Lung Cancer 137 (2019) 129–135

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Prognostic factors of acinar- or papillary-predominant adenocarcinoma of the lung

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ARTICLE INFO

Keywords:
Adenocarcinoma
Acinar
Papillary
Prognosis
Spread through air spaces
PD-L1

ABSTRACT

Objective: Histologic types are correlated with prognosis in lung adenocarcinoma. The acinar/papillary type is most common, although this group comprises heterogeneous tumor types. However, the prognostic factors in this group have not been well studied. Therefore, we investigated the prognostic factors of acinar/papillary lung adenocarcinomas and attempted to define the so-called "intermediate grade" group of lung adenocarcinoma.
Materials and methods: We classified surgically resected invasive non-mucinous adenocarcinomas of the lung and analyzed their clinicopathological features and prognostic factors, focusing on the acinar/papillary type.
Results: A total of 301 cases with stage I-III lung adenocarcinoma were enrolled, of which 193 were acinar/papillary type (64.1%). In survival analysis of the entire cohort, acinar/papillary types showed intermediate survival compared with lepidic and micropapillary/solid types. In the univariate survival analysis for acinar/papillary types, stage, age, lymphovascular invasion, spread through air spaces, presence of micropapillary or solid pattern, and programmed death-ligand 1 (PD-L1) positivity were associated with poor recurrence-free survival and overall survival. In multivariate analysis, spread through air spaces and PD-L1 expression were independent poor prognostic factors of recurrence-free survival and overall survival in the acinar/papillary cohort, respectively.
Conclusions: Evaluation of spread through air spaces and PD-L1 expression may be useful to stratify patients with acinar/papillary lung adenocarcinomas in terms of prognosis.

1. Introduction

Adenocarcinoma is the most common type of lung cancer [1]. According to the 2015 World Health Organization classification, invasive lung adenocarcinomas can be further subclassified into lepidic, acinar, papillary, micropapillary, and solid types [1,2]. Previous studies have consistently shown that lung adenocarcinomas with a predominant lepidic pattern have good prognosis [3–5]. In contrast, predominantly micropapillary or solid adenocarcinomas are associated with poor prognosis [6–8]. Acinar/papillary predominant adenocarcinomas have "intermediate" prognosis; therefore, some studies categorize lepidic type as low-grade, acinar/papillary type as intermediate-grade, and micropapillary/solid type as high-grade lung adenocarcinomas [9,10]. Although acinar/papillary adenocarcinomas are the most common type, accounting for more than 50% of all lung adenocarcinomas, prognostic factors that correlate with their biological behavior and can be used for further stratification have not been well studied [10,11]. In this study, we investigated prognostic factors of acinar/papillary lung adenocarcinomas and attempted to define the so-called "intermediate grade" group of lung adenocarcinomas.

2. Materials and methods

2.1. Patients

We included 301 chemotherapy-naïve patients who underwent surgical resection for invasive adenocarcinoma of the lung between 2005 and 2011. No adenocarcinomas in situ or no minimally invasive adenocarcinomas were included in this cohort. Cases were selected from consecutive surgeries on the basis of availability of archival slides and tissue, and medical records and archival slides were retrospectively analyzed. Detailed clinical data including age, sex, smoking history, and tumor size and stage were retrieved from the medical records. Tumor stage was classified according to the eighth edition of the standards of the American Joint Committee on Cancer. The institutional review board approved this retrospective study. Written informed consent from the patients was waived due to the retrospective nature of the study.

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https://doi.org/10.1016/j.lungcan.2019.09.026
Received 28 May 2019; Received in revised form 7 September 2019; Accepted 28 September 2019

Table 1
Patient characteristics according to lung adenocarcinoma subtypes.

Variables	All (n = 301)	Predominant subtype					p-value
		Lepidic	Acinar	Papillary	Micropapillary	Solid	
Age, years (mean ± SD)	61.7 ± 10.1	65.5 ± 8.1	61.6 ± 10.3	61.2 ± 9.8	64.1 ± 9.9	59.8 ± 10.5	0.193
Sex							0.004
Male	152 (50.4%)	7 (30.4%)	70 (46.1%)	19 (46.3%)	16 (55.2%)	40 (71.4%)	
Female	149 (49.6%)	16 (69.6%)	82 (53.9%)	22 (53.7%)	13 (44.8%)	16 (28.2%)	
Smoking							0.004
Yes	115 (38.2%)	3 (13.0%)	55 (36.2%)	15 (36.6%)	10 (34.5%)	32 (57.1%)	
No	186 (61.8%)	20 (87.0%)	97 (63.8%)	26 (63.4%)	19 (65.5%)	24 (42.9%)	
LVI							< 0.001
Yes	117 (38.8%)	1 (4.3%)	56 (36.8%)	16 (39.0%)	21 (72.4%)	23 (41.1%)	
No	184 (61.2%)	22 (95.7%)	96 (63.2%)	25 (61.0%)	8 (27.6%)	33 (58.9%)	
STAS							< 0.001
Yes	154 (51.1%)	1 (4.3%)	75 (49.3%)	19 (46.3%)	23 (79.3%)	36 (64.3%)	
No	147 (48.9%)	22 (95.7%)	77 (50.7%)	22 (53.7%)	6 (20.7%)	20 (35.7%)	
***EGFR* mutation**							< 0.001
Yes	155 (51.4%)	17 (73.9%)	91 (59.9%)	22 (53.7%)	15 (51.7%)	10 (17.9%)	
No	146 (48.6%)	6 (26.1%)	61 (40.1%)	19 (46.3%)	14 (48.3%)	46 (82.1%)	
***KRAS* mutation**							0.416
Yes	20 (6.7%)	2 (8.7%)	8 (5.3%)	1 (2.4%)	3 (10.3%)	6 (10.7%)	
No	281 (93.3%)	21 (91.3%)	144 (94.7%)	40 (97.6%)	26 (89.7%)	50 (89.3%)	
***ALK* fusion**							0.502
Yes	20 (6.7%)	0 (0.0%)	10 (6.6%)	2 (4.9%)	2 (6.9%)	6 (10.7%)	
No	281 (93.3%)	23 (100.0%)	142 (93.4%)	39 (95.1%)	27 (93.1%)	50 (89.3%)	
***ROS1* fusion**							0.729
Yes	7 (2.3%)	0 (0.0%)	3 (2.0%)	2 (4.9%)	1 (3.4%)	1 (1.8%)	
No	294 (97.7%)	23 (100.0%)	149 (98.0%)	39 (95.1%)	28 (96.6%)	55 (98.2%)	
PD-L1							< 0.001
Positive	60 (19.9%)	1 (4.3%)	22 (14.5%)	3 (7.3%)	9 (31.0%)	25 (44.6%)	
Negative	241 (80.1%)	22 (95.7%)	130 (85.5%)	38 (92.7%)	20 (69.0%)	31 (55.4%)	
Aberrant p53 expression							< 0.001
Yes	106 (35.3%)	4 (17.4%)	45 (29.6%)	8 (19.5%)	6 (20.7%)	43 (76.8%)	
No	195 (64.7%)	19 (82.6%)	107 (70.4%)	33 (80.5%)	23 (79.3%)	13 (23.2%)	
Recurrence							0.001
Yes	127 (42.1%)	2 (13.0%)	57 (37.5%)	21 (51.2%)	19 (65.5%)	28 (50.0%)	
No	174 (57.9%)	20 (87.0%)	95 (62.5%)	20 (48.6%)	10 (34.5%)	28 (50.0%)	
TNM stage							0.001
Stage I	168 (55.8%)	20 (87.0%)	91 (59.9%)	23 (56.1%)	8 (27.6%)	26 (46.4%)	
Stage II	54 (17.9%)	3 (13.0%)	21 (13.8%)	5 (12.2%)	10 (34.5%)	15 (26.8%)	
Stage III	79 (26.2%)	0 (0.0%)	40 (26.3%)	13 (31.7%)	11 (37.9%)	15 (26.8%)	

SD, standard deviation; LVI, lymphovascular invasion; STAS, spread through air spaces; *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; *ROS1*, ROS proto-oncogene 1; PD-L1, programmed death-ligand 1; TNM, tumor node metastasis.

2.2. Pathological evaluation

The surgical specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin blocks. The entire tumor was submitted for microscopic examination in small (< 3 cm) tumors. At least 3 representative sections (one section per 1 cm of tumor) were usually submitted for larger tumors. For each formalin-fixed, paraffin-embedded (FFPE) tissue block, 4-μm sections were cut and stained with hematoxylin and eosin. Two pathologists (MK and HSS) reviewed all available hematoxylin-eosin–stained slides, which included an average of 4.2 slides per case (range, 2–12). Analyzed pathologic characteristics included histologic subtype, lymphovascular invasion (LVI), and tumor spread through air space (STAS). Subtypes were classified according to the 2015 World Health Organization classification, and the percentage of each subtype (lepidic, acinar, papillary, micropapillary, and solid) was recorded in 5% increments. In addition, we analyzed and scored cribriform pattern [12]. Discrepancies in classification were resolved by consensus discussion. Comprehensive subtyping of the tumor was performed according to the predominant histologic pattern. The second most predominant pattern was also recorded, and we divided acinar/papillary group into lepidic versus non-lepidic subgroup based on the second most predominant pattern. Verhoeff's elastic stain was applied to samples in which it was difficult to determine pleural invasion histologically. STAS was defined as tumor cells within air spaces in the lung parenchyma beyond the edge of the main tumor [13].

2.3. Immunohistochemistry (IHC) and interpretation

After histologic review, two or three 3-mm diameter cores were taken from FFPE tissue blocks, and tissue microarrays (TMAs) were constructed. TMA blocks were sectioned at a thickness of 4 μm and stained with antibodies for programmed death-ligand 1 (PD-L1) (rabbit anti-human monoclonal, clone SP142, 1:100, Spring Bioscience, Pleasanton, CA, USA) and p53 (mouse anti-human monoclonal, clone DO-7, 1:100, Dako, Glostrup, Denmark) using a Ventana automated immunostainer (Ventana, Tucson, AZ, USA) according to the manufacturer's protocol. Signals were detected using ChromoMap DAB kit (Ventana) for PD-L1 and DAB Map kit (Ventana) for p53.

Stained slides were reviewed by two pathologists (M.K. and H.S.S.) blinded to the clinicopathological features. Discrepancies in interpretation were resolved by discussion. The final IHC score was taken as the average score from all cores of each tumor. Tissues were considered positive for PD-L1 if ≥5% of tumor cells had membranous staining, as previously described [14,15]. p53 staining was defined as "aberrant" if ≥60% of tumor cells showed either nuclear expression or complete absence of staining and "normal" if tumor cells showed no aberrant expression (1–59% staining), as previously described [16,17].

2.4. Molecular and genetic analysis

For each case, the representative slide containing the largest tumor

Table 2
Univariate and multivariate analysis for recurrence-free survival.

	Univariate analysis HR (95% CI)	p	Multivariate analysis HR (95% CI)	p
Younger age (< 62 years)	1.59 (1.01-2.51)	0.041	1.83 (1.15-2.90)	0.011
LVI	5.28 (3.29-8.46)	< 0.001		
STAS	3.53 (2.16-5.76)	< 0.001	2.73 (1.64-4.55)	< 0.001
Non-lepidic as the second most predominant pattern	2.97 (1.29-6.83)	0.01		
Presence of micropapillary pattern	2.49 (1.49-4.12)	< 0.001		
Presence of solid pattern	1.99 (1.23-3.20)	0.003		
Higher stage	6.51 (3.93-10.76)	< 0.001	5.04 (3.00-8.48)	< 0.001

HR, hazard ratio; CI, confidence interval; LVI, lymphovascular invasion; STAS, spread through air spaces.

Table 3
Univariate and multivariate analysis for overall survival.

	Univariate analysis HR (95% CI)	p	Multivariate analysis HR (95% CI)	p
Older age (> = 62 years)	1.84 (1.08-3.14)	0.023	1.88 (1.08-3.28)	0.025
Male	1.79 (1.06-3.02)	0.027		
Smoking history	2.08 (1.24-3.50)	0.005		
LVI	4.89 (2.77-8.64)	< 0.001		
STAS	1.75 (1.03-2.97)	0.036		
Presence of solid pattern	1.80 (1.04-3.13)	0.026		
Aberrant p53 expression	1.86 (1.10-3.16)	0.019		
PD-L1 positivity	2.83 (1.51-5.32)	0.001	2.14 (1.10-4.15)	0.025
Higher stage	6.51 (3.47-12.21)	< 0.001	6.65 (3.52-12.59)	< 0.001

HR: hazard ratio; CI, confidence interval; LVI, lymphovascular invasion; STAS, spread through air spaces; PD-L1: programmed death-ligand 1.

volume (at least 30%) was selected for molecular tests. To determine the epidermal growth factor receptor (*EGFR*) and *KRAS* mutation status, DNA was extracted from FFPE tissue using a DNeasy isolation kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. For *EGFR*, direct DNA sequencing of exons 18 through 21 was performed or the PNAClamp™ EGFR Mutation Detection Kit (PANAGENE, Daejeon, Korea) was used. For *KRAS*, direct DNA sequencing of codons 12 and 13 was performed. To identify *ALK* and *ROS1* rearrangements, IHC and fluorescence *in situ* hybridization were performed, as previously described [18].

2.5. Statistical analysis

Relationships between clinicopathologic parameters were evaluated using the Chi-square test for categorical parameters and Fisher's exact test for parameters with an expected frequency of less than 5. In univariate analysis, recurrence-free survival (RFS) and overall survival (OS) were evaluated using the Kaplan–Meier method. Statistical differences in survival times were determined using the log-rank test. In multivariate analysis, Cox proportional hazards regression analysis was applied to examine independent predictive factors among significantly correlated variables. Differences were considered significant at $p < 0.05$. All statistical analyses were conducted using IBM SPSS Statistics v.23 (IBM, Armonk, NY, USA).

3. Results

3.1. Clinicopathologic and molecular characteristics among different lung adenocarcinoma subtypes

Patient characteristics according to subtype are summarized in Table 1. Samples were derived from 152 men and 149 women aged 34 to 82 years (mean age: 61.7 years). There was a high degree of inter-observer agreement in classifying the predominant histologic types between two pathologists (the κ value was 0.860). Among 301 cases, 23 cases (7.6%) were classified as predominantly lepidic, 152 (50.4%) cases as predominantly acinar, 41 (13.6%) cases as predominantly papillary, 29 (9.6%) cases as predominantly micropapillary, and 56 (18.6%) cases as predominantly solid (Supplementary Fig. 1). A total of 17 cases (8.8%) were classified as cribriform predominant type in acinar/papillary group (n = 193). There were significant differences in sex ($p = 0.004$), smoking history ($p = 0.003$), and pathologic stage ($p = 0.001$) among the lung adenocarcinoma subtypes. Rates of aberrant p53 expression ($p < 0.001$), *EGFR* mutation ($p < 0.001$), PD-L1 positivity ($p < 0.001$), LVI ($p < 0.001$), and STAS ($p < 0.001$) were also significantly different among the subtypes.

3.2. Survival according to tumor grade

We further categorized lung adenocarcinomas as low grade (lepidic-predominant), intermediate grade (acinar/papillary-predominant), and high grade (micropapillary/solid-predominant). Kaplan-Meier curves of RFS and OS showed significant differences among the low-, intermediate-, and high-grade groups (Supplementary Figure 2). However, there were no differences in RFS and OS between patients with acinar- and papillary-predominant adenocarcinoma ($p = 0.142$ and $p = 0.116$, respectively).

3.3. Prognostic factors in acinar/papillary-predominant lung adenocarcinoma

We performed univariate and multivariate Cox regression analysis to determine factors associated with RFS and OS in acinar/papillary-predominant lung adenocarcinoma (Tables 2 and 3). In the univariate analysis, the poor prognostic factors of RFS were younger age ($p = 0.041$), LVI ($p < 0.001$), STAS ($p < 0.001$), non-lepidic pattern as the second most predominant type ($p = 0.010$), presence of micropapillary pattern ($p < 0.001$), presence of solid pattern ($p = 0.003$), and higher stage ($p < 0.001$). The poor prognostic factors of OS were older age ($p = 0.023$), male sex ($p = 0.027$), smoking history ($p = 0.005$), LVI ($p < 0.001$), STAS ($p = 0.036$), presence of solid pattern ($p = 0.026$), aberrant p53 expression ($p = 0.019$), PD-L1 positivity ($p = 0.001$), and higher stage ($p < 0.001$). In multivariate analysis, in addition to higher stage, STAS and younger age were

Table 4
Clinicopathologic characteristics of acinar/papillary-predominant lung adenocarcinoma cases according to STAS and PD-L1 status.

Variables	All (n = 193)	STAS status		P-value	PD-L1 status		P-value
		Positive	Negative		Positive	Negative	
Age				0.825			0.013
≥65	98 (50.7%)	49 (49.5%)	49 (52.1%)		19 (76.0%)	79 (47.0%)	
< 65	95 (49.3%)	45 (47.9%)	50 (50.5%)		6 (24.0%)	89 (53.0%)	
Sex				0.137			0.088
Male	89 (46.1%)	49 (52.1%)	40 (40.4%)		16 (64.0%)	73 (43.5%)	
Female	104 (53.9%)	45 (47.9%)	59 (59.6%)		9 (36.0%)	95 (56.5%)	
Smoking				0.055			0.523
Yes	70 (36.2%)	41 (43.6%)	29 (29.3%)		11 (44.0%)	59 (35.1%)	
No	124 (63.8%)	53 (56.4%)	70 (70.7%)		14 (56.0%)	109 (64.9%)	
LVI				< 0.001			0.064
Yes	72 (37.3%)	48 (51.1%)	24 (24.2%)		14 (56.0%)	58 (34.5%)	
No	121 (62.7%)	11 (44.0%)	75 (75.8%)		11 (44.0%)	110 (65.5%)	
STAS							0.154
	94 (48.7%)				16 (64.0%)	78 (46.4%)	
	99 (52.3%)				9 (36.0%)	90 (53.6%)	
***EGFR* mutation**				0.005			> 0.99
Yes	113 (58.5%)	45 (47.9%)	68 (68.7%)		15 (60.0%)	98 (58.3%)	
No	80 (41.5%)	49 (52.1%)	31 (31.3%)		10 (40.0%)	70 (41.7%)	
***KRAS* mutation**				> 0.99			0.175
Yes	9 (4.6%)	4 (4.3%)	5 (5.1%)		3 (12.0%)	6 (3.6%)	
No	184 (95.4%)	90 (95.7%)	94 (94.9%)		22 (88.0%)	162 (96.4%)	
***ALK* fusion**				0.005			0.961
Yes	12 (6.2%)	11 (11.7%)	1 (1.0%)		1 (4.0%)	11 (6.5%)	
No	181 (93.8%)	83 (88.3%)	98 (99.0%)		24 (96.0%)	157 (93.5%)	
***ROS1* fusion**				0.953			0.729
Yes	5 (2.5%)	3 (3.2%)	2 (2.0%)		0 (0.0%)	5 (3.0%)	
No	188 (97.5%)	91 (96.8%)	97 (98.0%)		25 (100.0%)	163 (97.0%)	
PD-L1 positivity				0.154			
Yes		16 (17.0%)	9 (9.1%)				
No		78 (83.0%)	90 (90.9%)				
Aberrant p53 expression				0.919			0.007
Yes	53 (27.4%)	25 (26.6%)	28 (28.3%)		13 (52.0%)	40 (23.8%)	
No	140 (72.6%)	69 (73.4%)	71 (71.7%)		12 (48.0%)	128 (76.2%)	
Lepidic as the second most predominant				0.001			0.022
Yes	36 (18.6%)	1 (1.1%)	35 (35.4%)		0 (0.0%)	36 (21.4%)	
No	157 (91.4%)	93 (98.9%)	64 (64.6%)		25 (100.0%)	132 (78.6%)	
Presence of micropapillary pattern				< 0.001			0.836
Yes	116 (60.1%)	74 (78.7%)	42 (42.4%)		16 (64.0%)	100 (59.5%)	
No	77 (39.9%)	20 (21.3%)	57 (57.6%)		9 (36.0%)	68 (40.5%)	
Presence of solid pattern				0.015			0.003
Yes	44 (22.8%)	29 (30.9%)	15 (15.2%)		12 (48.0%)	32 (19.0%)	
No	149 (77.2%)	65 (69.1%)	84 (84.8%)		13 (52.0%)	136 (81.0%)	
TNM stage				0.001			0.006
Stage I	114 (59.1%)	44 (46.8%)	70 (70.7%)		9 (36.0%)	105 (62.5%)	
Stage II	26 (13.4%)	13 (13.8%)	13 (13.2%)		8 (32.0%)	18 (10.7%)	
Stage III	53 (27.5%)	37 (39.4%)	16 (16.2%)		45 (26.8%)	8 (32.0%)	

PD-L1, programmed death-ligand 1; LVI, lymphovascular invasion; STAS, spread through air spaces; *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; *ROS1*, ROS proto-oncogene 1; TNM, tumor node metastasis.

independent poor prognostic factors of RFS, and PD-L1 expression and older age were independent poor prognostic factors of OS.

We also performed Cox regression analysis in the three subgroups; first group (n = 176) except for cribriform predominant adenocarcinomas, second group (n = 152) composed of acinar predominant type only, and third group (n = 41) composed of papillary predominant type only. STAS was an independent poor prognostic factor of RFS in all three groups (Supplementary Tables). PD-L1 expression was an independent poor prognostic factor of RFS in first group and of OS in first and third group (Supplementary Tables).

We additionally compared the prognosis by classifying the first subgroup (n = 176) into three categories according to the second most predominant pattern. There was a difference in RFS between the three categories (p = 0.013). The lepidic pattern had a good prognosis when compared with the acinar/papillary pattern (p = 0.047) and the micropapillary/solid pattern (p = 0.002). In the comparison between acinar/papillary and micropapillary/solid as the second most predominant pattern, the acinar/papillary group had a better prognosis, but it was not statistically significant (p = 0.091).

3.4. Clinicopathologic parameters associated with STAS and PD-L1 status in acinar/papillary-predominant lung adenocarcinoma

We further categorized acinar/papillary-predominant lung adenocarcinomas according to STAS and PD-L1 status (Table 4 and Fig. 1). STAS was observed in 94 of 193 cases (48.7%). STAS was frequently associated with the presence of micropapillary (p < 0.001) or solid pattern (p = 0.015), absence of *EGFR* mutation (p = 0.005), and presence of *ALK* fusion (p = 0.005). STAS was also significantly correlated with LVI (p < 0.001) and TNM stage (p = 0.001). However, STAS was infrequently associated with lepidic pattern as the second most predominant pattern (p < 0.001).

PD-L1 positivity was observed in 41 of 193 cases (21.2%). PD-L1 positivity was significantly associated with aberrant p53 expression (p = 0.007) and presence of solid pattern (p = 0.003). Old age (p = 0.013) and TNM stage (p = 0.006) were also significantly related to PD-L1 positivity. Conversely, presence of lepidic pattern as the second most predominant pattern was infrequently observed with PD-L1 positivity (p = 0.022).

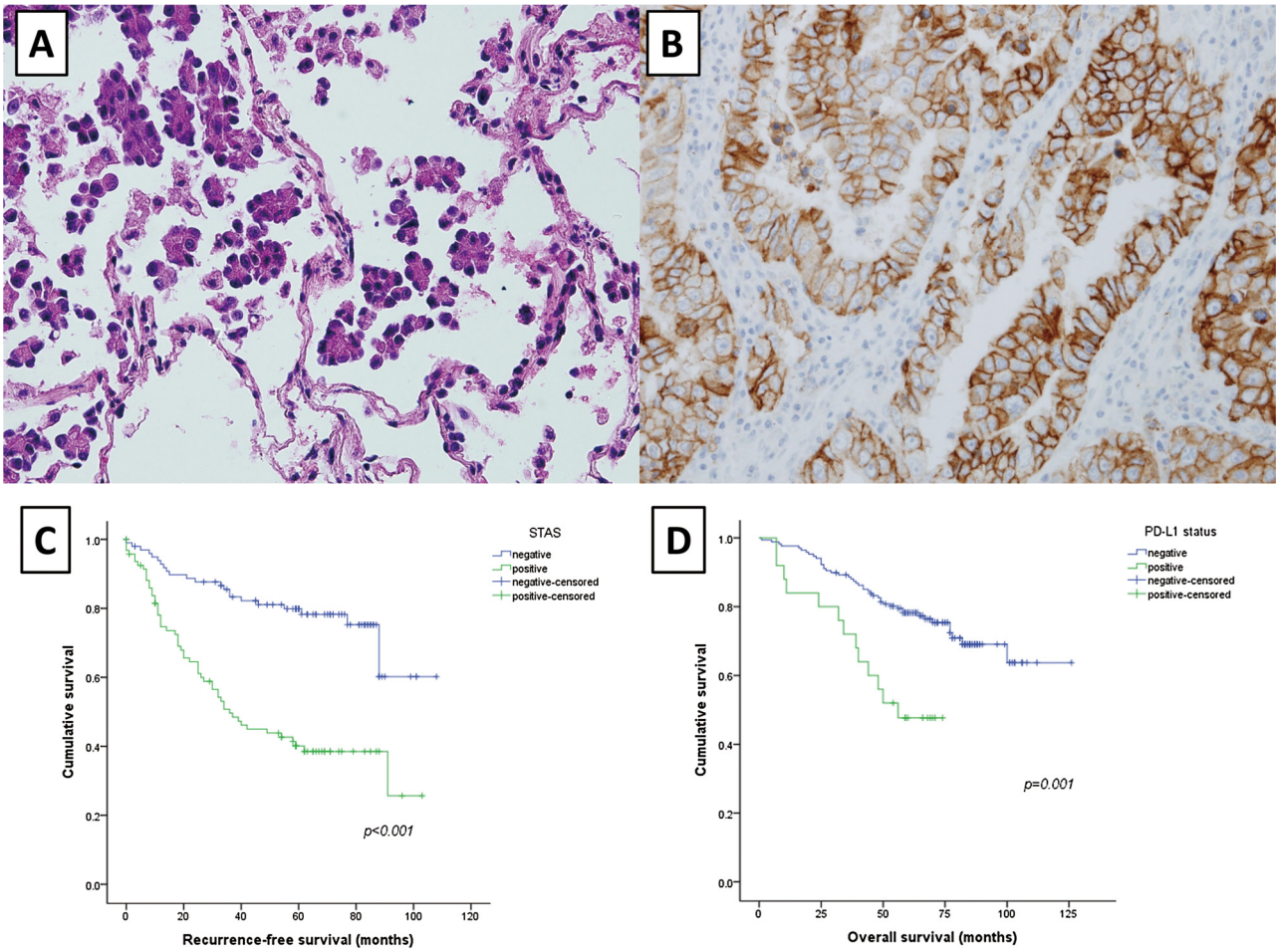

Fig. 1. Prognostic significance of spread through air spaces and programmed death-ligand 1 (PD-L1) expression. A: a representative photograph showing spread through air spaces (original magnification ×200), B: a representative photograph showing PD-L1 expression in tumor cells (original magnification ×200), C: Recurrence-free survival curve according to spread through air spaces ($p < 0.001$), D: Overall survival curve according to PD-L1 expression ($p = 0.001$).

4. Discussion

In this study, we categorized lung adenocarcinomas according to the predominant subtype and demonstrated that STAS and PD-L1 expression can be used to stratify prognosis in acinar/papillary adenocarcinoma, which is the most common and heterogeneous group.

First, we demonstrated that lung adenocarcinoma subtypes show distinct clinicopathologic characteristics. In our cohort, male sex, smoking history, and higher TNM stage were associated with high-grade lung adenocarcinoma (micropapillary and solid predominant type). Aberrant p53 expression, PD-L1 positivity, STAS, and LVI were also frequently observed in high-grade lung adenocarcinoma. In contrast, *EGFR* mutation was associated with the lepidic-predominant subtype and infrequently found in the solid-predominant subtype. These clinicopathologic findings are consistent with those of previous studies [2,9,19–21]. Therefore, our cohort reflects the general characteristics of lung adenocarcinomas subtypes.

Although acinar/papillary-predominant lung adenocarcinoma accounts for more than half of lung adenocarcinoma cases, there have been few studies regarding prognostically relevant stratification of these subtypes. Ito et al subclassified acinar/papillary lung adenocarcinomas into acinar/papillary-lepidic type and acinar/papillary-non-lepidic type according to whether the second most predominant component was a lepidic or invasive component [11]. They demonstrated that non-lepidic type as the second most predominant pattern was related to worse prognosis. Tsubokawa et al classified acinar/papillary-predominant lung adenocarcinoma based on vascular invasion and revealed a clear division into favorable and unfavorable prognostic subgroups [10]. However, there have been no previous studies including STAS and PD-L1 status in the risk stratification of intermediate-grade lung adenocarcinoma. In this study, although higher stage was the most important factor for shorter RFS and OS in multivariate analysis, we additionally found that STAS was an independent prognostic factor of RFS, and PD-L1 was an independent prognostic factor of OS.

To confirm the prognostic significance of STAS and PD-L1 expression in acinar/papillary-related cohort, we subcategorized our acinar/papillary group into three subgroups; first group except for cribriform predominant adenocarcinomas, second group composed of acinar predominant type only, and third group composed of papillary predominant type only. In this further analysis, we excluded cribriform predominant type from our cohort because cribriform pattern was recognized as high grade morphology associated with poor survival [12,22,23]. We confirmed that these factors still had a poor prognostic impact in the acinar/papillary subgroups.

STAS is a unique invasion pattern of lung cancer defined as spread of lung cancer cells into air spaces in the lung parenchyma adjacent to the main tumor [13,24,25]. Our previous study reported that STAS was significantly related to LVI, lymph node metastasis, higher stage, and high-grade histologic subtypes [26]. In addition to those findings, in this study, we further found that STAS can be used as a prognostic factor to stratify acinar/papillary-predominant lung adenocarcinoma. STAS is well known for its association with post-surgical recurrence, especially in patients who undergo sublobar resection [27]. In addition, it has been reported that STAS is associated with worse RFS and OS in

patients who undergo lobectomy [19]. In this study, we demonstrated the association of STAS with poor RFS in acinar/papillary lung adenocarcinoma. In our cohort, STAS-positive acinar/papillary-predominant lung adenocarcinomas were associated with frequent LVI and presence of micropapillary or solid pattern. Additionally, wild-type *EGFR* and *ALK* rearrangements were frequently observed in STAS-positive cases, and this molecular finding is consistent with that in our previous study [26].

Programmed death 1 (PD-1), an immune checkpoint regulator expressed on immune cells, is a receptor for PD-L1. The PD-1/PD-L1 pathway is involved in evasion of the anti-tumor immune response [28,29]. Previous studies have suggested an association between PD-L1 expression and worse prognosis in non-small cell lung cancers [20,30,31]. Song et al suggested an association between the presence of driver mutations and PD-L1 status [32]. However, Zhang et al conversely demonstrated that increased PD-L1 expression was correlated with wild-type *EGFR* [33], which is consistent with our findings. When subdividing acinar/papillary-predominant lung adenocarcinoma according to PD-L1 status, PD-L1 positivity was related with worse prognostic factors, such as aberrant p53 expression, presence of solid pattern, and higher stage [34–36].

In this study, we examined p53 status using IHC as surrogate marker, considering expression percentage [16,17]. Other studies included the intensity of expression in the assessment of p53 expression to correlate with TP53 mutations [37,38]. In our previous study, we demonstrated that PD-L1 positivity was more significantly observed in tumors with p53 aberrant expression [31]. This may indicate that PD-L1 might be more expressed in TP53 mutated lung adenocarcinomas and this relationship can be involved in poor prognosis.

There are some limitations to our study. First, this study was limited by its retrospective design. Second, although we removed the cribriform predominant type in further analysis, high-grade patterns may still remain in our intermediated group. In addition to the cribriform pattern, what is considered a high-grade pattern is a fused gland pattern and a filigree pattern [23,39]. On fused gland pattern, there have not been many reports on the diagnostic criteria and prognostic significance compared with the cribriform pattern [23]. On the filigree pattern, it has recently been described [39]. The clinical and pathological significances of these patterns may require further study. Third, we evaluated PD-L1 expression using TMAs. These tumor samples may be inadequate or not representative of the entire tumor mass. It is noteworthy that our cohort was composed of East Asians, had a high frequency of never smokers, and thus had a high frequency of *EGFR* mutations, unlike studies performed in Caucasian patients.

In conclusion, STAS status and PD-L1 expression were associated with poor prognosis in acinar/papillary-predominant adenocarcinoma of the lung. Pathological evaluation of STAS or PD-L1 expression may be useful to stratify acinar/papillary lung adenocarcinomas in terms of prognosis.

Funding

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2018R1D1A1B07047811).

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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.2019.09.026.

References

[1] W.D. Travis, E. Brambilla, A.G. Nicholson, Y. Yatabe, J.H.M. Austin, M.B. Beasley, et al., The 2015 World Health Organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification, J. Thorac. Oncol. 10 (2015) 1243–1260.
[2] M.S. Tsao, S. Marguet, G. Le Teuff, S. Lantuejoul, F.A. Shepherd, L. Seymour, et al., Subtype classification of lung adenocarcinoma predicts benefit from adjuvant chemotherapy in patients undergoing complete resection, J. Clin. Oncol. 33 (2015) 3439–3446.
[3] N. Yanagawa, S. Shiono, M. Abiko, S.Y. Ogata, T. Sato, G. Tamura, New IASLC/ATS/ERS classification and invasive tumor size are predictive of disease recurrence in stage I lung adenocarcinoma, J. Thorac. Oncol. 8 (2013) 612–618.
[4] W.D. Travis, E. Brambilla, M. Noguchi, A.G. Nicholson, K.R. Geisinger, Y. Yatabe, et al., International association for the study of lung cancer/American thoracic society/European respiratory society international multidisciplinary classification of lung adenocarcinoma, J. Thorac. Oncol. 6 (2011) 244–285.
[5] A. Mansuet-Lupo, A. Bobbio, H. Blons, E. Becht, H. Ouakrim, A. Didelot, et al., The new histologic classification of lung primary adenocarcinoma subtypes is a reliable prognostic marker and identifies tumors with different mutation status: the experience of a French cohort, Chest 146 (2014) 633–643.
[6] A. Yoshizawa, N. Motoi, G.J. Riely, C.S. Sima, W.L. Gerald, M.G. Kris, et al., Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases, Mod. Pathol. 24 (2011) 653–664.
[7] A. Yoshizawa, S. Sumiyoshi, M. Sonobe, M. Kobayashi, M. Fujimoto, F. Kawakami, et al., Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: analysis of 440 Japanese patients, J. Thorac. Oncol. 8 (2013) 52–61.
[8] M. Ma, Y. She, Y. Ren, C. Dai, L. Zhang, H. Xie, et al., Micropapillary or solid pattern predicts recurrence free survival benefit from adjuvant chemotherapy in patients with stage IB lung adenocarcinoma, J. Thorac. Dis. 10 (2018) 5384–5393.
[9] G. Sica, A. Yoshizawa, C.S. Sima, C.G. Azzoli, R.J. Downey, V.W. Rusch, et al., A grading system of lung adenocarcinomas based on histologic pattern is predictive of disease recurrence in stage I tumors, Am. J. Surg. Pathol. 34 (2010) 1155–1162.
[10] N. Tsubokawa, T. Mimae, Y. Miyata, S. Sasada, T. Yoshiya, K. Kushitani, et al., Prognostic significance of vascular invasion in intermediate-grade subtype of lung adenocarcinoma, Jpn. J. Clin. Oncol. (2016).
[11] M. Ito, Y. Miyata, T. Yoshiya, Y. Tsutani, T. Mimura, S. Murakami, et al., Second predominant subtype predicts outcomes of intermediate-malignant invasive lung adenocarcinomadagger, Eur. J. Cardiothorac. Surg. 51 (2017) 218–222.
[12] K. Kadota, Y.C. Yeh, C.S. Sima, V.W. Rusch, A.L. Moreira, P.S. Adusumilli, et al., The cribriform pattern identifies a subset of acinar predominant tumors with poor prognosis in patients with stage I lung adenocarcinoma: a conceptual proposal to classify cribriform predominant tumors as a distinct histologic subtype, Mod. Pathol. 27 (2014) 690–700.
[13] K. Kadota, J. Nitadori, C.S. Sima, H. Ujiie, N.P. Rizk, D.R. Jones, et al., Tumor spread through air spaces is an important pattern of invasion and impacts the frequency and location of recurrences after limited resection for small stage I lung adenocarcinomas, J. Thorac. Oncol. 10 (2015) 806–814.
[14] A. Calles, X. Liao, L.M. Sholl, S.J. Rodig, G.J. Freeman, M. Butaney, et al., Expression of PD-1 and its ligands, PD-L1 and PD-L2, in smokers and never smokers with KRAS-mutant lung cancer, J. Thorac. Oncol. 10 (2015) 1726–1735.
[15] A. D'Incecco, M. Andreozzi, V. Ludovini, E. Rossi, A. Capodanno, L. Landi, et al., PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients, Br. J. Cancer 112 (2015) 95–102.
[16] A. Yemelyanova, R. Vang, M. Kshirsagar, D. Lu, M.A. Marks, M. Shih Ie, et al., Immunohistochemical staining patterns of p53 can serve as a surrogate marker for TP53 mutations in ovarian carcinoma: an immunohistochemical and nucleotide sequencing analysis, Mod. Pathol. 24 (2011) 1248–1253.
[17] S.H. Lee, H. Kim, W.Y. Kim, H.S. Han, S.D. Lim, W.S. Kim, et al., Genetic alteration and immunohistochemical staining patterns of ovarian high-grade serous adenocarcinoma with special emphasis on p53 immnnostaining pattern, Pathol. Int. 63 (2013) 252–259.
[18] Y.J. Cha, J.S. Lee, H.R. Kim, S.M. Lim, B.C. Cho, C.Y. Lee, et al., Screening of ROS1 rearrangements in lung adenocarcinoma by immunohistochemistry and comparison with ALK rearrangements, PLoS One 9 (2014) e103333.
[19] S. Shiono, N. Yanagawa, Spread through air spaces is a predictive factor of recurrence and a prognostic factor in stage I lung adenocarcinoma, Interact. Cardiovasc. Thorac. Surg. 23 (2016) 567–572.
[20] J. Ma, D. Chi, Y. Wang, Y. Yan, S. Zhao, H. Liu, et al., Prognostic value of PD-L1 expression in resected lung adenocarcinoma and potential molecular mechanisms, J. Cancer 9 (2018) 3489–3499.
[21] T. Bian, D. Jiang, J. Feng, J. Liu, L. Qian, Q. Zhang, et al., Lepidic component at tumor margin: an independent prognostic factor in invasive lung adenocarcinoma, Hum. Pathol. 83 (2019) 106–114.
[22] K. Kadota, Y. Kushida, S. Kagawa, R. Ishikawa, E. Ibuki, K. Inoue, et al., Cribriform subtype is an independent predictor of recurrence and survival after adjustment for the eighth edition of TNM staging system in patients with resected lung adenocarcinoma, J. Thorac. Oncol. 14 (2019) 245–254.
[23] A.L. Moreira, P. Joubert, R.J. Downey, N. Rekhtman, Cribriform and fused glands are patterns of high-grade pulmonary adenocarcinoma, Hum. Pathol. 45 (2014) 213–220.
[24] A. Warth, T. Muley, C.A. Kossakowski, B. Goeppert, P. Schirmacher, H. Dienemann, et al., Prognostic impact of intra-alveolar tumor spread in pulmonary

adenocarcinoma, Am. J. Surg. Pathol. 39 (2015) 793–801.
[25] K. Kadota, Nitadori J-i, C.S. Sima, H. Ujiie, N.P. Rizk, D.R. Jones, et al., Tumor spread through air spaces is an important pattern of invasion and impacts the frequency and location of recurrences after limited resection for small stage I lung adenocarcinomas, J. Thorac. Oncol. 10 (2015) 806–814.
[26] J.S. Lee, E.K. Kim, M. Kim, H.S. Shim, Genetic and clinicopathologic characteristics of lung adenocarcinoma with tumor spread through air spaces, Lung Cancer 123 (2018) 121–126.
[27] K. Masai, H. Sakurai, A. Sukeda, S. Suzuki, K. Asakura, K. Nakagawa, et al., Prognostic impact of margin distance and tumor spread through air spaces in limited resection for primary lung cancer, J. Thorac. Oncol. 12 (2017) 1788–1797.
[28] W. Zou, J.D. Wolchok, L. Chen, PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations, Sci. Transl. Med. 8 (2016) 328rv4.
[29] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, Nat. Rev. Cancer 12 (2012) 252–264.
[30] C.Y. Mu, J.A. Huang, Y. Chen, C. Chen, X.G. Zhang, High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation, Med. Oncol. 28 (2011) 682–688.
[31] Y.J. Cha, H.R. Kim, C.Y. Lee, B.C. Cho, H.S. Shim, Clinicopathological and prognostic significance of programmed cell death ligand-1 expression in lung adenocarcinoma and its relationship with p53 status, Lung Cancer 97 (2016) 73–80.
[32] Z. Song, X. Yu, G. Cheng, Y. Zhang, Programmed death-ligand 1 expression associated with molecular characteristics in surgically resected lung adenocarcinoma, J. Transl. Med. 14 (2016) 188.
[33] M. Zhang, G. Li, Y. Wang, Y. Wang, S. Zhao, P. Haihong, et al., PD-L1 expression in lung cancer and its correlation with driver mutations: a meta-analysis, Sci. Rep. 7 (2017) 10255.
[34] J. Gu, Y. Zhou, L. Huang, W. Ou, J. Wu, S. Li, et al., TP53 mutation is associated with a poor clinical outcome for non-small cell lung cancer: evidence from a meta-analysis, Mol. Clin. Oncol. 5 (2016) 705–713.
[35] R. Rami-Porta, W.E.E. Eberhardt, Clinical implications of the innovations in the primary tumour and metastasis of the 8(th) edition of the TNM classification for lung cancer, J. Thorac. Dis. 10 (2018) S2682–S2685.
[36] A.C. Borczuk, Prognostic considerations of the new World Health Organization classification of lung adenocarcinoma, Eur. Respir. Rev. 25 (2016) 364–371.
[37] J. Alsner, V. Jensen, M. Kyndi, B.V. Offersen, P. Vu, A.L. Borresen-Dale, et al., A comparison between p53 accumulation determined by immunohistochemistry and TP53 mutations as prognostic variables in tumours from breast cancer patients, Acta Oncol. 47 (2008) 600–607.
[38] K.L. McGraw, J. Nguyen, R.S. Komrokji, D. Sallman, N.H. Al Ali, E. Padron, et al., Immunohistochemical pattern of p53 is a measure of TP53 mutation burden and adverse clinical outcome in myelodysplastic syndromes and secondary acute myeloid leukemia, Haematologica 101 (2016) e320–3.
[39] K. Emoto, T. Eguchi, K.S. Tan, Y. Takahashi, R.G. Aly, N. Rekhtman, et al., Expansion of the concept of micropapillary adenocarcinoma to include a newly recognized filigree pattern as well as the classical pattern based on 1468 stage I lung adenocarcinomas, J. Thorac. Oncol. (2019) [Epub ahead of print].