Radiological Features of IDO1⁺/PDL1⁺ Lung Adenocarcinoma: A Retrospective Single-institution Study

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Abstract. *Aim: A combination of immune-checkpoint inhibitors that target the programmed cell death 1 (PD1)/programmed cell-death ligand 1 (PDL1) pathway and indoleamine 2,3-dioxygenase 1 (IDO1) is a promising treatment for non-small-cell lung cancer. Herein, we investigated clinical features of IDO1⁺/PDL1⁺ primary lung adenocarcinoma. Materials and Methods: IDO1 and PDL1 expression in 388 resected primary lung adenocarcinoma samples was evaluated using immunohistochemistry, and the radiological features of patients with IDO1⁺/PDL1⁺ lung adenocarcinoma were analyzed. Results: Of 388 specimens, 229 (59.0%) were IDO1⁺, 131 (33.8%) were PDL1⁺, and 109 (28.1%) were IDO1⁺/PDL1⁺. In multivariate analysis, vascular convergence and the absence of surrounding ground glass opacity were significantly associated with IDO1⁺/PDL1⁺ tumors. Fisher's exact test showed high consolidation/tumor ratio was also significantly associated with IDO1⁺/PDL1⁺ tumors. Moreover, maximum standardized uptake in ¹⁸F-fluorodeoxyglucose positron-emission tomography/computed tomography was significantly higher in patients with IDO1⁺/PDL1⁺ tumors than in those with IDO1⁻ or PDL1⁻ tumors. Conclusion: IDO1/PDL1 co-expression was significantly related to radiological invasiveness and malignancy in lung adenocarcinoma. This study may help select patients likely to benefit from combination therapy using immune-checkpoint inhibitors.*

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Recent immunotherapy advances that target the immune checkpoint factors, programmed cell death-1 (PD1) and programmed cell death-ligand 1 (PDL1), have induced a paradigm shift in the management of non-small-cell lung cancer (NSCLC) (1-6). However, some patients who initially respond to this immunotherapy acquire resistance, and several resistance mechanisms have been reported (7-9). Therefore, combinations of PD1/PDL1 pathway inhibitors with other therapeutic methods, such as chemotherapy, radiation therapy, and other immunotherapy, have been explored to improve response rate and to overcome resistance.

The combination of inhibitors that target indoleamine 2,3-dioxygenase-1 (IDO1) and the PD1/PDL1 pathway is a promising new treatment option. IDO1 is a rate-limiting enzyme expressed on antigen-presenting cells and tumor cells which catabolizes tryptophan (an essential amino acid) into a stable metabolite under the kynurenine pathway (10). IDO1 exerts several immunosuppressive effects, such as inducing dysfunction and apoptosis of cytotoxic T-cells, converting naive T-cells into regulatory T-cells, and impairing natural killer T-cell function by depleting tryptophan and generating kynurenine (11, 12); IDO1 inhibitor thus has an antitumor effect.

The combination of inhibitors of IDO1 and PD1/PDL1 is currently attracting much attention. For example, many clinical trials of an IDO1 inhibitor (epacadostat) and a PD1 inhibitor (pembrolizumab) in patients with various types of solid tumor, including NSCLC, are ongoing (13-18). A clinical phase I trial of another combination of IDO1 and PDL1 inhibitors (INCB024360 and atezolizumab, respectively) in previously treated NSCLC is also ongoing (19). Although the clinical features of IDO1⁺/PDL1⁺ NSCLC have not been fully clarified, doing so may provide insights into their optimal use.

We recently investigated the relationship between IDO1 expression in lung adenocarcinoma and patient prognosis and clinicopathological features, including PDL1 expression (20).

We also evaluated associations between PDL1 expression and clinical features, including computed tomographic (CT) characteristics in 394 patients with primary lung adenocarcinoma (21), and the metabolic characteristics of lung cancer by using ^{18}F -fluorodeoxyglucose positron-emission tomography/CT (^{18}F -FDG PET/CT) with regard to PDL1 expression (22). In the current study, we analyzed the radiological features of IDO1⁺/PDL1⁺ lung adenocarcinoma, which may help select patients who are likely to benefit from the combination therapy.

Materials and Methods

Patients and samples. We retrospectively examined the data for patients who underwent surgical resection of primary lung adenocarcinoma between January 2003 and December 2012 at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University. Previously, we had analyzed the relationships between PDL1 expression and clinical features in 394 patients who had undergone preoperative thin-section CT at our Institution (21). Among them, 388 whose formalin-fixed and paraffin-embedded (FFPE) tumor tissue sections were available for immunohistochemistry (IHC) of IDO1 were enrolled in this study. Their clinicopathological features, including age at surgery, sex, smoking history, pathological tumor-node-metastasis stage (American Joint Committee on Cancer Lung Cancer Staging System, seventh edition) (23), histological subtype (World Health Organization Classification, 2015) (24), and mutation status of epidermal growth factor receptor (*EGFR*) gene were examined. *EGFR* status had previously been determined in tumor tissue using the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp method (Mitsubishi Chemical Medience, Tokyo, Japan) in 230 specimens (25). Clinical information was obtained from medical records. This study was approved by our Institutional Review Board (Kyushu University, IRB No. 29-318).

Chest CT. Patients underwent chest CT in the supine position during inspiratory breath-hold using various multi-detector row scanners: Aquilion 4 (Toshiba), Aquilion 64 (Toshiba), Aquilion One (Toshiba), Aquilion One Vision (Toshiba), Somatom Plus4 Volume Zoom (Siemens), Briliance CT (Phillips), and Briliance iCT (Phillips). The imaging parameters for thin-section CT were tube voltage: 100-500 mA; tube current: 120 kVp; scan field of view: 320-360 mm; slice thickness: 2 mm. Real-time exposure control (Toshiba) or automatic exposure control (Siemens and Phillips) was added in each study. All of the CT data sets were transferred to a Picture Archiving and Communication System, which was accessible at workstations (Volume Analyzer, Synapse-Vincent; Fujifilm, Tokyo, Japan) with a specialized application for lungs. The consolidation diameter of each tumor (C) and the diameter of the whole tumor (T) including ground glass opacity (GGO) were measured manually with axial 2-dimensional CT data at 2-mm sections; the C/T ratio was then calculated. Three thoracic oncologists evaluated all CT images; if their independent assessments did not agree, the CT images were reviewed together to achieve consensus judgments, which were adopted as final results.

^{18}F -FDG PET/CT. ^{18}F -FDG PET/CT was performed using various scanners: Secat Exact HR⁺ (Siemens), Biograph mCT (Siemens),

Table I. Clinicopathological characteristics of all patients.

Factors	Value
Age, years	
Median (range)	69 (29-85)
Gender, n	
Male	193
Female	195
Smoking status, n	
Never-smoker	197
Smoker	191
Pathological stage, n	
IA	210
IB	72
IIA	32
IIB	28
IIIA	36
IIIB	5
IV	5
Histological subtype, n	
AAH/AIS/MIA	38
Lepidic predominant	24
Papillary predominant	288
Acinar predominant	6
Micropapillary predominant	1
Solid predominant	25
Variants	6
<i>EGFR</i> , n*	
Wild-type	119
Mutant	111
IDO1, n	
Negative	159
Positive	229
PDL1, n	
Negative	257
Positive	131
IDO1/PDL1, n	
Other	279
Co-expression	109

AAH: Atypical adenomatous hyperplasia; AIS: adenocarcinoma *in situ*; *EGFR*: epidermal growth factor receptor gene; IDO1: indoleamine 2,3-dioxygenase 1; MIA: minimally invasive adenocarcinoma; PDL1: programmed cell-death ligand 1. *Cases for which data were available.

Discovery STElite16 (General Electric Healthcare, Milwaukee, WI, USA). Each tumor's maximum standardized uptake value (SUV_{max}) was evaluated, and statistically analyzed.

IHC analysis. FFPE tumor-tissue sections were used to determine IHC expression of IDO1 and PDL1 in primary lung adenocarcinomas. IHC analysis was conducted using commercially available antibodies against IDO1 at 1:200 dilution (mouse monoclonal, clone UMAB126; Origene Technologies, Rockville, MD, USA), and PDL1 at 1:100 dilution (rabbit monoclonal, clone SP142; Spring Bioscience, Ventana, Tucson, AZ, USA). IHC staining for PDL1 was performed as previously described (26). IHC staining for IDO1 was performed as follows: 4- μm sections were cut, dewaxed with xylene, and rehydrated through an ethanol

Figure 1. Representative images of immunohistochemical staining for indoleamine 2,3-dioxygenase 1 (IDO1) (A) and programmed cell-death ligand 1 (PDL1) (B) in surgically resected specimens from patients with primary lung adenocarcinoma. A: Positive cytoplasmic and membranous staining for IDO1. B: Positive membranous staining for PDL1. Scale bar=100 µm.

gradient. After inhibiting endogenous peroxidase activity for 30 min with 3% H₂O₂ in methanol, sections were pretreated with EDTA (pH 8.0) in a decloaking chamber at 110°C for 15 min, and then incubated with monoclonal antibodies at 4°C overnight. The immune complex was detected with a Dako EnVision Detection System (Dako, Glostrup, Denmark). The sections were finally reacted in 3,3'-diaminobenzidine, counterstained with hematoxylin, and mounted. Sections from human placentas were used as positive controls for IDO1.

The tumor proportion score (TPS) was independently estimated as the proportion of positively stained cells as a percentage that of total carcinoma cells in whole sections by three investigators (KT, KK, and YK) who were blinded to patients' clinical status. Final scores were decided by consensus. IHC evaluation for PDL1 was conducted as previously described (26); samples with less than 1% tumor (membranous) staining were considered negative in this study. For IDO1, cytoplasmic and membranous immunostaining on tumor cells was quantified; a cut-off value at 1% was set in this study, with reference to a previous report (27).

Statistical analysis. Associations between co-expression of IDO1/PDL1 and patient characteristics or C/T ratios were analyzed using Fisher's exact test. Univariate and multivariate analyses of relationships between IDO1/PDL1 co-expression and CT features, such as vascular convergence, surrounding GGO, air bronchogram, notching, pleural indentation, spiculation, and cavitation, were performed by logistic regression analysis with the backward elimination method. The association between IDO1/PDL1 co-expression and SUV_{max} in preoperative ¹⁸F-FDG PET/CT was examined using Student's *t*-test. All statistical analyses were performed using JMP Statistical Discovery Software (version 11.0; SAS Institute, Cary, NC, USA). Values of *p*<0.05 were considered significant.

Results

IDO1⁺/PDL1⁺ lung adenocarcinoma and patient clinicopathological characteristics. Of the 388 patients in the present study, 193 (49.7%) were male; 197 (50.8%) had never smoked; their median age was 69 years (range=29-85 years; Table I). Of the 230 patients for whom *EGFR* status was available, 119 (51.7%) had wild-type *EGFR*, and 111 (48.3%) had mutant *EGFR*.

IHC staining for IDO1 was detected in cytoplasm and membrane, and that for PDL1 was detected on cancer-cell membranes (Figure 1). Of the 388 specimens, 229 (59.0%) were IDO1⁺, 131 (33.8%) were PDL1⁺, and 109 (28.1%) were IDO1⁺/PDL1⁺. IDO1/PDL1 co-expression was significantly associated with male sex, smoking history, advanced-stage disease, predominantly micropapillary or solid histological subtypes, and wild-type *EGFR* (Fisher's exact test; Table II).

IDO1/PDL1 co-expression and CT features in primary lung adenocarcinoma. Figure 2 shows representative images of CT features, vascular convergence, surrounding GGO, air bronchogram, notching, pleural indentation, spiculation, and cavitation. Among the 388 patients, vascular convergence was found in 262 (67.5%), surrounding GGO in 188 (48.4%), air bronchogram in 308 (79.4%), notching in 139 (35.8%), pleural indentation in 306 (78.9%), spiculation in 175 (45.1%), and cavitation in 73 (18.8%; Table III). In univariate analysis, IDO1/PDL1 co-expression was

Figure 2. Representative images showing computed tomographic features in patients with primary lung adenocarcinoma. A: Vascular convergence and notching. B: Surrounding ground glass opacity and pleural indentation. C: Air bronchogram. D: Spiculation. E: Cavitation.

Figure 3. Maximum standardized uptake value (SUV_{max}) in ^{18}F -fluorodeoxyglucose positron-emission tomography/computed tomography according to "Indoleamine 2,3-dioxygenase 1 (IDO1)/programmed cell-death ligand 1 (PDL1) co-expression. SUV_{max} was significantly higher in patients with IDO1⁺/PDL1⁺ specimens than those with IDO1⁻ or PDL1⁻ specimens ($p < 0.0001$). Data are presented as box-and-whisker plots. The box spans the interquartile range, the median is indicated by a line inside the box, and the upper and lower whiskers indicate the third quartile plus $1.5 \times IQR$ and the first quartile minus $1.5 \times IQR$.

significantly associated with the presence of vascular convergence, notching, spiculation, and cavitation, and the absence of surrounding GGO and air bronchogram. In multivariate analysis, the presence of vascular convergence and the absence of surrounding GGO were significantly associated with IDO1/PDL1 co-expression (Table III).

IDO1⁺/PDL1⁺ expression was positively associated with C/T ratio ($p = 0.0002$; Table IV).

IDO1⁺/PDL1⁺ expression and ^{18}F -FDG PET/CT results in primary lung adenocarcinoma. The metabolic characteristics of primary lung adenocarcinoma were evaluated using ^{18}F -FDG PET/CT, an essential imaging modality in lung cancer diagnosis and staging, with regard to IDO1/PDL1 co-expression in 222 patients for whom data were available. The average SUV_{max} of patients with IDO1⁺/PDL1⁺ tumors (8.41; range=0.80-28.3) was significantly higher than that of patients with IDO1⁻ or PDL1⁻ tumors (4.45; range=0.00-30.4; $p < 0.0001$; Figure 3).

Discussion

In the present study, we examined the relationships between IDO1/PDL1 co-expression and features of chest CT and ^{18}F -FDG PET/CT in patients with primary lung adenocarcinoma.

Table II. Association between indoleamine 2,3-dioxygenase 1 (IDO1)/programmed cell-death ligand 1 (PDL1) expression and clinicopathological factors.

Factor	N (%)	IDO1/PDL1, N (%)		p-Value
		Other	Co-expression	
Age				
<70 Years	205 (52.8)	148 (53.1)	57 (52.3)	0.9103
≥70 Years	183 (47.2)	131 (46.9)	52 (47.7)	
Gender				
Male	193 (49.7)	129 (46.2)	64 (58.7)	0.0318
Female	195 (50.3)	150 (53.8)	45 (41.3)	
Smoking status				
Never-smoker	197 (50.8)	155 (55.6)	42 (38.5)	0.0032
Smoker	191 (49.2)	124 (44.4)	67 (61.5)	
Radiological tumor diameter				
≤2.0 cm	176 (45.4)	133 (47.7)	43 (39.5)	0.1733
>2.0 cm	212 (54.6)	146 (52.3)	66 (60.5)	
Pathological stage				
I	282 (72.7)	214 (76.7)	68 (62.4)	0.0054
≥II	106 (27.3)	65 (23.3)	41 (37.6)	
Histological subtype				
Micropapillary/solid	26 (6.7)	8 (2.9)	18 (16.5)	<0.0001
Other	362 (93.3)	271 (97.1)	91 (83.5)	
EGFR*				
Wild-type	119 (51.7)	81 (46.0)	38 (70.4)	0.0019
Mutant	111 (48.3)	95 (54.0)	16 (29.6)	

EGFR: Epidermal growth factor receptor gene. *Cases for which data were available.

The results of this study were similar to those of our previous reports on PDL1 expression (21, 22, 28). Moreover, both radiological and clinicopathological features of IDO1⁺/PDL1⁺ lung adenocarcinoma were similar to those of PDL1⁺ lung adenocarcinoma in accordance with our previous reports (21, 26). We recently showed that IDO1 expression was significantly associated with PDL1 expression in primary lung adenocarcinoma; all patients in an earlier study cohort with strong PDL1 expression (PDL1 TPS ≥50%) were positive for IDO1 (20). Expression of both IDO1 and PDL1 increases through responses to interferon- γ and transforming growth factor- β released in the tumor microenvironment, and immunotherapies that target these proteins might be effective against T-cell-inflamed tumors (19). Therefore, the relationship between IDO1/PDL1 co-expression and the infiltration by immune-related cells such as CD3⁺, CD4⁺, and CD8⁺ cells should be examined in future studies in order to confirm the above points. Our present results indicate that the combination of inhibitors against IDO1 and the PD1/PDL1 pathway might be more effective against non-squamous cell cancer with no *EGFR* mutation, in patients with smoking history, which is expected

Table III. Univariate and multivariate analyses for relationships between indoleamine 2,3-dioxygenase 1 (IDO1)/programmed cell-death ligand 1 (PDL1) expression and computed tomographic (CT) features.

CT feature		N (%)	IDO1/PDL1, N (%)		Univariate analysis			Multivariate analysis		
			Other	Co-expression	OR	95% CI	p-Value	OR	95% CI	p-Value
Vascular convergence	Absent	126 (32.5)	111 (39.8)	15 (13.8)	1.00			1.00		
	Present	262 (67.5)	168 (60.2)	94 (86.2)	4.14	2.34-7.77	<0.0001	2.10	1.10-4.18	0.0251
Surrounding GGO	Absent	200 (51.6)	114 (40.9)	86 (78.9)	1.00			1.00		
	Present	188 (48.4)	165 (59.1)	23 (21.1)	0.18	0.11-0.31	<0.0001	0.25	0.14-0.43	<0.0001
Air bronchogram	Absent	80 (20.6)	49 (17.6)	31 (28.4)	1.00					
	Present	308 (79.4)	230 (82.4)	78 (71.6)	0.54	0.32-0.91	0.0200			
Notching	Absent	249 (64.2)	193 (69.2)	56 (51.4)	1.00					
	Present	139 (35.8)	86 (30.8)	53 (48.6)	2.12	1.35-3.35	0.0011			
Pleural indentation	Absent	82 (21.1)	64 (22.9)	18 (16.5)	1.00					
	Present	306 (78.9)	215 (77.1)	91 (83.5)	1.50	0.86-2.74	0.1557			
Spiculation	Absent	213 (54.9)	171 (61.3)	42 (38.5)	1.00					
	Present	175 (45.1)	108 (38.7)	67 (61.5)	2.53	1.61-4.00	<0.0001			
Cavitation	Absent	315 (81.2)	236 (84.6)	79 (72.5)	1.00					
	Present	73 (18.8)	43 (15.4)	30 (27.5)	2.08	1.22-3.54	0.0076			

CI: Confidence interval; GGO: ground glass opacity; OR: odds ratio.

to show greater sensitivity to anti-PD1/PDL1 treatment than to monotherapy with PD1/PDL1 inhibitors (1-4, 6).

Vascular convergence of surrounding structures on chest CT is a sign of malignancy, and is considered to reflect fibrosis in the tumor; surrounding GGO in tumors, especially adenocarcinomas, indicates a replacement growth pattern of alveolar-lining cells (29). A predominant GGO patterns suggest tumor noninvasiveness, as the Japan Clinical Oncology Group 0201 study defined tumors with a C/T ratio ≤ 0.25 on thin-section CT as being radiologically noninvasive lung adenocarcinoma, since such tumors correspond well to pathologically noninvasive adenocarcinomas with very high sensitivity (30). We recently reported that the presence of vascular convergence, the absence of surrounding GGO, and a high C/T ratio were significantly associated with PDL1⁺ lung adenocarcinoma, which was likely to be radiologically invasive and malignant (21, 28). In this study, IDO1⁺/PDL1⁺ lung adenocarcinoma was also significantly associated with the presence of vascular convergence, absence of surrounding GGO, and high C/T ratio. Therefore, IDO1⁺/PDL1⁺ lung adenocarcinoma may also be radiologically invasive and malignant, similarly to PDL1⁺ lung adenocarcinoma. Moreover, IDO1⁺/PDL1⁺ lung adenocarcinomas had higher SUV_{max} in ¹⁸F-FDG PET/CT than IDO1⁻ and PDL1⁻ tumors, which indicates that IDO1/PDL1 co-expression is related to malignant features associated with high glucose metabolism, similarly to PDL1 expression (22).

We also examined the association between IDO1/PDL1 co-expression and clinicopathological features including age at surgery, sex, smoking history, pathological tumor-node-

Table IV. Indoleamine 2,3-dioxygenase 1 (IDO1)/programmed cell-death ligand 1 (PDL1) expression according to consolidation diameter/tumor diameter (C/T) ratio on computed tomography.

C/T ratio	IDO1/PDL1, N (%)		p-Value
	Other	Co-expression	
0 (Pure GGO)	33 (11.8)	6 (5.5)	0.0002
0.10-0.25	19 (6.8)	1 (0.9)	
0.26-0.50	43 (15.4)	7 (6.4)	
≥ 0.51	184 (66.0)	95 (87.2)	

GGO: Ground glass opacity.

metastasis stage, histological subtype, and EGFR mutation status. In this study, IDO1/PDL1 co-expression was significantly associated with male sex, smoking history, advanced-stage disease, predominantly micropapillary or solid histological subtypes, and wild-type *EGFR*. Our previous reports showed that PDL1 expression was also significantly associated with these factors in lung adenocarcinoma (21, 26). These data suggest that both PDL1⁺ and IDO1⁺/PDL1⁺ lung adenocarcinomas are pathologically invasive and malignant, and imply that clinical features of IDO1⁺/PDL1⁺ lung adenocarcinoma are similar to those of PDL1⁺ lung adenocarcinoma in both clinicopathological and radiological features.

There are several limitations associated with the present study. Firstly, this was a retrospective single-institution study

and not a trial-based correlative study; however, 388 patients were examined for associations between IDO1/PDL1 co-expression, for the features of two imaging modalities, chest CT and ¹⁸F-FDG PET/CT, in addition to their clinicopathological characteristics. The data obtained might help identify patients who would benefit from combination therapies that target IDO1 and the PD1/PDL1 pathway. Validation cohort studies should be conducted to confirm our findings. Secondly, we conducted PDL1 IHC using only one antibody, SP142, which was used in clinical trials on atezolizumab (1, 4). Some recent studies showed that the positivity rate of PDL1 expression using SP142 was lower than that for other antibodies, such as 28-8, 22C3, and SP263 (31-33). However, a clinical phase I trial on combination therapy using the IDO1 inhibitor INCB024360, and the PDL1 inhibitor atezolizumab, in previously treated NSCLC is ongoing (19). This present study may be a useful reference in understanding the results of that clinical trial. PDL1 expression should be evaluated using other antibodies, such as 22C3, as used in the ongoing clinical trials with the combination therapy of IDO1 inhibitor epacadostat, and PD1 inhibitor pembrolizumab, in patients with various types of solid tumors, including NSCLC (13-18). Thirdly, no definitive guidelines for antibody use or quantification of IDO1 expression in NSCLC exist, and no comparative data for different IDO1 antibodies are available. We used clone UMAB126 and set the cut-off value for positivity at 1% staining of tumor-cell cytoplasm and membrane in this study. However, this antibody has not been evaluated in a clinical setting. Therefore, IDO1 expression should be further evaluated using other antibodies and cut-off values. The fourth limitation is the lack of analysis for advanced, late-stage disease because we examined associations between IDO1/PDL1 co-expression and clinical features using surgical specimens. An analysis of advanced cases is warranted to validate our findings here.

To our knowledge, this is the first report to demonstrate a relationship between IDO1/PDL1 co-expression and clinicopathological characteristics, including features of imaging modalities in resected lung adenocarcinomas. We found IDO1/PDL1 co-expression to be associated with radiological invasiveness and malignancy in lung adenocarcinoma. This study may be a useful reference in selecting patients who are likely to benefit from combined IDO1/PDL1 inhibitor therapy.

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Conflicts of Interest

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

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