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Original Article

Prognostic significance of PD-L1 expression and tumor infiltrating lymphocytes in large cell neuroendocrine carcinoma of lung

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Abstract: Objectives: Since large cell neuroendocrine carcinoma (LCNEC) is a relatively rare histologic type of primary lung cancer, little is known about the immunological status of patients with LCNEC. We aimed to clarify the expression and prognostic impact of programmed cell death ligand 1 (PD-L1), CD8, CD4, and Forkhead box protein P3 (Foxp3) in LCNEC. Methods: We retrospectively analyzed PD-L1, CD8, CD4, and Foxp3 expressions in 95 surgically resected LCNEC. PD-L1 positive staining was determined in tumors with more than 1% of tumor cells stained to any intensity, and CD8, CD4, and Foxp3 positivity was determined in tumors with more than 5% of lymphocytes stained. Results: Positive expression of PD-L1, CD8, CD4, and Foxp3 was observed in 70 (74%), 52 (55%), 76 (80%), and 43 (45%) tumors, respectively. The expression of PD-L1 was significantly correlated with positive lymphatic permeation. Positive correlations were mutually observed among tumor infiltrating immune cells. Univariate and multivariate analyses showed that positive pleural invasion and Foxp3 negative expression were independent unfavorable prognostic factors for overall survival (OS). Advanced pathological stage, positive pleural invasion, CD4 negative expression in cancer stroma, and Foxp3 negative expression were identified as independent unfavorable prognostic factors for recurrence free survival (RFS). Conclusions: Foxp3 positive tumor infiltrating lymphocytes (TILs) were an independent favorable prognostic factor for both OS and RFS, whereas CD4 positive TILs were an independent significant unfavorable prognostic factor for RFS. The high frequency of PD-L1 expression could support the use of anti-programmed cell death 1 antibody in the treatment of LCNEC.

Keywords: Large cell neuroendocrine carcinoma (LCNEC), lung cancer, PD-L1, Foxp3, tumor infiltrating lymphocyte (TIL)

Introduction

Large cell neuroendocrine carcinoma (LCNEC) is a relatively rare tumor, which accounts for approximately 2-4% of all lung cancers [1]. LCNEC has been initially classified as a subgroup of large cell carcinoma, but is now pathologically classified as a high grade neuroendocrine tumor (NET), all together with small cell lung cancer (SCLC) [2]. Furthermore, patients with LCNEC are clinically treated with the conventional chemotherapeutic regimen administered to those with SCLC [3]. Because of its rarity, there has been no established biomarker to predict the outcome after treatment.

Anti-programmed cell death-1 (PD-1) antibody has brought a transformative change in the

treatment of advanced or metastatic non-small cell lung cancer (NSCLC), and has drastically changed the survival of patients with metastatic NSCLC [4-7]. Immune checkpoint inhibitors have been identified as one of the principal therapies in addition to cytotoxic chemotherapy and tyrosine kinase inhibitors in NSCLC. The expression of programmed cell death-ligand 1 (PD-L1) within cancer cells is thought as a significant biomarker to predict efficacy of anti-PD-1 treatment. Indeed, the chemotherapeutic strategy using anti-PD-1 antibody has been considered for metastatic or recurrent NSCLC according to the expression status of PD-L1. Several researchers have described the relationship between the prognostic impact and the expression levels of PD-L1 in patients with SCLC [3, 8-10]. Three of these studies have reported that patients with positive expression of PD-L1 yielded significantly favorable survival than those with PD-L1 negative expression [3, 8, 10], whereas one study has demonstrated that the expression of PD-L1 was closely related to poor prognosis in SCLC [9]. This difference might be associated with the sample size, the various antibodies for immunohistochemistry, and the threshold for PD-L1 positivity. Nevertheless, the expression of PD-L1 seems to be a useful marker to predict a good outcome in SCLC. Recently, Tsuruoka *et al.* described that the overall survival (OS) time of LCNEC and SCLC tended to be longer in patients with positive expression of PD-L1 than in those with PD-L1 negative expression [11]. Inamura *et al.* also reported same tendency in LCNEC [12]. However, it remains unclear whether PD-L1 could predict a good outcome in patients with LCNEC. In their analysis, tissue microarray (TMA) blocks were used for immunohistochemistry [11, 12]; however, surgically resected tumor specimens are actually suitable for the correct assessment of PD-L1 expression because of delicate cut-off values, such as 1% or 5%, and the heterogeneous staining pattern of PD-L1 expression in whole tumor tissues.

Tumor infiltrating lymphocytes (TILs) have been also used as a prognostic marker in NSCLC, and recent meta-analysis has demonstrated that high level of CD8 T cells infiltration in tumor stroma or tumor nest and high level of CD4 T cells infiltration in tumor stroma are linked to a good prognosis in lung cancer, whereas high level of Foxp3 T cells infiltration in tumor stroma is identified as a poor prognostic predictor [13]. Little is known about the relationship between TILs and prognostic significance in patients with NET of the lung, such as LCNEC.

Based on this background information, we conducted this study to evaluate the relationship between the clinicopathological significance and immunological expression of PD-L1, CD8, CD4, and Foxp3 in surgically resected LCNEC.

Methods

Patient selection and follow-up

This was a multi-institutional joint retrospective study conducted by researchers at Gunma University Hospital, Gunma Prefectural Cancer Center, Maebashi Red Cross Hospital, National Hospital Organization Takasaki General Medical Center, and National Hospital Organization Shibukawa Medical Center. The study protocol was approved by the Institutional Review Board of each participating institution according to the Helsinki Declaration.

Ninety-eight patients underwent surgical resection of LCNEC at the participating institutions between April 2000 and March 2016. After excluding 2 patients with inadequate clinicopathological data and 1 patient with poor quality specimen, we enrolled 95 patients with LCNEC into this study.

The histology of LCNEC was confirmed at Gunma University according to the World Health Organization criteria. The stages of pathological tumor-node-metastasis were established using the International System for Staging Lung Cancer adopted by the American Joint Committee on Cancer and the Union Internationale Centre le Cancer. The follow-up period for censored cases ranged from 16 days to 5131 days (median, 1113 days).

Immunohistochemical staining

For PD-L1, CD8, CD4, and Foxp3, immunohistochemical staining was performed according to the procedures described in a previous study [14]. All sections were deparaffinized in xylene, rehydrated, and then incubated with fresh 0.3% hydrogen peroxide in 100% methanol for

Table 1. Patient characteristics

	N (%)
Age: median (range)	74 (36-88)
Gender	
Male/Female	82/13 (86/14)
Smoking history	
No/Yes	4/91 (4/96)
Histology	
LCNEC only	77 (81)
Combined LCNEC	18 (19)
Pathological stage	
IA (IA1, IA2, IA3)	29 (31)
IA1	3 (3)
IA2	17 (18)
IA3	9 (9)
IB	25 (27)
IIA	8 (8)
IIB	16 (17)
IIIA	14 (15)
IV	3 (3)
Lymphatic permeation	
No/Yes	31/62 (33/67)
Vascular invasion	
No/Yes	25/67 (27/73)
Pleural invasion	
No	57 (61)
Yes	37 (39)
PL1	18 (19)
PL2	14 (15)
PL3	5 (5)
Adjuvant therapy	
No/Yes	72/23 (76/24)

PL: pleural invasion.

30 min at room temperature to block endogenous peroxidase activity. After rehydration through a graded series of ethanol treatments, PD-L1 was retrieved using the universal HIER antigen retrieval reagent (Abcam, ab208572) at 120°C for 20 min in autoclave, and then, sections were passively cooled to room temperature. Antigen retrieval was performed using Immunosaver (NJ15T, NEM) at 98-100°C for 30 min for CD4, CD8, and Foxp3 staining. After rinsing in 0.1 M phosphate buffered saline (PBS, pH 7.4) to block non-specific binding sites, sections were incubated with protein block serum-free reagent (DAKO, Carpinteria, CA, USA) for 30 min at room temperature. Rabbit monoclonal antibodies against PD-L1 (Cell Signaling, E1L3N^R, 1:200 dilution), CD8 (Abcam ab4055, 1:600 dilution), CD4 (Abcam ab133616, 1:200 dilution), and Foxp3 (Abcam ab20034, 1:200 dilution) were used as primary antibodies. PD-L1 expression on each cell was considered positive when membrane staining was observed. A semi-quantitative scoring method was used for PD-L1 expression, as follows: 0 ≤ 1%, 1 = 1-5%, 2 = 6-10%, 3 = 11-25%, 4 = 26-50%, 5 ≥ 51% of cells were positive according to a previous report [14]. Tumors with score ≥ 1 were graded as PD-L1 positive according to previous studies [4, 11]. CD8, CD4, and Foxp3 expressions were semi-quantitatively evaluated on the extent of positive lymphocytes infiltrating with tumor specimens. CD8 and CD4 expressions on lymphocytes were considered positive when membrane staining was observed. The CD8 and CD4 expressions were examined in a whole cancer tissue, as well as separately in cancer nest only and cancer stroma only in order to clarify the prognostic impact of their localization. Foxp3 expression on lymphocytes was considered positive when nuclear staining was observed. Foxp3 positive TILs were present only in cancer stroma. CD8, CD4, and Foxp3 positivity was defined in tumors with more than 5% of positive lymphocytes, according to a previous report [14]. The tissue sections were examined in a blinded fashion using light microscopy by at least two of the authors (Y.O and K.K). In case of any discrepancies, both investigators evaluated the slides simultaneously, until reaching a consensus on their final assessment. Neither of the investigators had any knowledge of the patient outcomes.

Statistical analysis

OS was defined as the time interval between the date of tumor resection and the date of death from any cause or censored date. Recurrence free survival (RFS) was defined as the time interval between the date of tumor resection and the date of any recurrence detected or death from other cause than cancer death or the last follow-up. For univariate analyses, survival rates were estimated by the Kaplan-Meier method, and differences in survival between subgroups were compared by the log-rank test. Multivariate analyses were performed using the Cox proportional hazard model. Forward and backward stepwise proce-

Table 2. PD-L1, CD8, CD4 and Foxp3 expression rate

Factors	Cut off value	N (%)
PD-L1 expression		
Negative	< 1%	25 (26)
Positive	≥ 1%	70 (74)
CD8 expression in whole tumor		
Negative	< 5%	43 (45)
Positive	≥ 5%	52 (55)
CD8 expression in cancer nest		
Negative	< 5%	24 (25)
Positive	≥ 5%	71 (75)
CD8 expression in cancer stroma		
Negative	< 5%	45 (47)
Positive	≥ 5%	50 (53)
CD4 expression in whole tumor		
Negative	< 5%	19 (20)
Positive	≥ 5%	76 (80)
CD4 expression in cancer nest		
Negative	< 5%	80 (84)
Positive	≥ 5%	15 (16)
CD4 expression in cancer stroma		
Negative	< 5%	17 (18)
Positive	≥ 5%	78 (82)
Foxp3 expression		
Negative	< 5%	52 (55)
Positive	≥ 5%	43 (45)

dures were performed to determine the prognostic effect of combined factors. Chi-squared test was performed to evaluate the relationship between categorical valuables, and Student's *t*-test was used to evaluate continuous variables. All of the reported *P* values were two-sided, and the significance level was set at less than 0.05. All statistical analyses were performed using SPSS Statistics 20 statistical software (Dr. SPSS II for Windows; standard version 20.0; SPSS Inc., Chicago, IL, USA).

Results

Patients' characteristics

The patients' characteristics are listed in **Table 1**. Of the 95 patients, 82 were men and 13 were women, with a median age of 74 years. Ninety-one patients (96%) had smoking history. Histologically, 77 patients (81%) had pure LCNEC and 18 patients (19%) had combined LCNEC. Lymphatic permeation, vascular invasion, and pleural invasion were observed in 67%, 73%, and 39% of patients, respectively.

PD-L1 expression and TILs

Positive expression of PD-L1, CD8, CD4, and Foxp3 was observed in 70 (74%), 52 (55%), 76 (80%), and 43 (45%) tumors, respectively (**Table 2**). Representative images for PD-L1, CD8, CD4, and Foxp3 expressions are shown in **Figure 1**. Although the rate of PD-L1 positivity was high, only 7 patients (7%) had tumors with more than 50% of tumor cells expressing PD-L1 (score 5+) (Table S1). CD8 positive TILs were abundant in cancer nest, whereas most of CD4 positive TILs were present in cancer stroma.

Clinicopathological characteristics according to PD-L1 expression and TILs

The clinicopathological features according to PD-L1 expression and TILs are listed in **Table 3**. The expression of PD-L1 was significantly correlated with lymphatic permeation. For correlation among immunological expressions, positive correlations were observed between PD-L1 and CD4, PD-L1 and Foxp3, CD8 and CD4, CD8 and Foxp3, and CD4 and Foxp3. Except PD-L1 and CD8 expressions, there were significant mutually positive correlations among tumor infiltrating immune cells.

Univariate and multivariate survival analyses

The 5-year OS rate of all patients was 51.6%. Of 95 patients, 40 patients died and 36 patients developed recurrent diseases. Mean survival time is 1207 days (3.3 years). The OS and RFS curves according to the expression of PD-L1, CD8, CD4, and Foxp3 are shown in **Figure 2**. The RFS of the patients with PD-L1 positive expression tended to be higher than those with negative expression, without statistical significance (**Figure 2A**, **2B**). There were no survival differences in the expression status of CD8 (**Figure 2C**, **2D**), irrespective of the location (Figure S1), neither of CD4 in the whole tumor tissue (**Figure 2E**, **2F**), nor of CD4 in cancer nest (Figure S2). When the evaluation of CD4 expression was limited to cancer stroma, the patients with CD4 positive TILs in cancer stroma had significantly worse RFS than those with CD4 negative TILs in cancer stroma (**Figure 2G**, **2H**). The patients with tumors with Foxp3

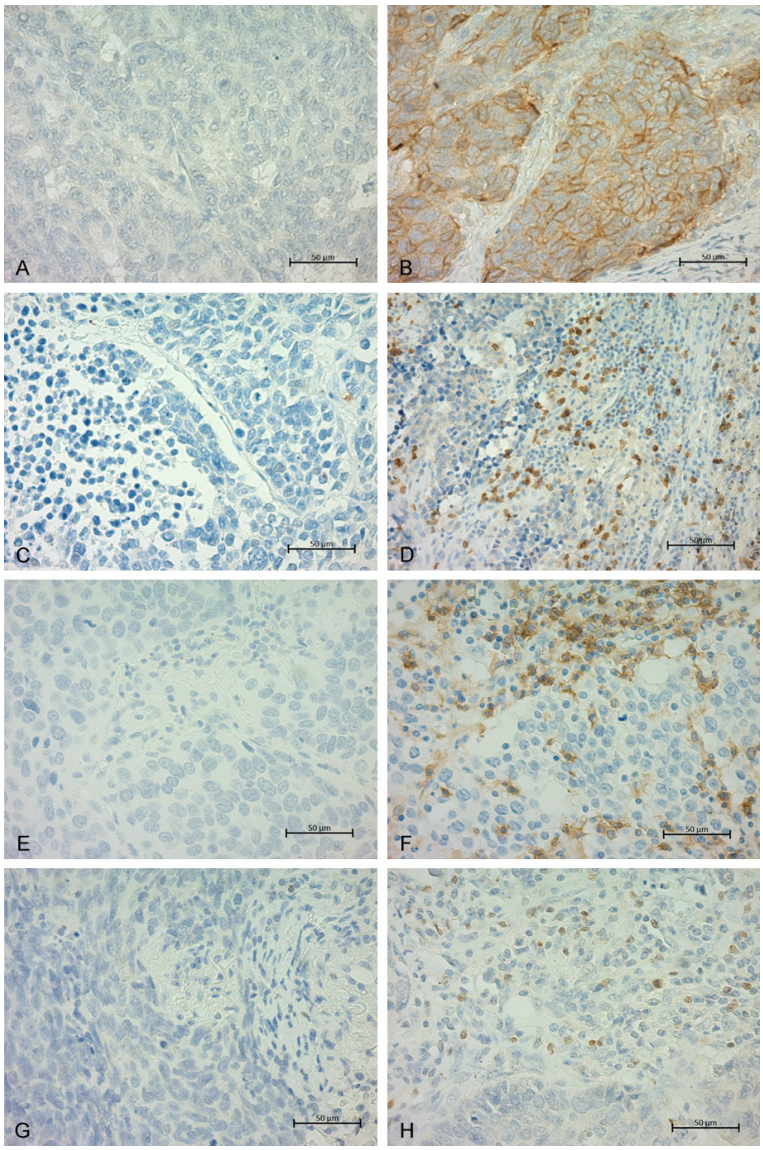

Figure 1. Representative immunohistochemical findings of LCNEC are shown. PD-L1 negative (A) and PD-L1 positive are shown (B). CD8 negative (C), CD8 positive (D), CD4 negative (E), CD4 positive (F), Foxp3 negative (G) and Foxp3 positive (H) are shown.

positive TILs had significantly better prognosis than those with Foxp3 negative LCNEC, for both OS and RFS (**Figure 2I**, **2J**).

Table 4 shows univariate and multivariate analyses of OS and RFS. Univariate and multivariate analyses showed that positive pleural invasion and Foxp3 negative expression were independent unfavorable prognostic factors for OS. For RFS, advanced pathological stage, positive lymphatic permeation, positive pleural invasion, CD4 positive expression in cancer stroma, and Foxp3 negative expression were identified as significant unfavorable prognostic factors in univariate analysis. Of them, multivariate analysis showed that besides advanced pathological stage and positive pleural invasion, CD4 positive expression in cancer stroma, and Foxp3 negative expression were identified as independent unfavorable prognostic factors for RFS.

Discussion

In this study, we revealed the prognostic impact of PD-L1 expression and TILs in LCNEC. Until now, there have been limited reports about the prognostic impact of immune cells infiltration in LCNEC. Of immune-related cells, infiltration of Foxp3 negative TILs was an independent unfavorable prognostic factor for OS and RFS. The presence of CD4 positive TILs in cancer stroma was also identified as an independent unfavorable prognostic factor for RFS in patients with LCNEC.

In terms of Foxp3 expression, our results were contradictory to a previous meta-analysis of lung cancer, reporting that low number of Foxp3 T cells infiltration in tumor stroma was identified as a good prognostic factors [13]. One of the reasons might be the difference of tumor histology among the population. In most of former studies, adenocarcinoma or NSCLC were examined. Even though these studies included patients with LCNEC, the total numbers of patients was small. The condition of immune cell infiltration might be different according to tumor histology between NET and the others, especially in LCNEC. Another reason is that a previous meta-analysis has included only a few studies on Foxp3 expression. The sample size seems to be too small to conclude the prognostic impact of Foxp3 in whole lung cancer.

Table 3. Correlation among immunological expression and clinicopathological factors

	N	PD-L1 expression			CD8 expression			CD4 expression			Foxp3 expression		
		Negative (N = 25)	Positive (N = 70)	*p*-value[a]	Negative (N = 52)	Positive (N = 43)	*p*-value[a]	Negative (N = 19)	Positive (N = 76)	*p*-value[a]	Negative (N = 52)	Positive (N = 43)	*p*-value[a]
Age													
< 74	46	11	35	0.61	27	19	0.45	12	34	0.15	26	20	0.74
≥ 74	49	14	35		25	24		7	42		26	23	
Gender													
Male	82	19	63	0.08	43	39	0.26	14	68	0.07	44	38	0.60
Female	13	6	7		9	4		5	8		8	5	
Smoking history													
Yes	91	23	68	0.27	50	41	0.85	18	73	0.80	50	41	0.85
No	4	2	2		2	2		1	3		2	2	
Stage													
I	54	14	40	0.60	29	25	0.82	8	46	0.15	27	27	0.29
II-IV	41	11	40		23	18		11	30		25	16	
Lymphatic permeation													
No	31	4	27	0.04[b]	17	14	0.88	3	28	0.10	15	16	0.46
Yes	62	20	42		33	29		15	47		35	27	
Vascular invasion													
No	25	6	19	0.89	17	8	0.08	4	21	0.60	17	8	0.11
Yes	67	17	50		32	35		14	53		33	34	
Pleural invasion													
No	57	13	44	0.45	33	24	0.38	9	48	0.30	29	28	0.42
Yes	37	11	26		18	19		9	28		22	15	
CD8 expression													
Negative	52	16	36	0.28	-	-		-	-		-	-	
Positive	43	9	34		-	-		-	-		-	-	
CD4 expression													
Negative	19	7	12	< 0.01[b]	6	1	< 0.01[b]	-	-		-	-	
Positive	76	18	58		0	42		-	-		-	-	
Foxp3 expression													
Negative	52	18	34	0.04[b]	39	13	< 0.01[b]	17	35	< 0.01[b]	-	-	
Positive	43	7	36		13	30		2	41		-	-	

[a]Peason's chi-square test, [b]denotes significance.

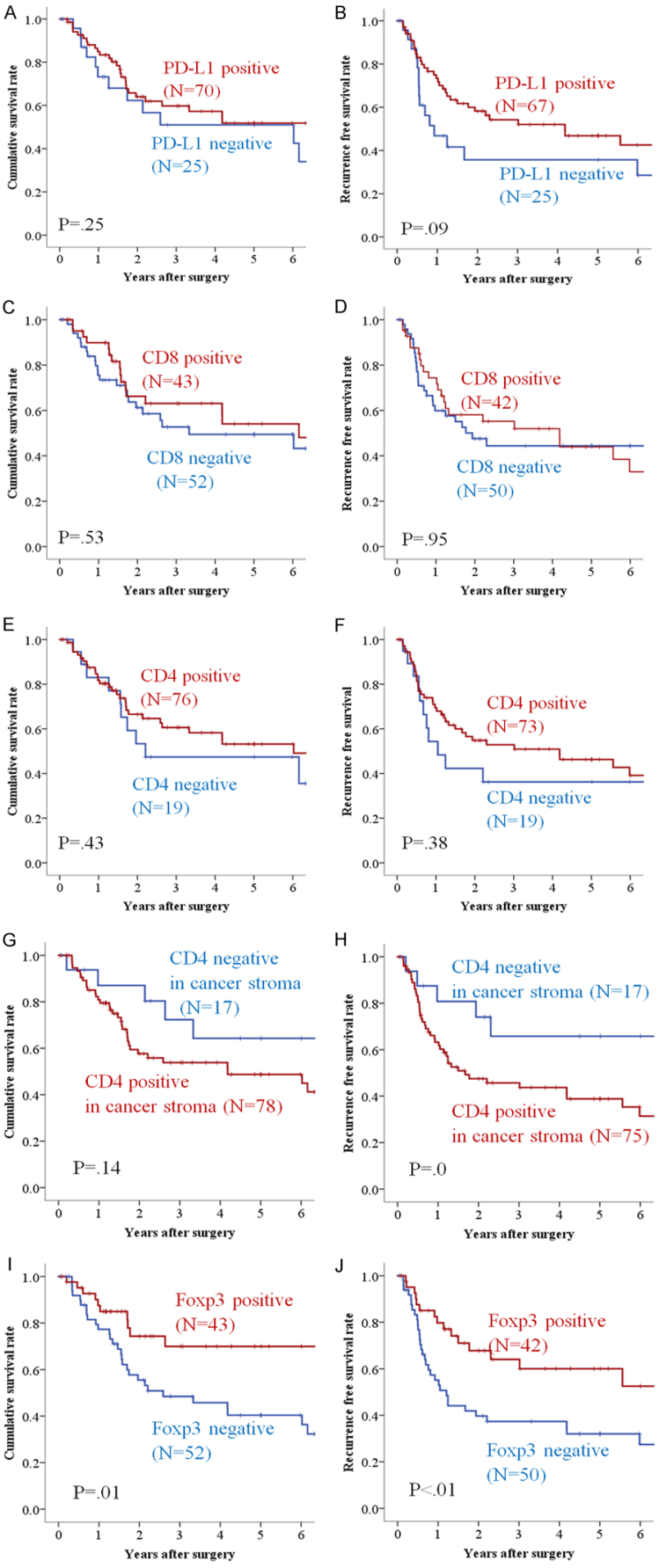

Figure 2. Overall survival (OS) and recurrence free survival (RFS) curves according to PD-L1 (A, B), CD8 (C, D), CD4 (E, F), CD4 in cancer stroma (G, H) and Foxp3 (I, J) are shown.

Foxp3 positive regulatory T cells (Treg) are potent mediators of dominant self-tolerance in the periphery and abundant CD4 positive T cells expressing Foxp3 are responsible for suppressing the anti-tumor immune response. Abundant Foxp3 positive T cells have been thought to be a poor prognostic factor in various cancers; however, some studies have shown favorable prognostic impact of Foxp3 positive Treg infiltration in some neoplasms. In a meta-analysis on gastric cancer, Zheng *et al.* described that intra-tumoral Foxp3 positive T cells were associated with poor survival, whereas extra-tumoral Foxp3 positive T cells invasion was associated with better survival [15]. Authors suggested that Foxp3 T cells have opposite functions in the intra- and extra-tumoral environment; our result was consistent with the extra-tumoral (i.e. stromal) Foxp3 expression observed in their study. Some reports on colorectal cancer have also shown favorable impact of Foxp3 expression on patient outcome [16-18]. Saito *et al.* revealed that colorectal cancer, which is commonly infiltrated by suppression-competent Foxp3-positive Treg cells, can be classified into two types by the degree of non-suppressive T cells with low Foxp3 expression [19]. Colorectal cancer with abundant infiltration of Foxp3 positive but low expressing T cells showed significantly better prognosis than those with predominantly Foxp3 highly expressing Treg cell infil-

Table 4. Univariate and Multivariate analyses

	OS				RFS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	N = 95	*p*-value[a]	HR	*p*-value[b]	N = 92	*p*-value[a]	HR	*p*-value[b]
Age								
≥ 74/< 74	49/46	0.12			48/44	0.13		
Gender								
Male/Female	82/13	0.32			79/13	0.22		
Smoking history								
Yes/No	4/91	0.50			3/89	0.81		
Stage								
II-IV/I	41/54	0.13			38/54	0.02[c]	1.97 (1.05-3.71)	0.03[c]
Lymphatic permeation								
Yes/No	62/31	0.10			29/61	< 0.01[c]	1.93 (0.94-3.97)	0.07
Vascular invasion								
Yes/No	67/25	0.72			66/23	0.09		
Pleural invasion								
Yes/No	37/57	0.02[c]	2.82 (1.21-3.93)	< 0.01[c]	35/56	< 0.01[c]	2.23 (1.23-4.04)	< 0.01[c]
Adjuvant therapy								
No/Yes	72/23	0.08			70/22	0.71		
PD-L1 expression								
Negative/Positive	25/70	0.25			25/67	0.09		
CD8 expression in whole tumor								
Negative/Positive	52/43	0.53			50/42	0.95		
CD8 expression in cancer nest								
Negative/Positive	71/24	0.67			69/23	0.61		
CD8 expression in cancer stroma								
Negative/Positive	45/50	0.44			43/49	0.82		
CD4 expression in whole tumor								
Negative/Positive	19/76	0.43			19/73	0.38		
CD4 expression in cancer nest								
Negative/Positive	80/15	0.69			77/15	0.68		
CD4 expression in cancer stroma								
Negative/Positive	17/78	0.14			17/75	0.03[c]	0.31 (0.11-0.82)	0.02[c]
Foxp3 expression								
Negative/Positive	52/43	0.01[c]	1.97 (1.06-3.68)	0.03[c]	50/42	< 0.01[c]	1.97 (1.05-3.71)	0.04[c]

[a]Log-rank test, [b]Cox proportional hazard model, [c]denotes significance.

tration. Authors described that functionally distinct subpopulations of tumor-infiltrating Foxp3 positive T cells contribute in opposing ways to determining prognosis. We did not investigate the expressing levels and relationship between other markers, but many T cells expressing low Foxp3 levels might be included within the Foxp3 positive T cells.

Regarding CD4 positive TILs, one meta-analysis has shown that high number of CD4 positive T cells infiltration in tumor stroma was identified as a good prognostic factor. In contrast, another meta-analysis has shown that high number of CD4 positive T cells infiltration in the whole tumor tissue was associated with a good prognosis for OS of patients with lung cancer [20]. The discrepancy between their analysis and our result might be explained by the same reason as that for Foxp3, such as histology and small sample size. Generally, CD4 positive T cells were thought to suppress anti-tumor immune response [21], but the prognostic impact has not been clarified in a large patient size. In LCNEC, stromal CD4 positive T cells might suppress the anti-tumor immune response, as demonstrated in previous reports in other cancers [22, 23].

Until now, many studies have investigated the frequency and prognostic impact of PD-L1 expression in lung cancer [9, 24-35]. Several

reports have shown that PD-L1 expression was an independent unfavorable factor for survival in lung adenocarcinoma [25, 29, 32, 36], lung squamous cell carcinoma [27, 28], SCLC [9], and NSCLC [34]. However, some reports have shown no significant differences between PD-L1 positive and negative tumors [26], while others have shown that PD-L1 was an independent favorable prognostic factor in NSCLC [10, 35, 37, 38]. It is controversial whether high PD-L1 expression is prognostic or non-prognostic, and whether favorable or unfavorable. Because tumor immune microenvironment might be different depending on histologic variations, we examined the outcome limited to LCNEC. In our study, patients with PD-L1 positive tumor had better but not significant RFS, which was consistent with a previous report on LCNEC [11, 12]. Despite similar prognosis and same cut-off value of PD-L1 used in Tsuruoka *et al.*, PD-L1 expression positive rate was much lower than that of our study (10.4% vs 73.7%). Although Inamura *et al.* used 5% as a cut-off value of PD-L1 positivity, PD-L1 expression positive rate (26.8%) was also lower than that of ours. This differences might be caused by the different methodology; those authors used TMA for evaluation and they enrolled patients for a long-term period (1982-2010 [11], and 1990-2014 [12]). However, considering our data that showed high PD-L1 expression in LCNEC, anti-PD-1 antibody might be effective to LCNEC.

This study had several limitations. First of all, we did not observe the effect of anti-PD-1 antibody treatment and whether PD-L1 expression is predictive for the therapeutic outcome. In this study, we only examined the prognostic impact of immune-related markers including PD-L1. Another limitation is that we collected surgically resected tumor samples for a relatively long period. PD-L1 expression might be different between new and old samples, as it has been previously noted [39].

Conclusions

In conclusion, we showed the prognostic impact of PD-L1 expression and TILs in LCNEC. Foxp3 positive TILs were an independent significant good prognostic factor for both OS and RFS. CD4 positive TILs were conversely an independent significant poor prognostic factor for RFS. The high frequency of PD-L1 positive expression could further support the use of anti-PD-1 antibody in the treatment of LCNEC and a good tumor response following treatment, same as in other NSCLC subtypes.

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Disclosure of conflict of interest

None.

Abbreviations

CI, Confidence interval; HR, Hazard ratio; LCNEC, Large cell neuroendocrine carcinoma; ND, Nodal dissection; NSCLC, Non-small cell lung cancer; OS, Overall survival; PD-L1, Programmed death-ligand 1; RFS, Recurrence free survival; TIL, Tumor infiltrating lymphocyte.

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Table S1. PD-L1 expressional distributions in LCNEC

Score	0	1+	2+	3+	4+	5+
PD-L1 expression rate	< 1%	1-5%	6-10%	11-25%	26-50%	51%-
N	25	28	17	12	6	7
%	26	30	18	12	6	7

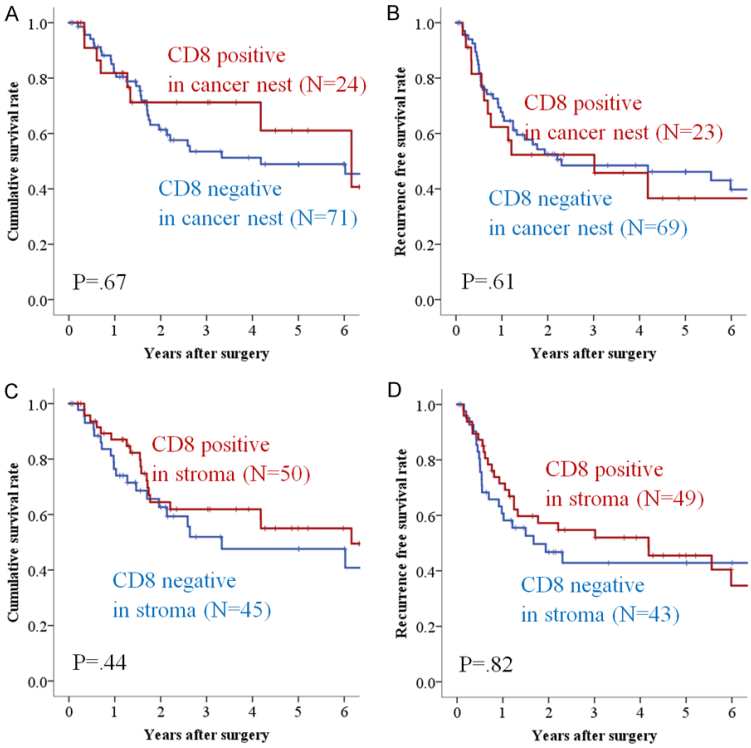

Figure S1. OS and RFS curves according to CD8 expression in cancer nest (A, B) and cancer stroma (C, D) are shown. There were not any significant differences.

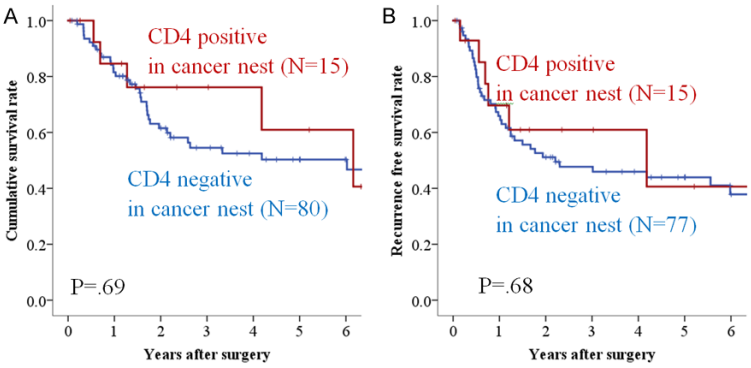

Figure S2. OS and RFS curves according to CD4 expression in cancer nest are shown (A, B). There were no significant survival differences between the groups.