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ORIGINAL RESEARCH

Clinicopathological and Prognostic Significance of *EML4-ALK* Rearrangement in Patients with Surgically Resected Lung Adenocarcinoma: A Propensity Score Matching Study

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Objective: The echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (*EML4-ALK*) fusion gene is a key oncogenic driver in non-small cell lung cancer (NSCLC). This study analyzed the clinicopathological characteristics and prognostic significance of *EML4-ALK* fusion gene in patients with surgically resected adenocarcinoma.

Methods: The clinicopathological characteristics of 1056 consecutive patients with surgically resected stage I–IIIA adenocarcinoma were collected from February 2014 to October 2014, and *EML4-ALK* rearrangement was detected using real-time polymerase chain reaction (RT-PCR) technology. To compare the imaging and pathological features, a propensity score matching (PSM) method was performed. The follow-up information was collected to evaluate the long-term outcomes of patients with *EML4-ALK* rearrangement.

Results: The prevalence of *EML4-ALK* rearrangement was 6.6% in 1056 consecutive patients. A total of 70 *EML4-ALK*-positive and 210 *EML4-ALK*-negative patients were identified after PSM. Imaging and pathological analyses showed that *EML4-ALK* rearrangement was significantly associated with less ground-glass opacity (GGO) (adjusted OR=1.38, 95% CI=1.03–1.85, P_{trend}=0.029) and higher prevalence of non-invasive mucinous adenocarcinoma mucin-laden adenocarcinomas (non-IMA MLA, adjusted OR=6.79, 95% CI=2.69–17.17, P<0.001). *EML4-ALK* rearrangement was found to be an unfavorable prognostic factor for disease-free survival (DFS) in female patients (HR=2.26, 95% CI=1.13–4.53, P=0.021).

Conclusion: Our results suggest that adenocarcinomas harboring *EML4-ALK* fusion gene exhibit specific radiological and pathological characteristics compared with *EML4-ALK*-negative adenocarcinomas. In female patients, *EML4-ALK* rearrangement was associated with shorter DFS.

Keywords: adenocarcinoma, non-small cell lung cancer, NSCLC, anaplastic lymphoma kinase, survival

Introduction

Tyrosine kinase inhibitors (TKIs) are a class of pharmaceutical drugs that show beneficial effects in non-small cell lung cancer (NSCLC) patients harboring specific molecular alterations with less toxicity compared to chemotherapy and radiotherapy. They function as anticancer agents by targeting oncogene-driven signaling pathways and thus improving patients' survival and quality of life.[1,2] The

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investigations on TKIs contribute to the development of therapeutic interventions for NSCLC patients.

The translocation and inversion of anaplastic lymphoma kinase (*ALK*) have been detected in a subset of NSCLC patients, especially the ones diagnosed with adenocarcinoma. Echinoderm microtubule-associated protein-like 4 (*EML4*) is the most common fusion partner for *ALK* in NSCLC.[3,7] As the first TKI approved for *ALK*-positive NSCLC, Crizotinib treatment improved the survival of patients at advanced stages compared to standard chemotherapy.[4] Therefore, *ALK* rearrangement detection is recommended for lung adenocarcinoma patients according to the guideline published by the International Association for the Study of Lung Cancer (IASLC).[5]

Patients harboring *ALK* rearrangement share certain clinicopathological features.[6] *ALK* rearrangement is associated with younger age, never or light smoker, solid nodules, adenocarcinoma and excessive mucin production.[8–10] However, the prognosis of patients with *ALK*-positive surgically resected adenocarcinoma in the absence of TKIs treatment remains controversial. Previous data suggested that *ALK* positivity was an unfavorable prognostic factor for disease-free survival (DFS).[11–13] However, other studies failed to prove that *ALK* rearrangement was associated with the prognosis of patients with surgically resected NSCLC.[14–17] The conflicting data on the prognostic significance of *ALK* positivity may be due to baseline difference. Some clinical characteristics, such as gender, smoking status and age might be the confounders in evaluating the prognostic significance of *ALK* rearrangement in surgically resected NSCLC.

In the current study, we assessed the prognostic significance of *EML4-ALK* rearrangement in patients underwent curative resection for lung adenocarcinoma. To minimize selection bias and reduce baseline differences, a propensity score matching (PSM) analysis was performed to compare the computed tomography (CT) features, pathological characteristics and DFS between *EML4-ALK*-positive and *EML4-ALK*-negative patients.

Materials and Methods

Patients and Follow-Up

A total of 1056 patients who were diagnosed with stage I-IIIA lung adenocarcinoma and underwent curative resection were included from February 2014 to October 2014 in Shanghai Pulmonary Hospital. They were screened for *EML4-ALK* fusion gene using real-time polymerase chain reaction (RT-PCR) (Figure 1). This retrospective study was approved by the Review Board of Shanghai Pulmonary Hospital and performed in accordance with the Declaration of Helsinki. The written informed consent was obtained from all patients before surgery. Patients who received preoperative chemotherapy, radiation therapy or TKI targeted therapy were excluded. None of the 1056 patients received ALK-targeted TKIs before disease recurrence. The medical records including age, gender, smoking history, tumor size and

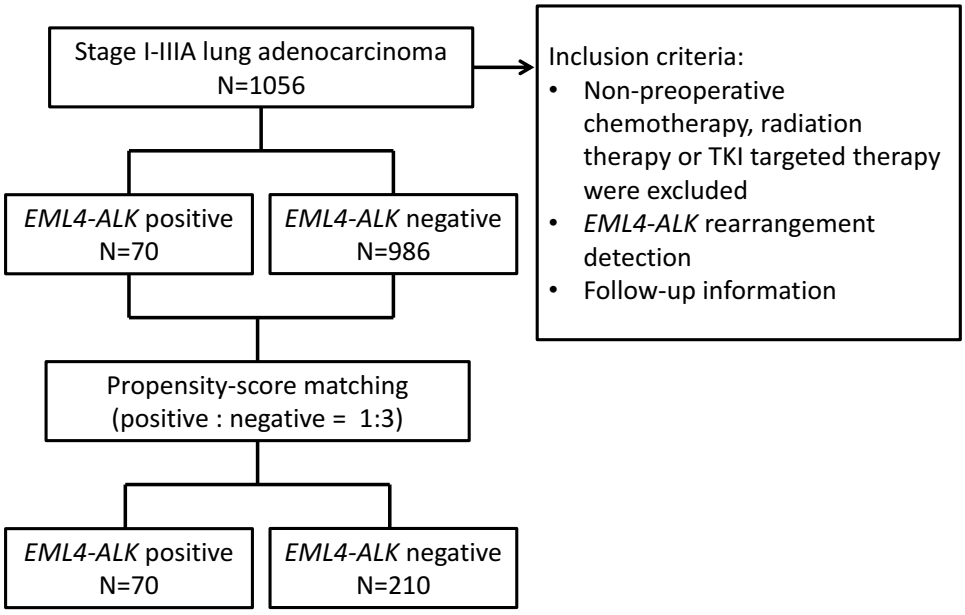

Figure 1 Flow diagram for this study.
Abbreviations: EML4, echinoderm microtubule-associated protein-like 4; ALK, anaplastic lymphoma kinase; TKI, tyrosine kinase inhibitors.

location, pathological Tumor-Node-Metastasis (pTNM) stage, histological type, preoperative carcino-embryonic antigen (CEA) level and postoperative therapy were collected from all patients. Pathologic staging was determined according to the 8th edition of TNM classification.

All patients received regular follow-up, mainly at our outpatient clinic or by telephone. The examination results were recorded in the health system. The radiological images of patients who visited our outpatient department for postoperative follow-up were reviewed by two radiologists and one physician. The disease-free survival (DFS) was defined as the time from the date of operation to the day of tumor recurrence or metastasis.

CT Examinations, Pathologic Diagnosis and *EML4-ALK* Fusion Gene Detection

All patients underwent preoperative chest CT in our hospital one month before surgery using SIEMENS Somatom Definition AS (Siemens Medical Systems, Erlangen, Germany). CT images were acquired at tube voltage of 120kVp, tube current of 300mA, section width of 2.0mm, reconstruction interval of 1.0mm, slice acquisition of 128×0.6 mm and rotation time of 0.5 s. Two radiologists reviewed the images and the following characteristics were recorded: estimated greatest diameter, percentage of ground-glass opacity (GGO) component, border (smooth or lobulated), speculation, cavity, bubble-like lucency, and air bronchogram. The percentage of GGO component was calculated as: (the greatest diameter of the tumor-the greatest diameter of the solid component)/(the greatest diameter for the tumor) ×100%.

Formalin-fixed and paraffin-embedded tissue sections (FFPE) were examined by two pathologists. The subtypes of adenocarcinoma were determined according to the International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS)/European Respiratory Society (ERS) International Multidisciplinary Classification of Lung Adenocarcinoma.[18]

To detect *EML4-ALK* rearrangement, total RNA was extracted from fresh tissues using QIAamp RNeasy Kit (Qiagen, Germany). *EML4-ALK* rearrangement was detected using a commercially available kit (ACCB Diagnostics, Beijing, China) according to the manufacturers' protocol. There are nine known *EML4-ALK* fusion transcript variants could be detected, including: E13;A20, E20;A20, E6a;A20, E6b;A20, E14ins11;del49A20, E2;A20, E2;ins117A20, E13;ins69A20, E14;del12A20 ("E": *EML4* and "A": *ALK*).

Statistical Analysis and Propensity Score Matching (PSM)

Pearson's Chi-square test ($\chi 2$ test) or the Fisher exact test was used to compare the differences in categorical variables. The differences between the means of continuous variables were compared using independent *t*-test. Univariate logistic regression was performed to investigate the association between the imaging and pathological features and *EML4-ALK* rearrangement. Multivariate logistic regression was used to determine the independent variables that could distinguish *EML4-ALK*-positive adenocarcinomas from the negative ones.

Kaplan–Meier curves were generated to compare the survival outcomes, and log-rank test was used to compare the survival differences between *EML4-ALK* -positive *and* *EML4-ALK*-negative groups. Cox regression model was performed to determine the independent prognostic factors after complete tumor resection. A *P*-value of less than 0.05 was considered statistically significant. All statistical analyses were processed by SPSS version 17.0 (SPSS Inc., Chicago, IL).

To minimize the selection bias, PSM method was used to balance the confounding factors with a 1:3 matching ratio between *EML4-ALK*-positive and negative groups, using a logistic regression model.

Results

Clinical Characteristics of Patients Harboring *EML4-ALK* Fusion Gene

The baseline characteristics of patients with surgically resected lung adenocarcinoma (n = 1056) are retrospectively analyzed in Table 1. Results showed that 6.6% (70/1056) of these patients harbored *EML4-ALK* fusion gene. The mean age of *EML4-ALK*-positive patients was younger than that of *EML4-ALK*-negative patients (mean±SD, 59.94±9.30 vs 55.96 ±11.35, *P*=0.005). Significant differences were observed in the distribution of pathological T-stage and the level of preoperative CEA between the two groups (*P*=0.047 and *P*=0.006, respectively), whereas no significant differences were found in gender, smoking history, tumor size, pathological N-stage, tumor location, and postoperative chemotherapy (*P*>0.05). PSM was used to reduce the selection bias in this study. It balanced the potential confounders between the two groups, including age, gender, pTNM stage, smoking history, preoperative CEA level and postoperative chemotherapy. After matching, no significant differences were observed in age, pathological T-stage and preoperative CEA level.

Table 1 Clinicopathologic Characteristics of 1056 Consecutive Patients and 280 Propensity Score-Matched Patients Screened for *EML4-ALK* Rearrangement

Variables	Total (N=1056)	*EML4-ALK* Rearrangement (Before PSM)		*P*-value	Total (N=280)	*EML4-ALK* Rearrangement (After PSM)		*P*-value
		Negative (N=986)	Positive (N=70)			Negative (N=210)	Positive (N=70)	
Age(mean±SD, years)		59.94±9.30	55.96±11.35	0.005[a]		58.35±8.70	55.96±11.35	0.11
Gender				0.439				0.725
Male	454	427(43.3)	27(38.6)		113	86(41.0)	27(38.6)	
Female	602	559(56.7)	43(61.4)		167	124(59.0)	43(61.4)	
Smoking history				0.187				0.579
Ever	315	299(30.3)	16(22.9)		71	55(26.2)	16(22.9)	
Never	741	687(69.7)	54(77.1)		209	155(73.8)	54(77.1)	
Tumor size (mean±SD, mm)		23.95±12.35	22.61±10.32	0.378		22.87±11.98	22.61±10.32	0.875
Pathologic T-stage				0.047				0.592
T1	438	398(40.4)	40(57.1)		145	105(50.0)	40(57.1)	
T2	572	543(55.1)	29(41.4)		128	99(47.1)	29(41.4)	
T3	25	24(2.4)	1(1.4)		7	6(2.9)	1(1.4)	
T4	21	21(2.1)	0(0)		/	/	/	
Pathologic N-stage				0.694				0.785
N0	836	783(79.4)	53(75.7)		217	164(78.1)	53(75.7)	
N1	43	40(4.1)	3(4.3)		15	12(5.7)	3(4.3)	
N2	177	163(16.5)	14(20.0)		48	34(16.2)	14(20.0)	
Tumor location				0.491				0.61
Upper/middle	684	636(64.5)	48(68.6)		185	137(65.2)	48(68.6)	
Lower	372	350(35.5)	22(31.4)		95	73(34.8)	22(31.4)	
Preoperative CEA (ng/mL)	1033[b]			0.006				0.414
<5	736	676(70.2)	60(85.7)		231	171(81.4)	60(85.7)	
≥5	297	287(29.8)	10(14.3)		49	39(18.6)	10(14.3)	
Postoperative chemotherapy				0.249				0.58
Yes	538	507(51.4)	31(44.3)		132	101(48.1)	31(44.3)	
No	518	479(48.6)	39(55.7)		148	109(51.9)	39(55.7)	

Notes: [a]*P*-value for independent *t*-test. [b]A total of 1033 patients underwent blood tests for CEA in our hospital before surgery.
Abbreviations: *EML4*, echinoderm microtubule-associated protein-like 4; *ALK*, anaplastic lymphoma kinase; CEA, carcino-embryonic antigen; PSM, propensity score matching.

Baseline characteristics were well balanced between *EML4-ALK*-negative (N=210) and *EML4-ALK*-positive (N=70) patients.

Comparison of Radiological and Pathological Characteristics

The mutation status analysis is critical for the diagnosis and treatment of NSCLC. However, the detection of molecular alteration in tumor tissue using biopsy has some limitations considering tumor location or tumor size. CT images are widely used in the prediction of pathological characteristics in many diseases, and the correlation between the image patterns and gene mutations has been widely investigated. To identify the variables that might differentiate *EML4-ALK*-positive lung cancer from the negative ones, the imaging and pathological features of 280 propensity score-matched patients were collected and summarized in Table 2. The CT patterns such as nodule type, lobulated border, speculated margin, bubble-like lucency and mucin-laden adenocarcinomas (MLA) in *EML4-ALK*-positive group were significantly

Table 2 Association Analysis Between Imaging and Pathological Features of 280 Propensity Score-Matched Patients and *EML4-ALK* Rearrangement

Variables	*EML4-ALK* Rearrangement		*P*-value for χ^2 Test	Univariate Analysis			Multivariate Analysis		
	Negative (N=210)	Positive (N=70)		OR	95% CI	*P*-value	OR	95% CI	*P*-value
Nodule type			0.016						
Pure GGO	17(8.1)	2(2.9)		Ref			Ref		
≥50% of GGO	18(8.6)	3(4.3)		2.00	0.29–13.91	0.484	2.54	0.35–18.51	0.357
<50% of GGO	49(23.3)	8(11.4)		0.85	0.15–4.83	0.855	0.99	0.17–5.89	0.992
Solid	126(60.0)	57(81.4)		3.59	0.80–16.14	0.095	3.23	0.68–15.41	0.141
Trend	/	/	/	1.52	1.15–2.00	0.003	1.38	1.03–1.85	0.029
Lobulated border	74(35.2)	36(51.4)	0.016	1.95	1.13–6.37	0.02	1.50	0.83–2.72	0.181
Spiculated margin	80(38.1)	40(57.1)	0.005	2.17	1.25–3.75	0.01	1.62	0.88–2.96	0.119
Cavity	12(5.7)	5(7.1)	0.665	0.43	0.11–1.76	0.24	1.03	0.33–3.26	0.958
Bubble-like lucency	27(11.8)	3(3.9)	0.046	0.32	0.09–1.08	0.07	0.37	0.10–1.29	0.118
Air bronchogram	59(28.1)	13(18.6)	0.114	0.58	0.30–1.15	0.12	0.73	0.35–1.51	0.732
Histologic subtype			<0.001						
Non-MLA	185(88.1)	46(65.7)		Ref			Ref		
Non-IMAMLA	9(4.3)	19 (27.1)		8.49	3.61–19.99	<0.001	6.79	2.69–17.17	<0.001
IMA	16(7.6)	5(7.1)		1.26	0.44–3.61	0.67	1.03	0.35–3.10	0.953
Trend	/	/	/	1.77	1.16–2.69	0.01	1.52	0.97–2.38	0.070
Visceral pleural invasion	84(40.0)	26(37.1)	0.672	0.89	0.51–1.55	0.67	0.75	0.41–1.39	0.362

Abbreviations: *EML4*, echinoderm microtubule-associated protein-like 4; *ALK*, anaplastic lymphoma kinase; OR, odds ratio; CI, confidence interval; Ref, reference GGO, ground grass opacity; MLA, mucin-laden adenocarcinomas; IMA, invasive mucinous adenocarcinoma.

different from those in *EML4-ALK*-negative patient ($P<0.05$). The results of univariate logistic regression analysis demonstrated that all positive variables in chi-squared test were significantly associated with an increased risk of *EML4-ALK* rearrangement (OR>1.00, $P<0.05$), except bubble-like lucency. The nodule type (adjusted OR=1.38, 95% CI=1.03–1.85, $P_{trend}=0.029$) and non-invasive mucinous adenocarcinoma MLA(non-IMA MLA, adjusted OR =6.79, 95% CI =2.69–17.17, $P<0.001$ compared with non-MLA) were found to be important predictors of *EML4-ALK*-positive lung adenocarcinoma.

Prognostic Significance of *EML4-ALK* Fusion Gene in Surgically Resected Adenocarcinoma

To investigate the association between *EML4-ALK* rearrangement and survival outcomes in 280 matched patients, the analysis of DFS was performed. The median follow-up time was 49 (3–57) months. The survival curve analysis showed that there was no significant association between *EML4-ALK* rearrangement and DFS in the general population (log rank $P=0.161$) (Figure 2A). Subgroup analysis by gender showed that female patients harboring *EML4-ALK* fusion gene had a shorter DFS (log rank $P=0.022$) (Figure 2B). No significant association was found between *EML4-ALK* rearrangement and DFS in male patients (log rank $p=0.63$) (Figure 2C).

The cox regression analysis showed that *EML4-ALK* rearrangement was a significant prognostic factor for decreased DFS in female patients with adenocarcinoma (HR=2.10, 95% CI=1.10–4.04, $P=0.026$). Other variables including tumor size, pathological T-stage, pathological N-stage, preoperative CEA level, and postoperative chemotherapy were shown to be associated with a worse DFS in all groups (HR>1.00, $P<0.05$; Table 3). Multivariate analysis suggested that lymphatic metastasis was an unfavorable prognostic factor for DFS in all groups after adjusted for confounders (adjusted HR>1.00, $P_{trend}<0.05$). *EML4-ALK* rearrangement remained to be an independent prognostic factor for a shortened DFS in the female population (adjusted HR=2.26, 95% CI=1.13–4.53, $P=0.021$; Table 4).

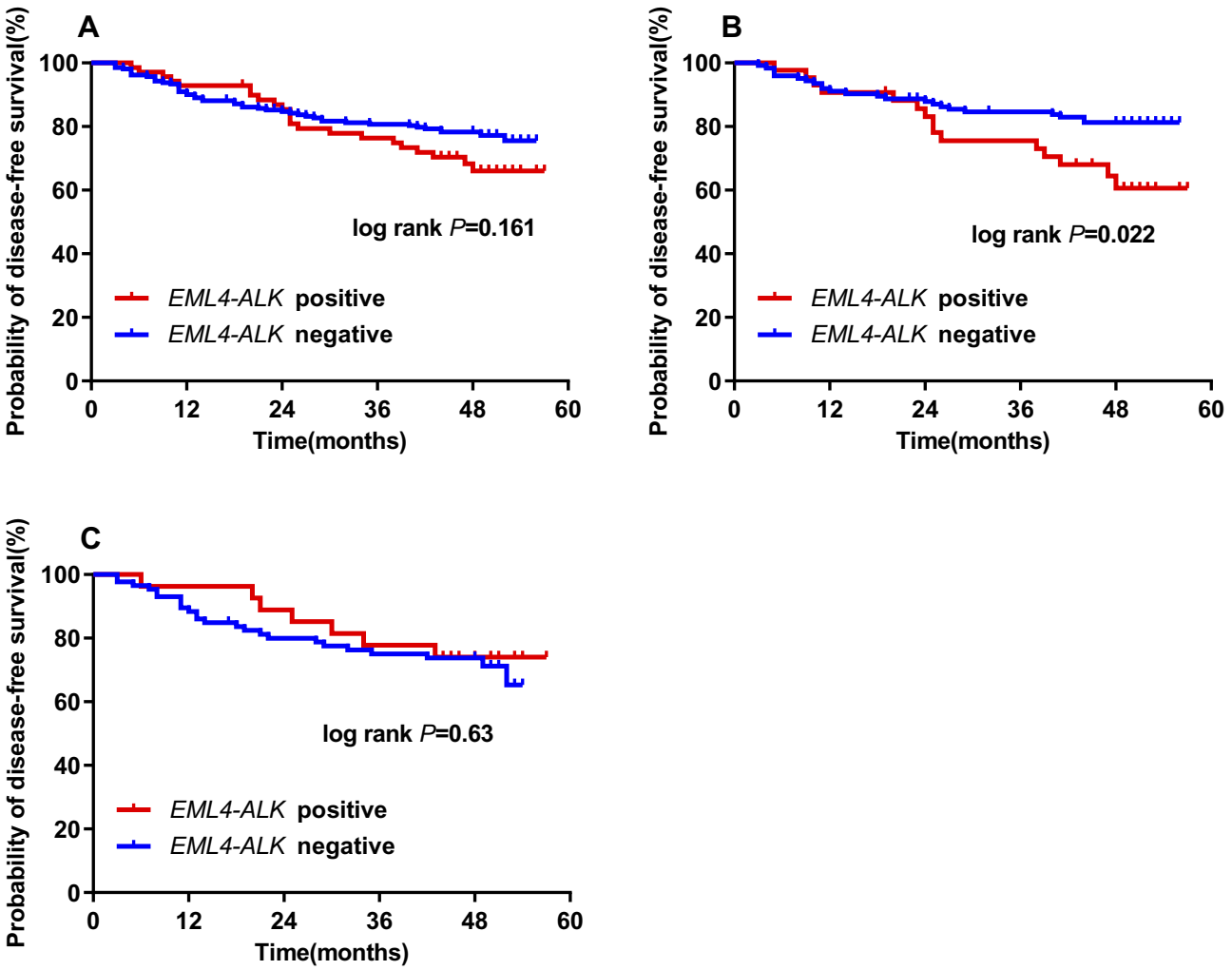

Figure 2 Disease-free survival curve analyses for surgically resected adenocarcinoma after propensity score matching, stratified by detection for *EML4-ALK* fusion gene.
Notes: (**A**) DFS curves in all patients. (**B**) DFS curves in female patients. (**C**) DFS curves in male patients
Abbreviations: EML4, echinoderm microtubule-associated protein-like 4; ALK, anaplastic lymphoma kinase.

Discussion

The identification of *EML4-ALK* rearrangement, an important biomarker in NSCLC, is highly recommended for patients with lung adenocarcinoma.[5] The imaging characteristics of tumors are shown to have significant clinical values in pathological and survival analyses. In this retrospective study, we reported that surgically resected *EML4-ALK*-positive adenocarcinomas had a significantly higher proportion of solid component on CT images and were more likely to produce excessive mucin. Our data also showed that *EML4-ALK* rearrangement was significantly associated with decreased DFS in female patients.

The prevalence of *EML4-ALK* fusion genes in adenocarcinomas ranges from 3.4% to 10.2% when using the RT-PCR method.[19,20] Here, we showed that 70 (6.6%) of 1056 patients with completely resected adenocarcinoma harbored *EML4-ALK* fusion gene, which was consistent with previously reported mutation rate (6.1%) in Asian ethnicity groups.[31] Patients harboring *ALK* rearrangement appear to be younger and have a light or never smoking history.[21] Consistently, our patients with *EML4-ALK* fusion gene were relatively younger compared to *EML4-ALK*-negative patients before PSM. In non-smokers, the incidence of *EML4-ALK* rearrangement was higher than *EML4-ALK*-negative adenocarcinomas (22.9% vs 30.3%), but not statistically significant. The negative association might be resulted from the study population and adenocarcinoma subtypes.

The use of CT image features to predicate the epidermal growth factor receptor (*EGFR*) mutations in NSCLC has been previously studied. Most studies showed that nodules with higher GGO proportion were more likely to harbor *EGFR*

Table 3 Univariate Analysis of Prognostic Factors for Disease-Free Survival in Propensity Score-Matched Patients Screened for *EML4-ALK* Rearrangement

Variables	All Patients (N=280)			Female Patients (N=113)			Male Patients (N=167)		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age	1.02	0.99–1.04	0.241	1.01	0.97–1.04	0.655	1.02	0.99–1.06	0.216
Gender									
Female	Ref			/			/		
Male	1.30	0.81–2.07	0.281	/			/		
Smoking history									
Never	Ref			Ref			Ref		
Ever	1.38	0.77–2.48	0.279	3.20	0.44–23.32	0.251	1.65	0.82–3.34	0.164
Tumor size	1.05	1.03–1.06	<0.001	1.04	1.01–1.07	0.003	1.05	1.03–1.07	<0.001
Pathological T-stage									
T1	Ref			Ref			Ref		
T2	2.21	1.33–3.65	0.002	2.02	1.04–3.91	0.038	2.42	1.10–5.32	0.028
T3	6.80	2.59–17.85	<0.001	3.60	0.82–15.75	0.089	17.23	4.58–64.83	<0.001
Trend	2.40	1.58–3.65	<0.001	1.96	1.14–3.39	0.016	3.27	1.64–6.54	0.001
Pathological N-stage									
N0	Ref			Ref			Ref		
N1	2.73	1.06–7.01	0.037	3.31	0.98–11.20	0.054	2.08	0.47–9.22	0.336
N2	7.62	4.66–12.45	<0.001	7.29	3.72–14.28	<0.001	7.90	3.80–16.45	<0.001
Trend	2.76	2.16–3.53	<0.001	2.70	1.94–3.78	<0.001	2.82	1.95–4.08	<0.001
Preoperative CEA									
<5	Ref			Ref			Ref		
≥5	3.00	1.83–4.91	<0.001	2.94	1.39–6.21	0.005	3.10	1.54–6.25	0.002
Postoperative chemotherapy									
No	Ref			Ref			Ref		
Yes	3.61	2.07–6.31	<0.001	3.55	1.72–7.31	0.001	3.59	1.48–8.74	0.005
EML4-ALK rearrangement									
Negative	Ref			Ref			Ref		
Positive	1.43	0.86–2.37	0.164	2.10	1.10–4.04	0.026	0.81	0.35–1.89	0.632

Abbreviations: *EML4*, echinoderm microtubule-associated protein-like 4; *ALK*, anaplastic lymphoma kinase; CEA, carcino-embryonic antigen; HR, hazard ratio; CI, confidence interval; Ref, reference.

mutations.[22,23] However, other studies reported a negative or contrary correlation between *EGFR* mutations and CT features,[24,25] which might be explained by the difference in demographic characteristics.[26,27] A recent PSM study of a large Asian population showed that patients with lung adenocarcinomas harboring *EGFR* exon 21 missense had the most inner GGO component, followed by the ones harboring *EGFR* exon 19 deletion, when compared to patients with *EGFR* wild-type tumors.[28] However, the imaging features of *EML4-ALK*-positive NSCLC were rarely reported – due to its low incidence in lung cancer patients.[10] We further used PSM method to minimize the potential selection bias caused by demographic confounders. Tumors harboring *EML4-ALK* rearrangement were more likely to appear as a pure solid nodule on CT scans in comparison to *EML4-ALK*-negative tumors (81.4% vs 60.0%). Also, less GGO proportion was significantly associated with *EML4-ALK* rearrangement in multivariate logistic regression analysis (adjusted OR=1.38, 95% CI=1.03–1.85, P_{trend}=0.029). It has been revealed that solid component may be the result of abundant mucin production,[29] which is an important pathological feature in lung adenocarcinomas. Here, we also found that abundant mucin production was closely related to *EML4-ALK* fusion gene. Therefore, our data indicated that the radiological and pathological characteristics of tumor were closely correlated with the molecular alteration.

Table 4 Multivariate Analysis of Prognostic Factors for Disease-Free Survival in Propensity Score-Matched Patients Screened for *EML4-ALK* Rearrangement

Variables	All Patients (N=280)			Female Patients (N=113)			Male Patients (N=167)		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age	1.02	0.99–1.05	0.26	1.01	0.97–1.04	0.962	1.03	0.99–1.08	0.135
Gender									
Female	Ref			/			/		
Male	1.20	0.69–2.06	0.475	/			/		
Smoking history									
Never	Ref			Ref			Ref		
Ever	1.53	0.79–2.98	0.217	1.95	0.26–14.79	0.518	1.49	0.72–3.10	0.283
Tumor size	1.01	0.98–1.03	0.546	0.99	0.95–1.03	0.554	1.02	0.99–1.05	0.182
Pathological T-stage									
T1	Ref			Ref			Ref		
T2	1.25	0.23–6.71	0.798	1.03	0.42–2.57	0.944	1.16	0.44–3.05	0.760
T3	4.01	1.25–12.85	0.019	4.15	0.71–24.42	0.115	9.64	1.82–51.12	0.008
Trend	1.39	0.78–2.49	0.261	1.37	0.60–3.16	0.458	1.93	0.80–4.62	0.142
Pathological N-stage									
N0	Ref			Ref			Ref		
N1	2.30	0.86–6.17	0.097	2.85	0.77–10.62	0.118	1.24	0.23–6.54	0.802
N2	4.75	2.65–8.50	<0.001	5.37	2.40–12.00	<0.001	3.89	1.58–9.63	0.003
Trend	2.18	1.63–2.91	<0.001	2.31	1.55–3.45	<0.001	1.95	1.24–3.07	0.004
Preoperative CEA									
<5	Ref			Ref			Ref		
≥5	1.49	0.84–2.64	0.170	1.85	0.69–4.98	0.225	1.90	0.86–4.24	0.115
Postoperative chemotherapy									
No	Ref			Ref			Ref		
Yes	1.50	0.76–2.96	0.238	1.57	0.62–3.97	0.344	1.76	0.59–5.20	0.310
EML4-ALK rearrangement									
Negative	Ref			Ref			Ref		
Positive	1.49	0.89–2.48	0.129	2.26	1.13–4.53	0.021	0.89	0.38–2.12	0.894

Abbreviations: *EML4*, echinoderm microtubule-associated protein-like 4; *ALK*, anaplastic lymphoma kinase; CEA, carcino-embryonic antigen; HR, hazard ratio; CI, confidence interval; Ref, reference.

The detection of *ALK* rearrangement insurgically resected tumors is superior to biopsy considering the number of tested cancer cells received for molecular analyses.[30] Some studies showed that *ALK*-positive adenocarcinomas were more common in patients at advanced stages and with early lymph node metastasis.[16,31] Shin and colleagues[32] reported the incidence of more regional lymph node recurrence was higher than that of distant metastasis in patients with *ALK*-positive stage IA adenocarcinoma. In the 22 *EML4-ALK*-positive patients who developed recurrence after PSM in this study, 13 had regional recurrence, including mediastinal lymph node and pleural metastasis (not shown). These findings indicated that *ALK*-positive tumors may be more aggressive compared to *ALK*-negative lung cancer. In contrast, some previous studies failed to demonstrate the prognostic significance of *ALK* rearrangement in postoperative NSCLC patients.[13,15–17] To minimize the inherent selection bias from the confounding variables such as age, gender, smoking history, tumor stage, and postoperative chemotherapy, we performed this PSM study in a cohort of 70 *ALK*-positive patients to investigate the prognostic value of *EML4-ALK* fusion gene in surgically resected adenocarcinomas. Our results demonstrated that *EML4-ALK* rearrangement was an unfavorable prognostic factor for a decreased DFS in female patients with adenocarcinoma (HR=2.26, 95% CI=1.13–4.53, *P*=0.021)

after adjusted for other confounders, while not in the males (HR=0.89, 95% CI=0.38–2.12, *P*=0.894). Some studies reported that patients with *EML4-ALK*-positive NSCLC had a significantly worse prognosis compared with *EML4-ALK*-negative ones in non-smoking population.[11,14] Given that most female patients are non-smokers, our data supported the prognostic significance of *EML4-ALK* rearrangement in lung adenocarcinoma.

Previous evidence showed that *EGFR* mutations and *EML4-ALK* rearrangement were mutually exclusive,[33] and approximately 70% of female non-smoker Asian patients with adenocarcinoma harbored *EGFR* mutations.[34] Chaft and colleagues[36] reported that the postoperative DFS in *ALK*-positive patients was shorter compared to *EGFR*-mutant patients. A study also found that most *EML4-ALK*–negative female patients harbored *EGFR* mutations and patients with *EGFR*-positive adenocarcinomas were more likely to have a shortened postoperative DFS.[35] All these findings may explain the association between *EML4-ALK* rearrangement with unfavorable survival outcomes, and the high risk of disease recurrence in female patients with adenocarcinoma.

This study had some limitations. First, this was a retrospective study and all clinical data were collected from a single center. Further retrospective analysis including multiple centers will be needed. Second, although a large number of patients with *EML4-ALK*-positive adenocarcinomas were included, the ones with inoperable tumors harboring *EML4-ALK* fusion gene at advanced stages were excluded. Third, more radiological and pathological characteristics, such as the presence of signet-ring cell, need to be analyzed in the future.[8] Last, the fluorescence in situ hybridization and immunohistochemistry could be used to assess *ALK* rearrangement, as rare or unknown ALK fusion variants might not be detected by the RT-PCR method. Considering that this study was an observational research, and the commercially available kits used had covered common variant type of *ALK* rearrangement, our results were more practicable to be retrospectively analyzed in multiple centers.

Conclusion

In conclusion, this study suggested that *EML4-ALK* -positive adenocarcinoma was significantly associated with specific radiological and pathological features, including less GGO component and higher incidence of non-IMA MLA. Also, *EML4-ALK* rearrangement was identified as an unfavorable prognostic factor for a decreased DFS in female patients with resected adenocarcinomas.

Disclosure

The authors report no conflicts of interest in this work.

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