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ORIGINAL RESEARCH

EGFR mutations are significantly associated with visceral pleural invasion development in non-small-cell lung cancer patients

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Objectives: A retrospective study was performed to investigate the association between *EGFR* mutations and visceral pleural invasion (VPI), and evaluate the prognostic value of *EGFR* in resected non-small-cell lung cancer (NSCLC) patients with VPI.

Materials and methods: Clinicopathological characteristics and follow-up information were collected from 508 consecutive patients with surgically resected stage I–III NSCLC, and *EGFR* mutations were detected based on real-time PCR technology. Significant results ($P<0.05$) from univariate logistic regression analysis were involved as covariates to adjust confounding factors in the analysis of independent factors.

Results: VPI and *EGFR* mutations were detected in 229 (45.1%) and 243 (47.8%) cases in NSCLC, respectively. There was a significant association between *EGFR* mutations and VPI development. Both 19-del (adjusted OR =2.13, 95%CI =1.13–3.99, $P=0.019$) and L858R (adjusted OR =2.89, 95%CI =1.59–5.29, $P=0.001$) could significantly increase the risk of VPI development compared with *EGFR* wild-type. Higher frequency of L858R (adjusted OR =2.63, 95%CI =1.42–4.88, $P=0.002$) was detected in VPI patients compared with non-VPI patients. 19-del (adjusted HR =0.31, 95%CI =0.12–0.80, $P=0.015$) was an independent prognostic factor for a better disease-free survival (DFS) in non-VPI patients. No significant association was shown between *EGFR* mutations and DFS in VPI patients.

Conclusion: *EGFR* mutations were significantly associated with VPI development in NSCLC, but no significant association was observed between *EGFR* mutations and DFS in the patients with VPI. 19-del was a favorable prognostic factor for DFS in non-VPI patients.

Keywords: *EGFR* mutations, visceral pleural invasion, non-small-cell lung cancer, association study

Introduction

Lung cancer remains the most common cause of cancer-related mortality worldwide.[1] Non-small-cell lung cancer (NSCLC) accounts for ~85% of lung cancer.[2] Adenocarcinoma (ADC) and squamous cell carcinoma (SCC) are two major histological subtypes of NSCLC. Although conventional treatment strategies including surgery, chemotherapy, and radiotherapy have improved the prognosis of NSCLC patients, the side effects on life quality should not be ignored. In the past few decades, studies on signaling pathways involved in the onset and progression of NSCLC have acquired great achievements, especially the ectopic activation of *EGFR* which plays a crucial role in the tumor growth and invasiveness. About 40% of NSCLC patients presented the dysregulation of *EGFR*.[3] The somatic mutations of *EGFR* prominently locate in

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the exons 19–23, which encode the tyrosine kinase domain.[4] Approximately 70% of Asian female nonsmoker patients with ADC harbored *EGFR* mutations.[5] Exon 19 deletions (19-del) and exon 21 missense mutation (L858R) are the two predominant mutant subtypes of *EGFR* in NSCLC. Tyrosine kinase inhibitors (TKIs), which specifically target *EGFR* mutations (19-del and L858R), could improve the prognosis of NSCLC patients harboring *EGFR* mutations, and have been recommended as the first-line therapy in lung cancer patients with *EGFR* mutations.[6] Some recent studies presented that patients harboring 19-del had a better overall survival compared with those with L858R following TKIs treatment especially in advanced-stage NSCLC, while others failed to reach this conclusion.[7–10] As a result, the difference of 19-del and L858R in the prognosis of patients harboring *EGFR* mutations remains controversial.

The condition that tumors adjacent to the pleural might be an unfavorable prognostic factor was first observed by Brewer et al in 1958.[11] Compared with tumors growing in the mid-lung zone, tumors under pleural surface had an adverse influence on survival. Since that, visceral pleural invasion (VPI) has been identified as an adverse factor for the survival of patients who underwent NSCLC resection.[12] In the eighth edition of AJCC TNM classification for lung cancer, VPI was an essential factor for the T descriptors – tumors ≤3cm will be upstaged to T2 stage if they invade the visceral pleural.[13]

According to the previous researches, tumors with VPI presented more aggressive invasiveness, which may lead to the dissemination of tumor cells in the pleural cavity and mediastinal lymph node metastasis.[14] A great number of studies have focused on the prognostic value of VPI stratified by the tumor size, especially the necessity of chemotherapy for postoperative patients with VPI in early stage NSCLC.[12,15,16] Computer tomography (CT) is an important imaging method in diagnosis of NSCLC. Three types of tumor–pleural relationship could be observed on CT images including no contacting, abutting pleural, and pleural tag. Some studies suggested that tumors abutting the pleural surface can predict 77% of VPI in accuracy.[17] Pleural tag, which represents the stripes stretching from the tumor margin to the pleural surface, is formed from thickened interlobular septa. This important CT feature could be classified into three types (pleural tag type I, type II, and type III) according to Hsu et al's study. Tumors with pleural tags may be an important clue to prejudge VPI, and the positive predictive value is up to 76.2% according to different types observed on CT images.[18] Although it is commonly recognized that tumors that contact the pleural presented on CT scans have large potential of VPI, it is still difficult to diagnose VPI from CT images accurately.

Numerous researches focused on the prognostic value of VPI stratified by tumor size, while limited studies shed light on the correlation of *EGFR* mutations and VPI. In order to clarify their relationship in NSCLC, a retrospective study was conducted to investigate the association between *EGFR* mutations and VPI, and evaluate their prognostic value in NSCLC patients who underwent primary tumor resection.

Materials and methods

Patient selection and follow-up

Seven hundred eleven consecutive patients who underwent primary tumor lobectomy and *EGFR* mutation detection were included in our current study to analyze the association between *EGFR* mutations and VPI as well as their roles in the prognosis of NSCLC patients in Shanghai Pulmonary Hospital from November 2013 to May 2014. The exclusion criteria were 1) pathological stage IV diagnosed after surgery; 2) administration of preoperative chemotherapy, radiation therapy, or EGFR-TKIs; 3) patients who died of surgical complications in perioperative period. Written informed consent was obtained from all patients before surgery, and the study was approved by the Review Board of Shanghai Pulmonary Hospital. This study was conducted in accordance with the Declaration of Helsinki. Finally, 508 patients with resected NSCLC were included in this study; clinicopathologic characteristics including gender, age, smoking status, tumor size, histological type, preoperative carcinoembryonic antigen (CEA) level, pathological TNM (pTNM) stage, and postoperative therapy were collected. Pathologic staging was performed according to the eighth edition of TNM classification.

Each patient received regular follow-up mainly in outpatient department or by telephone, and latest examination reports were recorded for rechecking. Consultant doctors checked radiological images of all patients who visited our outpatient department for postoperative follow-up and compared with the former ones. The examination reports for these patients were recorded in our hospital health system. For a minority of patients living far away, they would choose to be followed in the local hospital, and we would keep in touch with them by telephone every 3 months and enquired about their examination reports then recorded. For these patients, we checked their radiological reports instead. The disease-free survival (DFS) was defined as the time from the day of operation to the day of confirmation of recurrence according to clinical and radiological findings.

CT imaging, pathologic diagnosis, and EGFR mutation analysis

All patients underwent chest CT in our hospital within 1 month before surgery. CT images were performed using CT scanner systems (SIEMENS Somatom Definition AS; Siemens Medical Systems, Erlangen, Germany) according to the parameters as follows: section width, 2.0 mm; reconstruction interval, 1.0 mm; slice acquisition, 128×0.6 mm; rotation time, 0.5 seconds; tube voltage, 120 kVp; tube current, 300 mA. CT images were assessed by two experienced radiologists using standard lung (window width, 1,600 HU; window level, −600 HU) and mediastinal (window width, 350 HU; window level, 50 HU) window settings. The relationship of tumor and pleural on CT scans was recorded for each patient. For tumors showing pleural tags on lung window, we classified them into three types as previously reported by Hsu et al.[18] Surgical specimens were reviewed by two experienced pathologists. For *EGFR* mutation analysis, genomic DNA was extracted from fresh tissues using QIAamp DNA Tissue Kit (Qiagen, Hilden, Germany). Mutations of *EGFR* were detected using commercially available kits from ACCB Diagnostics (Beijing, China). The procedure was based on amplification refractory mutation system real-time PCR technology. All experiments were performed according to the manufacturer's instructions.

Statistical analysis

The differences in distribution of categorical variables were assessed by Pearson's chi-squared test or the Fisher's exact test. Independent *t*-test was carried out to evaluate the difference of mean value for continuous variables. Univariate logistic regression was performed to investigate the association between clinicopathologic characteristics and VPI, and multivariate logistic regression was carried out to analyze the independent risk factors for VPI.

Kaplan–Meier method was adopted to generate survival curve, and log-rank test was performed to compare the differences of survival curves between patients' group. Cox regression model was used to assess the independent prognostic factors of VPI. Two-sided *P*-value <0.05 was considered statistically significant. All analyses were performed with SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Patients' characteristics

To investigate the association between clinicopathologic characteristics and *EGFR* mutations as well as VPI, 508 NSCLC patients who underwent primary tumor resection in one tertiary care hospital were included. The patients' characteristics are listed in Table 1. The median age was 61 years old (range 25–91), and 293 patients (57.7%) were >60 years when diagnosed. Male patients accounted for 52% of all cases. Sixty-one percent of patients were never smokers, and 39% of patients had a history of smoking. The mean tumor size was 27.3 mm, and most tumors were no more than 30 mm (70.1%), and only 7.1% of tumors were >50 mm. 77.6% of tumors did not contain ground-glass opacity (GGO). According to eighth edition of pathological TNM stage, 67.5% of patients were in stage I, 10.6% of patients were in stage II, and 21.9% of patients were in stage III. Twenty-two percent of patients (intrapulmonary lymph node-N1 4.7%, and mediastinal lymph node-N2 17.9%) were detected lymphatic metastasis. 80.9% of patients were diagnosed as ADC, 16.1% of patients were SCC. Based on the CT image, five different types describing tumor–pleural relationship were presented: no contacting (28.9%), abutting pleural (32.1%), pleural tag type I (13.6%), pleural tag type II (19.5%), and pleural tag type III (5.9%; Figure S1). According to pathological diagnosis, 45.1% of patients showed VPI. The results of *EGFR* mutation status showed that 19-del and L858R accounted for 19.5% and 24% of all the subtypes, respectively. 55.3% of patients received postoperative adjuvant therapy. The median follow-up time was 50 (range 3–57) months. The median DFS time was 49 (range 2–57) months.

Association between VPI and clinicopathologic characteristics

In order to investigate the potential correlation between VPI and clinicopathologic characteristics, the following data including age, gender, smoking status, tumor size, pTNM stage, histological type, tumor location, tumor–pleural relationship, lymphatic metastasis and preoperative CEA were collected to uncover the association between VPI and clinical factors. The results are listed in Table 2.

The distribution of gender, smoking status, nodule type, histological type, preoperative CEA level, *EGFR* mutations, pTNM stage, lymphatic metastasis, and tumor–pleural relationship showed significant differences between VPI and non-VPI groups. Significant difference in tumor size was also observed in the two groups (*P*=0.022). The results of univariate logistic regression analysis showed that all positive characteristics presented in chi-squared test were significantly associated with the increased risk of VPI development (OR >1.00, *P*<0.05), except histological type; SCC could significantly reduce the risk of VPI occurrence (OR =0.18, 95%CI =0.10–0.33, *P*<0.001) compared with ADC. After adjusting

Table 1 Clinicopathologic characteristics and prognosis of patients

Patient characteristics	Total	Number	%
Total patients	508		
Age, years (median, range)	508	61 (25–91)	
<60		215	42.3
≥60		293	57.7
Gender	508		
Male		264	52
Female		244	48
Smoking status	508		
Ever		198	39
Never		310	61
Tumor size, mm (mean ± SD)	508	27.3±14.97	
≤30		356	70.1
30–50		116	22.8
>50		36	7.1
Nodule type	508		
Non-solid		114	22.4
Solid		394	77.6
pTNM stage (eighth edition)	508		
I		343	67.5
II		54	10.6
III		111	21.9
Lymphatic metastasis	508		
N0		393	77.4
N1		24	4.7
N2		91	17.9
Histological type	508		
ADC		411	80.9
SCC		82	16.1
Others[a]		15	3
VPI	508		
Yes		229	45.1
No		279	54.9
Tumor–pleural relationship	508		
No contacting		147	28.9
Pleural tag type I		69	13.6
Pleural tag type II		99	19.5
Pleural tag type III		30	5.9
Abutting pleural		163	32.1
EGFR mutation status	508		
Wild-type		265	52.2
19-del		99	19.5
L858R		122	24
Others[b]		22	19.1
Preoperative CEA (ng/mL)	499[c]		
<5		378	75.8
≥5		121	24.2
Postoperative therapy	508		
No		227	44.7
Yes		281	55.3
Follow-up time, months (median, range)		50 (3–57)	
DFS, months (median, range)		49 (2–57)	

Notes: [a]Other histological types include large cell lung cancer and adenosquamous carcinoma. [b]Other mutation types include 20-ins, G719X, T790M, and L861Q. [c]A total of 499 patients underwent preoperative CEA examination in this study.
Abbreviations: ADC, adenocarcinoma; CEA, carcinoembryonic antigen; DFS, disease-free survival; pTNM, pathological TNM; SCC, squamous cell carcinoma; VPI, visceral pleural invasion.

the covariates in multivariate logistic regression analysis, tumor size (OR =1.02, 95%CI =1.01–1.04, *P*=0.041), *EGFR* mutations (adjusted OR =2.13, 95%CI =1.13–3.99, *P*=0.019 for 19-del, and adjusted OR =2.89, 95%CI =1.58–5.29, *P*=0.001 for L858R compared to *EGFR* wild-type), and tumor–pleural relationship (adjusted OR =2.54, 95%CI =2.14–3.02, P_{trend}<0.001) showed independent risk factors for VPI. SCC was an independent protective factor for the development of VPI (adjusted OR =0.17, 95%CI =0.06–0.43, *P*<0.001 compared with ADC).

Association between EGFR mutations and clinicopathologic characteristics

To further investigate the distribution of *EGFR* mutations in VPI and non-VPI patients, chi-squared test and logistic regression analysis were carried out to analyze the association between clinicopathologic characteristics and *EGFR* mutations. The covariate factors including age, gender, smoking status, tumor size, nodule type, pTNM stage, histological type, tumor–pleural surface relationship, lymphatic metastasis, and preoperative CEA were collected in this study. The results showed that gender, smoking status, tumor size, nodule type, histological type, tumor–pleural relationship, and VPI presented significant association with *EGFR* mutations (Table S1), including 19-del (Table S2) and L858R (Table S3).

The prevalence of *EGFR* mutations was 47.8% in NSCLC in this study. More female patients (61.7% vs 38.3%, *P*<0.001) and never smokers (74.1% vs 25.9%, *P*<0.001) harbored *EGFR* mutations. *EGFR*-mutant nodules tended to be much smaller (24.47±12.92 vs 29.90±16.23 mm, *P*<0.001) and contained GGO component (*P*<0.001; Table S1). Compared with nodules containing GGO component, the mutation rate of *EGFR* was significantly decreased in solid tumors (OR =0.39, 95%CI =0.24–0.65, *P*<0.001), and only L858R presented significant association with nodule type after adjusting covariates (adjusted OR =0.33, 95%CI =0.18–0.59, *P*<0.001) in subgroup analysis (Table 3). Analysis for histological type showed that 96.7% of *EGFR* mutations presented in ADC and the mutation rate of *EGFR* was 57.2% in ADC (Table S1). Compared with ADC, the mutation rates of both 19-del (adjusted OR =0.05, 95%CI =0.01–0.41, *P*<0.001) and L858R (adjusted OR =0.05, 95%CI =0.01–0.34, *P*=0.003) were significantly decreased in SCC (Table 3). VPI also showed significant association with *EGFR* mutations (adjusted OR =2.21, 95%CI =1.36–3.59, *P*=0.001), and higher frequency of *EGFR* mutations occurred in tumors with VPI (adjusted OR =2.21, 95%CI =1.36–3.59, *P*=0.001). Subgroup analysis

Table 2 Association analysis between clinicopathologic characteristics and VPI

Characteristics	Total	VPI(–)	VPI(+)	*P*-value for χ^2	Univariate analysis		Multivariate analysis	
		n (%)	n (%)		OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
	508	279 (54.9)	229 (45.1)					
Gender				<0.001				
Male	264	165 (59.1)	99 (42.1)		1.00 (reference)		1.00 (reference)	
Female	244	114 (40.9)	130 (56.8)		1.90 (1.33–2.71)	<0.001	1.66 (0.88–3.12)	0.119
Smoking status				<0.001				
Ever	198	131 (47.0)	67 (29.3)		1.00 (reference)		1.00 (reference)	
Never	310	148 (53.0)	162 (70.7)		2.14 (1.48–3.10)	<0.001	1.29 (0.68–2.44)	0.430
Tumor size, mm (mean ± SD)		25.92±14.73	28.98±15.13	0.022[a]	1.01 (1.00–1.03)	0.023	1.02 (1.01–1.04)	0.041
Nodule type				0.015				
Non-solid	114	74 (26.5)	40 (17.5)		1.00 (reference)		1.00 (reference)	
Solid	394	205 (73.5)	189 (82.5)		1.71 (1.11–2.63)	0.016	1.48 (0.81–2.72)	0.205
Histological type				<0.001				
ADC	411	200 (71.7)	211 (92.1)		1.00 (reference)		1.00 (reference)	
SCC	82	69 (24.7)	13 (5.7)		0.18 (0.10–0.33)	<0.001	0.17 (0.06–0.43)	<0.001
Preoperative CEA (ng/mL)	499[b]			0.042				
<5	378	218 (79.3)	160 (71.4)		1.00 (reference)		1.00 (reference)	
≥5	121	57 (20.7)	64 (28.6)		1.53 (1.01–2.31)	0.043	0.88 (0.51–1.54)	0.660
EGFR mutation status				<0.001				
Wild-type	265	174 (62.4)	91 (39.7)		1.00 (reference)		1.00 (reference)	
19-del	99	41 (14.7)	58 (25.3)		2.71 (1.69–4.34)	<0.001	2.13 (1.13–3.99)	0.019
L858R	122	54 (19.4)	68 (29.7)		2.41 (1.55–3.73)	<0.001	2.89 (1.58–5.29)	0.001
Tumor–pleural relationship				<0.001				
No contacting	147	147 (52.7)	0 (0.0)		/	/	/	/
Pleural tag type I	69	43 (15.4)	26 (11.4)		1.00 (reference)		1.00 (reference)	
Pleural tag type II	99	33 (15.4)	66 (28.8)		3.31 (1.74–6.28)	<0.001	4.19 (2.03–8.65)	<0.001
Pleural tag type III	30	12 (4.3)	18 (7.9)		2.48 (1.03–5.97)	0.043	2.54 (0.98–6.55)	0.054
Abutting pleural	163	44 (15.8)	119 (52.0)		4.47 (2.46–8.13)	<0.001	6.52 (3.26–13.05)	<0.001
Trend					2.35 (2.03–2.72)	<0.001	2.54 (2.14–3.02)	<0.001
Lymphatic metastasis				0.005				
N0	393	231 (82.8)	162 (70.7)		1.00 (reference)		1.00 (reference)	
N1	24	8 (3.2)	15 (6.6)		2.38 (1.02–5.56)	0.046	1.25 (0.38–4.10)	0.719
N2	91	39 (14.0)	52 (22.7)		1.90 (1.20–3.02)	0.006	0.55 (0.16–1.94)	0.354

Notes: [a]*P*-value for independent *t*-test. [b]A total of 499 patients underwent preoperative CEA examination in this study.
Abbreviations: ADC, adenocarcinoma; CEA, carcinoembryonic antigen; SCC, squamous cell carcinoma; VPI, visceral pleural invasion.

Table 3 Multivariate logistic regression analysis between *EGFR* mutations and clinicopathologic characteristics

Characteristics	*EGFR* mutations		*EGFR* 19-del		*EGFR* L858R	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Nodule type						
Non-solid	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Solid	0.39 (0.24–0.65)	<0.001	0.56 (0.29–1.07)	0.080	0.33 (0.18–0.59)	<0.001
Histological type						
ADC	1.00 (reference)		1.00 (reference)		1.00 (reference)	
SCC	0.08 (0.03–0.24)	<0.001	0.05 (0.01–0.41)	0.005	0.05 (0.01–0.34)	0.003
VPI						
No	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Yes	2.21 (1.36–3.59)	0.001	1.83 (0.99–3.37)	0.053	2.63 (1.42–4.88)	0.002
Lymphatic metastasis						
N0	/		1.00 (reference)		/	
N1	/	/	5.02 (1.59–15.81)	0.006	/	/
N2	/	/	1.34 (0.66–2.84)	0.393	/	/
Trend	/	/	1.24 (0.87–1.77)	0.225	/	/

Abbreviations: ADC, adenocarcinoma; SCC, squamous cell carcinoma; VPI, visceral pleural invasion.

showed that only L858R showed significant association with VPI (adjusted OR =2.63, 95%CI =1.42–4.88, *P*=0.002), 19-del presented potential significance level of *P*<0.05 with VPI (*P*=0.053). Lymphatic metastasis analysis showed that only 19-del was an independent risk factor for intrapulmonary lymph node (N1) metastasis (adjusted OR =5.02, 95%CI =1.59–15.81, *P*=0.006; Table 3).

Disease-free survival analysis in non-VPI and VPI patients

To investigate the association between survival and VPI as well as *EGFR* mutations, DFS analysis were carried out in this study. Survival curve analysis showed that VPI was significantly associated with DFS (log-rank *P*=0.047; Figure 1A). *EGFR* mutations presented better DFS compared with *EGFR* wild-type, but did not reach the significance level of 0.05 (Figure 1B). Subgroup analysis in VPI and non-VPI showed that whether 19-del or L858R was significantly associated with DFS (log-rank *P*=0.004 for 19-del, and log-rank *P*=0.024 for L858R, respectively; Figure 1C) in non-VPI group; however, no significant association was found between *EGFR* mutations and DFS (log-rank *P*>0.05) in VPI group (Figure 1D). There was no significant difference between VPI and non-VPI in *EGFR* wild-type group (Figure 1E). Survival curve analysis in *EGFR* mutation groups showed that the significant difference between VPI and non-VPI was present only in 19-del group (log-rank *P*=0.002), but not in L858R (log-rank *P*=0.090; Figure 1F).

The Cox regression analysis showed that after adjusting the significant factors in univariate analysis, *EGFR* 19-del (adjusted HR =0.31, 95%CI =0.12–0.80, *P*=0.015) could significantly decrease the risk of DFS, and was the independent prognosis factor for DFS in non-VPI group; preoperative CEA level (adjusted HR =2.32, 95%CI =1.40–3.85, *P*=0.001) and lymphatic metastasis (adjusted HR =1.78, 95%CI =1.36–2.32, P_{trend}<0.001) were independent risk factors for DFS in non-VPI group. In VPI group, only lymphatic metastasis (adjusted HR =2.36, 95%CI =1.78–3.14, P_{trend}<0.001) was the independent risk factor for DFS (Table 4).

Discussion

EGFR is one of the most well-studied mutant genes in NSCLC. According to a recent meta-analysis, the average mutation rate of *EGFR* ranged from 9.6% to 82.2% worldwide, which was higher in Asian population than in other races on average (43.5% vs 37.9%).[19] VPI was considered as an adverse prognostic factor in NSCLC; for this reason, tumors <3 cm will be upstaged to T2 stage if they invade the visceral pleural according to the eighth edition of AJCC TNM classification for lung cancer.[13] However, limited researches were focused on the association between *EGFR* mutations and VPI. In this retrospective study, 508 patients who underwent

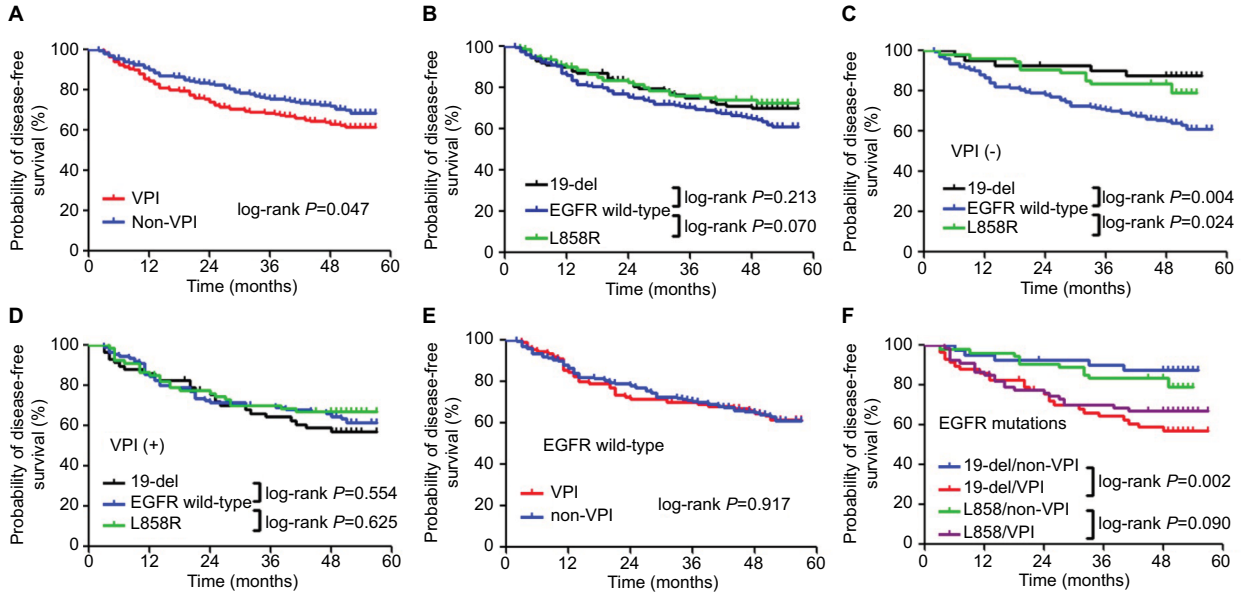

Figure 1 DFS curve analysis for postoperative patients based on VPI and EGFR mutations.
Notes: (**A**) DFS curves in VPI and non-VPI groups. (**B**) DFS curves in EGFR wild-type and EGFR mutation groups. (**C**) DFS curves in non-VPI patients stratified by EGFR mutations. (**D**) DFS curves in VPI patients stratified by EGFR mutations. (**E**) DFS curves in EGFR wild-type patients stratified by VPI. (**F**) DFS curves in EGFR mutation patients stratified by VPI.
Abbreviations: DFS, disease-free survival; VPI, visceral pleural invasion.

Table 4 DFS analysis in non-VPI and VPI groups

Variables	Non-VPI				VPI			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
Gender								
Male	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
Female	0.47 (0.29–0.77)	0.003	0.80 (0.45–1.41)	0.440	0.56 (0.36–0.86)	0.007	0.71 (0.45–1.13)	0.150
Tumor size (mm)								
≤30	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
30–50	2.61 (1.63–4.16)	<0.001	1.40 (0.81–2.44)	0.230	1.95 (1.21–3.13)	0.006	0.92 (0.54–1.56)	0.742
>50	2.67 (1.26–5.66)	0.011	1.32 (0.57–3.05)	0.513	3.77 (1.99–7.14)	<0.001	1.92 (0.94–3.94)	0.075
Trend	1.87 (1.39–2.53)	<0.001	1.21 (0.83–1.76)	0.316	1.94 (1.45–2.61)	<0.001	1.25 (0.87–1.80)	0.237
Nodule type								
Non-solid	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
Solid	3.97 (1.91–8.24)	<0.001	2.05 (0.91–4.60)	0.083	5.33 (1.95–14.55)	0.001	2.31 (0.80–6.66)	0.121
Histological type								
ADC	1.00 (reference)		1.00 (reference)		1.00 (reference)			
SCC	2.17 (1.38–3.41)	0.001	1.58 (0.87–2.86)	0.131	1.28 (0.56–2.93)	0.564		
Preoperative CEA (ng/mL)								
<5	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
≥5	2.55 (1.62–4.02)	<0.001	2.32 (1.40–3.85)	0.001	2.10 (1.36–3.23)	0.001	1.10 (0.69–1.77)	0.688
Lymphatic metastasis								
N0	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
N1	0.96 (0.23–3.95)	0.956	0.57 (0.14–2.38)	0.437	5.24 (2.58–10.67)	<0.001	3.37 (1.56–7.28)	0.002
N2	5.27 (3.32–8.36)	<0.001	3.24 (1.93–5.43)	<0.001	6.73 (4.60–11.69)	<0.001	5.48 (3.08–9.74)	<0.001
Trend	2.27 (1.80–2.87)	<0.001	1.78 (1.36–2.32)	<0.001	2.69 (2.14–3.37)	<0.001	2.36 (1.78–3.14)	<0.001
EGFR mutation status								
Wild-type	1.00 (reference)		1.00 (reference)		1.00 (reference)		/	
19-del	0.29 (0.12–0.71)	0.007	0.31 (0.12–0.80)	0.015	1.17 (0.69–1.97)	0.565	/	/
L858R	0.49 (0.26–0.92)	0.028	0.96 (0.47–1.94)	0.899	0.88 (0.51–1.50)	0.631	/	/
Postoperative therapy								
No	1.00 (reference)				1.00 (reference)			
Yes	2.91 (1.85–4.58)	<0.001	1.40 (0.81–2.40)	0.225	2.07 (1.20–3.56)	0.009	0.89 (0.48–1.65)	0.720

Abbreviations: ADC, adenocarcinoma; CEA, carcinoembryonic antigen; DFS, disease-free survival; SCC, squamous cell carcinoma; VPI, visceral pleural invasion.

primary tumor lobectomy were enrolled to analyze the association between *EGFR* mutations and VPI as well as their roles in the prognosis of NSCLC patients. Our results showed that 47.8% NSCLC patients harbored *EGFR* mutations, among which 19-del and L858R are the two main subtypes. 45.1% of patients presented VPI. *EGFR* mutations were significantly associated with VPI, and higher frequency of *EGFR* mutations was found in VPI patients. Tumor–pleural relationship indicated to be an important CT feature as it showed great association with VPI. In 19-del group, VPI showed significant association with DFS and was identified to be an independent risk factor. At the same time, 19-del was found to have an important prognostic value for a better DFS in non-VPI patients. Lymphatic metastasis was an independent risk factor for DFS in both VPI and non-VPI patients.

Tumor that abuts the pleural surface does not necessarily mean pleural invasion. The possibility of VPI can be further predicted by 1) the obtuse angle between pleural surface and lesion; 2) contact length >3 cm, and 3) thickening of adjacent pleural. The combination of all these image markers can reach a sensitivity of 87% and a specificity of 68%.[20] Hsu et al[18] first reported that for tumors that do not abut the pleural surface, pleural tags might be a sensitive predictor of VPI on CT image. The authors classified pleural tags into three types in their study, and showed that the pleural tag increased its accuracy of VPI invasion from the order of type I, type III, and type II, which was consistent with ours. Our study also showed that no VPI was diagnosed in the tumors without pleural contact on CT scans, and tumors abutted the pleural surface was an independent risk factor for the development of VPI.

The influence of VPI on the prognosis of resected NSCLC patients has not reached an agreement. Some previous studies revealed that VPI was recognized as an adverse independent

factor for the prognosis in NSCLC patients who underwent tumor resection.[15,21] David et al excluded the association between VPI and OS or DFS in tumors <5 cm.[22] Yanagawa et al found that VPI failed to be an independent predictor for decreased survival in resected stage I patient.[23] In our study, we observed that VPI had an adverse effect on the DFS in NSCLC patients. Further stratified analysis in VPI and non-VPI subgroups identified that only regional lymph node metastasis actually decreased survival independently in VPI group. In non-VPI group, *EGFR* mutation was an independent factor for a better DFS.

Patients with *EGFR* mutation are recommended for TKIs target therapy; however, the prognostic value of *EGFR* mutations in resected NSCLC patients is still under debate. Kim et al suggested that *EGFR* mutation is not a prognostic factor for patients after tumor resection,[24] whereas the research published by Takamochi et al[8] demonstrated that *EGFR* mutations contributed to better DFS and OS. Lee et al[25] retrospectively reviewed 117 patients who underwent curative resection and pointed out that *EGFR* mutations may benefit patients' DFS. D'Angelo et al[26] studied 1,118 postoperative patients with stage I–III lung cancers and showed that *EGFR* mutations lowered death rate significantly. The underlying mechanism for this prognostic significance of *EGFR* mutations in NSCLC remains unclear. It is speculated that the progression of *EGFR*-mutated lung cancer cells was less likely to be interacted with other oncogenes, making the survival of these patients better than those who exhibit higher nonsynonymous mutation burden.[27]

Exon 19 (19-del) and Exon 21 (L858R) account for >80% of *EGFR* gene mutations. Li et al reported that mutation frequencies in exon 19 were significantly higher in early stage lung cancer (I/II), whereas L858R were more common in tumors having lymph node metastasis and late stage (III/IV).[28] Renaud et al analyzed 108 19-del and 88 L858R surgically treated NSCLC patients and concluded that exon19 confers a better OS than exon 21 in stage II and III NSCLC.[7] However, Takamochi et al compared the prognosis between L858R and 19-del in resected tumors, and no differences in OS and DFS were observed.[8] Our data showed that both 19-del and L858R could significantly increase the DFS in non-VPI patients. However, after adjusting for covariates, only 19-del was identified as an independent prognostic factor for better DFS in non-VPI patients. No significant results were found in VPI group. It is possible that in VPI patients, the merit of *EGFR* mutations in improving DFS could be eliminated by strong invasive potential to visceral pleural; whereas, the favorable prognostic significance of *EGFR* mutations, especially 19-del, could be displayed in patients without VPI.

EGFR mutations occur more frequently in patients with malignant pleural effusion than those without,[29] which may indicate that *EGFR* mutation is closely related to the invasiveness of NSCLC. To the best of our knowledge, few studies have analyzed the relationship between VPI and *EGFR* mutations. Le et al[30] suggested that *EGFR* signaling pathway may promote the development of VPI through its downstream effector miR-135b. Hence, the clinical meaning of *EGFR* mutations correlated with VPI should be studied. Lin et al observed no association between *EGFR* mutations and visceral pleural surface invasion in 172 patients with tumors no more than 2 cm.[31] In our study, we found that both 19-del and L858R were significantly associated with VPI as well as independent risk factors for VPI. Furthermore, we identified that the frequency of *EGFR* mutations was increased from the order of pleural tag type I, type III, and type II, which consisted with the predictive accuracy of VPI occurrence. This result infers that *EGFR* mutations might play important roles in the development of VPI. We also demonstrated that only 19-del was an independent factor for the increased DFS in non-VPI patient.

Our study also has several limitations. The data were based on a retrospective study in a single center, which may cause selection bias inevitably. We did not show the depth of VPI infiltration using elastic staining. VPI-positive patients in our study were defined as PL1 and PL2.[32] Besides, we did not show the overall survival in this study. A variety of methods and therapeutic strategies after disease recurrence including chemotherapy, radiotherapy, target therapy, and palliative care were decided by patients, and we considered that this discrepancy may interfere with the overall survival.

Conclusion

In this study, we identified that there was a significant association between *EGFR* mutations and VPI. *EGFR* mutations could significantly increase the risk of VPI, and higher frequency of L858R was detected in VPI patients. *EGFR* mutations might not benefit the survival of patients with VPI, but conversely 19-del was an independent favorable prognostic factor for DFS in patients without VPI.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

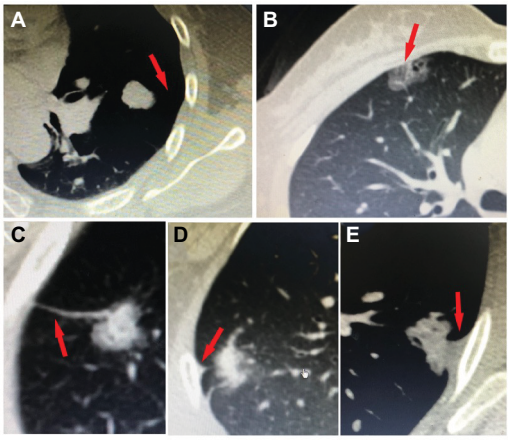

Figure S1 Five types of tumor–pleural surface relationship.
Notes: (**A**) No contacting, (**B**) abutting pleural, (**C**) pleural tag type I, (**D**) pleural tag type II, and (**E**) pleural tag type III. The red arrows illustrate the five types of tumor–pleural surface relationship on CT images.

Table S1 Association analysis between *EGFR* mutations and clinicopathologic characteristics

Characteristics	Total	*EGFR* wild-type		*EGFR* mutant		χ^2	*P*-value	Univariate analysis	
		n	%	n	%			OR (95%CI)	*P*-value
	508	265	52.2	243	47.8				
Age, years (mean ± SD)		60.82±10.21		61.82±9.05			0.241[a]	1.01 (0.99–1.03)	0.243
<60	215	120	45.3	95	39.1	1.99	0.158		
≥60	293	145	54.7	148	60.9				
Gender						35.01	<0.001		
Male	264	171	64.5	93	38.3			1.00 (reference)	
Female	244	94	35.5	150	61.7			2.93 (2.05–4.21)	<0.001
Smoking status						33.36	<0.001		
Ever	198	135	50.9	63	25.9			1.00 (reference)	
Never	310	130	49.1	180	74.1			2.97 (2.04–4.32)	<0.001
Tumor size, mm (mean ± SD)		29.90±16.23		24.47±12.92			<0.001[a]	0.97 (0.96–0.99)	<0.001
≤30	356	164	61.9	192	79	18.36	<0.001	1.00 (reference)	
30–50	116	75	28.3	41	16.9			0.47 (0.30–0.72)	0.001
>50	36	26	9.8	10	4.1			0.33 (0.15–0.70)	0.004
Trend								0.52 (0.39–0.71)	<0.001
Nodule type						24.96	<0.001		
Non-solid	114	36	13.6	78	32.1			1.00 (reference)	
Solid	394	229	86.4	165	67.9			0.33 (0.21–0.52)	<0.001
pTNM stage						2.19	0.335		
I	343	173	65.3	170	70			1.00 (reference)	
II	54	33	12.5	21	8.6			0.65 (0.36–1.16)	0.147
III	111	59	22.3	52	21.4			0.90 (0.58–1.38)	0.619
Trend									
Histological type						77.71	<0.001		
ADC	411	176	66.4	235	96.7			1.00 (reference)	
SCC	82	78	29.4	4	1.6			0.04 (0.01–0.11)	<0.001
Others[b]	15	11	4.2	4	1.6			0.27 (0.09–0.87)	0.028

(*Continued*)

Table S1 (*Continued*)

Characteristics	Total	*EGFR* wild-type		*EGFR* mutant		χ^2	*P*-value	Univariate analysis	
		n	%	n	%			OR (95%CI)	*P*-value
VPI						25.81	<0.001		
No	279	174	65.7	105	43.2			1.00 (reference)	
Yes	229	91	34.3	138	56.8			2.51 (1.76–3.60)	<0.001
Preoperative CEA (ng/mL)	499[c]					1.47	0.225		
<5	378	202	78	176	73.3			1.00 (reference)	
≥5	121	57	22	64	26.7			1.29 (0.86–1.94)	0.226
Lymphatic metastasis						2.17	0.338		
N0	393	208	78.5	185	76.1			1.00 (reference)	
N1	24	9	3.4	15	6.2			1.87 (0.80–4.38)	0.147
N2	91	48	18.1	43	17.7			1.01 (0.64–1.59)	0.975
Trend								1.03 (0.83–1.29)	0.778
Tumor–pleural relationship						15.62	**0.004**		
No contacting	147	95	35.8	52	21.4			1.00 (reference)	
Pleural tag type I	69	33	12.5	36	14.8			1.99 (1.12–3.56)	**0.02**
Pleural tag type II	99	40	15.1	59	24.3			2.70 (1.59–4.56)	**<0.001**
Pleural tag type III	30	14	5.3	16	6.6			2.09 (0.95–4.61)	0.069
Abutting pleural	163	83	31.3	80	32.9			1.76 (1.12–2.78)	**0.015**
Trend								1.13 (1.01–1.25)	**0.032**

Notes: [a]*P*-value for independent *t*-test. [b]Other histological types including large cell lung cancer and adenosquamous carcinoma. [c]A total of 499 patients underwent preoperative CEA examination in this study.
Abbreviations: ADC, adenocarcinoma; CEA, carcinoembryonic antigen; pTNM, pathological TNM; SCC, squamous cell carcinoma; VPI, visceral pleural invasion.

Table S2 Association analysis between *EGFR* 19-del and clinicopathologic characteristics

Characteristics	Total	*EGFR* wild-type		*EGFR* 19-del		*P*-value	Univariate analysis	
		n	%	n	%		OR (95%CI)	*P*-value
	508	265	52.2					
Age, years (mean ± SD)		60.82±10.21		60.36±9.68		0.92[a]	0.99 (0.97–1.02)	0.703
<60	215	120	45.3	43	43.4	0.752		
≥60	293	145	54.7	56	56.6			
Gender						<0.001		
Male	264	171	64.5	35	35.4		1.00 (reference)	
Female	244	94	35.5	64	64.6		3.33 (2.05–5.39)	<0.001
Smoking status						<0.001		
Ever	198	135	50.9	26	26.3		1.00 (reference)	
Never	310	130	49.1	73	73.7		2.92 (1.75–4.85)	<0.001
Tumor size, mm (mean ± SD)		29.90±16.23		24.00±12.84		0.001[a]	0.97 (0.96–0.99)	0.002
≤30	356	164	61.9	78	78.8	0.008	1.00 (reference)	
30–50	116	75	28.3	17	17.2		0.48 (0.26–0.86)	0.014
>50	36	26	9.8	4	4		0.32 (0.11–0.96)	0.042
Trend							0.52 (0.34–0.81)	0.003
Nodule type						0.001		
Non-solid	114	36	13.6	28	28.3		1.00 (reference)	
Solid	394	229	86.4	71	71.7		0.40 (0.23–0.70)	0.001
pTNM stage						0.933		
I	343	173	65.3	65	65.7		1.00 (reference)	
II	54	33	12.5	11	11.1		0.89 (0.42–1.86)	0.751
III	111	59	22.3	23	23.2		1.04 (0.59–1.82)	0.897
Trend							1.01 (0.77–1.33)	0.952
Histological type						<0.001		
ADC	411	176	66.4	97	98		1.00 (reference)	
SCC	82	78	29.4	1	1		0.02 (0.01–0.17)	<0.001
Others[b]	15	11	4.2	1	1		0.17 (0.02–1.30)	0.087

(*Continued*)

Table S2 (*Continued*)

Characteristics	Total	*EGFR* wild-type		*EGFR* 19-del		*P*-value	Univariate analysis	
		n	%	n	%		OR (95%CI)	*P*-value
VPI						<0.001		
No	279	174	65.7	41	41.4		1.00 (reference)	
Yes	229	91	34.3	58	58.6		2.71 (1.69–4.34)	<0.001
Preoperative CEA (ng/mL)	499[c]					0.617		
<5	378	202	78	74	75.5		1.00 (reference)	
≥5	121	57	22	24	24.5		1.15 (0.67–1.99)	0.617
Lymphatic metastasis						0.102		
N0	393	208	78.5	73	73.7		1.00 (reference)	
N1	24	9	3.4	9	9.1		2.85 (1.09–7.45)	0.033
N2	91	48	18.1	17	17.2		1.01 (0.55–1.87)	0.977
Trend							1.07 (0.79–1.43)	0.676
Tumor–pleural relationship						0.018		
No contacting	147	95	35.8	18	18.2		1.00 (reference)	
Pleural tag type I	69	33	12.5	14	14.1		2.24 (1.01–5.00)	0.049
Pleural tag type II	99	40	15.1	24	24.2		3.17 (1.55–6.47)	0.002
Pleural tag type III	30	14	5.3	5	5.1		2.42 (1.28–4.55)	0.275
Abutting pleural	163	83	31.3	38	38.4		2.42 (1.28–4.55)	0.006
Trend							1.19 (1.03–1.37)	0.016

Notes: [a]*P*-value for independent *t*-test. [b]Other histological types including large cell lung cancer and adenosquamous carcinoma. [c]A total of 499 patients underwent preoperative CEA examination in this study.
Abbreviations: ADC , adenocarcinoma; CEA, carcinoembryonic antigen; pTNM, pathological TNM; SCC, squamous cell carcinoma; VPI, visceral pleural invasion.

Table S3 Association analysis between *EGFR* L858R and clinicopathologic characteristics

Characteristics	Total	*EGFR* wild-type		*EGFR* L858R		*P*-value	Univariate analysis	
		n	%	n	%		OR (95%CI)	*P*-value
	508	265	52.2					
Age, years (mean ± SD)		60.82±10.21		62.58±8.47		0.177[a]	1.02 (0.99–1.04)	0.097
<60	215	120	45.3	44	36.1	0.088		
≥60	293	145	54.7	78	63.9			
Gender						<0.001		
Male	264	171	64.5	45	36.9		1.00 (reference)	
Female	244	94	35.5	77	63.1		3.11 (1.99–4.86)	<0.001
Smoking status						<0.001		
Ever	198	135	50.9	30	24.6		1.00 (reference)	
Never	310	130	49.1	92	75.4		3.19 (1.98–5.13)	<0.001
Tumor size, mm (mean ± SD)		29.90±16.23		23.99±12.62		<0.001[a]	0.97 (0.96–0.99)	0.001
≤30	356	164	61.9	100	82	<0.001	1.00 (reference)	
30–50	116	75	28.3	19	15.6		0.42 (0.24–0.73)	0.002
>50	36	26	9.8	3	2.5		0.19 (0.06–0.64)	0.008
Trend							0.42 (0.28–0.66)	<0.001
Nodule type						<0.001		
Non-solid	114	36	13.6	44	36.1		1.00 (reference)	
Solid	394	229	86.4	78	63.9		0.28 (0.17–0.46)	<0.001
pTNM stage						0.06		
I	343	173	65.3	90	73.8		1.00 (reference)	
II	54	33	12.5	6	4.9		0.35 (0.14–0.87)	0.023
III	111	59	22.3	26	21.3		0.85 (0.50–1.44)	0.537
Trend							0.87 (0.67–1.13)	0.299
Histological type						<0.001		
ADC	411	176	66.4	118	96.7		1.00 (reference)	
SCC	82	78	29.4	1	0.8		0.02 (0.01–0.14)	<0.001
Others[b]	15	11	4.2	3	2.5		0.41 (0.11–1.49)	0.174

(*Continued*)

Table S3 (*Continued*)

Characteristics	Total	*EGFR* wild-type		*EGFR* L858R		*P*-value	Univariate analysis	
		n	%	n	%		OR (95%CI)	*P*-value
VPI						<0.001		
No	279	174	65.7	54	44.3		1.00 (reference)	
Yes	229	91	34.3	68	55.7		2.41 (1.55–3.73)	<0.001
Preoperative CEA (ng/mL)	499[c]					0.341		
<5	378	202	78	89	73.6		1.00 (reference)	
≥5	121	57	22	32	26.4		1.27 (0.77–2.10)	0.342
Lymphatic metastasis						0.902		
N0	393	208	78.5	94	77		1.00 (reference)	
N1	24	9	3.4	5	4.1		1.23 (0.40–3.77)	0.718
N2	91	48	18.1	23	18.9		1.06 (0.61–1.84)	0.836
Trend							1.04 (0.79–1.36)	0.789
Tumor–pleural relationship						0.054		
No contacting	147	95	35.8	28	23		1.00 (reference)	
Pleural tag type I	69	33	12.5	21	17.2		2.16 (1.08–4.31)	0.029
Pleural tag type II	99	40	15.1	29	23.8		2.46 (1.30–4.65)	0.006
Pleural tag type III	30	14	5.3	7	5.7		1.51 (0.85–2.68)	0.3
Abutting pleural	163	83	31.3	37	30.3		1.51 (0.85–2.68)	0.157
Trend							1.08 (0.94–1.23)	0.278

Notes: [a]*P*-value for independent *t*-test. [b]Other histological types including large cell lung cancer and adenosquamous carcinoma. [c]A total of 499 patients underwent preoperative CEA examination in this study.

Abbreviations: ADC, adenocarcinoma; CEA, carcinoembryonic antigen; pTNM, pathological TNM; SCC, squamous cell carcinoma; VPI, visceral pleural invasion.