Analyzing EGFR mutations and their association with clinicopathological characteristics and prognosis of patients with lung adenocarcinoma

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Received September 18, 2017; Accepted March 29, 2018

DOI: 10.3892/ol.2018.8681

Abstract. Epidermal growth factor receptor (EGFR) is an important gene in the development of lung adenocarcinoma. However, there is controversy regarding the association between EGFR mutations and survival time of patients with lung adenocarcinoma. In the present study, tissue specimens and clinical data were collected from 219 patients with lung adenocarcinoma who had not undergone prior radiotherapy or chemotherapy. EGFR mutations were detected using a fluorescence polymerase chain reaction method, and the association between EGFR mutations and clinicopathological characteristics was analyzed. Overall survival (OS) curves were constructed using the Kaplan-Meier method and the influence of clinicopathological characteristics on OS was analyzed using the Cox regression model. The EGFR mutation rate was 50.7%, and the most common mutations were the L858R substitution mutation in exon 21 (L858R; 54.9%) and the deletion mutation in exon 19 (19-Del; 36%). The presence of EGFR mutations varied significantly with sex, smoking history, T stage, vascular invasion and adenocarcinoma subtypes (P<0.05). The survival time was significantly longer for female, young (<60 years-old), non-smokers or patients exhibiting EGFR mutations (G719X, 19-Del, L858R and L861Q). The survival time was also significantly longer for patients with a 19-Del mutation, early stage tumors, tyrosine kinase inhibitors targeted therapy-treated patients, for those not exhibiting nerve or vascular invasion, and for those without disease recurrence (P<0.05). Multivariate analysis revealed that tumor pathological Tumor-Node-Metastasis (pTNM) stage, nerve invasion, vascular invasion, EGFR mutation and the 19-Del mutation were independent predictors (P<0.05). Therefore, tumor pTNM stage, nerve invasion, vascular invasion and EGFR mutation status, particularly that of 19-Del, were independent prognostic factors for patients with lung adenocarcinoma.

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Abbreviations: EGFR, epidermal growth factor receptor; ARMS, amplification refractory mutation system; CT, computed tomography; F-PCR, fluorescence-polymerase chain reaction; G719X, point mutations in exon 18; 19-Del, deletion mutations in exon 19; 20-Ins, insertion mutations in exon 20; L858R and L861Q, two base-pair substitution mutations in exon 21; MIA, minimally invasive adenocarcinoma; IA, invasive adenocarcinoma; IAV, invasive adenocarcinoma variant; IASLC/ATS/ERS, International Association for the Study of Lung Cancer, American Thoracic Society and European Respiratory Society; MST, median survival time; NSCLC, non-small cell lung cancer; OS, overall survival; pTNM, pathological tumor-node-metastasis; TKIs, tyrosine kinase inhibitors

Key words: lung adenocarcinoma, epidermal growth factor receptor, mutation, clinicopathological characteristics, tyrosine kinase inhibitors, prognosis

Introduction

With the rapid development of industrialization, lung cancer has become the most common type of malignant tumor, with high rates of morbidity and mortality (1). It has been reported that >730,000 cases were diagnosed, and ~610,000 mortalities due to lung cancer occurred in China in 2015 (2). Non-small cell lung cancer (NSCLC) accounts for ~85% lung cancer cases, and the majority of patients are diagnosed at a late stage of NSCLC, and thus have a poor prognosis (3). Lung adenocarcinoma is the most common subtype of NSCLC, with a high recurrence rate and short survival time (4). According to previous research, oncogenes serve an important role in the

occurrence and development of lung adenocarcinomas, and may be potential therapeutic targets (4-6).

Epidermal growth factor receptor (EGFR) is an important driving gene in lung adenocarcinoma, and it has been reported that EGFR mutations are more common in Asian patients, non-smokers and females (6). Previous studies have demonstrated that mutation of EGFR is a positive predictor of prognosis for patients with lung adenocarcinoma (7-10). Patients with EGFR mutations have been indicated to respond well to EGFR tyrosine kinase inhibitors (EGFR-TKIs) (11). However, it has been demonstrated that patients with different EGFR mutation subtypes experience different outcomes following EGFR-TKI treatment (8,12,13). Therefore, the association between EGFR mutations and survival time of patients with lung adenocarcinoma requires further investigation. Furthermore, in developing countries with limited economic conditions, including China, EGFR mutations of patients with lung adenocarcinoma often go undetected (14). This highlights the importance of characterizing the significance of EGFR mutations in lung adenocarcinoma and the associated clinicopathological characteristics. The International Association for the Study of Lung Cancer, American Thoracic Society and European Respiratory Society (IASLC/ATS/ERS) classification system (2011 version) is often used to classify lung adenocarcinoma (13,15). However, there is some controversy over its effectiveness (15-17).

Using IASLC/ATS/ERS classification, EGFR mutation detection was performed using tissues from 219 patients with lung adenocarcinoma. The associations between EGFR mutation status and clinical characteristics were analyzed, and the significance was evaluated in the context of survival time to provide empirical and theoretical foundations for the improvement of the clinical treatment of lung cancer.

Materials and methods

Patients and clinical data. A total of 435 patients with primary lung adenocarcinoma, who underwent surgical resection between October 2012 and March 2013 at the Affliated Hospital of Binzhou Medical University (Yantai, China) or the Yuhuangding Hospital (Yantai, China), were invited to participate in the present study. However, 216 patients were excluded due to the lack of follow-up information or accurate classification following surgery. The final 219 participants included 105 females and 114 males, with a mean age of 60 years (range, 30-88 years).

Biopsy materials were selected in accordance with the National Comprehensive Cancer Network guidelines of 2011 (18). The tissues were classified by 2 experienced pathologists of the Affliated Hospital of Binzhou Medical University (Yantai, China) and the Yuhuangding Hospital (Yantai, China), using the IASLC/ATS/ERS system (19). Pathological Tumor-Node-Metastasis (pTNM) classification was performed according to the international lung cancer staging system (20). Adenocarcinoma subtypes included minimally invasive adenocarcinoma (MIA), invasive adenocarcinoma (IA) and invasive adenocarcinoma variant (IAV).

The present study was approved by the Medical Research Ethics Committee of Binzhou Medical University (Yantai, China), and all patients provided written informed consent for their participation in the present study. No patients had received prior radiotherapy or chemotherapy. The postoperative treatment was as follows: Pemetrexed and cisplatin for patients without EGFR mutations or with mutations in exon 20; the first-line TKI, gefitinib, for patients with 19-Del and L858R mutations, and the second-line TKI, afatinib, for patients with other EGFR mutations. All patients were followed-up by telephone or hospital appointment, including a computed tomography scan of the chest and upper abdomen. Tumor-enlargement or identification of distant metastasis was considered indicative of disease recurrence. Non-smokers were defined as smoking <100 cigarettes in lifetime. The final follow-up took place on April 30th, 2017. The overall survival (OS) time was defined as the period from surgery to the last day of follow-up, or the occurrence of mortality.

EGFR mutation detection. All surgical specimens were fixed in formalin and embedded in paraffin. The sample DNA was obtained using a paraffin tissue DNA Extraction kit (cat. no. 56404; Qiagen GmbH, Hilden, Germany). The concentration of DNA was adjusted to 1 ng/μl, and EGFR mutations were detected using the amplification refractory mutation system (ARMS) with human EGFR Mutations Detection kit (cat. no. ADx-EG01; Amoy Diagnostics, Co., Ltd., Xiamen, China), and the assay was performed according to the manufacturer's protocol and as previously described (21). In brief, the ARMS-PCR assay was performed in a 50-μl volume containing 5 μl PCR buffer, 10 pM forward and reverse primers, 20 pM probe and 12.5 μM dNTPs. The thermocycling conditions were as follows: 95˚C for 5 min, then 15 cycles of 95˚C for 25 sec, 64˚C for 20 sec and 72˚C for 20 sec, followed by 31 cycles of 93˚C for 25 sec, 60˚C for 35 sec and 72˚C for 20 sec. The human EGFR Mutations Detection kit is able to detect 29 EGFR mutations from exon 18 to exon 21, including 3-point mutations in exon 18 (G719X), 19 19-Del mutations, 3 insertion mutations in exon 20 (20-Ins), T790M, S768I, L858R, and another base-pair substitution mutation in exon 21 (L861Q).

Statistical analysis. The associations between EGFR mutations and clinical characteristics were analyzed using χ^2. OS curves, which were constructed using the Kaplan-Meier method, and further evaluation was performed using the log-rank test. The association between clinical characteristics and OS time was analyzed using Cox regression. All statistical analyses were performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Descriptive statistics. The patients' clinical data are presented in Table I. Of the 219 lung adenocarcinoma patients enrolled in the present study, 54 patients (24.7%) were current or former smokers, 171 patients (78.1%) had pTNM stage I tumors, 170 patients (77.6%) had T1 stage tumors, 184 patients (84%) were classified with N0 stage lung adenocarcinoma, and 202 patients (92.2%) were classified with M0 stage lung adenocarcinoma. A total of 29 patients (13.2%) exhibited nerve invasion, 33 patients (15.1%) exhibited vascular invasion and 209 patients (95.4%) were diagnosed with invasive adenocarcinoma. Following surgery,

Table I. Associations between EGFR mutation status and clinical characteristics.

Characteristics	No. (%)	EGFR, number (%)		P-value
		Wild type	Mutation	
Sex				
Female	105 (47.9)	31 (29.5)	74 (70.5)	<0.001
Male	114 (52.1)	77 (67.5)	37 (32.5)	
Age, years				
<60	99 (45.2)	44 (44.4)	55 (55.6)	0.190
≥60	120 (54.8)	64 (53.3)	56 (46.7)	
Smoking status				
Non-smoker	165 (75.3)	72 (43.6)	93 (56.4)	0.003
Smoker	54 (24.7)	36 (66.7)	18 (33.3)	
T stage				
T1	170 (77.6)	77 (45.3)	93 (54.7)	0.027
T2	49 (22.4)	31 (63.3)	18 (36.7)	
N stage				
N0	184 (84.0)	88 (47.8)	96 (52.2)	0.540
N1	27 (12.3)	16 (59.3)	11 (40.7)	
N2	8 (3.7)	4 (50.0)	4 (50.0)	
M stage				
M0	202 (92.2)	100 (49.5)	102 (50.5)	0.856
M1a	3 (1.4)	1 (33.3)	2 (66.7)	
M1b	14 (6.4)	7 (50.0)	7 (50.0)	
pTNM stage				
I	171 (78.1)	82 (48.0)	89 (52.0)	0.607
II	30 (13.7)	17 (56.7)	13 (43.3)	
III	1 (0.5)	1 (100.0)	0 (0.0)	
IV	17 (7.8)	8 (47.1)	9 (52.9)	
Nerve invasion				
No	190 (86.8)	89 (46.8)	101 (53.2)	0.061
Yes	29 (13.2)	19 (65.5)	10 (34.5)	
Vascular invasion				
No	186 (84.9)	86 (46.2)	100 (53.8)	0.031
Yes	33 (15.1)	22 (66.7)	11 (33.3)	
Recurrence				
No	197 (90.0)	99 (50.3)	98 (49.7)	0.406
Yes	22 (10.0)	9 (40.9)	13 (59.1)	
Histologic subtypes				
MIA	3 (1.4)	0 (0.0)	3 (100.0)	<0.001
IA	209 (95.4)	101 (48.3)	108 (51.7)	
IAV	7 (3.2)	7 (100.0)	0 (0.0)	

EGFR, epidermal growth factor receptor; pTNM, pathological Tumor-Node-Metastasis; MIA, minimally invasive adenocarcinoma; IA, invasive adenocarcinoma; IAV, invasive adenocarcinoma variant.

110 patients received chemotherapy and 109 cases underwent TKI targeted therapy (Table II).

Association between EGFR mutations and clinicopathological characteristics. Of the 219 patients, EGFR mutations were identified in 111 patients (50.7%), including 61 cases of L858R mutations (55%), 40 cases of 19-Del (36%), 5 cases of L861Q (4.5%), 3 cases of G719X (2.7%) and 2 cases of 20-Ins (1.8%). Double mutations were not detected. EGFR mutations were more common in females compared with males (70.5% vs. 32.5%; P<0.001), and the mutation rate was increased in non-smokers compared with smokers (56.4% vs. 33.3%; P=0.003). The EGFR mutation rate in MIA cases was significantly increased compared with IA and IAV (100% vs. 51.7% and 0%, respectively; P<0.001). EGFR mutations were more common in patients with T1 stage tumors compared with other stages (54.7%; P=0.027) and in patients without vascular invasion compared with patients exhibiting vascular invasion (53.8%; P=0.031).

Association between clinicopathological characteristics and survival time. A total of 151 mortalities occurred prior to

Table II. EGFR mutation types and treatment of 219 patients.

Mutation type	Total, no. (%)	Treatment
Wild type	108 (49.3)	Chemotherapy[a]
G719X	3 (2.7)	Second-line TKI[c]
19-Del	40 (36.0)	First-line TKI[b]
L858R	61 (55.0)	First-line TKI[b]
L861Q	5 (4.5)	Second-line TKI[c]
20-Ins	2 (1.8)	Chemotherapy[a]

Treatment with [a]cisplatin plus pemetrexed or [b]gefitinib or [c]afatinib. EGFR, epidermal growth factor receptor; G719X, point mutation in exon 18; 19-Del, deletion mutation in exon 19; 20-Ins, insertion mutation in exon 20; L858R and L861Q, 2 base-pair substitution mutation in exon 21.

March 30th, 2017. The mean follow-up time was 30.9 months (range, 4.7-53.8 months). The 1-, 2- and 3-year survival rates of the patients were 83.6, 54.8 and 42.9%, respectively, and the median survival time (MST) was 27.2 months (data not shown). The survival time was significantly increased in female patients, patients <60 years-old and non-smokers compared with male patients, patients ≥60 years-old and smokers (Fig. 1A-C). Patients with pTNM stage I tumors were associated with increased OS time compared with those with stage II or III tumors (39.6 months vs. 11.3 months and 10.4 months, respectively; P<0.001; Fig. 1D). The survival time was significantly increased in patients without nerve invasion or vascular invasion or clinical recurrence compared with patients with nerve invasion or vascular invasion or clinical recurrence, respectively (Fig. 1E-G). There was no significant difference in OS time among adenocarcinoma subtypes (P=0.112; Fig. 1H). The survival time was markedly increased in patients with EGFR mutations or 19-Del compared with patients without EGFR mutations or 19-Del (Fig. 2A and B). There was no significant difference in OS time between patients with or without the L858 mutation (P=0.074; Fig. 2C), or with or without other mutations of EGFR (P=0.222; Fig. 2D).

A total of 110 patients received cisplatin-based chemotherapy, while 109 patients received TKI targeted therapies (Table II). The present study suggested that TKI targeted therapies could prolong survival time compared with cisplatin-based chemotherapy (47.3 months vs. 15.8 months, P<0.001). However, there was no significant difference in terms of survival time with first- or second-line TKI treatment (45.7 months vs. 49.2 months; Fig. 1I). Cox's multiple regression analysis was used to analyze the association of various clinical characteristics and patient prognosis. Multivariate analysis revealed that tumor pTNM stage, nerve invasion, vascular invasion and EGFR mutation types were independent predictors for patient prognosis. The results also suggested that patients with the 19-Del mutation were associated with a relatively good prognosis, while patients with an L858R mutation were not (Table III).

Discussion

In the present study, EGFR mutations were detected in 111/219 patients with lung adenocarcinoma (50.7%), and the most common mutations were L858R (54.9%) and 19-Del (36%), accounting for 90.9% of all EGFR mutations. Sex, age, smoking status, pTNM stage, nerve invasion, vascular invasion, EGFR mutation status, recurrence and therapeutic regimen were all associated with OS time. Multivariate analysis revealed that pTNM stage, nerve invasion, vascular invasion and EGFR mutations were independent predictors for patient prognosis.

The detection of EGFR mutations in lung adenocarcinoma patients has been widely performed worldwide (5-7,10,21). A number of methods for detecting EGFR mutations now, exist, with direct sequencing and F-PCR being the most common clinically used methods (22-24). Direct sequencing can detect unknown gene mutations and is the 'gold standard' for detecting gene mutations. However, the low sensitivity, the requirement for large specimen size, the complexity and duration of the protocol, the high cost and difficult interpretation of results are disadvantages of direct sequencing (22,23). The F-PCR method combines specific primers with a double loop probe technique. The amplified products are detected by double ring probes, and the mutation status of sample DNA are observed using a PCR platform, specific reaction procedures and highly specific Taq DNA polymerases (22,24). This method has the advantages of high specificity and sensitivity for detecting rare mutations, a simple and rapid protocol, simple data interpretation, and suitability for large-scale screening in clinical laboratories (22,24). In the present study, EGFR mutations in lung adenocarcinoma patients were detected using the F-PCR method.

The EGFR gene is located on the short arm of human chromosome 7, composed of 188,307 bases and 28 exons, and its tyrosine kinase functional domain is encoded by exons 18-24 (5). Previous studies have demonstrated that mutations in exons 18-21 in patients with lung cancer were associated with patient-responsiveness to EGFR-TKIs. This is likely due to changes in the structure of the EGFR ATP binding area, enhancing the combining capacity of EGFR-TKIs (5,7,21). To date, >30 mutations of EGFR have been reported, including 19-Del (~45% all EGFR mutations), L858R (~40-45%), G719X (~5%), 20-Ins (~1%) (5,12,21). Studies have suggested that EGFR mutation rates in patients with lung adenocarcinoma differ among countries and ethnicities, between sexes, and with smoking status (25-27). The overall mutation rate of EGFR in Chinese patients with lung adenocarcinoma in the present study was 50.7%, which is consistent with previous reports (6,25-27). The mutation rate in female patients was significantly higher than that of male patients (P<0.001) while the mutation rate in smokers was low compared with non-smokers (P=0.003), which was also consistent with previous reports (25,26,28). It was also demonstrated that EGFR mutations were more common in patients with T1 stage tumors or without vascular invasion compared with T2 stage patients, or those with vascular invasion (P=0.027 and P=0.031, respectively; Table I).

Previous studies have revealed that EGFR mutations are predictors of TKI treatment response and prognosis of patients with lung adenocarcinoma (10,20,27,29). However, clinical studies indicated that patients with different EGFR mutations were associated with different outcomes (10,29). Patients with 19-Del or L858R have been demonstrated to be associated with a relatively good prognosis following TKI treatment compared

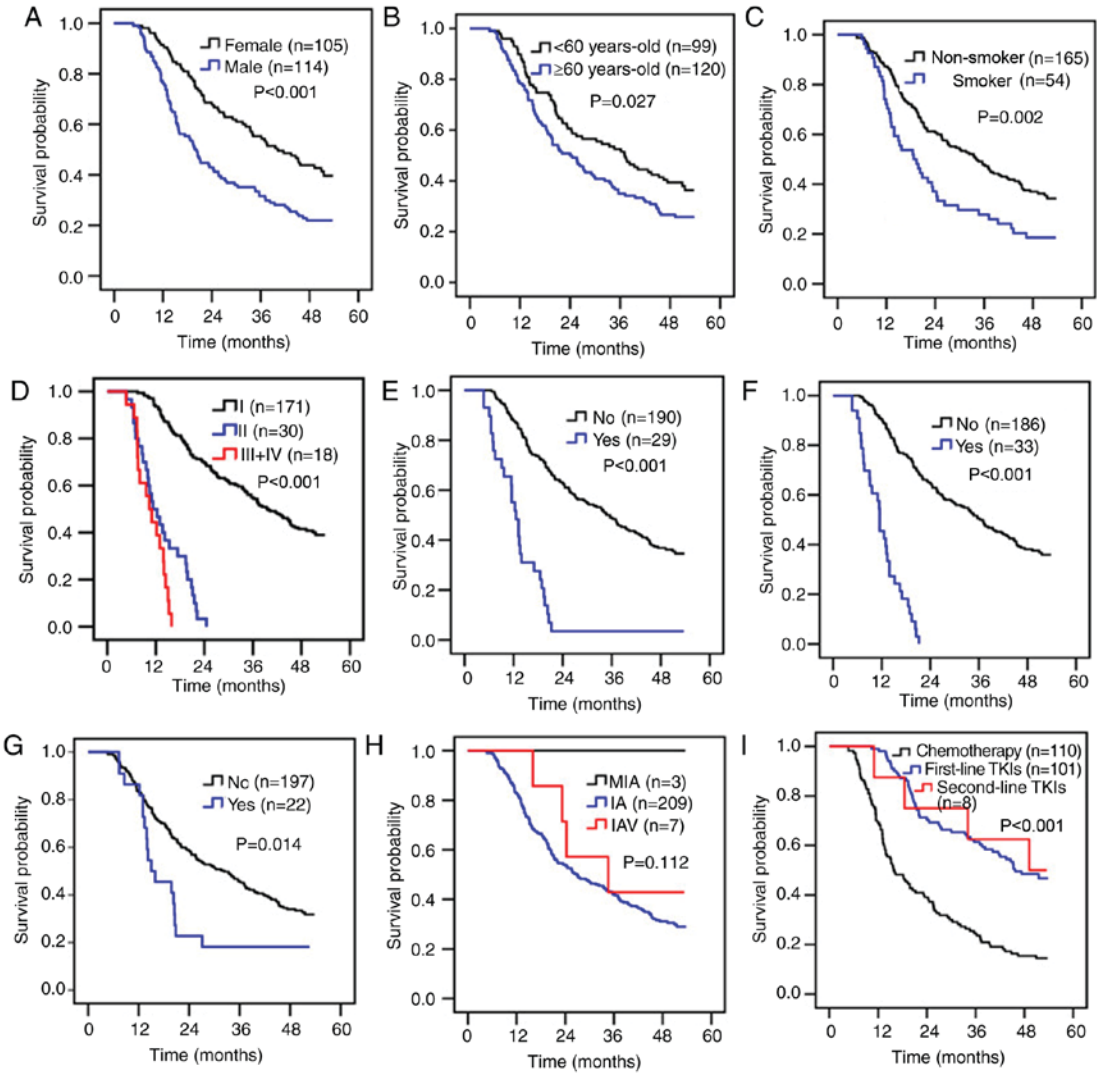

Figure 1. Survival analysis of 219 patients with lung adenocarcinoma. Analysis of the association of OS time and (A) sex, (B) age, (C) smoking history (D) pTNM stage, (E) nerve invasion status, (F) vascular invasion status, (G) clinical recurrence, (H) adenocarcinoma subtypes, and (I) post-surgical therapeutic regimen. OS, overall survival; pTNM pathological Tumor-Node-Metastasis; TKI, tyrosine kinase inhibitor; n, number.

with other mutations (5). A number of studies have suggested that 19-Del or L858R mutations do not have different effects on prognosis (21,30), while others have indicated that patients with 19-Del survived significantly longer than patients with L858R (31,32). In the present study, the survival time of patients with EGFR mutations was significantly higher than patients without EGFR mutations (P<0.001; Fig. 2A), and multivariate analyses demonstrated that the presence of an EGFR mutation was a predictor of favorable prognosis for patients with lung adenocarcinoma (P<0.001; Table III). These results were consistent with previous reports (10,29). Patients with 19-Del were associated with an improved prognosis compared with those without (Fig. 2B; P<0.001), and 19-Del was demonstrated to be a predictor of good outcome (HR, 0.463; 95% CI, 0.241-0.889; P=0.021). L858R was not demonstrated to be an independent predictor of lung adenocarcinoma prognosis, although the survival time of patients with L858R was longer than those without (37.6 months vs. 24.5 months; P=0.074; Fig. 2C). This may be associated with the differences in the sequences and structures of exons 19 and 21, and the differences in TKI activity in patients with different mutations (33,34).

A number of rare EGFR mutations were also detected, including 3 cases of G719X (2.7%), 5 cases of L861Q (4.5%) and 2 cases of 20-Ins (1.8%), a rate which was consistent with previous reports (35,36). Patients with G719 or L861Q had an MST of 49.2 months following second-line TKI treatment, which was longer than that of patients without G719 or L861Q mutations (49.2 months vs. 26.9 months; P=0.222; Fig. 2D). In the present study, only 10 cases of rare mutations were detected (9.0%), and only 8 of these patients were treated with second-line TKIs. Further larger scale studies are required.

It has been reported that ~5% EGFR mutations occur in exon 20, and patients with these mutations are often insensitive to TKI treatment (37). Clinical studies have confirmed that patients with NSCLC acquired resistance to TKIs following a period of treatment. A mutation in T790M in exon 20 was detected in ~50% patients with acquired resistance (37,38). Other studies have demonstrated that T790M mutations existed in a minority of untreated tumor cells prior to treatment, and that the mutation rates increased significantly following treatment (21,38). No T790M mutation was detected in exon 20, which may be due to all specimens being collected

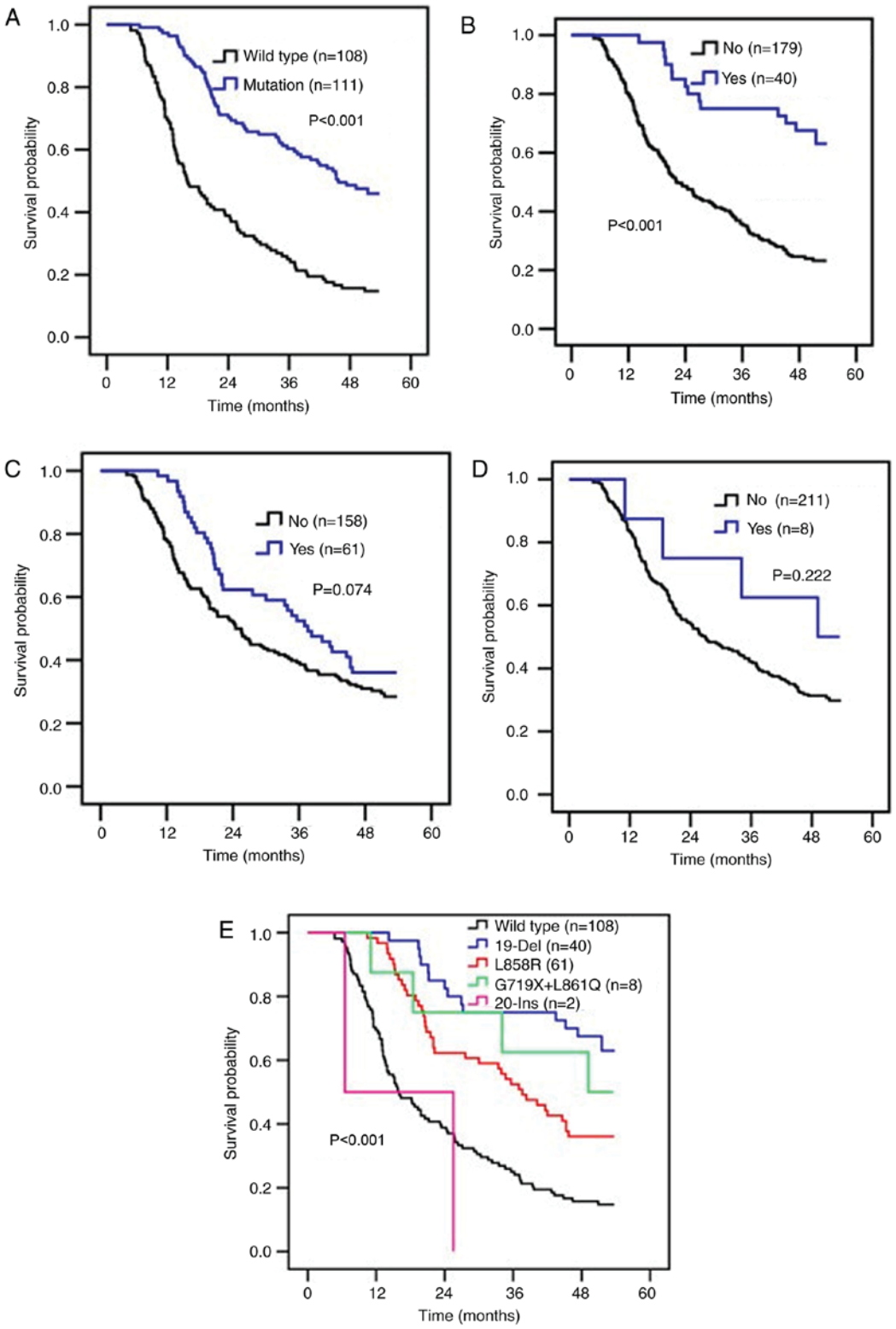

Figure 2. Kaplan-Meier survival analysis for EGFR mutation subtypes of the patients. Overall survival time for patients with or without (A) EGFR mutations, (B) 19-Del, (C) L858R, (D) rare mutations of EGFR, and (E) different EGFR mutation types. EGFR, epidermal growth factor receptor; 19-Del, deletion mutations in exon 19; L858R, base-pair substitution mutation in exon 21; n, number.

from surgical patients, or because no patients were treated with chemotherapeutic drugs prior to surgery. Alternatively, the number of tumor cells exhibiting the T790M mutation may have been too low for detection. In following studies, it is necessary to perform analyze T790M in patients with acquired drug-resistant lung adenocarcinoma.

Previous studies have indicated that IASLC/ATS/ERS classification of lung adenocarcinoma may be an independent prognostic factor (13,15,20). In the present study, it was demonstrated that EGFR mutations were more common in MIA (100%) than in IA (51.7%) and IAV (0%). The survival time of patients with MIA was longer than that of patients with IA and

Table III. Univariate and multivariate analysis of clinical characteristics and the overall survival time of patients with lung adenocarcinoma.

	Univariate analysis			Multivariate analysis		
Variable	Number, (MST, months)	95% CI	P-value	HR	95% CI	P-value
Sex						
Female	105 (40.2)	29.976-50.424	<0.001			
Male	114 (19.8)	16.487-23.113				
Age, years						
<60	99 (37.1)	28.325-45.875	0.027			
≥60	120 (24.0)	18.249-29.751				
Smoking status						
Non-smoker	165 (34.1)	26.695-41.505	0.002			
Smoker	54 (18.7)	12.579-24.821				
pTNM stage						
I	171 (39.6)	32.872-46.328	<0.001	1		
II	30 (11.3)	7.945-14.655		8.904	6.175-12.840	<0.001[c]
III+IV	18 (10.4)	7.490-13.310				
Nerve invasion						
No	190 (34.7)	28.081-41.319	<0.001	1		
Yes	29 (12.6)	10.358-14.842		6.692	3.591-12.470	<0.001[c]
Vascular invasion						
No	186 (36.2)	29.517-42.883	<0.001	1		
Yes	33 (11.5)	9.706-13.294		3.579	1.961-6.533	<0.001[c]
Recurrence						
No	197 (32.3)	24.899-39.701	0.014			
Yes	22 (15.0)	8.335-21.664				
Histologic subtypes						
MIA	3 (NR)	-	0.112			
IA	209 (26.9)	-				
IAV	7 (34.6)	-				
EGFR mutation						
Wild type	108 (15.8)	11.472-20.128	<0.001[a]	1		
Mutation	111 (45.7)	36.326-57.290				
19-Del	40 (NR)	-	<0.001[b]	0.432	0.101-1.855	0.259[c]
L858R	61 (37.6)	29.182-46.018[b]		0.051	0.011-0.244	<0.001[c]
G719X+L861Q	8 (49.2)	28.500-54.146[b]		0.110	0.025-0.493	0.004[c]
20-Ins	2 (6.5)	6.500-[b]		0.066	0.011-0.390	0.003[c]
EGFR 19-Del mutation						
No	179 (22.2)	17.853-26.547	<0.001	1		
Yes	40 (NR)	-		0.463	0.241-0.889	0.021[d]
EGFR L858R mutation						
No	158 (24.5)	19.804-29.196	0.074			
Yes	61 (37.6)	29.182-46.018				
EGFR rare mutatione						
No	211 (26.9)	19.906-33.894	0.222			
Yes	8 (49.2)	29.501-52.199				
Treatment						
Chemotherapy	110 (15.8)	11.432-20.168	<0.001			
TKI	109 (47.3)	21.500-1.711				
First-line	101 (45.7)	21.500-1.678				
Second-line	8 (49.2)	18.500-14.146				

CI, confidence intervals; EGFR, epidermal growth factor receptor; HR, hazard ratio; pTNM, pathological Tumor-Node-Metastasis; 19-Del, deletion mutations in exon 19; L858R, base-pair substitution mutation in exon 21; MIA, minimally invasive adenocarcinoma; IA, invasive adenocarcinoma; IAV, invasive adenocarcinoma; MST, median survival time; m, month; NR, median OS was not reached; TKI, tyrosine kinase inhibitor. [a]Result of Cox univariate analysis of the wild-type group and the mutant group as dichotomous variables. [b]Cox univariate analysis of wild-type, 19-Del, L858R, G719X+L861Q and 20-Ins as polytomous variables. [c]Results of multivariable analysis with EGFR mutation types (wild-type, 19-Del, L858R, G719X+L861Q and 20-Ins) as a polytomous variable and other characteristics (including sex, age, smoking status, pTNM stage, nerve invasion, vascular invasion and recurrence) as binary variables. [d]Results of multivariable analysis using with or without 19-Del mutation as a binary variable and other characteristics (including sex, age, smoking status, pTNM stage, nerve invasion, vascular invasion and recurrence) also as binary variables. eG719X and L861Q mutation types of EGFR.

IAV, however, this was not statistically significant (26.9 months vs. 34.6 months, P=0.112; Fig. 1I). The present study has a number of limitations: ~95.4% specimens were collected from patients with IA. Furthermore, the majority of cases of lung adenocarcinoma were at an early stage. All patients who participated in the present study were treated in a single area (Yantai, China), which may cause selection bias. Furthermore, the histological categories of lung adenocarcinoma, including MIA, IA and IAV, were considered in the present study, but the histological subtypes of lung adenocarcinoma, including lepidic, acinar, papillary, micropapillary, solid and mucinous predominant subtypes, were not considered. A further study with a more even ratio of all lung adenocarcinoma subtypes and stages is required.

Sumiyoshi *et al* (39) indicated that nerve invasion and clinical recurrence could serve as independent predictors for patients with lung adenocarcinoma, and Matsumura *et al* (12) suggested that vascular invasion could also function as an independent predictor (40,41). In the present study, multivariate analysis demonstrated that nerve invasion, vascular invasion and clinical recurrence were independent predictors for patients with lung adenocarcinoma (Fig. 1E-G). Previous studies have suggested that high pTNM stage is associated with poor prognosis (41-43). In the present study, high pTNM stage was associated with a relatively short survival time, and pTNM stage was demonstrated to be a predictor of prognosis (Fig. 1D).

To conclude, the present study suggests that prognosis of patients with lung adenocarcinoma is associated with pTNM staging, nerve invasion, vascular invasion and EGFR mutation status. Patients exhibiting 19-Del were associated with a good prognosis compared with those exhibiting L858R following TKI targeted therapy. Overall, the present study demonstrated that EGFR mutation detection is conducive for selecting a favorable therapeutic regimen for patients with lung adenocarcinoma.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Science and Technology Program of Shandong Province (grant no. J15LK02) and the Scientific Research Project of Yantai (grant no. 2016ZH081).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

XZ, LC and JL were major contributors toward data collection, data analysis and manuscript writing. XH, YZ and HZ performed the histological examination of lung adenocarcinoma. BW, BL and PG, were responsible for manuscript preparation, study design, data analysis and article finalization. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethics approval was granted by the Medical Ethics Committee of Binzhou Medical University (reference no. 2012-37). Written informed consent was obtained from each participant.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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