〈Regular Article〉

The biological Characteristics of Solid Components are Different between Part-Solid-Type and Solid-Type in Lung Adenocarcinoma

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ABSTRACT Numerous studies have been conducted to determine the clinical significance of the ground-glass opacity and solid-components in lung adenocarcinomas, however, few biological analyses of the two components have been carried out. This study was aimed at clarifying the biological characteristics of solid components in part-solid-type and solid-type lung adenocarcinomas. Data of a total of 112 cases of cT1b/cN0M0 lung adenocarcinoma treated by surgical resection were analyzed. We compared clinicopathological characteristics and prognosis between part-solid-type and solid-type tumors. In addition, we performed immunohistochemical analysis to determine the Ki-67 labeling index (LI), programmed cell death-1 ligand 1 (PD-L1) expression status, and CD8-positive tumor-infiltrating T lymphocyte (CD8+ TIL) count for the solid component of each tumor. Five-year recurrence-free survival (RFS) risks were significantly worse for patients with solid-type tumor than for those with part-solid-type tumor (51.7% vs. 83.2%; $p < 0.001$). The percentages of lymphovascular invasion, lymph node metastasis, high Ki-67 LI, and high PD-L1 expression were higher in the patient group with solid-type tumors. Univariate analysis identified Ki-67 LI, PD-L1 expression status, and CD8+ TIL count were identified as predictors of RFS in the entire subject population. Separate analyses in the two groups identified only the Ki-67 LI as an independent predictor of the RFS in the group with part-solid-type tumors, whereas in the group with solid-type tumors, the PD-L1 expression status and CD8+ TIL count were identified as independent predictors of the RFS. Clear differences in the biological characteristics of the solid-component were identified between part-solid-type and solid-type lung adenocarcinomas.

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Key words：Solid-type lung adenocarcinoma, Part-solid-type lung adenocarcinoma, Solid component, Ki-67, PD-L1, CD8+ TIL

INTRODUCTION

According to the 8th edition of the tumor-node-metastasis (TNM) classification for lung cancer, the clinical T category is determined according to

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the size of the solid component on high-resolution computed tomography (HRCT)[1]. Numerous reports have described the presence of a ground-glass opacity (GGO) component in the tumor on HRCT as a favorable prognostic factor in patients with early-stage non-small cell lung cancer (NSCLC)[2, 3]. Conversely, tumors with only a solid component have been reported to be associated with a higher likelihood of lymphovascular invasion or nodal metastasis, and to be associated with a relatively poor prognosis as compared to tumors with a GGO component[4, 5]. In particular, in the analysis of pairs matched for the size of the solid component, solid-type tumors were associated with significantly higher incidences of lymphatic, vascular, and pleural invasion and poorer prognosis as compared to part-solid tumors[6]. While extensive research has been conducted on the clinical significance of the GGO and solid components in early-stage NSCLC, few biological analyses have been carried out to date.

In this study, to clarify the biological characteristics of the solid component between part-solid-type and solid-type tumors, we examined the Ki-67 labeling index (LI), programmed cell death-1 ligand 1 (PD-L1) expression status and CD8-positive tumor-infiltrating T lymphocyte (CD8+ TIL) count in the solid component of resected lung adenocarcinoma tissues. In addition, we analyzed the correlation between the results of these biomarker analyses and prognosis of the patient groups with part-solid-type and solid-type adenocarcinomas.

PATIENTS AND METHODS

Patients

A total of 112 consecutive patients who underwent lobectomy with systematic lymph node dissection for clinical stage IA2 (ie, T1bN0M0) or IA3 (ie, T1cN0M0) lung adenocarcinoma according to the Union for International Cancer Control eighth TNM staging system[1] as the initial treatment at Kawasaki Medical School Hospital between January 2007 and December 2014 were included in this study. Patients who had not been evaluated preoperatively by both HRCT and F-18-fluorodeoxyglucose positron emission tomography (FDG-PET) were excluded from the study. The TNM classification was determined according to the revised criteria published in 2017 (TNM classification 8th edition)[1]. The histological diagnosis of the tumors was based on the International Association for the Study of Lung Cancer (IASLC) / American Thoracic Society (ATS) / European Respiratory Society (ERS) Classification of Lung Adenocarcinoma in 2011[7]. Clinical N0 was defined as non-enlarged lymph nodes measuring < 10 mm in diameter in the short axis on CT and no uptake by lymph nodes on FDG-PET. Written informed consent for the study of the excised tissue samples was obtained from each patient at the time of surgery. This study was conducted with the approval of the Ethics Committee of Kawasaki Medical School (No. 5024-00; approved on September 16, 2020).

HRCT assessment and data collation

The overall tumor size and size of the solid component were determined preoperatively on HRCT images. The consolidation tumor ratio (CTR) was defined as the ratio of the maximum size of the solid component to the maximum tumor size. Lung tumors with both a GGO and solid component were defined as part-solid-type tumors ($0 < CTR < 1.0$), while tumors showing only a solid component without any GGO component were defined as solid-type tumors ($CTR = 1.0$). Patients with pure GGO lesions ($CTR = 0$) on HRCT were excluded from this study.

Data on age, sex, smoking status (Brinkman index), preoperative serum carcinoembryonic antigen (CEA) concentration, maximum standardized uptake value (SUVmax) on FDG-PET, clinical and pathological stages of the tumor, histological subtype of the tumor, and epidermal

growth factor receptor (EGFR) mutation status were retrieved from the medical records.

Immunohistochemical analysis and assessment

Immunohistochemical (IHC) analyses of the resected, paraffin-embedded lung cancer tissue specimens were performed. After microtome sectioning (4 μm), slides were processed for staining using an automated immunostainer (Nexes; Ventana, Tucson, AZ, USA). The following primary antibodies were used according to the instructions from the manufacturer using a previously described protocol[8-10]: mouse monoclonal anti-Ki-67 antibody (1 : 50, clone MIB-1; Dako), anti-PD-L1 antibody (1 : 100, clone SP142; Spring Bioscience, Pleasanton, CA, USA), and anti-CD8 antibody (1 : 50, clone C8 / 144B; Dako; Agilent Technologies, Santa Clara, CA, USA). The expression status of each marker was evaluated based on previously reported protocols[8-10]. Tumor PD-L1 expression was categorized as positive when the tumor cell membrane showed positive staining of any intensity. Positive PD-L1 expression for a section was observed at a prespecified expression level of 10% of all cells in a section that included at least 100 evaluable tumor cells[8]. For IHC analysis of CD8+ TIL, 10 digital high-power-field images of the tumor were selected, and the absolute numbers of CD8+ TIL in these images were counted[9]. The Ki-67 LI was measured by determining the percentage of cells showing positively stained nuclei in a section that included at least 100 evaluable tumor cells showing positive staining[10]. A cut-off value of the Ki-67 LI of 14% to discriminate between breast cancers showing high and low values of the index was reported at the Sankt Gallen consensus meeting in 2011, but the most appropriate cutoff value among values between 14% - 20% still remains under debate[11]. In this study, the Ki-67 LI was defined as high in tumors with values of the LI of > 14%.

Statistical analyses

All statistical analyses were performed using the SPSS statistical software (version 17.0; SPSS, Chicago, IL). Frequencies were compared using the chi-square test for categorical variables, with Fisher's exact test applied to small samples; Mann-Whitney's U test and t test were used to compare continuous variables. Overall survival (OS) was defined as the time from the date of surgery until the date of death from any cause, and recurrence-free survival (RFS) was defined as the time from the date of surgery until the date of either diagnosis of lung cancer recurrence or non-lung cancer death. The Kaplan-Meier method was used to draw survival curves, and differences in the survival were compared by the log-rank testing. Potential recurrent risk factors were first analyzed by univariate analysis in all patients, and several factors (age, gender, and biological factors) were analyzed by multivariate Cox regression models to identify independent risk factors for recurrence in each group. Two-sided p-values of less than 0.05 were considered as denoting statistical significance.

RESULTS

The characteristics of the 112 patients are summarized in Table 1. The mean patient age was 68.5 years (range, 44-88 years). The duration of postoperative follow-up ranged from 9.7 to 98.2 months (median, 52.3 months). The histological subtypes of the tumors were as shown in Table 1, with 87 patients with a lepidic growth component and 39 patients with poorly-differentiated components (solid component or micropapillary component).

Representative cases and IHC finding

Case 1 was a 75-year-old woman with part-solid-type tumor measuring 2.4 cm in diameter (diameter of the solid component, 1.8 cm; CTR = 0.75) and SUVmax (on FDG-PET) of 2.70. The

histological subtype was lepidic-dominant invasive adenocarcinoma (Fig. 1). Case 2 was a 70-year-old man with a solid-type tumor measuring 2.4 cm in diameter (CTR = 1.0) and SUVmax of 12.20. The histological subtype was acinar-dominant invasive adenocarcinoma (Fig. 2).

Table 1. Characteristics of Patients Enrolled in This Study (n = 112)

		N	%
Age	< 70 years	63	56.2
	≥ 70 year	49	43.8
Sex	Male	53	47.3
	Female	59	52.8
Smoking history	Current / Former	55	49.1
	Never	57	50.9
Histological subtype	Lepidic	32	28.6
	Acinar	37	33.0
	Papillary	32	28.6
	Solid	10	8.9
	Micropapillary	1	0.9
Pathological nodal status	n0	93	83.0
	n1	8	7.1
	n2	11	9.8
Pathological stage	IA (IA1/IA2/IA3)	53	47.3
	IB	35	31.2
	IIA/IIB	10	8.9
	IIIA	14	12.5
EGFR mutation status	Mutant	35	31.3
	Wild type	30	26.8
	Unknown	47	41.9

Clinicopathological and biological characteristics of the patients in accordance with the HRCT findings

The clinicopathological and biological characteristics of the patients are shown in Table 2. The patient group with solid-type tumors showed a higher Brinkman index ($p = 0.020$), higher serum CEA level ($p = 0.038$), higher SUVmax ($p < 0.001$) and higher percentages of patients with lymph node metastasis ($p = 0.002$), lymphatic invasion ($p = 0.005$) and vascular invasion ($p < 0.001$). Patients with part-solid-type tumors showed larger maximum tumor diameter ($p < 0.001$) and higher proportion of patients with lepidic-dominant adenocarcinoma ($p < 0.001$). With regard to the IHC characteristics, solid-type tumors showed a higher tumor Ki-67 LI ($p < 0.001$) and higher percentage of tumors showing high PD-L1 expression ($p = 0.005$), while

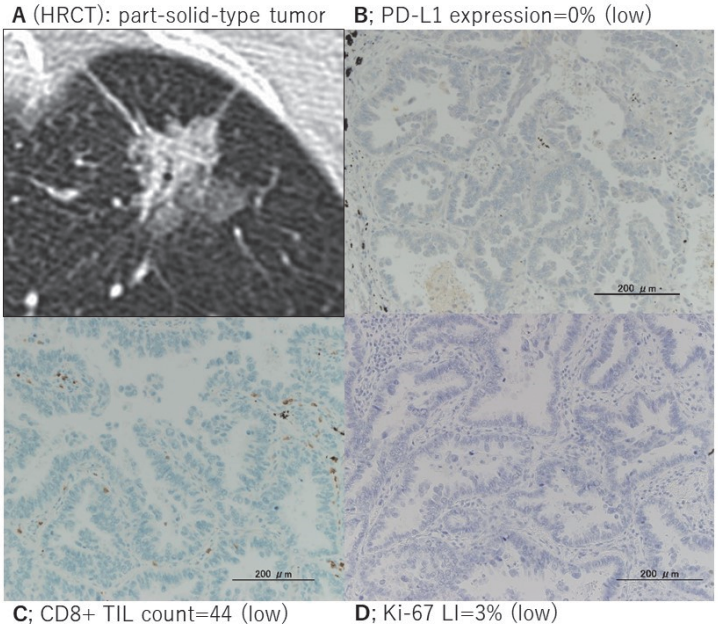

Fig. 1. Case-1 with part-solid-type tumor; representative images of HRCT and IHC staining
(A) HRCT image of part-solid-type tumor. (B) PD-L1 expression in solid part = 0% of all cells (negative). (C) CD8+ TIL count in solid part = 44. (D) Ki-67 LI in solid part = 20.

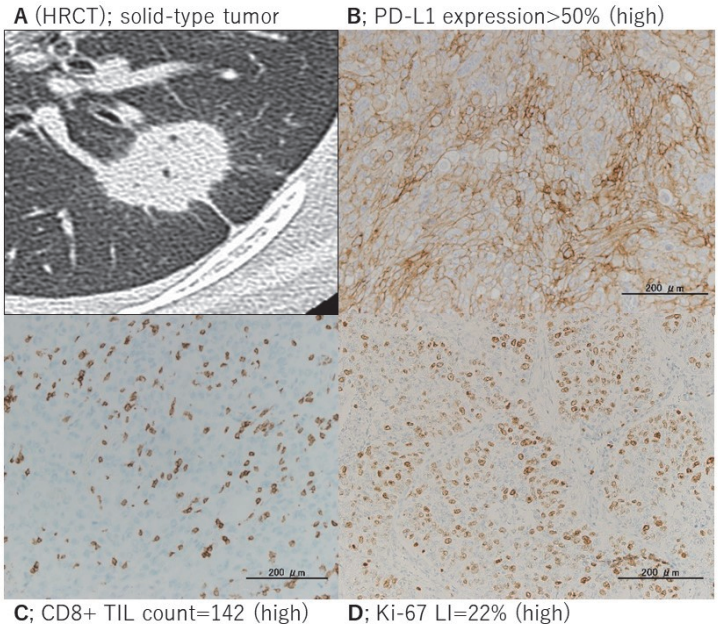

Fig. 2. Case-2 with solid-type tumor; representative images of HRCT and IHC staining
(A) HRCT image of solid-type tumor. (B) PD-L1 expression > 50% of all cells (positive). (C) CD8+ TIL count = 142. (D) Ki-67 LI = 120.

no significant differences in the CD8+ TIL count or EGFR mutation status was observed between the two types of tumors.

Survival analysis

In the 112 patients overall, the 5-year OS was 82.7% and the 5-year RFS rate was 67.5%. The Kaplan-Meier curves for OS and RFS according to the HRCT findings are shown in Fig. 3. The 5-year OS and RFS rates were significantly higher in the patient group with part-solid-type tumors than in the patient group with solid-type tumor (OS; $p = 0.039$, RFS; $p < 0.001$).

In this study, prognostic evaluation was performed based on the RFS. In a univariate analysis of the entire subject population, the HRCT findings, serum CEA levels, SUVmax, histological subtype, presence / absence of lymph node metastasis, tumor Ki-67 LI, tumor PD-L1 expression status, and CD8+ TIL count were identified as predictors of RFS. (Table 3). In the group of patients with part-solid-type tumors, multivariate analysis identified tumor Ki-67 LI ($p < 0.001$) as independent predictors of the RFS (Table 4A-C). In the group of patients with solid-type tumors, multivariate analysis identified tumor PD-L1 expression status ($p = 0.015$) and CD8+ TIL count ($p = 0.036$) as independent predictors of the RFS (Table 5A-C).

Prognostic impact of tumor Ki-67 LI

In the overall subject population, the RFS was significantly worse in the patient group with a high tumor Ki-67 LI than in the patient group with a low tumor Ki-67 LI ($p < 0.001$) (Fig. 4A). Among the patients with part-solid tumor, the RFS of the patient group with a high tumor Ki-67 LI was significantly inferior to that in the patient group with a low tumor Ki-67 LI ($p < 0.001$) (Fig. 4B). In contrast, among the patients with solid tumors, the RFS of the patient group with a high tumor Ki-67 LI was not inferior to that in the patient group with a low tumor Ki-67 LI ($p = 0.369$) (Fig. 4C).

Table 2. Clinicopathological and Biological Characteristics of the Patients in Accordance with the HRCT Findings

Characteristics	Part-Solid-Type n = 54	Solid-Type n = 58	*P*-value
Clinical Findings			
Age (years) *	68.4 ± 7.7	68.5 ± 8.2	0.645
< 70 years	29	31	
≥ 70 years	25	27	
Sex			0.003
Male	18	36	
Female	36	22	
Smoking status			< 0.001
Smoker	17	38	
Non-smoker	37	20	
Brinkman index (pack-year) *	691 ± 385	1,026 ± 647	0.02
Serum CEA (ng/mL) *	3.79 ± 4.14	6.81 ± 9.85	0.038
≤ 5.0 ng/mL	42	39	
> 5.0 ng/mL	12	19	
HRCT Findings			
Maximum tumor size (mm) *	27.2 ± 7.9	21.1 ± 5.0	< 0.001
Solid component size (mm) *	19.1 ± 6.1	21.1 ± 5.0	0.071
Consolidation tumor ratio *	0.71 ± 0.17	1	< 0.001
PET-CT Findings			
SUVmax *	3.74 ± 2.54	8.22 ± 4.36	< 0.001
≤ 5.0	42	16	
> 5.0	12	42	

Characteristics	Part-Solid-Type n = 54	Solid-Type n = 58	*P*-value
Pathological Findings			
Histological subtypes			
Lepidic	29	3	
Acinar	18	19	
Papillary	6	26	
Solid	1	9	
Micropapillary	0	1	
Poorly-differentiated components			0.029
Yes	13	26	
No	41	32	
Lepidic-growth components			< 0.001
Yes	54	33	
No	0	25	
Lymph node metastasis			0.002
n0	51	42	
n1/n2	3	16	
Pleural involvement			0.011
pl0	41	30	
pl1 / pl2 / pl3	13	28	
Lymphatic invasion			0.005
ly0	47	37	
ly1	7	21	
Vascular invasion			< 0.001
v0	46	31	
v1 / v2	8	27	
Biomarker Findings			
Ki-67 labeling index *	8.93 ± 7.32	20.14 ± 11.89	< 0.001
High (≥ 14%)	9	37	
Low (< 14%)	45	21	
PD-L1 expression			0.005
High (> 10%)	7	21	
Low (≤ 10%)	47	37	
CD8+TIL count *	80.3 ± 59.4	78.4 ± 53.9	0.86
High (> 80)	18	24	
Low (≤ 80)	36	34	
EGFR mutation			0.204
Wild type	9	21	
Mutant	17	18	
unknown	28	19	

* mean ± SD

Prognostic impact of tumor PD-L1 expression

In the overall subject population, the RFS of the patient group with high tumor PD-L1 expression was significantly inferior to that in the patient group with low tumor PD-L1 expression (p = 0.002) (Fig. 5A). Among the patients with part-solid tumor, the RFS in the patient group with high tumor PD-L1 expression was equivalent to that in the patient group with low tumor PD-L1 expression (p = 0.486) (Fig. 5B). In the patients with solid-type tumor, in contrast, the RFS in the patient group with high tumor PD-L1 expression was significantly inferior to that in the patient group with low tumor PD-L1 expression (p = 0.040) (Fig. 5C).

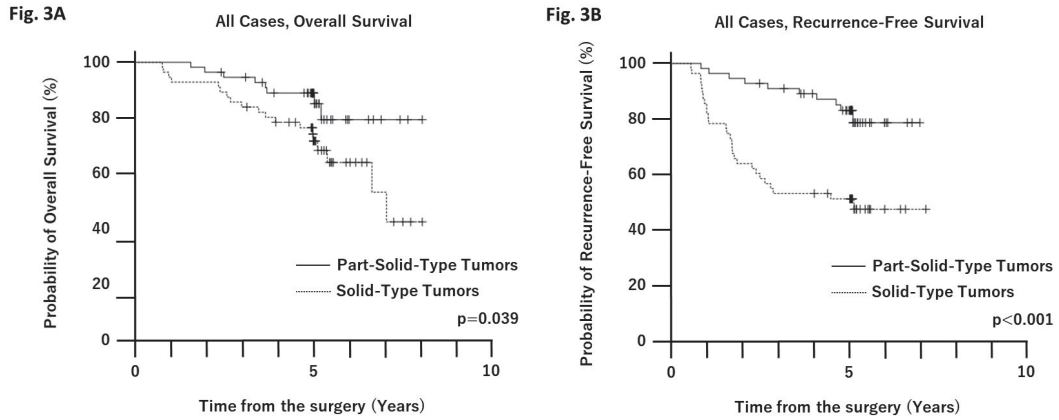

Fig. 3. Kaplan-Meier curves for overall survival (OS) and recurrence-free survival (RFS) in lung adenocarcinoma with part-solid-type tumor (solid line) and solid-type tumor (dotted line).
(A) 5-year OS rates between patients with part-solid-type tumor and solid-type tumor are 89.0% vs. 76.5% (p = 0.039, log-rank test).
(B) 5-year RFS rates between patients with part-solid-type tumor and solid-type tumor are 83.2% vs. 51.7% (p < 0.001, log-rank test).

Table 3. Results of Univariate Cox Regression Analyses of Factors Influencing Recurrence-Free Survival in All Cases

Variable	HR	95% CI	*P*-value
Clinical factors			
Age	1	0.96 - 1.04	0.869
Sex (female vs. male)	1.52	0.80 - 2.88	0.199
Serum CEA level	1.04	1.02 - 1.06	< 0.001
HRCT finding (part-solid vs. solid)	3.67	1.78 - 7.56	< 0.001
SUVmax	1.12	1.06 - 1.18	< 0.001
Pathological factors			
Histology (lepidic vs. non-lepidic)	3	1.31 - 6.82	0.009
LN metastasis (negative vs. positive)	5.16	2.63 - 10.09	< 0.001
Biological factors			
Ki-67 labeling index (low vs. high)	3.61	1.84 - 7.07	< 0.001
PD-L1 expression (low vs. high)	2.7	1.41 - 5.19	0.003
CD8+ TIL count (low vs. high)	0.45	0.21 - 0.96	0.033

CI, confidence interval; HR, hazard ratio.

Prognostic impact of the CD8+ TIL count

In the overall subject population, the RFS in the patient group with a low CD8+ TIL count tended to be inferior to that in the patient group with a high CD8+ TIL status (p = 0.033) (Fig. 6A). Among the patients with part-solid tumor, the RFS in the patients with a low CD8+ TIL count was equivalent to that in the patient group with a high CD8+ TIL count (p = 0.575) (Fig. 6B). Conversely, among in the patients with solid tumor, the RFS in the patient group with a low CD8+ TIL count was significantly inferior to that in the patient group with a high CD8+ TIL count (p =0.017) (Fig. 6C).

DISCUSSION

Several previous studies have been conducted to compare the characteristics of solid tumors and part-solid tumors with a GGO component in patients with clinical stage IA lung adenocarcinoma. These studies have clearly demonstrated differences in

Table 4. Results of Univariate and Multivariate Cox Regression Analyses of Factors Influencing Recurrence-Free Survival in 54 Cases with Part-Solid-Type Tumor

(A) Model 1; Age / Gender / Ki-67 labeling index

Variable	Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age	1.01 (0.94 - 1.09)	0.644	1.01 (0.92 - 1.10)	0.789
Gender (female vs. male)	1.20 (0.39 - 3.71)	0.743	1.75 (0.53 - 5.72)	0.349
Ki-67 labeling index (high vs. low)	0.15 (0.05 - 0.47)	< 0.001	0.13 (0.04 - 0.44)	< 0.001

(B) Model 2; Age / Gender / PD-L1 expression

Variable	Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age	1.01 (0.94 - 1.09)	0.644	1.02 (0.94 - 1.10)	0.596
Gender (female vs. male)	1.20 (0.39 - 3.71)	0.743	1.13 (0.33 - 3.82)	0.84
PD-L1 expression (high vs. low)	0.73 (0.16 - 3.35)	0.689	0.73 (0.13 - 3.85)	0.712

(C) Model 3; Age / Gender / CD8+ TILs count

Variable	Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age	1.01 (0.94 - 1.09)	0.644	1.02 (0.94 - 1.10)	0.571
Gender (female vs. male)	1.20 (0.39 - 3.71)	0.743	1.17 (0.37 - 3.71)	0.782
CD8+ TIL count (high vs. low)	0.86 (0.28 - 2.64)	0.797	0.79 (0.23 - 2.68)	0.717

CI, confidence interval; HR, hazard ratio.

Table 5. Results of Univariate and Multivariate Cox Regression Analyses of Factors Influencing Recurrence-Free Survival in 58 Cases with Solid-Type Tumor

(A) Model 1; Age / Gender / Ki-67 labeling index

Variable	Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age	1.00 (0.95 - 1.04)	0.99	1.00 (0.95 - 1.04)	0.977
Gender (female vs. male)	1.28 (0.64 - 2.57)	0.477	1.34 (0.66 - 2.73)	0.412
Ki-67 labeling index (high vs. low)	0.74 (0.37 - 1.50)	0.413	0.71 (0.35 - 1.45)	0.355

(B) Model 2; Age / Gender / PD-L1 expression

Variable	Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age	1.00 (0.95 - 1.04)	0.99	1.01 (0.96 - 1.06)	0.606
Gender (female vs. male)	1.28 (0.64 - 2.57)	0.477	0.82 (0.36 - 1.87)	0.649
PD-L1 expression (high vs. low)	0.42 (0.21 - 0.82)	0.011	0.37 (0.17 - 0.83)	0.015

(C) Model 3; Age / Gender / CD8+ TILs count

Variable	Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age	1.00 (0.95 - 1.04)	0.99	1.00 (0.96 - 1.05)	0.749
Gender (female vs. male)	1.28 (0.64 - 2.57)	0.477	1.70 (0.81 - 3.56)	0.159
CD8+ TIL count (high vs. low)	1.77 (0.88 - 3.52)	0.099	2.20 (1.05 - 4.62)	0.036

CI, confidence interval; HR, hazard ratio.

the clinicopathological characteristics, such as the serum CEA level, SUVmax, and rate of pathological lymph node metastasis and lymphovascular invasion between patients with solid-tumor and part-solid tumor, and also demonstrated significant differences in the prognosis between these two groups of patients[6, 12]. For peripheral-type adenocarcinomas, most part-solid tumors with a GGO component on HRCT images are histologically adenocarcinomas with a lepidic growth component, which is

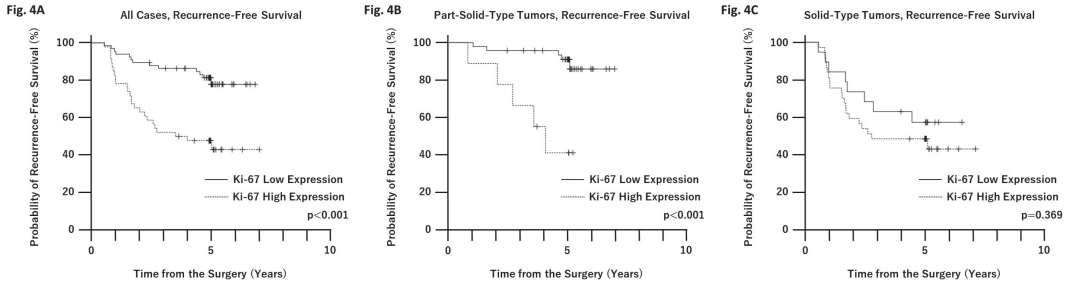

Fig. 4. Comparisons of recurrence-free survival (RFS) curves of patients with lung adenocarcinoma according to tumor Ki-67 LI.
(A) 5-year RFS rates for entire lung adenocarcinoma patients with low (solid line) and high (dotted line) tumor Ki-67 expression: 81.3% vs. 47.7% ($p < 0.001$, log-rank test).
(B) 5-year RFS rates for lung adenocarcinoma patients with part-solid-type tumors showing low (solid line) and high (dotted line) tumor Ki-67 expression: 91.1% vs. 41.7% ($p < 0.001$, log-rank test).
(C) 5-year RFS rates for lung adenocarcinoma patients with solid-type tumors showing low (solid line) and high (dotted line) tumor Ki-67 expression: 57.4% vs. 48.6% ($p = 0.369$, log-rank test).

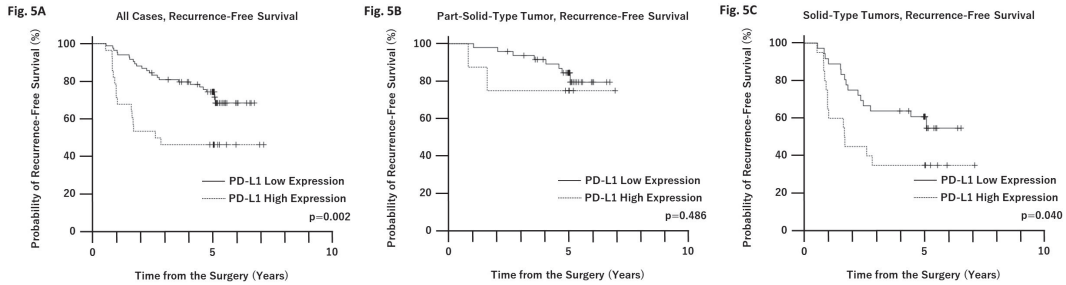

Fig. 5. Comparisons of recurrence-free survival (RFS) curves of patients with lung adenocarcinoma according to tumor PD-L1 expression.
(A) 5-year RFS rates for entire lung adenocarcinoma patients with low (solid line) and high (dotted line) tumor PD-L1 expression: 74.4% vs. 46.4% ($p = 0.002$, log-rank test).
(B) 5-year RFS rates for lung adenocarcinoma patients with part-solid-type tumors with low (solid line) and high (dotted line) tumor PD-L1 expression: 84.5% vs. 75.0% ($p = 0.486$, log-rank test).
(C) 5-year RFS rates for lung adenocarcinoma patients with solid-type tumors with low (solid line) and high (dotted line) tumor PD-L1 expression: 60.8% vs. 35.0% ($p = 0.040$, log-rank test).

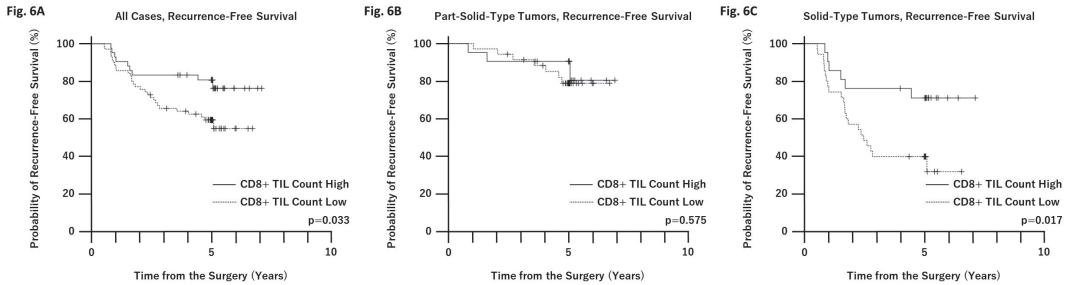

Fig. 6. Comparisons of recurrence-free survival (RFS) curves of patients with lung adenocarcinoma according to CD8+ TILs count.
(A) 5-year RFS rates for entire lung adenocarcinoma patients with high (solid line) and low (dotted line) CD8+ TIL count: 80.7% vs. 59.4% ($p = 0.033$, log-rank test).
(B) 5-year RFS rates for lung adenocarcinoma patients with part-solid-type tumors with high (solid line) and low (dotted line) CD8+ TIL count: 78.8% vs. 80.4% ($p = 0.575$, log-rank test).
(C) 5-year RFS rates for lung adenocarcinoma patients with solid-type tumors with high (solid line) and low (dotted line) CD8+ TIL count: 71.1% vs. 40.0% ($p = 0.017$, log-rank test).

thought to represent a stepwise progression of carcinogenesis[13, 14]. On the other hand, solid tumors without a GGO component are mostly invasive adenocarcinoma without a lepidic growth component, and tumors of this type are thought as being de novo tumors[15].

The clinical T category is now defined according to the size of the solid component of HRCT images, according to the TNM classification for lung cancer 8th edition. However, whether the solid components of a part-solid tumor and solid tumor exhibit different biological characteristics remains controversial. In this study, we attempted to determine the biological characteristics influencing the outcomes between patients with solid-type and part-solid-type lung adenocarcinomas, by conducting IHC analysis for three biomarkers of Ki-67, PD-L1, and CD8+ TIL count. We demonstrated, for the first time, clear differences in the tumor Ki-67 LI, tumor PD-L1 expression, and CD8+ TIL count between part-solid-type and solid-type lung adenocarcinomas.

Ki-67 is known to be observed in proliferating tumor cells, and the KI-67 expression level has been used to evaluate the proliferative activity of the tumor in breast cancers, NSCLC, and other cancers. Several studies have suggested the Ki-67 LI as a strong prognostic factor in patients with NSCLC[16–19]. Recently, Wen S *et al*. reported the results of a meta-analysis of 32 studies, and reported that a high tumor Ki-67 LI was associated with a poorer outcome in NSCLC patients, particularly in Asian patients with early-stage adenocarcinoma[11]. In this study, the Ki-67 LI was identified as a prognostic factor only in patients with part-solid-type tumors, even though the tumor Ki-67 LI was significantly lower than in the part-solid-type tumors; despite the higher value, the Ki-67 LI was not identified as a significant prognostic factor in the patients with the solid-type of tumors. Part-solid-type tumors are considered to represent stepwise progression of carcinogenesis[13, 14], therefore the malignant behavior and prognosis would be expected to be highly dependent on the proliferative activity of the tumor cells. In contrast, solid-type tumors are thought to be *de novo* tumors[15], and contain highly proliferative tumor cells from the early stage of carcinogenesis. Therefore, the prognosis of solid-type tumors does not depend solely on the proliferative ability of the tumor cells.

Previous studies have investigated the association between PD-L1 expression and clinicopathological features such as the gender, smoking status, histological differentiation grade, presence / absence of lymph node metastases, and TNM stage in patients with NSCLC, and reported a significant association of high tumor PD-L1 expression with a poor differentiation grade, positive lymph node metastasis, and advanced disease stage[20]. Mori *et al*. reported that high tumor PD-L1 expression in lung adenocarcinoma was a poor prognostic factor, particularly in smokers[21]. The importance of lymphocytic infiltration as a predictor of the outcome has also been shown in patients with several types of cancers. Previous studies in NSCLC patients have indicated the existence of an association between the number of CD8+ TIL and the patient outcome[9, 22, 23]. In 2017, we demonstrated, in patients with lung adenocarcinoma treated by resection, the outcomes of patients with low tumor PD-L1 expressions and high CD8+ TIL counts were significantly better than those with other patients[24]. In this study, the proportion of smokers and the Brinkman index were significantly higher in the patient group with solid-type tumors. In addition, the PD-L1 expression level was significantly higher in the patient group with solid-type tumors than in the patient group with part-solid-type tumors. We also found that high tumor PD-L1 expression and a low CD8+ TIL count were identified as independent unfavorable prognostic factors in the patient group with solid-type tumors.

In lung adenocarcinoma, the relationship between cigarette smoking and carcinogenesis, malignant progression, and histological differentiation have not yet been elucidated. However, there is convincing evidence that smoking increases the risk of lung adenocarcinoma in the Japanese population, with the relative risk for current smokers as compared to that in never smokers being around 2.30 for men and 1.37 for women[25]. There are thought to be two carcinogenic processes of lung adenocarcinoma: smoking-related carcinogenesis and smoking-unrelated carcinogenesis[26]. Differences in the mechanisms underlying the carcinogenesis result in differences in the histological characteristics. That is, morphologically, adenocarcinoma with lepidic growth features are more common in never-smokers than in smoker. Considering these clinical background and biological characteristics, in patients with invasive and poorly-differentiated adenocarcinoma without lepidic growth components, smoking, one of the environmental factors, might have differentially influenced the carcinogenesis, biological characteristics, and consequently the prognosis.

This study had several limitations that should be considered when interpreting the results. First, the retrospective study design and relatively small number of enrolled patients were among the major limitations of the study. Second, we used SP142 as the PD-L1 antibody in the Blueprint PD-L1 IHC Assay Comparison Project[27]. It has been reported that the SP142 assay yields lower tumor cell staining rates as compared to the 22C3, 28-8, and SP263 assays. However, since the same assay method was used in both groups, we consider that the assay method used is unlikely to have had any significant influence on the results of tumor PD-L1 expression.

CONCLUSIONS

The present results demonstrated that the biological characteristics of the solid component clearly differed between part-solid-type and solid-type tumors among patients with lung adenocarcinomas. In patients with part-solid-type tumors, which are considered to be the result of stepwise progression, the outcomes were influenced by the Ki-67 LI in the solid component. In contrast, among patients with solid-type tumors, which are considered to be de novo tumors, the outcomes were influenced by the tumor PD-L1 expression status and the tumor CD8+ TIL count.

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COMPETING INTERESTS

All authors have no conflicts of interest to declare.

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