ≥30 pack-years in patients with SCC because almost all patients with SCC were smokers) and a high SUVmax.

Discussion

In the present study, we examined PD-L1 protein expression in patients with primary small-sized (≤2 cm) lung cancer and investigated the association between PD-L1 protein expression and other patient characteristics. The SUVmax on preoperative ^{18}F-FDG PET/CT was significantly higher in patients with NSCLC with than in those without PD-L1 protein expression. Moreover, the multivariate analysis revealed that smoking and a high SUVmax on ^{18}F-FDG PET/CT were predictors of PD-L1 protein expression in patients with NSCLC. These results are very similar to those obtained in the analysis that did not exclude the bias of partial volume effect and suggest that PD-L1-positive NSCLC has high glucose metabolism regardless of tumor size.

Although ^{18}F-FDG PET/CT may be useful for evaluating PD-L1 protein expression in patients with NSCLC regardless of the tumor size, the mechanism of the association between FDG uptake and PD-L1 protein expression remains unclear. FDG uptake by lung carcinoma cells involves glucose metabolism, hypoxia, angiogenesis, and the mammalian target of rapamycin (mTOR) signaling pathway, and mTOR complex 1 activity affects the amount of FDG uptake by lung carcinoma cells (14). Moreover, a previous report

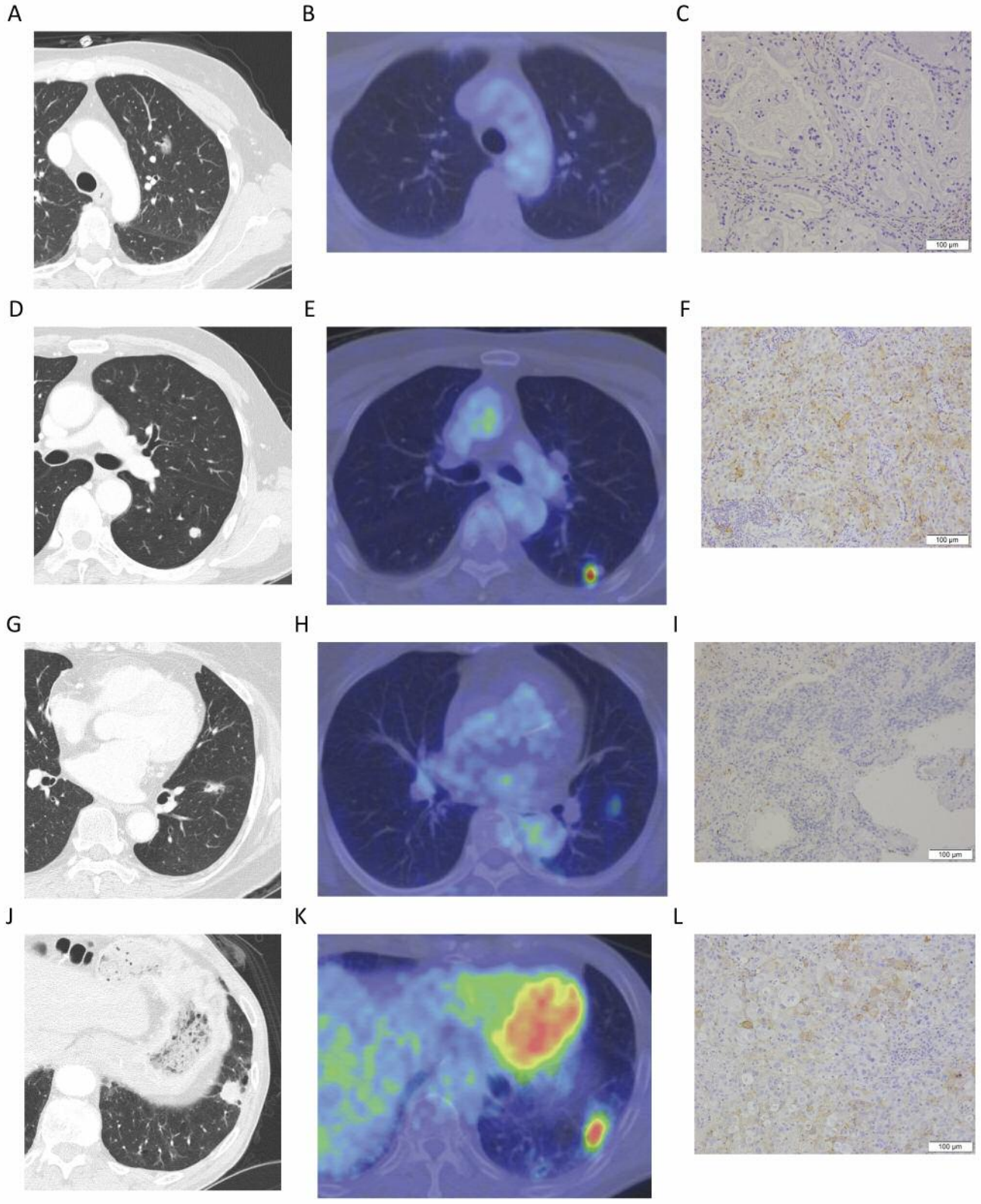

Figure 1. *Representative images of computed tomography, ^{18}F-fluorodeoxyglucose positron-emission tomography/computed tomography and immunohistochemistry in patients without (A-C, G-I) and with (D-F, J-L) expression of programmed cell death ligand 1 protein in (A-F) adenocarcinoma and (G-L) squamous cell carcinoma. The maximum standardized uptake values were: B: 0.00, E: 6.23, H: 2.18 and K: 14.87. Scale bar: 100 µm.*

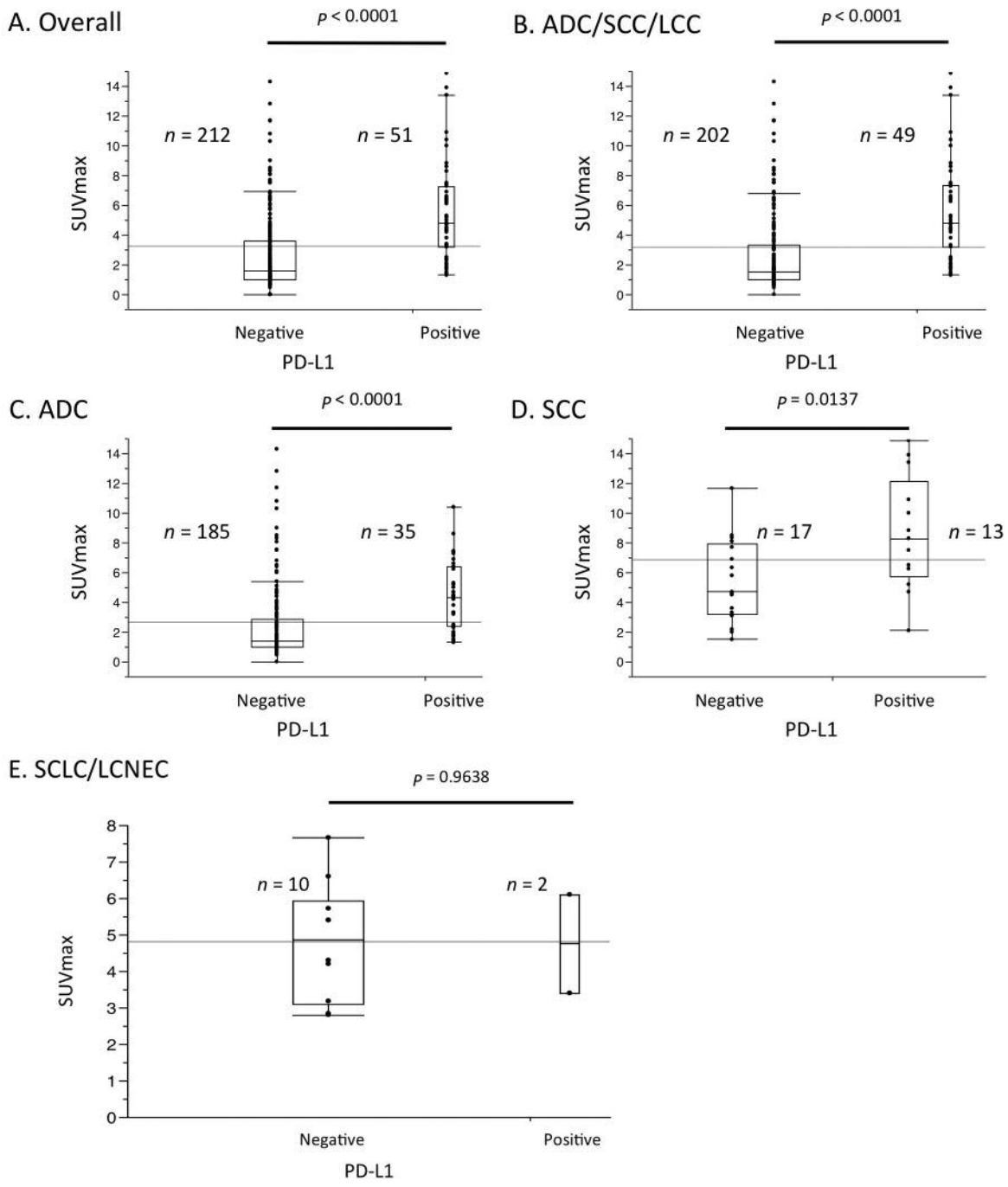

Figure 2. *Maximum standardized uptake value (SUVmax) according to expression of programmed cell death ligand 1 (PD-L1) protein. The SUVmax was significantly higher in patients with than in those without PD-L1 protein expression in the analyses of cancer overall (A), adenocarcinoma/squamous cell carcinoma/large cell carcinoma (ADC/SCC/LCC) (B), ADC (C) and SCC (D). However, there was no correlation between the SUVmax and PD-L1 protein expression in patients with neuroendocrine tumors [small cell lung carcinoma (SCLC), large cell neuroendocrine carcinoma (LCNEC).*

showed that activation of the protein kinase B (AKT)–mTOR pathway increased PD-L1 protein expression in NSCLC (24). These findings suggest that a correlation between high FDG uptake and PD-L1 protein expression may reflect the activation of the AKT–mTOR pathway. The AKT molecule has been shown to be necessary for cell proliferation (25). Given these findings, PD-L1 protein expression of tumor cells appears to be associated with oncogenic signal

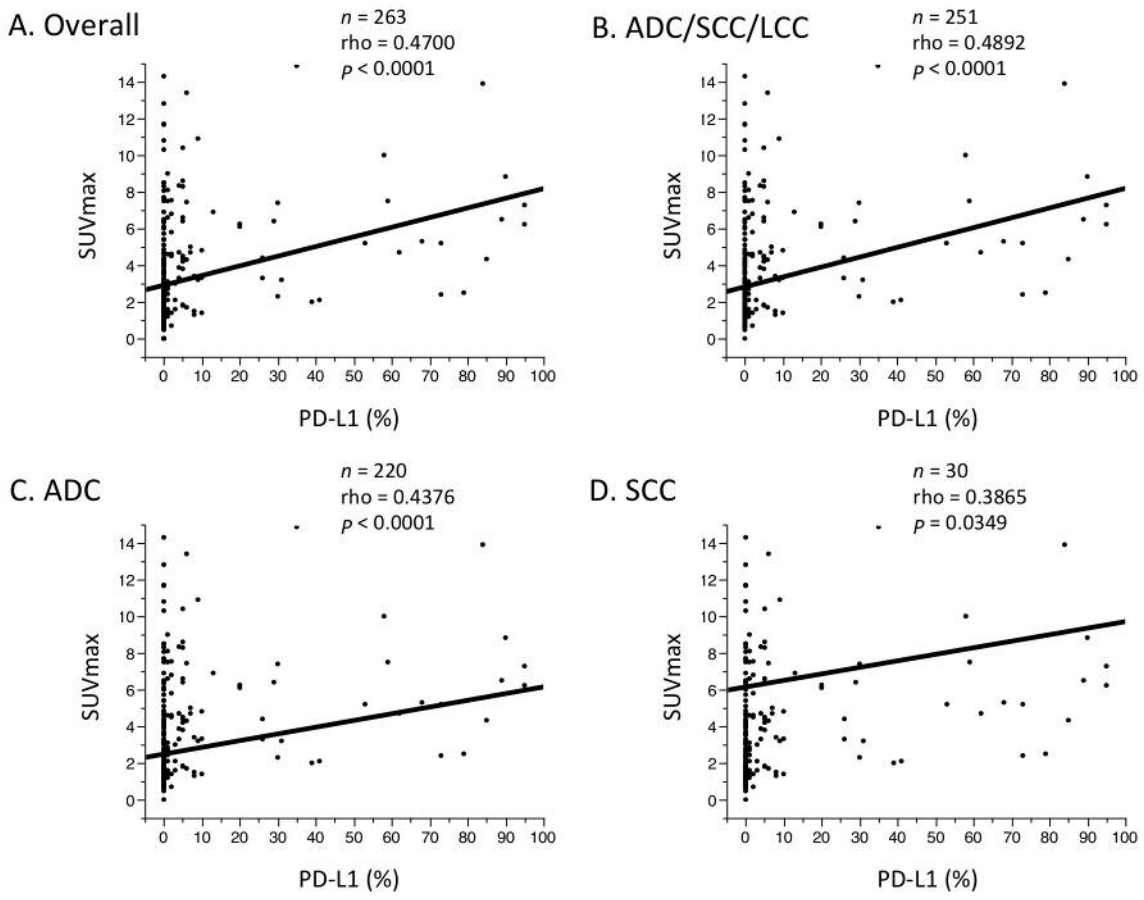

Figure 3. *Association between the proportion of programmed cell death ligand 1 (PD-L1)-positive carcinoma cells and the maximum standardized uptake value (SUVmax) in the analyses of cancer overall (A), adenocarcinoma/squamous cell carcinoma/large cell carcinoma (ADC/SCC/LCC) (B), ADC (C) and SCC (D).*

Table II. *Summary of the frequency of programmed cell death ligand 1 (PD-L1) protein expression and maximum standardized uptake value (SUVmax) in preoperative ^{18}F-fluorodeoxyglucose positron-emission tomography/computed tomography.*

Histology	n	Mean SUVmax, (range)	PD-L1 n (%)		Mean SUVmax according to PD-L1 expression, (range)		
			Negative	Positive	Negative	Positive	*p*-Value
Overall	263	3.23 (0.00-14.87)	212 (80.6)	51 (19.4)	2.68 (0.00-14.30)	5.54 (1.30-14.87)	<0.0001
ADC	220	2.65 (0.00-14.30)	185 (84.1)	35 (15.9)	2.30 (0.00-14.30)	4.44 (1.30-10.40)	<0.0001
SCC	30	6.84 (1.50-14.87)	17 (56.7)	13 (43.3)	5.46 (1.50-11.66)	8.65 (2.10-14.87)	0.0137
LCC	1	4.81	0 (0.0)	1 (100.0)	-	4.81	-
SCLC	7	4.64 (3.18-6.60)	6 (85.7)	1 (14.3)	4.85 (3.18-6.60)	3.40	0.3146
LCNEC	5	5.02 (2.80-7.66)	4 (80.0)	1 (20.0)	4.76 (2.80-7.66)	6.10	0.6468

ADC, Adenocarcinoma; SCC, squamous cell carcinoma; LCC, large cell carcinoma; SCLC, small cell lung carcinoma; LCNEC, large cell neuroendocrine carcinoma.

activation, including the AKT–mTOR pathway, that would also promote high tumor cell proliferation.

Smoking was also a predictor of PD-L1 protein expression in patients with NSCLC. According to recent genetic analyses, smoking-associated lung cancer, such as SCC and smoking-associated ADC, without mutations in the epidermal growth factor receptor gene (*EGFR*) had larger somatic mutation burdens than NSCLC in never-smokers

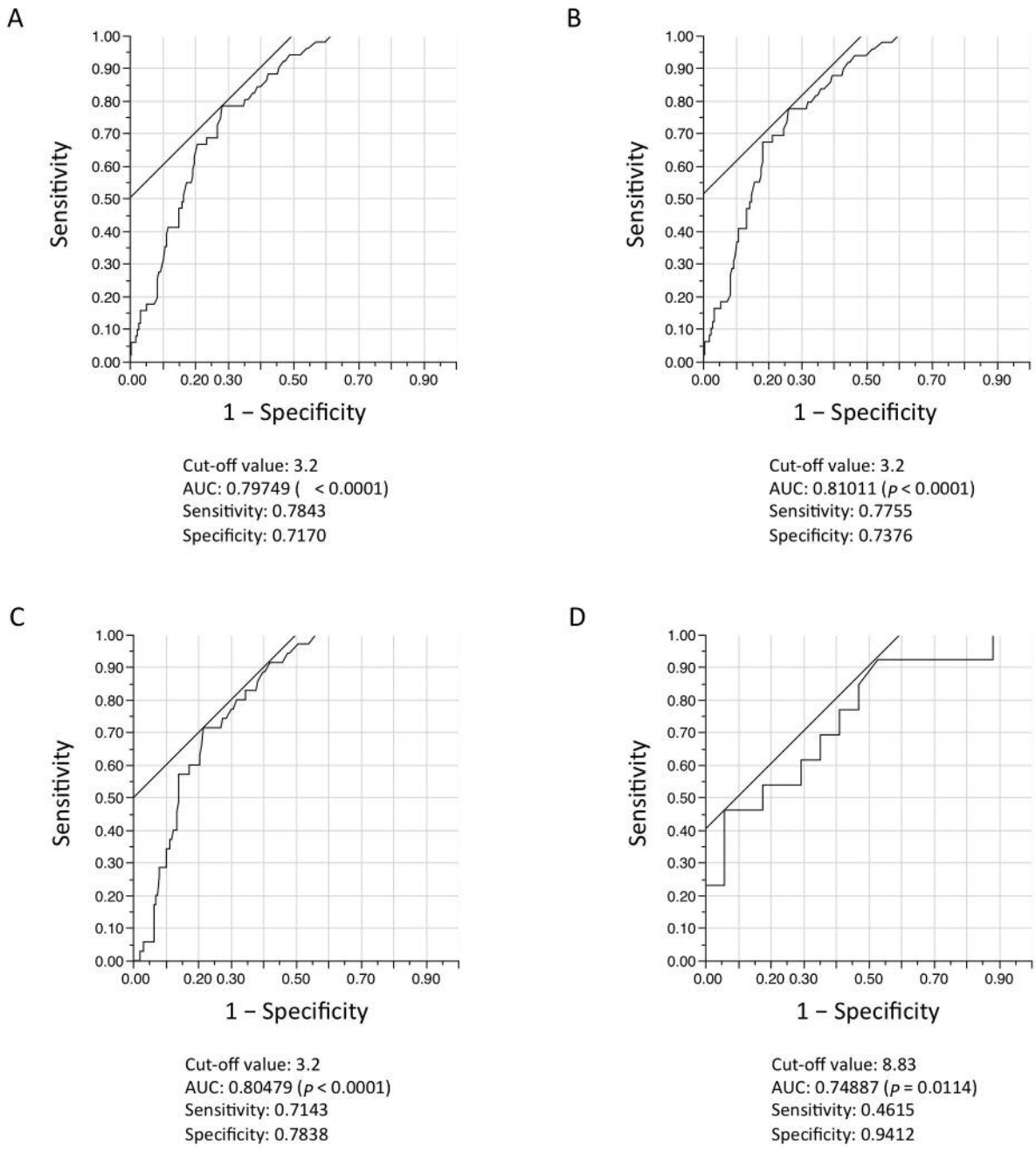

Figure 4. *Representative receiver operating characteristic curves in the analyses of cancer overall (A), adenocarcinoma/squamous cell carcinoma/large cell carcinoma (ADC/SCC/LCC) (B), ADC (C) and SCC (D). AUC: Area under the curve.*

(26, 27). Moreover, tumors with a larger number of somatic mutations were more sensitive to immune checkpoint inhibitors targeting PD-1 and PD-L1 (28). Recent studies based on The Cancer Genome Atlas project revealed that melanoma and NSCLC, which showed significant sensitivity to PD-1/PD-L1 inhibitors, had a higher mutational burden than other solid tumors (26, 29). Additionally, patients with high PD-L1 protein expression experienced greater sensitivity to anti-PD-1/PD-L1 inhibitors (3, 30, 31). These findings indicate that smoking-associated lung cancer, such as SCC and smoking-associated ADC, without *EGFR* mutations tend to exhibit higher PD-L1 protein expression than cancer in nonsmokers; thus, these types of cancer may show greater sensitivity to anti-PD-1/PD-L1 inhibitors.

Table III. *Univariate and multivariate analyses of the relationship between the likelihood programmed cell death ligand 1 (PD-L1) protein expression and other patient characteristics.*

Factor	Overall[a]				ADC/SCC/LCC			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value
Age (years)								
≥69/<69	0.96 (0.52-1.78)	0.8999			0.88 (0.47-1.65)	0.7005		
Gender								
Male/female	4.67 (2.26-10.67)	<0.0001			4.72 (2.27-10.83)	<0.0001		
Smoking status								
Smoker/never-smoker	4.80 (2.26-11.45)	<0.0001	3.41 (1.53-8.45)	0.0023	4.93 (2.31-11.80)	<0.0001	3.75 (1.66-9.35)	0.0011
Stage								
≥II/I	4.64 (2.07-10.34)	0.0003			4.85 (2.09-11.25)	0.0003		
Pleural invasion								
Present/absent	3.95 (1.87-8.25)	0.0004			4.96 (2.29-10.74)	<0.0001		
Lymphatic invasion								
Present/absent	0.75 (0.11-2.89)	0.7001			0.45 (0.02-2.46)	0.4032		
Vascular invasion								
Present/absent	2.45 (1.13-5.13)	0.0242			2.65 (1.18-5.77)	0.0192		
SUVmax[b]								
High/low	9.21 (4.57-19.97)	<0.0001	7.68 (3.75-16.85)	<0.0001	9.71 (4.77-21.23)	<0.0001	8.32 (4.02-18.44)	<0.0001

ADC: Adenocarcinoma; SCC: squamous cell carcinoma; LCC: large cell carcinoma; OR: odds ratio; CI: confidence interval; SUVmax: maximum standardized uptake value. [a]ADC, SCC, LCC, small cell lung carcinoma, and large cell neuroendocrine carcinoma. [b]Cut-off values of 3.20 and 3.20 in analyses of cancer overall and ADC/SCC/LCC, respectively.

The present study had several limitations. Firstly, this was a single-institution retrospective study, not a trial-based correlative study; however, 263 patients with small-sized lung cancer were examined for associations between PD-L1 protein expression and the SUVmax on preoperative ^{18}F-FDG PET/CT. These data may help to identify patients with PD-L1 expression who would benefit from anti-PD-1/PD-L1 inhibitors. The second limitation is that PD-L1 immunohistochemistry was conducted using only one antibody and one cut-off value. McLaughlin *et al*. recently reported discordance in PD-L1 expression within a sample when using different antibodies (32). Furthermore, the Blueprint Working Group showed that the positive staining rate using clone SP142 was lower than that using other antibodies including 28-8, 22C3, and SP263 (33). However, we previously found that SP142 had a higher positivity rate in 40 patients with small cell lung cancer than other antibodies, including 28-8 and E1L3N (21). Several antibodies and cut-off values should be evaluated in future studies. The third limitation is the lack of analysis of patients with advanced cancer such as stage IV or recurrent tumors because we examined the association between PD-L1 protein expression and the SUVmax on ^{18}F-FDG PET/CT using surgically resected primary lung cancer specimens. Further studies with larger sample sizes are warranted because the definitive cut-off for SUVmax has not been established.

Table IV. *Frequency of programmed cell death ligand 1 (PD-L1) protein expression and maximum standardized uptake value (SUVmax) in preoperative ^{18}F-fluorodeoxyglucose positron-emission tomography/computed tomography.*

Histology	Smoking history	SUVmax[b]	Percentage of t umors with positive PD-L1 expression (%)
Overall[a]	Never-smoker	Low	2.4 (2/82)
	Smoker	Low	11.1 (9/81)
	Never-smoker	High	23.1 (6/26)
	Smoker	High	45.9 (34/74)
ADC/SCC/LCC	Never-smoker	Low	2.5 (2/81)
	Smoker	Low	11.4 (9/79)
	Never-smoker	High	23.1 (6/26)
	Smoker	High	49.2 (32/65)
ADC	Never-smoker	Low	2.5 (2/81)
	Smoker	Low	10.8 (8/74)
	Never-smoker	High	21.7 (5/23)
	Smoker	High	47.6 (20/42)
SCC	<30 pack-years	Low	0.0 (0/0)
	≥30 pack-years	Low	20.0 (1/5)
	<30 pack-years	High	25.0 (1/4)
	≥30 pack-years	High	52.4 (11/21)

ADC: Adenocarcinoma; SCC: squamous cell carcinoma; LCC: large cell carcinoma. [a]ADC, SCC, LCC, small cell lung carcinoma, and large cell neuroendocrine carcinoma. [b]Cut-off values of 3.20, 3.20, 3.20, and 8.83 in analyses of cancer overall, ADC/SCC/LCC, ADC, and SCC, respectively.

In conclusion, PD-L1 protein expression was related to high glucose metabolism in small-sized NSCLC. Medical examination including assessment of the patient's smoking history and radioisotope examination by ^{18}F-FDG PET/CT, which are non-invasive or minimally-invasive tools, may be useful to predict PD-L1 protein expression in patients with small-sized NSCLC and consequently provide information on the suitability of checkpoint inhibitors for their therapy.

Conflicts of Interest

The Authors have no conflict of interest in regard to this study.

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