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Co-expression of IDO1 and PD-L1 in lung squamous cell carcinoma: Potential targets of novel combination therapy

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ABSTRACT

Objectives: Combination therapy with an inhibitor of indoleamine 2, 3-dioxygenase 1 (IDO1) and an agent targeting programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) is expected to be a novel and effective treatment option for various solid tumors including non-small cell lung cancer (NSCLC). Therefore, it is important to elucidate the clinical and pathological features of tumors with IDO1/PD-L1 co-expression and the association between IDO1/PD-L1 co-expression and efficacy of combination therapy in NSCLC patients. In this study, we examined the prognostic impact of IDO1/PD-L1 co-expression and its relationship with tumor-infiltrating lymphocytes (TILs) in primary lung squamous cell carcinoma (SCC).

Materials and methods: The expression levels of IDO1, PD-L1, Ki-67, cluster of differentiation 3 (CD3), CD4, and CD8 in 202 patients with surgically resected primary lung SCC were evaluated by immunohistochemistry.

Results: Among 202 patients, 176 (87.1%) were positive for IDO1 expression, 106 (52.5%) were positive for PD-L1 expression, and 99 (49.0%) showed co-expression of IDO1/PD-L1 proteins. Fisher's exact test showed a significant association between IDO1 and PD-L1 tumor proportion scores ($P = 0.0011$). Kaplan–Meier curve showed that PD-L1 alone and co-expression of IDO1 and PD-L1 were significantly associated with shorter overall survival, but IDO1 alone was not (log rank test: $P = 0.0122$, $P = 0.0303$ and $P = 0.5168$, respectively). The Ki-67 labeling index was significantly higher in patients with co-expression of IDO1 and PD-L1 than in patients without co-expression (Student's *t*-test: $P = 0.0005$). Moreover, IDO1/PD-L1 co-expression was significantly associated with high CD3, CD4, and CD8 expression (Fisher's exact test: $P = 0.0033$, $P = 0.0003$, and $P < 0.0001$, respectively).

Conclusions: IDO1 expression correlated to PD-L1 expression, and co-expression of IDO1 and PD-L1 may be important targets for immunotherapy in lung SCC.

1. Introduction

Recently, many combination therapies involving an immune checkpoint inhibitor targeting the programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) pathway and another treatment option such as chemotherapy, radiation therapy, and various immunotherapy agents have been explored to improve the response rate and clinical outcomes in various solid tumors including non-small cell lung cancer (NSCLC) [1–8]. Especially, combination therapy with an indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor and a PD-1/PD-L1 inhibitor is expected to be a novel and effective treatment option and is currently attracting a lot of attention. Many clinical trials with combination therapy involving the IDO1 inhibitor epacadostat and a PD-1 inhibitor, either pembrolizumab or nivolumab, in patients with various solid tumors including NSCLC are ongoing [7–14].

IDO1 is a rate-limiting enzyme that catabolizes conversion of tryptophan, an essential amino acid necessary for cell survival, into a stable metabolite under the kynurenine pathway [15]. In normal tissues, IDO1 is expressed by endothelial cells in the placenta and lung, epithelial cells in the female genital tract, and inflammatory lesions; in the tumor

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microenvironment it is also expressed by antigen-presenting cells such as dendritic cells, macrophages, and tumor cells that are exposed to interferon-γ (IFN-γ) and other proinflammatory stimuli [15,16]. IDO1 plays an important role in immune tolerance; it induces dysfunction and apoptosis of cytotoxic T cells, converts naive T cells into regulatory T cells (Treg), and impairs the function of natural killer T cells through the depletion of tryptophan and generation of kynurenine, leading to a cancer immunosuppressive state [17,18]. Therefore, IDO1 is a potential immune-based therapeutic target, and combination therapy with an IDO1 inhibitor and an anti-PD-1/PD-L1 drug may be an effective treatment option. Elucidation of the clinical and pathological features associated with IDO1/PD-L1 co-expression in NSCLC may provide insights into effective strategies for combination therapy.

Recently, we investigated the relationship between IDO1 expression in lung adenocarcinoma and patient prognosis and clinicopathological features, including PD-L1 expression. In this translational study, we examined the prognostic impact of IDO1/PD-L1 co-expression and its relationship with tumor-infiltrating lymphocytes (TILs) to elucidate the clinicopathological impact of IDO1/PD-L1 co-expression in primary lung squamous cell carcinoma (SCC). This study may be a useful reference for the selection of patients likely to benefit from combination therapy for lung SCC.

2. Materials and methods

2.1. Patients and samples

We retrospectively examined patients with primary lung SCC who underwent complete surgical resection between January 1990 and December 2010 at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University. Previously, we conducted immunohistochemical analysis of PD-L1 and Ki-67 to analyze the relationship between PD-L1 expression and clinical features and proliferative capacity in 205 patients with primary lung SCC [19]. We enrolled 202 of these patients with available formalin-fixed and paraffin-embedded (FFPE) tumor tissue sections for immunohistochemistry of IDO1, cluster of differentiation 3 (CD3), CD4, and CD8 in this study. The patients who had the past history of SCC of the head and neck or esophagus and who received neoadjuvant therapy were excluded from this study because the possibility of metastatic carcinoma from these cancers and the possibility of inconsistency in the tumor microenvironment before and after neoadjuvant therapy cannot be denied. Clinicopathological features, including age at surgery (< 70 or ≥ 70 years), sex (male or female), smoking history (< 30 or ≥ 30 pack years), tumor differentiation (non-keratinizing type or keratinizing type), pathologic tumor-node-metastasis (TNM) stage (seventh edition) (T1 or ≥ T2, N0 or ≥ N1, and stage I or ≥ stage II), pleural or lymphovascular invasion (absent or present), and surgical procedure (≥ lobectomy or sublobar resection) were examined. Systemic dissection of the hilar and mediastinal lymph nodes was performed during lobectomy. In sublobar resection, selected lymph node sampling was performed. Perioperative therapies, which were selected by the physician, were performed according to the Japanese clinical practice guidelines for lung cancer. After surgery, routine check-ups including a physical examination, blood tests (including evaluation of serum tumor marker levels), and a chest X-ray were performed at 3-month intervals for the first 3 years and at 6-month intervals thereafter. Computed tomography was performed twice a year for the first 3 years and at least annually thereafter. Adjuvant chemotherapy was administered to some patients when necessary. The eligibility criteria for patients receiving adjuvant chemotherapy were as follows: (i) p-stage IB to IIIB disease; (ii) < 76 years of age; (iii) performance status of 0 or 1; and (iv) written informed consent. The regimen for p-stage IB disease was uracil-tegafur, whereas that for p-stage IIA to IIIB disease was in principle a platinum-based combination regimen. The clinical information and follow-up data were obtained from the patients' medical records. This study was approved by our institutional review board (Kyushu University, IRB No. 29-318).

2.2. Immunohistochemical analysis

We used 4-μm thick FFPE tumor tissue sections to conduct immunohistochemistry of IDO1, PD-L1, Ki-67, CD3, CD4, and CD8 in 202 patients with surgically resected primary lung SCC. Immunohistochemical staining for PD-L1 and Ki-67 was performed as previously described [19]. Immunohistochemical staining of IDO1, CD3, CD4, and CD8 was performed as follows: 4-μm sections were cut, dewaxed with xylene, and rehydrated through a graded series of ethanol. After inhibition of endogenous peroxidase activity for 30 min with 3% H_2O_2 in methanol, the sections were pretreated with EDTA buffer (pH = 8.0) (IDO1), citrate buffer (pH = 6.0) (CD3), or Target Retrieval Solution (pH = 9.0) (DakoCytomation, Glostrup, Denmark) (CD4 and CD8) in a decloaking chamber at 110 °C for 15 min (IDO1) or a microwave at 100 °C for 20 min (CD3, CD4, and CD8), and then incubated with monoclonal antibodies at 4 °C overnight. The immune complex was detected with a DAKO EnVision Detection System (DakoCytomation). The sections were finally reacted in 3,3′-diaminobenzidine, counterstained with hematoxylin, and mounted. Sections from human placenta for IDO1 and human tonsil tissue for CD3, CD4, and CD8 were used as positive controls.

The immunohistochemical analysis was conducted using commercially available antibodies as follows: anti-IDO1 antibody at 1:200 dilution (mouse monoclonal, clone UMAB126, Origene Technologies, Rockville, MD), anti-PD-L1 antibody at 1:100 dilution (rabbit monoclonal, clone SP142, Spring Bioscience, Ventana, Tucson, AZ), anti–Ki-67 antibody at 1:100 dilution (mouse monoclonal, clone MIB-1, DakoCytomation, Glostrup, Denmark), anti-CD3 antibody (prediluted; mouse monoclonal, clone PS1, Nichirei Biosciences, Tokyo, Japan), anti-CD4 antibody at 1:100 dilution (mouse monoclonal, clone 4B12, DakoCytomation, Carpinteria, CA), and anti-CD8 antibody at 1:100 dilution (mouse monoclonal, clone 1 A5, BioGenex, Fremont, CA).

All immunohistochemical data were evaluated by two experienced observers (K.T. and K.K.) who were blinded to the clinical status of the patients. The final assessments were achieved by consensus. Regarding immunohistochemical evaluation for IDO1 and PD-L1, cytoplasmic IDO1 expression and membrane PD-L1 expression on tumor cells was defined by tumor proportion score (TPS) of $< 1\%$, 1%–49%, and $\geq 50\%$, and cases with TPS $< 1\%$ were considered negative in this study. Moreover, we defined the co-expression of IDO1 and PD-L1 as being when IDO1 TPS $\geq 1\%$ and PD-L1 TPS $\geq 1\%$. Immunohistochemical evaluation for Ki-67 was performed as previously described [19]. In addition, the number of CD3+, CD4+, and CD8 + TILs was counted and averaged over three high-power fields for each case, and the median numbers of CD3+, CD4+, and CD8 + TILs were determined as the cut-off points for CD3+, CD4+, and CD8 + TIL density, respectively [20,21]. Representative images of immunohistochemical staining for IDO1, PD-L1, Ki-67, CD3, CD4, and CD8 are shown in Fig. 1A and B and Supplementary Fig. 1.

2.3. Statistical analysis

Statistical analyses of categorical factors were performed using Fisher's exact test. Overall survival (OS) was defined as the time from surgery to last follow-up or death from any cause. Survival was analyzed using the Kaplan–Meier method and compared using the log-rank test. Cox proportional hazards regression analysis was performed to estimate the hazard ratios for the positive risk factors with the backward elimination method. The association between IDO1/PD-L1 co-expression and the Ki-67 labeling index was examined using Student's *t*-test. All statistical analyses were performed using JMP Statistical Discovery Software (version 11.0; SAS Institute, Cary, NC). Results were considered statistically significant at $P < 0.05$.

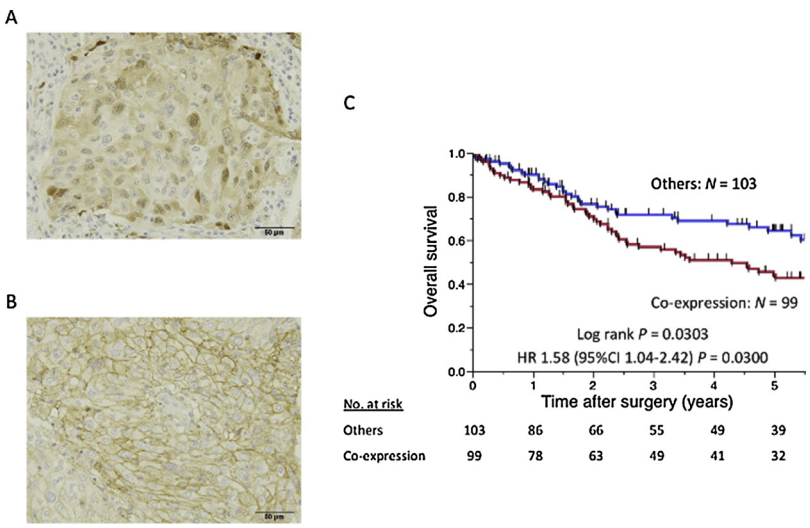

Fig. 1. Representative images of immunohistochemical staining for IDO1 and PD-L1 in surgically resected specimens from patients with primary lung SCC and Kaplan–Meier curve showing OS of patients with primary lung SCC according to IDO1 and PD-L1 expression. We adopted cut-off values of 1% for IDO1 and PD-L1 expression in this analysis. (A) Positive cytoplasmic staining for IDO1. (B) Positive membrane staining for PD-L1. (C) Patients with IDO1/PD-L1 co-expression had significantly shorter OS after surgery than patients without co-expression (log rank test value of $P = 0.0303$). Scale bar: 50 μm. IDO1, indoleamine 2,3-dioxygenase 1; PD-L1, programmed cell death-ligand 1; SCC, squamous cell carcinoma; OS, overall survival.

3. Results

3.1. Association between IDO1/PD-L1 co-expression and clinicopathological characteristics

The characteristics of the 202 patients in this study are listed in Table 1. The median age of all patients was 69 years (range, 39–87 years), 168 (83.2%) patients were male, and 171 (84.7%) were heavy smokers (≥ 30 pack years).

Among 202 patients, 176 (87.1%) were positive for IDO1 expression, 106 (52.5%) were positive for PD-L1 expression, and 99 (49.0%) patients showed co-expression of IDO1/PD-L1 proteins. The association between IDO1/PD-L1 co-expression and clinicopathological factors is described in **Supplementary** Table 1, and IDO1/PD-L1 co-expression was not associated with any of the clinicopathological features by Fisher's exact test. There was a significant association between IDO1 and PD-L1 TPS (Fisher's exact test: $P = 0.0011$; Table 2). All patients with strong PD-L1 expression (PD-L1 TPS ≥ 50%) were positive for IDO1, and 77 of 96 (80.2%) patients with negative PD-L1 expression (PD-L1 TPS < 1%) were positive for IDO1 expression (Table 2).

3.2. Prognostic impact of IDO1/PD-L1 co-expression

Survival analyses by the Kaplan–Meier method showed that no significant difference in OS was observed between IDO1-negative patients and IDO1-positive patients (log rank test: $P = 0.5168$; **Supplementary** Fig. 2A), but patients with PD-L1 expression had significantly shorter OS after surgery than did patients without PD-L1 expression (log rank test: $P = 0.0122$; **Supplementary** Fig. 2B). We assessed the association between IDO1/PD-L1 co-expression and postoperative survival. Survival analysis using a Kaplan–Meier method revealed that the postoperative OS of patients with IDO1/PD-L1 co-expression was significantly shorter than that of patients without co-expression (log rank test: $P = 0.0303$; Fig. 1C). Univariate and multivariate analyses of OS in all patients are shown in **Supplementary** Table 2. In multivariate analyses, older age ($P = 0.0347$), advanced stage ($P < 0.0001$), the presence of lymphatic invasion ($P = 0.0124$), and PD-L1 positivity ($P = 0.0269$) were independent prognostic factors for OS, but IDO1/PD-L1 co-expression was not.

3.3. Association between IDO1/PD-L1 co-expression and proliferative capacity

We examined the association between IDO1/PD-L1 co-expression and the Ki-67 labeling index to evaluate the proliferative capacity. The average Ki-67 labeling index of patients with IDO1/PD-L1 co-expression was 25.2 (range, 0–90), while that of patients without co-expression was 15.2 (range, 0–73). The Ki-67 labeling index in patients with IDO1/PD-L1 co-expression was significantly higher than that in patients

Table 1
Clinicopathological characteristics of patients.

Factors		Value or no. of patients (%)
Age (years)	Median	69
	Range	39-87
Sex	Male	168 (83.2)
	Female	34 (16.8)
Smoking history	< 30 pack years	31 (15.3)
	≥ 30 pack years	171 (84.7)
Grade	Non-keratinizing	33 (16.3)
	Keratinizing	169 (83.7)
T	T1	56 (27.7)
	T2	120 (59.4)
	T3	19 (9.4)
	T4	7 (3.5)
N	N0	145 (71.8)
	N1	43 (21.3)
	N2	14 (6.9)
	N3	0 (0.0)
M	M0	202 (100.0)
	M1	0 (0.0)
Stage	IA	49 (24.2)
	IB	58 (28.7)
	IIA	48 (23.8)
	IIB	25 (12.4)
	IIIA	19 (9.4)
	IIIB	3 (1.5)
	IV	0 (0.0)
pl	Absent	147 (72.8)
	Present	55 (27.2)
ly	Absent	170 (84.2)
	Present	32 (15.8)
v	Absent	137 (67.8)
	Present	65 (32.2)
Surgical procedure	Lobectomy	124 (61.4)
	Bilobectomy	30 (14.8)
	Pneumonectomy	22 (10.9)
	Sublobar resection	26 (12.9)
IDO1	Negative	26 (12.9)
	Positive	176 (87.1)
PD-L1	Negative	96 (47.5)
	Positive	106 (52.5)
IDO1/PD-L1	Others	103 (51.0)
	Co-expression	99 (49.0)

pl, pleural invasion; ly, lymphatic invasion; v, vascular invasion; IDO1, indoleamine 2,3-dioxygenase 1; PD-L1, programmed cell death-ligand 1.

Table 2
Association between IDO1 and PD-L1 tumor proportion scores.

	PD-L1 TPS, *N* (%)			
IDO1 TPS	**TPS < 1%**	**1% ≤ TPS < 50%**	**50% ≤ TPS**	***P* value**
TPS < 1%	19 (19.8)	7 (9.3)	0 (0.0)	0.0011
1% ≤ TPS < 50%	67 (69.8)	58 (77.3)	20 (64.5)	
50% ≤ TPS	10 (10.4)	10 (13.4)	11 (35.5)	

IDO1, indoleamine 2,3-dioxygenase 1; PD-L1, programmed cell death-ligand 1; TPS, tumor proportion score.

without co-expression (Student's *t*-test: $P = 0.0005$; Fig. 2).

3.4. Association between IDO1/PD-L1 co-expression and TIL density

The heterogeneity of immunoreactivity of IDO1 and PD-L1 within tumor area did exist, and the local TIL density strongly affected the difference in the expression levels of IDO1 and PD-L1. Therefore, we conducted immunohistochemical staining of CD3, CD4, and CD8 to analyze the association between IDO1/PD-L1 co-expression and TIL density. The median CD3, CD4, and CD8 densities were 34 (range, 0–388), 12 (range, 0–158), and 7 (range, 0–138), respectively, and 105 (52.0%), 102 (50.5%), and 101 (50.0%) patients were assigned to the CD3+, CD4+, and CD8 + TILs high groups, respectively. There was a low level of lymphocyte infiltration in cases that were IDO1 negative and PD-L1 negative, compared with high infiltration in cases that were IDO1-positive and PD-L1–positive (Fig. 3). Table 3 shows the associations between IDO1 expression, PD-L1 expression, and IDO1/PD-L1 co-expression and TIL density. Fisher's exact test showed that IDO1/PD-L1 co-expression was significantly associated with high CD3, CD4, and CD8 expression ($P = 0.0033$, $P = 0.0003$, and $P < 0.0001$, respectively; Table 3).

4. Discussion

The main findings from our study are as follows: IDO1 expression was significantly associated with PD-L1 expression; co-expression of IDO1 and PD-L1 was significantly associated with shorter OS; and co-expression was significantly associated with high CD3, CD4, and CD8 expression in primary lung SCC. Co-expression of IDO1 and PD-L1 was present in 99 (49.0%) patients in this study; this rate was considerably higher than previous reports of approximately 10% of NSCLC patients with IDO1/PD-L1 co-expression by Schalper et al. and 19% of SCC patients with IDO1/PD-L1 co-expression by Parra et al. [22,23]. These discrepancies may be due to heterogeneity in tumor specimens. Schalper et al. evaluated IDO1 and PD-L1 expression using quantitative immunofluorescence on tissue microarray (TMA) sections and Parra et al. used immunohistochemistry on TMA specimens, whereas we performed immunohistochemistry of FFPE tumor tissue sections from surgically resected lung SCC [22,23]. The expression of PD-L1 and IDO1 by tumor cells is induced by IFN-γ and other proinflammatory stimuli present in the tumor microenvironment [15,16]. Recently, we showed that IDO1 and PD-L1 were both upregulated in six lung cancer cell lines following treatment with IFN-γ and transforming growth factor-β (unpublished observation). Therefore, the tumor microenvironment can influence both IDO1 and PD-L1 expression, which might lead to the differences in IDO1/PD-L1 co-expression rates between our study and those of Schalper et al. and Parra et al. [22,23]. We believe that the

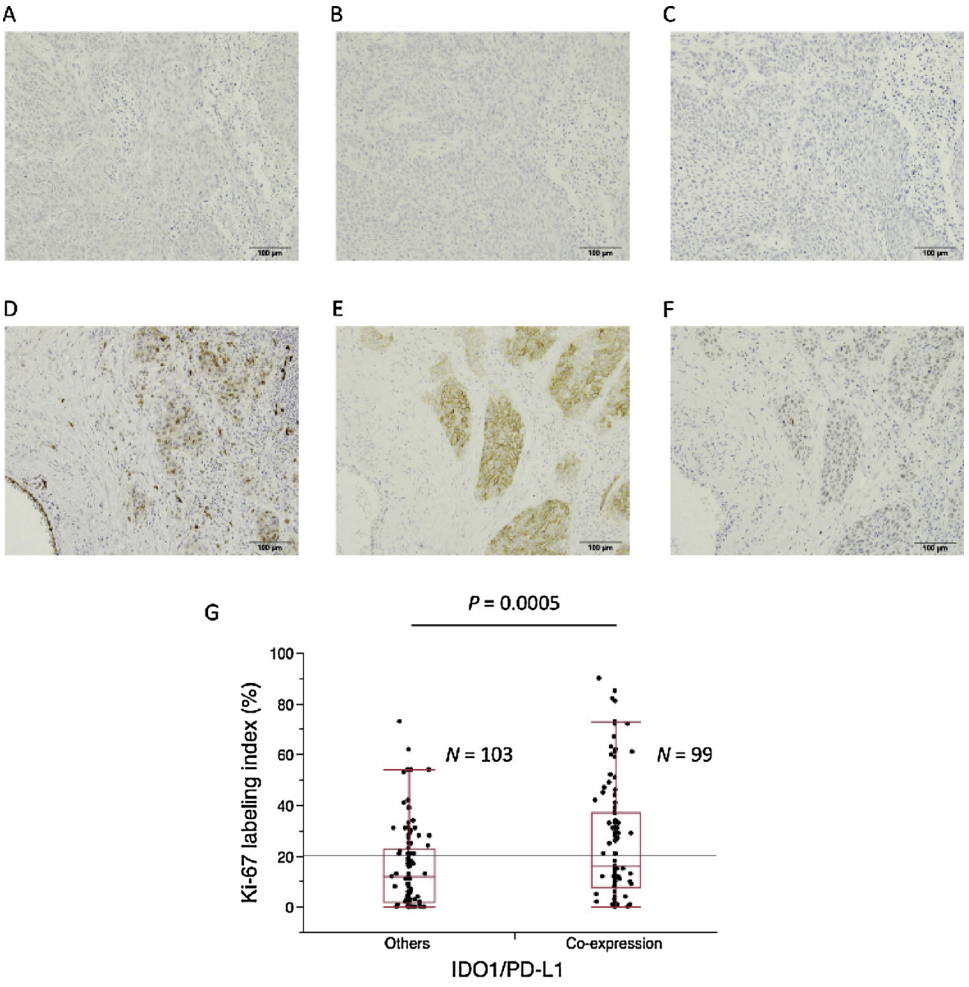

Fig. 2. Ki-67 labeling index according to IDO1 and PD-L1 expression in primary lung SCC. We adopted cut-off values of 1% for IDO1 and PD-L1 expression in this analysis. Cases with IDO1-negative (A) and PD-L1–negative (B) staining had low values of Ki-67 (C), whereas cases with IDO1-positive (D) and PD-L1–positive (E) staining had high values of Ki-67 (F). (G) The Ki-67 labeling index was significantly higher in patients with IDO1/PD-L1 co-expression than in patients without co-expression (Student's *t*-test: $P = 0.0005$). Scale bar: 100 μm. IDO1, indoleamine 2,3-dioxygenase 1; PD-L1, programmed cell death-ligand 1; SCC, squamous cell carcinoma.

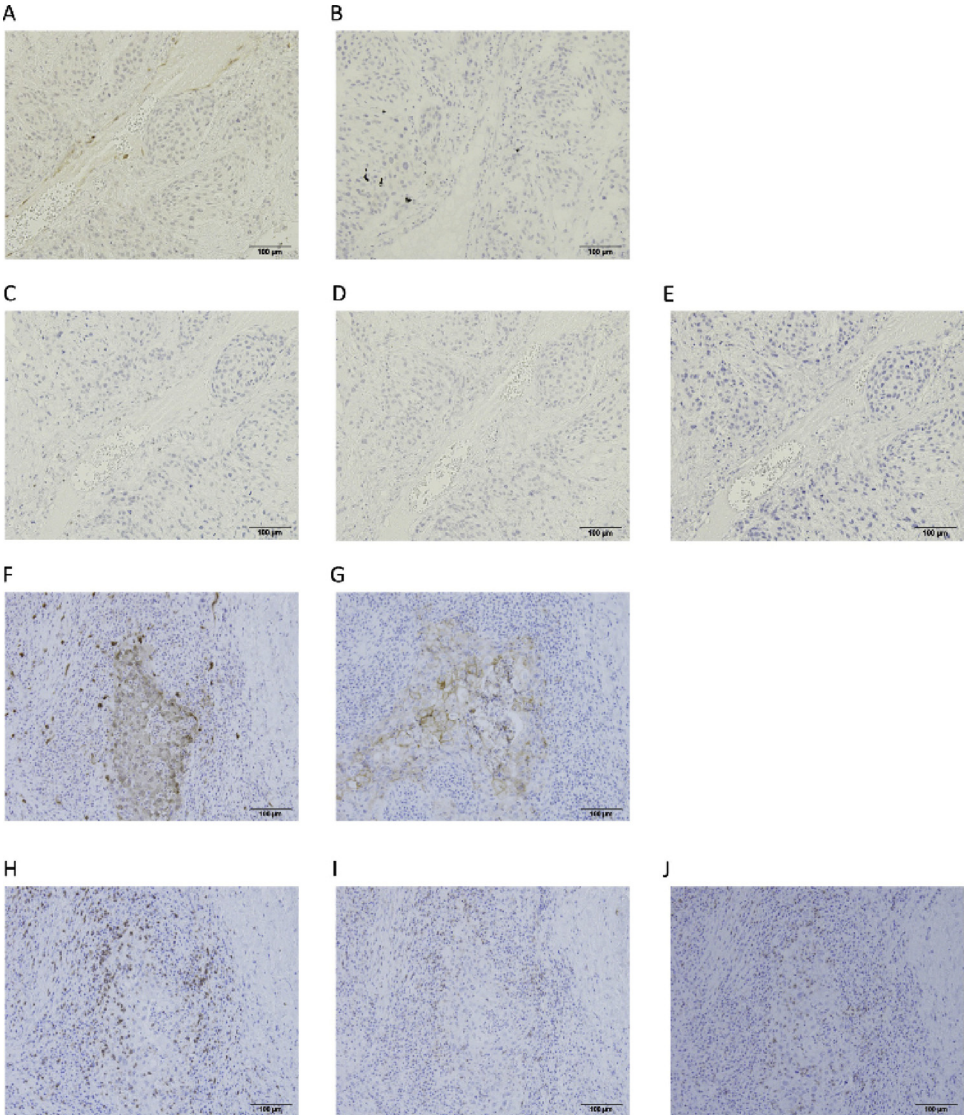

Fig. 3. Tumor-infiltrating lymphocytes according to IDO1 and PD-L1 expression in primary lung SCC. Representative images of immunohistochemical staining for IDO1 (A, F), PD-L1 (B, G), CD3(C, H), CD4 (D, I), and CD8 (E, J). A case with negative staining for IDO1 (A) and PD-L1 (B) showed scant infiltration of lymphocytes (C-E). In contrast, a case with positive staining for IDO1 (F) and PD-L1 (G) showed many infiltrating lymphocytes (H-J). Scale bar: 100 μm. IDO1, indoleamine 2,3-dioxygenase 1; PD-L1, programmed cell death-ligand 1; SCC, squamous cell carcinoma; CD3, cluster of differentiation 3; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8.

result of the present study is very important when considering combination therapy of IDO1 inhibitor and PD-1/PD-L1 inhibitor. In our study, all of the patients with strong PD-L1 expression (PD-L1 TPS ≥ 50%) were positive for IDO1, and the positive rate of IDO1 expression was very high (80.2%) even among the patients with negative PD-L1 expression (PD-L1 TPS < 1%). Pal et al. recently reported that high IDO1 expression was observed not only in the PD-L1-high category but also in the PD-L1-low category among 1467 unselected clinical cases with advanced cancer including breast, colon, lung, pancreatic, ovarian, brain, and prostate cancer [24]. Therefore, this novel combination therapy could be an effective treatment option as the positive rate of IDO1 expression is high even among patients with negative PD-L1 expression, and there are many ongoing clinical trials of combination therapy of IDO1 inhibitor and PD-1/PD-L1 inhibitor in various solid tumors (summarized in **Supplementary** Table 3). Long et al. recently reported that the addition of epacadostat to pembrolizumab did not result in greater clinical benefit than pembrolizumab alone [14]. The association between IDO1 expression and the efficacy of IDO1 inhibitor is unclear. Clinical trials are needed to clarify the association between the efficacy of IDO1 inhibitor and IDO1 expression, in addition to the association between the efficacy of combination therapy and IDO1/PD-L1 co-expression.

Table 3
Associations between IDO1 expression, PD-L1 expression, and IDO1/PD-L1 co-expression and TIL density.

TILs		*N* (%)	IDO1, *N* (%)		*P* value	PD-L1, *N* (%)		*P* value	IDO1/PD-L1, *N* (%)		*P* value
			Negative	Positive		Negative	Positive		Others	Co-expression	
CD3	Low	97 (48.0)	14 (53.9)	83 (47.2)	0.5368	55 (57.3)	42 (39.6)	0.0164	60 (58.3)	37 (37.4)	0.0033
	High	105 (52.0)	12 (46.1)	93 (52.8)		41 (42.7)	64 (60.4)		43 (41.7)	62 (62.6)	
CD4	Low	100 (49.5)	13 (50.0)	87 (49.4)	1.0000	59 (61.5)	41 (38.7)	0.0019	64 (62.1)	36 (36.4)	0.0003
	High	102 (50.5)	13 (50.0)	89 (50.6)		37 (38.5)	65 (61.3)		39 (37.9)	63 (63.6)	
CD8	Low	101 (50.0)	21 (80.8)	80 (45.5)	0.0012	75 (78.1)	26 (24.5)	< 0.0001	82 (79.6)	19 (19.2)	< 0.0001
	High	101 (50.0)	5 (19.2)	96 (54.5)		21 (21.9)	80 (75.5)		21 (20.4)	80 (80.8)	

IDO1, indoleamine 2,3-dioxygenase 1; PD-L1, programmed cell death-ligand 1; TIL, tumor infiltrating lymphocyte.

The Ki-67 labeling index was significantly higher in the patients with IDO1/PD-L1 co-expression than in those without co-expression, and survival analysis using a Kaplan–Meier method revealed that postoperative OS of the patients with IDO1/PD-L1 co-expression was significantly shorter than that of the patients without co-expression although IDO1/PD-L1 co-expression was not an independent prognostic factor of OS in multivariate analysis. The expression of both IDO1 and PD-L1 is upregulated mainly by various inflammatory cytokines such as IFN-γ [15,16], but expression is also induced by activation of signaling pathways such as the interleukin 6–signal transducer and activator of transcription 3 signaling pathway, NF-κB pathway, and the mammalian target of rapamycin signaling pathway, all of which result in tumor progression [16,25,26]. However, survival analysis by the Kaplan–Meier method showed that no significant difference in OS was observed between IDO1-negative patients and IDO1-positive patients. Moreover, in multivariate analyses, PD-L1 positivity was an independent prognostic factor for OS, but IDO1/PD-L1 co-expression was not. In this study, cases with TPS < 1% were considered negative, and only 26 (12.9%) were negative for IDO1 expression. Therefore, IDO1 expression should be further evaluated using other cut-off values in more cases.

Co-expression of IDO1 and PD-L1 was significantly associated with high CD3, CD4, and CD8 expression in this study. Some previous studies revealed that high IDO1 expression on tumor cells was associated with low CD3+ and CD8 + T cells in some types of cancer [27–29], which is in contrast to the result of this study. However, our results are in part consistent with those of recent studies on NSCLC by Schalper et al. and prostate cancer by Kolijn et al. [23,30]. IDO1 and PD-L1 expression increases through a response to IFN-γ, which is released mainly by CD8+ cytotoxic T cells [15,16], and the result of this study may reflect the tumor microenvironment state. With regard to CD4 + T cells, the CD4 + T-cell compartment in this study may include highly immunosuppressive Treg cells (CD4+ FoxP3+) and naive CD4 + T cells (CD4+ FoxP3–), and IDO1 converts naive CD4 + T cells into Tregs [16]. Therefore, most of the CD4 + T cells in patients of this study may be highly immunosuppressive Treg cells. We should therefore examine the relationship between IDO1/PD-L1 co-expression and the infiltration of Tregs (CD4+ FoxP3+). Based on the results of this study, combination therapy of an IDO1 inhibitor and an immune checkpoint inhibitor targeting the PD-1/PD-L1 pathway may be more effective against T-cell–inflamed tumors.

There are several limitations associated with this study. First, this was a single-institution retrospective study and not a trial-based correlative study; however, 202 patients with lung SCC were examined for the associations between IDO1/PD-L1 co-expression and both clinicopathological characteristics and TIL density. The data obtained from this study may help identify patients who would benefit from combination therapy targeting IDO1 and the PD-1/PD-L1 pathway. Validation cohort studies should be conducted to confirm our results. Second, we conducted PD-L1 immunohistochemistry with antibody SP142, which is used in atezolizumab clinical trials [31,32]. Some recent studies showed that the positive rate of PD-L1 expression using SP142 was lower than that for other antibodies such as 28-8, 22C3, and SP263 [33–36]. However, there is an ongoing phase I clinical trial on combination therapy with epacadostat and atezolizumab in previously treated NSCLC (**Supplementary** Table 3) and our study may be a useful reference to understand the results of this clinical trial. We should also evaluate PD-L1 expression using antibody 22C3 because many clinical trials of combination therapy with epacadostat and pembrolizumab in patients with various solid tumors including NSCLC are ongoing (**Supplementary** Table 3). Third, there are no definitive guidelines for antibody use or quantification of IDO1 expression in NSCLC, and no comparative data of different IDO1 antibodies are available. In this study we used clone UMAB126 and set the cut-off value for positivity as 1% cytoplasmic staining in tumor cells, but 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 of advanced cases because we used surgical specimens. Analysis in advanced cases should be performed to validate our results from resected tumors.

In conclusion, IDO1 expression correlated to PD-L1 expression, and co-expression of IDO1 and PD-L1 may be important targets for immunotherapy in lung SCC. Clinical trials are needed to clarify the association between IDO1/PD-L1 co-expression and efficacy of combination therapy with an IDO1 inhibitor and a PD-1/PD-L1 inhibitor.

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Disclosure

The authors have declared no conflicts of interest in association with this study.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2018.12.008.

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