

EGFR mutations subset in Chinese lung squamous cell carcinoma patients

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Abstract. Research has identified that epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) possess large benefits for adenocarcinoma (ADC), although little benefit for squamous cell carcinoma (SCC). The aim of the present study was to investigate the percentage of patients with SCC with the EGFR mutations subset and the benefits of EGFR TKIs in SCC. In the present study, the EGFR mutations subset was detected with an amplification refractory mutation system in 1,359 clinical SCC tissues. The association of the EGFR mutations subset with clinicopathological parameters was evaluated using the Mann-Whitney U test, and Kruskal-Wallis H. Kaplan-Meier survival analysis was used to estimate the effect of the EGFR mutations subset on SCC patient survival rates. A total of 94 out of 1,359 SCC patients were identified as having EGFR mutations, an EGFR mutation rate of 6.92%. The EGFR mutations subset in the 94 cases was identified as follows: 37.2% (35/94) in exon 19; 39.4% (37/94) in L858R; 5.3% (5/94) in T790M; 4.3% (4/94) in G719X; 2.1% (2/94) in L861Q; and 11.7% (11/94) in other mutations. Kaplan-Meier survival analysis identified that the differentiation, pathological tumor, node, metastasis stage, lymph node metastasis and distant metastases were significantly associated with

patients' survival ($P>0.05$; log-rank test), and no significant difference was observed between TKI therapy and chemotherapy in terms of patient survival rates ($P>0.05$). In addition, the overall discordant rate of the EGFR mutations subset in SCC patients was relatively low. Due to the non-significant difference between TKI therapy and chemotherapy in terms of patient survival and the lower discordance rate of the EGFR mutations subset in SCC patients, EGFR TKIs could be a recommended treatment for SCC.

Introduction

Epidermal growth factor receptor (EGFR), a trans-membrane glycoprotein, may be distinguished into three parts: an extracellular domain, a transmembrane domain and an intracellular domain, which possesses tyrosine kinase (TK) activity. Particular mutations of EGFR, for example EGFR exon 19 deletion and exon 21 point mutation, serve a key role in the development of non-small cell lung cancer (NSCLC), such as promoting cancer cell proliferation, differentiation, revascularization and metastasis (1). EGFR TK inhibitors (TKIs), mainly targeting EGFR TK activities, are able to effectively prolong the survival time of patients with cancer. In particular, a number of studies have demonstrated the efficacy of the two most widely available TKIs, erlotinib and gefitinib, in the first-line, maintenance and relapsed settings (2-4).

The response rate of EGFR mutation activation to EGFR TKI treatment reaches 70%; TKI treatment may additionally prolong the NSCLC patients' progression free survival (PFS) (5-8). EGFR exon 19 deletion (Del 19) and exon 21 point mutation (L858R), the main EGFR mutation activation, primarily exist in females, never-smokers, East Asians (~50%) and patients with lung adenocarcinoma (ADC, ~30%) (9-12). However, activation of EGFR mutations are rare in patients with squamous cell carcinoma (SCC) (<3%); the lack of reported mutations may limit the use of EGFR-TKIs in lung cancer patients with SCC (13-17). To date, the benefits of EGFR-TKIs in EGFR-mutated patients with SCC have not been well-studied.

In the present study, the mutation rate of SCC in all EGFR mutations was analyzed in our laboratory and the correlation of EGFR-mutations associated with SCC under clinicopathological parameters was conducted. A Kaplan-Meier survival analysis performed to estimate the effect of the EGFR mutations

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Abbreviations: EGFR, epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors; SCC, squamous cell carcinoma; ADC, lung adenocarcinoma; PFS, progression free survival; OS, overall survival; NSCLC, non-small cell lung cancer

Key words: epidermal growth factor receptor mutations, squamous cell carcinoma, non-small cell lung cancer, tyrosine kinase inhibitors therapy

in the survival rates of patient with SCC. Additionally, the potential implications of EGFR TKI treatment and the tumor biology of EGFR mutations in patients with SCC was investigated. The aim of the present study was to investigate the mutations of EGFR in lung SCC; the results of the present study may contribute to developments in treatments for lung SCC.

Materials and methods

Patients and samples. All SCC samples (tumor tissues, blood and pleural effusion) used in the present study were collected between 2010 and 2016 from clinical data or an archived thoracic oncology tissue repository at the Department of Thoracic Surgery of Tangdu Hospital affiliated with The Fourth Military Medical University (Xi'an, China; Fig. 1). Patients who had received preoperative chemotherapy, radiotherapy or EGFR-targeted therapy were excluded from the present study. Detailed clinicopathological information, including patient ages, sex, smoking history, tumor status, histological differentiation, nodal status, clinical manifestation, surgical method, postoperative treatment and follow-up information were collected and completed. The surgery day was considered to be the starting day for estimating postoperative survival time. The follow-up lasted until August 13, 2016, with a median follow-up period of 39.62 months (range, 2-63.28 months). The histological classification of the tumors was reviewed by pathologists. And all tumors were staged according to the pathological tumor, node, metastasis (pTNM) classification (7th edition) of the International Union against Cancer (18). The study protocol was approved by the Regional Ethics Committee for Clinical Research of The Fourth Military Medical University. All patients provided written informed consent for use of their medical records and tumor specimens for research purposes.

EGFR mutation testing. Genomic DNA was isolated and purified from fresh tumor specimens using TIANamp Genomic DNA kit (Taingen Biotech, Beijing, People's Republic of China) according to the manufacturer's instructions.

EGFR mutation analysis of DNA was performed using ADx-ARMS® technology, a technology based on amplified refractory mutation system (ARMS) (19). Quantitative polymerase chain reaction (qPCR) was conducted on the MX3005P qPCR system (Stratagene California; Agilent Technologies, Inc., Santa Clara, CA, USA) using the AmoyDx human EGFR Gene Mutation Detection kit (Amoy Diagnostics Co., Ltd., Xiamen, China) according to the manufacturer's protocols.

A total of 26 mutations in exon 18 (G719A, G719S, G719C), exon 20 (T790M, S768I), and exon 21 (L858R, L861Q), and exon 19 (deletions, n=19) were detected. The primer sequences of EGFR mutation testing were obtained from The Primer Express® Software v3.0.1 (Thermo Fisher Scientific, MA, Waltham, USA) was applied to design specific primers for these common mutations of the EGFR gene; the primer catalogue numbers of the 26 mutations of EGFR gene provided by the AmoyDx human EGFR Gene Mutation Detection kit are presented in Table I.

Table I. Primer sequences for quantitative polymerase chain reaction.

Primer	Catalogue number
18-F1	SEQ ID NO:1
18-F2	SEQ ID NO:2
18-F3	SEQ ID NO:3
18-R	SEQ ID NO:4
18-P	SEQ ID NO:5
19-F1	SEQ ID NO:6
19-F2	SEQ ID NO:7
19-F3	SEQ ID NO:8
19-F4	SEQ ID NO:9
19-F5	SEQ ID NO:10
19-P	SEQ ID NO:11
19-R	SEQ ID NO:12
20-F1	SEQ ID NO:13
20-F2	SEQ ID NO:14
20-F3	SEQ ID NO:15
20-F4	SEQ ID NO:16
20-F5	SEQ ID NO:17
20-P	SEQ ID NO:18
20-R	SEQ ID NO:19
21-F1	SEQ ID NO:20
21-F2	SEQ ID NO:21
21-P	SEQ ID NO:22
21-R	SEQ ID NO:23
CTRL-F	SEQ ID NO:24
CTRL-R	SEQ ID NO:25
CTRL-P	SEQ ID NO:26

The qPCR amplification program was performed as follows: Initial denaturation at 95°C for 5 min, 15 cycles of amplification (at 95°C for 25 sec, 64°C for 20 sec, and 72°C for 20 sec) and a final denaturation followed by 31 cycles of amplification (at 93°C for 25 sec, 60°C for 35 sec, and 72°C for 20 sec), and the FAM and HEX signals were collected at 60°C.

According to the manufacturer's protocols of the AmoyDx human EGFR Gene Mutation Detection kit described that samples were defined as EGFR mutation-negative when the Cq value ≥ 34 ; when the sample mutation Cq value < 31 , the samples were defined as EGFR mutation-positive. When the calculated ΔCq value [$\Delta Cq = Cq(\text{sample}) - Ct(\text{control})$], when ΔCq value $< \Delta Ct$ (Cut-off) was $31 \leq$ the sample mutation Cq value ≤ 33 , the samples were defined as EGFR mutation-positive, and when the ΔCq value $\geq \Delta Cq$ (Cut-off), the samples were defined as EGFR mutation-negative.

Discordance rate of EGFR mutations analysis. Patients with lung SCC (n=14) with EGFR activating mutations were used for discordance rate of EGFR mutations analysis. The tumor samples were fixed with 10% formaldehyde for 24 h at room temperature and embedded with paraffin. Sections were sliced to 4- μ m thickness, deparaffinized with a series of xylene and

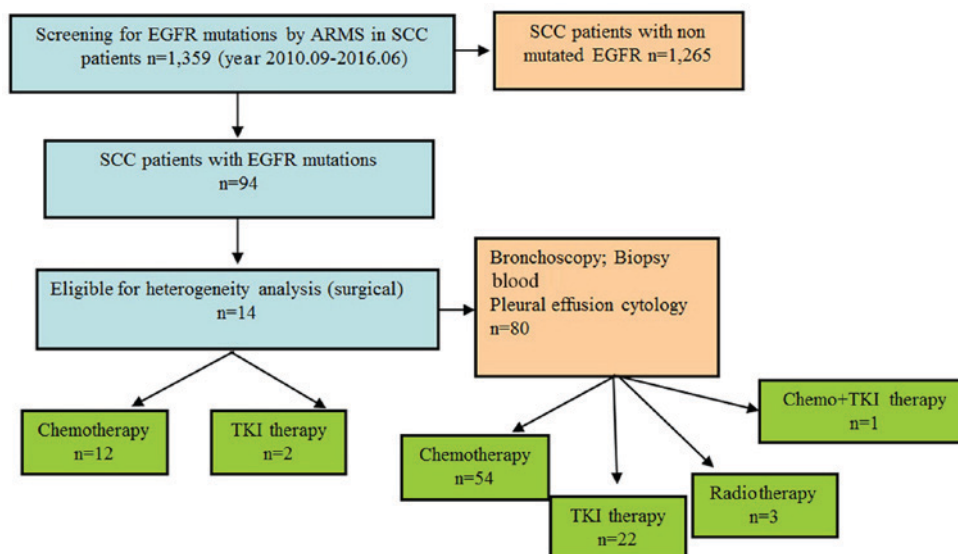


Figure 1. Enrolment and outcomes. ARMS, amplification refractory mutation system; SCC, squamous cell carcinoma; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

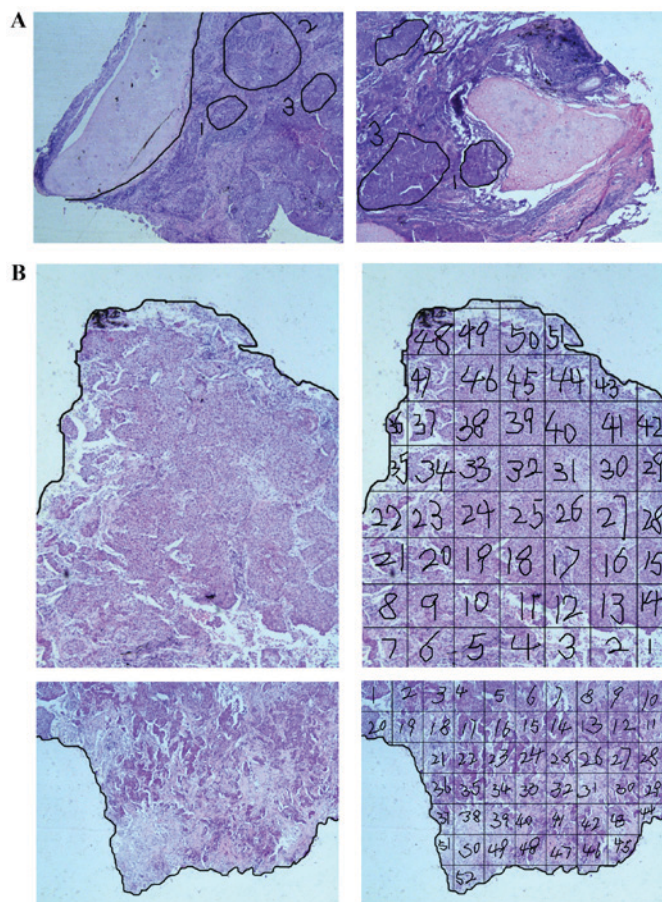


Figure 2. Distribution of the epidermal growth factor receptor mutation was examined in individual lung SCCs carrying the mutation. (A) First, three small areas from each of 14 lung SCCs were selected (magnification, x50). (B) Next, five SCCs were dissected into >100 pieces. The mutation status was assessed in all pieces, and identical mutation patterns were identified in each tumor (magnification, x50). SCC, squamous cell carcinoma.

rehydrated with a graded alcohol series. The sections were stained with hematoxylin for 40 sec, then counterstained with

eosin for 1 min at room temperature. Finally, the sections were dehydrated with a graded alcohol series and embedded in paraffin. Inclusion in the study was based on the presence of morphologically different tumor areas within the same tumor and a sufficient cancer cell content (>30%) in each defined area. Subsequently, three parts of each individual tumor were selected, and EGFR mutation detection was performed. For every tumor, three areas were identified by three pathologists to represent the most distinct and variable histological patterns (Fig. 2).

Similarly, each of five such SCCs was divided into >100 segments. The present study used two sections from each tumor. Thus, each section yielded 50 or more pieces, which were 3x3 mm on average (Fig. 2). The EGFR mutations of each piece were detected independently. To ensure that the results were collected in an unbiased manner, mutations were analyzed by a different technician from the one who had scratched the tissues (20).

Statistical analyses. The correlations of EGFR mutations with clinicopathological parameters were statistically analyzed using the Mann-Whitney U test, and Kruskal-Wallis H (mainly used to detect pathological differentiation). Kaplan-Meier survival analysis was used to estimate the effect of the type of EGFR mutation on the survival of patients with SCC. Statistical analyses were performed using SPSS software version 18.0 (SPSS, Inc., Chicago, IL, USA). P-values were adjusted for multiple testing, and $P > 0.05$ was considered to indicate a statistically significant difference.

Results

EGFR mutation. A total of 94 out of 1,359 patients with lung SCC had EGFR mutations (6.92%), and 1,265 patients did not. All EGFR mutations identified are present in Table II: Exon 19 (n=35, 37.2%); L858R (n=37, 39.4%); T790M (n=5, 5.3%); G719X (n=4, 4.3%); L861Q (n=2, 2.1%); and other mutations (n=11, 11.7%).

Table II. Tumors examined in the present study.

Variable	No. of tumors (n, %)	Exon 19 (%)	L858R (%)	T790M (%)	G719X (%)	L861Q (%)	Others (%)
EGFR-mutated SCC	94 (1,359, 6.92)	35 (37.2)	37 (39.4)	5 (5.3)	4 (4.3)	2 (2.1)	11 (11.7)
Trans-sectional analysis of number of areas in a tumor							
3	14 (94, 14.9)	4 (28.6)	7 (50)	2 (14.3)	0	0	1 (7.1)
100	5 (14, 35.7)	2 (40)	3 (60)	0	0	0	0

EGFR, epidermal growth factor receptor; SCC, squamous cell carcinoma.

Patient characteristics. The clinicopathological characteristics of the patients are summarized in Table III. In 94 SCCs with EGFR mutations, there were 18 female and 76 male patients, with a median age of 59 years (range, 36-84 years). Histopathological diagnoses included well-differentiated (10, 10.64%), moderate differentiation (60, 63.83%), and poor differentiation (24, 25.53%). Postoperative staging evaluation demonstrated stage I disease in 24 patients, stage II disease in 22 patients, stage III disease in 36 patients and stage IV disease in 12 patients. Metastatic sites included five brain metastases (5.3%), one liver metastasis (1.1%), eight bone metastases (8.5%), and one kidney metastasis (1.1%).

Clinical characteristics in smoker and non-smoker patients with lung SCC with EGFR mutation. When comparing smoker and non-smoker patients in terms of baseline clinical characteristics, significant differences were identified in sex (smoker vs. non-smoker: 100% male vs. 28%, respectively $P>0.001$), differentiation (smoker vs. non-smoker: 71.01% moderate vs. 44%; 18.84% poor vs. 44%, $P=0.036$) and pTNM stage (smoker vs. non-smoker: 55.1% I-II vs. 32%, $P=0.049$). There was no significant difference in age and lymph node metastasis (Table IV).

Clinical characteristics in patients with early- and advanced-stage lung SCC with EGFR mutation. When comparing patients with early (I-II) and advanced (III-IV) stage in baseline clinical characteristics, significant differences were identified in smoking history (patients with early vs. advanced stage: 82.6% smoker vs. 64.6%, $P=0.049$), differentiation (patients with early vs. advanced stage: 71.74% moderate vs. 56.25%; 8.7% poor vs. 41.67%, $P>0.001$) and lymph node metastasis (patients with early vs. advanced stage: 21.74% metastasis vs. 70.83%, $P>0.001$). There were no significant differences in age and sex (Table V).

Clinical characteristics in young, middle-aged, and elderly patients with lung SCC with EGFR mutation. When comparing young, middle-aged and elderly patients in terms of baseline clinical characteristics, no significant differences were identified in the clinical characteristics of the patients (Table VI).

Overall discordance rate of EGFR mutations in lung SCC. To determine the overall discordance rate of EGFR mutations,

Table III. Patient characteristics.

Patients (n=94)		
Age, years		
Median	59	
Range	36-84	
	No.	%
Sex		
Male	76	80.9
Female	18	19.1
Smoking history		
Smoker	69	73.4
Non-smoker	25	26.6
Differentiation		
Well	10	10.64
Moderate	60	63.83
Poor	24	25.53
pTNM stage		
I-II	46	48.9
III-IV	48	51.1
Primary tumor location		
Central	69	73.4
Peripheral	25	26.6
Primary tumor size, cm		
<4.2	45	47.9
≥4.2	49	52.1
Lymph node metastasis		
Yes	44	46.8
No	50	53.2
No. of metastatic sites analyzed		
Brain	5	5.3
Liver	1	1.1
Bone	8	8.5
Kidney	1	1.1

pTNM, pathological tumor, node, metastasis classification.

Table IV. Clinical characteristics in smoker and non-smoker patients with lung squamous cell carcinoma with EGFR mutation.

Variables	No. of cases, n=94 (%)	Smoker, n=69 (%)	Non-smoker, n=25 (%)	P-value
Age, years				0.913
<59	46 (48.9)	34 (49.3)	12 (48)	
≥59	48 (51.1)	35 (50.7)	13 (52)	
Sex				<0.001
Male	76 (80.9)	69 (100)	7 (28)	
Female	18 (19.1)	0 (0)	18 (72)	
Differentiation				0.036
Well	10 (10.64)	7 (10.15)	3 (12)	
Moderate	60 (63.83)	49 (71.01)	11 (44)	
Poor	24 (25.53)	13 (18.84)	11 (44)	
pTNM stage				0.049
I-II	46 (48.9)	38 (55.1)	8 (32)	
III-IV	48 (51.1)	31 (44.9)	17 (68)	
Lymph node metastasis				0.546
Yes	44 (46.8)	31 (44.9)	13 (52)	
No	50 (53.2)	38 (55.1)	12 (48)	

EGFR, epidermal growth factor receptor; pTNM, pathological tumor, node, metastasis classification.

Table V. Clinical characteristics in early stage and advanced stage lung squamous cell carcinoma patients with EGFR mutation.

Variables	No. of cases, n=94 (%)	TNM I-II, n=46 (%)	TNM III-IV, n=48 (%)	P-value
Age, years				0.149
<59	46 (48.9)	19 (41.3)	27 (56.3)	
≥59	48 (51.1)	27 (58.7)	21 (43.7)	
Sex				0.143
Male	76 (80.9)	40 (87)	36 (75)	
Female	18 (19.1)	6 (13)	12 (25)	
Smoking history				0.049
Smoker	69 (73.4)	38 (82.6)	31 (64.6)	
Non-smoker	25 (26.6)	8 (17.4)	17 (35.4)	
Differentiation				<0.001
Well	10 (10.64)	9 (19.56)	1 (2.08)	
Moderate	60 (63.83)	33 (71.74)	27 (56.25)	
Poor	24 (25.53)	4 (8.70)	20 (41.67)	
Lymph node metastasis				<0.001
Yes	44 (46.8)	10 (21.74)	34 (70.83)	
No	50 (53.2)	36 (78.26)	14 (29.17)	

EGFR, epidermal growth factor receptor; TNM, tumor, node, metastasis classification.

EGFR mutations in 14 SCCs were detected and analyzed. Three parts of each individual tumor were selected and examined for the EGFR mutations subset, and identical mutations were demonstrated in the three morphologically different tumor areas (Table VII).

As three parts may be insufficient to detect the discordance rate of the EGFR mutations, five tumors were dissected into

>100 pieces, and each piece was examined for EGFR mutations (Fig. 2). The results additionally revealed identical mutations throughout each individual tumor.

Clinical characteristic-associated prognosis of patients with lung SCC with EGFR mutations. Among clinico-pathological factors, including age, sex, smoking history,

Table VI. Clinical characteristics in young and elderly patients with lung squamous cell carcinoma with EGFR mutation.

Variables	No. of cases, ≤40 year of age, n=94 (%)	41-60 years of age, n=2 (%)	61-80 years of age, n=56 (%)	>80 years of age, n=33 (%)	>80 years of age, n=3 (%)	P-value
Sex						0.929
Male	76 (80.9)	0 (0)	48 (85.7)	25 (75.8)	3 (100)	
Female	18 (19.1)	2 (100)	8 (14.3)	8 (24.2)	0 (0)	
Smoking history						0.613
Smoker	69 (73.4)	0 (0)	45 (80.4)	21 (63.6)	3 (100)	
Non-smoker	25 (26.6)	2 (100)	11 (19.6)	12 (36.4)	0 (0)	
Differentiation						0.797
Well	10 (10.64)	0 (0)	7 (12.5)	3 (9.1)	0 (0)	
Moderate	60 (63.83)	1 (50)	37 (66.1)	19 (57.6)	3 (100)	
Poor	24 (25.53)	1 (50)	12 (21.4)	11 (33.3)	0 (0)	
pTNM stage						0.303
I-II	46 (48.9)	0 (0)	27 (48.2)	16 (48.5)	3 (100)	
III-IV	48 (51.1)	2 (100)	29 (51.8)	17 (51.5)	0 (0)	
Lymph node metastasis						0.108
Yes	44 (46.8)	2 (100)	28 (50)	14 (42.4)	0 (0)	
No	50 (53.2)	0 (0)	28 (50)	19 (57.6)	3 (100)	

EGFR, epidermal growth factor receptor; pTNM, pathological tumor, node, metastasis classification.

et al differentiation, pTNM stage and lymph node metastasis were significantly associated with patient survival rates. Patients with well or moderately differentiated tumors [n=70; 95% confidence interval (CI), 45.036-56.253 months] exhibited longer durations of survival compared with those with poorly differentiated tumors (n=24; 95% CI, 20.905-43.613 months; P=0.005) (Fig. 3A). Patients with pTNM I-II tumors (n=46; 95% CI, 49.091-60.002 months) exhibited a longer duration of survival compared with those with pTNM III-IV tumors (n=48; 95% CI, 29.621-45.614 months; P<0.001; Fig. 3B). Patients with no lymph node metastasis (n=50; 95% CI, 46.783-58.485 months) exhibited a longer duration of survival compared with those with lymph node metastasis (n=44; 95% CI, 30.236-46.535 months; P=0.005; Fig. 3C).

The prognosis of patients with lung SCC with EGFR mutations associated with distant metastases, EGFR mutations, and postoperative treatment (chemotherapy and EGFR TKI) were subsequently investigated. Patients with non-distant metastasis (n=79; 95% CI, 42.350-53.076 months) exhibited a longer duration of survival compared with those with distant metastasis (n=15; 95% CI, 19.069-47.515 months; P=0.014; Fig. 3D). A significant difference was not observed between patients with L858R (n=37; 95% CI, 41.678-57.284 months) and patients with Del 19 (n=35; 95% CI, 28.587-45.703 months; P>0.05; Fig. 3E). Additionally, a significant difference between patients with aged 41-60 years (n=56; 95% CI, 37.213-51.322 months) and patients with aged 61-80 years was not observed (n=33; 95% CI, 40.064-56.205 months; P>0.05; Fig. 3F). Furthermore, a significant difference was observed between patients treated with TKI (n=24; 95% CI, 33.099-51.624 months) and patients treated with chemotherapy (n=66; 95% CI, 38.160-51.387 months; P>0.05; Fig. 3G).

Discussion

ADC, SCC, and large-cell undifferentiated carcinoma are the principal subsets of non-small cell lung cancer (NSCLC), and approximately 20-30% of cases of NSCLC are SCC (22). Historically, the subtype of NSCLC has not been a major factor in determining patient therapy management, and there is not been well established regarding the fundamental difference in the molecular pathogenesis of ADC and SCC (23). It is only in recent years that driver oncogenes, including EGFR-activating mutations, and subsequent corresponding therapies have been identified (7,24-26). The majority of patients with NSCLC with EGFR mutations respond well to EGFR TKIs (including gefitinib and erlotinib). EGFR mutations are frequently observed in female, non-smoking, ADC and Asian patients, but rare in SCC (9-12). Research has identified that in pure SCC, there is the presence of fibroblast growth factor receptor 1, phosphatase and tensin homolog and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α /AKT serine/threonine kinase 1 mutations, and an absence of EGFR and KRAS proto-oncogene GTPase mutations (27). Compared with lung ADC, evidence about the efficacy of EGFR TKIs and treatment progress in patients with lung SCC is limited and controversial (4,28-30).

The present study performed ARMS analysis to investigate the EGFR mutations subset in clinical lung SCC samples. Statistical analysis revealed that 6.9% (94/1,359) of the tumor samples were EGFR-activating mutations. The EGFR mutated SCC samples were identified as follows: 37.2% (35/94) in exon 19; 39.4% (37/94) in L858R; 5.3% (5/94) in T790M; 4.3% (4/94) in G719X; 2.1% (2/94) in L861Q; and 11.7% (11/94) in other mutations (Table II). Due to the limited number in the

Table VII. Tumor cell content and epidermal growth factor receptor mutation status detected in the three histologically distinct tumor areas from each patient.

Case no.	Area	Tumor cell content, %	Predominant growth pattern ^a	Mutation
1	Tumor area 1	60	Keratinizing	Exon 19 del E746-A750
	Tumor area 2	60	Keratinizing	Exon 19 del E746-A750
	Tumor area 3	70	Keratinizing	Exon 19 del E746-A750
2	Tumor area 1	80	Non-keratinizing	Exon 21 L858R
	Tumor area 2	85	Non-keratinizing	Exon 21 L858R
	Tumor area 3	90	Keratinizing	Exon 21 L858R
3	Tumor area 1	55	Basaloid	Exon 21 L858R
	Tumor area 2	55	Warty	Exon 21 L858R
	Tumor area 3	50	Warty	Exon 21 L858R
4	Tumor area 1	60	Keratinizing	Exon 21 L858R
	Tumor area 2	65	Keratinizing	Exon 21 L858R
	Tumor area 3	65	Keratinizing	Exon 21 L858R
5	Tumor area 1	70	Basaloid	Exon 21 L858R
	Tumor area 2	75	Basaloid	Exon 21 L858R
	Tumor area 3	80	Basaloid	Exon 21 L858R
6	Tumor area 1	40	Basaloid	Exon 21 L858R
	Tumor area 2	50	Warty	Exon 21 L858R
	Tumor area 3	60	Basaloid	Exon 21 L858R
7	Tumor area 1	80	Keratinizing	Exon 19 del E746-A750
	Tumor area 2	70	Keratinizing	Exon 19 del E746-A750
	Tumor area 3	70	Non-keratinizing	Exon 19 del E746-A750
8	Tumor area 1	55	Basaloid	Exon 21 L858R
	Tumor area 2	45	Warty	Exon 21 L858R
	Tumor area 3	50	Basaloid	Exon 21 L858R
9	Tumor area 1	45	Keratinizing	Exon 20 Ins
	Tumor area 2	35	Keratinizing	Exon 20 Ins
	Tumor area 3	40	Keratinizing	Exon 20 Ins
10	Tumor area 1	70	Basaloid	Exon 21 L858R
	Tumor area 2	70	Basaloid	Exon 21 L858R
	Tumor area 3	70	Basaloid	Exon 21 L858R
11	Tumor area 1	55	Keratinizing	Exon 19 del E746-A750
	Tumor area 2	60	Keratinizing	Exon 19 del E746-A750
	Tumor area 3	60	Basaloid	Exon 19 del E746-A750
12	Tumor area 1	40	Non-keratinizing	Exon 19 del E746-A750
	Tumor area 2	30	Keratinizing	Exon 19 del E746-A750
	Tumor area 3	50	Keratinizing	Exon 19 del E746-A750
13	Tumor area 1	85	Warty	Exon 20 T790M
	Tumor area 2	85	Basaloid	Exon 20 T790M
	Tumor area 3	70	Basaloid	Exon 20 T790M
14	Tumor area 1	60	Non-keratinizing	Exon 20 T790M
	Tumor area 2	60	Keratinizing	Exon 20 T790M
	Tumor area 3	60	Keratinizing	Exon 20 T790M

^aPredominant growth pattern in analyzed area in accordance with the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society lung squamous cell carcinoma classification 2011 (21).

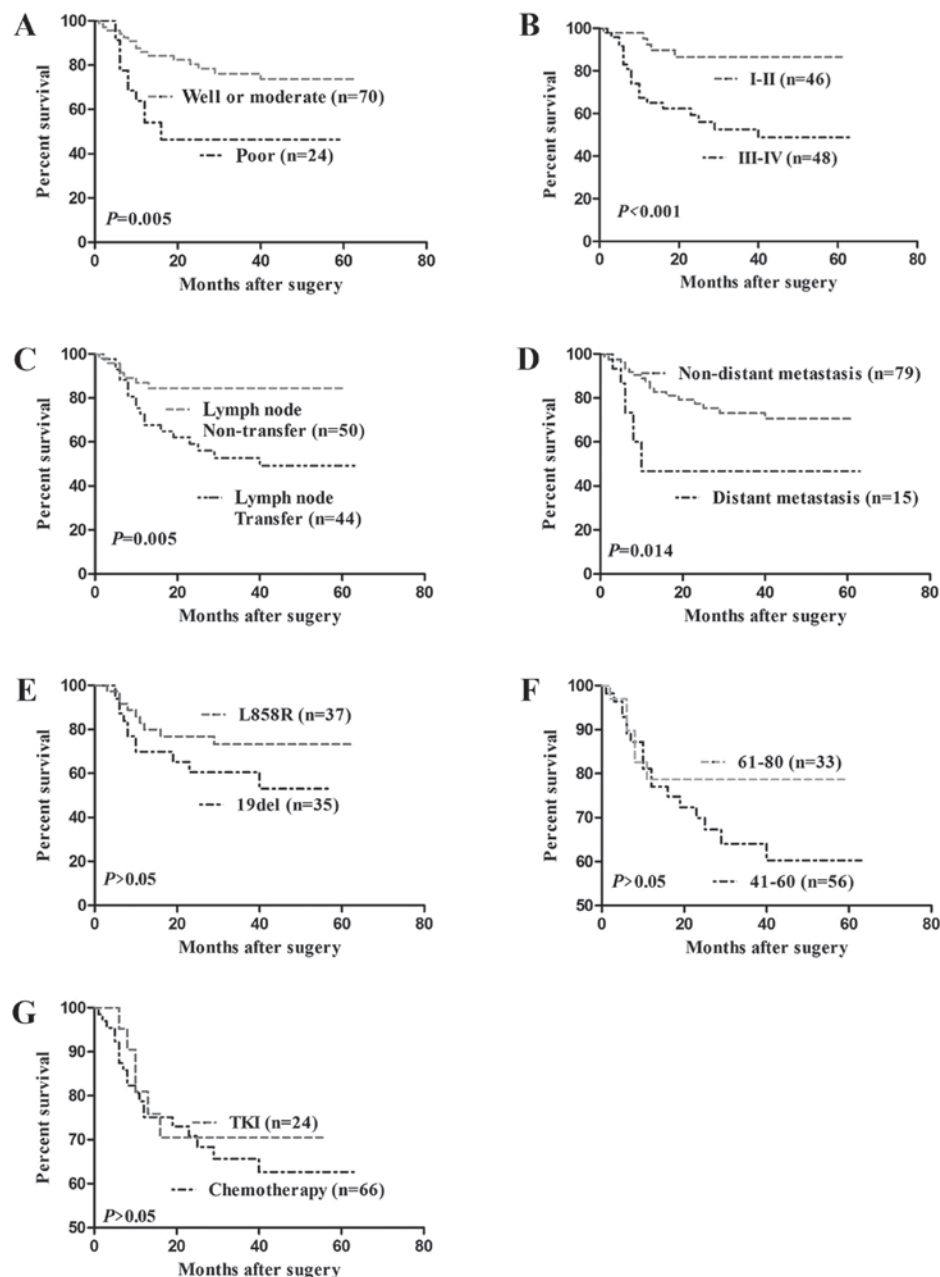


Figure 3. Kaplan-Meier survival analyses for patients with lung SCC. The P-value was determined using the log-rank test. (A) Comparison of OS between patients with well-differentiated or moderately and poorly differentiated lung SCC. (B) Comparison of the OS between patients with pTNM I/II and pTNM III/IV lung SCC. (C) Comparison of the OS between patients with lung lymph node non-metastatic and lymph node metastatic lung SCC. (D) Comparison of the OS between distant metastases and non-distant metastases of patients with lung SCC. (E) Comparison of the OS between patients with EGFR 19del and EGFR L858R lung SCC. (F) Comparison of the OS between young and elderly patients with lung SCC. (G) Comparison of the OS between different treatments in patients with lung SCC. OS, overall survival; pTNM, pathological tumor, node, metastasis classification; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; SCC, squamous cell carcinoma.

EGFR mutations subset, only the proportions of EGFR mutations in exon 19 (Del 19) and exon 21 (L858R) were larger (~76.6% of the total), although no significant difference in prognosis was observed between the EGFR Del 19 and L858R groups in SCC.

In the present study, there were significant differences between the smoking group, pTNM stage group and baseline clinical characteristics. Recently, along with extended life span, patients >80 years of age are increasing in number, and differences in prognosis are significant in the age range 28-30 years (31-33). However, due to the limited sample size,

no significant difference was observed between very young and very elderly patients.

Previous studies on the role of EGFR TKIs in SCC have identified that EGFR TKIs may be an option for the treatment of SCC, and the EGFR mutations subset may help to select a subgroup of patients with best response to TKIs (34-36). In the present study, among clinical characteristics, only the differentiation, pTNM stage, lymph node metastasis and distant metastases were significantly associated with patients' survival ($P>0.05$; log-rank test). The SCC patients identified as having EGFR activating mutations following surgery were

treated as follows: 70.2% (66/94) with chemotherapy; 25.5% (24/94) with EGFR TKIs; 3.2% (3/94) with radiotherapy; and 1.1% (1/94) with chemotherapy and EGFR TKIs. However, the difference in prognosis was not marked between the chemotherapy and TKI therapy groups.

There may be specific reasons to explain these results, and it was hypothesized that EGFR TKIs may prolong patient survival in a way comparable to the function of chemotherapy. However, the present study used a limited sample size, thus a study with an expanded sample size is required. In addition, EGFR TKIs are used for patients with EGFR mutation. Whether used in ADC or SCC, EGFR TKIs are recommended as long as the EGFR site is mutated. In the present study, the prognostic difference was not marked between the chemotherapy and the TKIs therapy groups, which indicated that EGFR TKIs were able to prolong patient survival in way comparable to the function of chemotherapy; therefore, it was hypothesized that EGFR TKIs may be an option for the treatment of SCC with EGFR mutations.

In certain individual tumors, EGFR mutations were not evenly distributed, and this may be one of the causes of drug-resistance to EGFR TKIs. However, in previous studies, the opposite results have been demonstrated in lung ADC (18,26). In the present study, identical EGFR mutations were identified throughout individual tumors by examining 14 tumors divided into three parts and five tumors divided into 100 parts. However, the limited sample size is a shortcoming of the present study, thus it is intended to expand the sample size in the future.

The results of the present study suggested that EGFR Del 19/L858R may be the main EGFR mutations subset in SCC. The effect of EGFR TKIs on SCC patients' prognoses is the same as the effect of chemotherapy, showing fewer complications and a higher quality of life, so EGFR TKIs could be a worthwhile option for the treatment of SCC. In addition, the heterogeneous distribution of EGFR mutations in SCC is extremely rare.

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Availability of data and materials

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

Authors' contributions

YS, XY and MW conducted the EGFR mutation test; JZ contributed to the follow-up; XW and JX interpreted the clinicopathological information statistics; YZ conducted the discordance rate of EGFR mutations analysis; and ZZ and XL performed statistical analysis. The manuscript was drafted

by YS and edited by XY. All authors read and approved the manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Regional Ethics Committee for Clinical Research of the Fourth Military Medical University. All patients provided written informed consent for use of their medical records and tumor specimens for research purposes.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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