Correlative analysis of gene mutation and clinical features in patients with non-small cell lung cancer

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Background: Lung cancer is the main reason for death associated with cancer all over the world. In most cases of non-small cell lung cancer (NSCLC), patients only express one type of gene mutation, each gene mutation population has different clinicopathological features, and each is expressed differently in different regions of the population. At present, there are few studies on multiple driver genes and clinicopathological features of the population in Hunan, China.

Methods: From February 2016 to December 2017, the Department of Geriatric Respiratory Medicine of Xiangya Hospital of Central South University diagnosed 113 cases of NSCLC. Genetic testing of next-generation sequencing (NGS) was completed, and it conformed to the inclusion criteria. All cases were pathologically confirmed as NSCLC, with the tumor staging being based on the 8th edition of TNM classification.

Results: In this study, we included a total of 113 NSCLC cases, including 78 males and 35 females. Histological distributions were mainly adenocarcinoma (ADC, 78.76%) and squamous cell carcinoma (SCC, 21.24%). We found 71 cases had gene-mutations. There was one concurrent mutation of *ALK* and *ROS1*, one concurrent mutation of epidermal growth factor receptor (*EGFR*) and *BRAF*, one concurrent mutation of *EGFR* and *MET*, one concurrent mutation of *MET* and *BRAF*, and one concurrent mutation of *EGFR* and *KRAS*; there were two cases of concurrent mutation of *EGFR* and *ERBB2*. The distribution of each of the mutated genes was as follows: *EGFR*, 62.82%; *ALK*, 8.97%; *ROS1*, 5.13%; *MET*, 5.13%; *ERBB2*, 5.13%; *RET*, 0.00%; *BRAF*, 2.56%; *KRAS*, 10.26%. Our study found that in patients with *EGFR* mutation, the mutation rate of males was 32.05%, and the rate in females was 68.57% (P<0.01); the mutation rate in people aged 60 or above was 40.00% while for those aged lower than 60 it was 46.55% (P>0.05); the mutation rate of ADC was 52.81% and in SCC was 8.33% (P<0.01); the mutation rate in smokers was 32.84% and in non-smokers was 58.70% (P<0.05); the mutation rate in patients of IV stage was 47.37% and the rate in patients of non-IV stage was 22.22% (P>0.05). Our study found that among patients with *ALK/ROS1/MET/ERBB2/BRAF/KRAS* mutations, the mutation rate in men was 7.69%, 2.60%, 3.85%, 2.56%, 0.00%, and 8.97% respectively, and for females it was 2.86%, 5.71%, 2.86%, 5.71%, 5.71%, and 2.86% respectively; the mutation rate in patients aged 60 and older was 3.64%, 5.45%, 3.64%, 1.82%, 1.82%, and 9.09% respectively; the rate in patients aged lower 60 was 8.62%, 1.72%, 3.45%, 5.17%, 1.72%, and 5.17% respectively; the mutation rate of ADC was 6.74%, 3.37%, 3.37%, 4.49%, 2.25%, and 6.74% respectively, while for SCC, it was 4.17%, 4.17%, 4.17%, 0.00%, 0.00%, and 8.33% respectively; the mutation rate in smokers was 8.96%, 1.49%, 4.48%, 1.49%, 0.00%, and 10.45% respectively, while in non-smokers, it was 2.17%, 6.52%, 2.17%, 6.52%, 4.35%, and 2.17% respectively; the mutation rate in patients of IV stage was 7.37%, 4.21%, 2.11%, 4.21%, 2.11%, and 7.37% respectively, and in patients of non-IV stage, it was 0.00%, 0.00%, 11.11%, 0.00%, 0.00%, and 5.56% respectively. In *ALK/ROS1/MET/ERBB2/BRAF/KRAS* mutations, there was no statistically significant difference in gender, age, tissue type, smoking history, and stage. Our research shows that the distribution of each mutant type of *EGFR* mutation was as follows: exon 2, 1/74 (1.35%); exon 4, 1/74 (1.35%); exon 6, 1/74

(1.35%); exon 18, 1/74 (1.35%); exon 19, 25/74 (33.78%); exon 20, 12/74 (16.22%); exon 21, 19/74 (25.68%); exon 22, 1/74 (1.35%); and *EGFR* amplification, 13/74 (17.57%).

Conclusions: (I) *EGFR* mutation was more common in non-smoking female patients with ADC and had no significant correlation with age and stage. (II) *EGFR* mutations were mainly concentrated in exon 19, 20, 21, and *EGFR* amplification. There was no significant statistical difference between mutations in exons 19, 20, 21, *EGFR* gene amplification and clinical features. (III) There was no statistically significant difference in the *ALK/ROS1/MET/ERBB2/BRAF/KRAS* mutations with gender, age, tissue type, smoking history, and tumor stage.

Keywords: Clinical analysis; gene mutation; individualized therapy

Introduction

Lung cancer is the main reason for death associated with cancer death all over the world (1). With the increase of morbidity and mortality, cancer has become the main cause of death and an important public health problem in China where cancer is the most common cancer (2). Nearly 733,300 new cases and 610,200 deaths of lung cancer were estimated to have occurred in China in 2015 (2).

More than 85% of lung cancer cases are non-small cell lung cancer (NSCLC) (3). In the 90s, patients with advanced NSCLC were usually treated with chemotherapeutic drugs, particularly platinum drugs, regardless of histological subtype (4). The criteria for treatment of NSCLC began to change at the beginning of this century. The discovery of epidermal growth factor receptor (*EGFR*) mutations in NSCLC laid the foundation for the development of targeted molecular therapies and predictive biomarkers (5-8). For instance, in advanced NSCLC patients with *EGFR* mutation-positive disease in Asia, afatinib significantly improves progression-free survival with tolerable and controlled safety (9). Also, Wang *et al.* found that erlotinib monotherapy is more cost-effective than platinum-based chemotherapy as first-line treatment of *EGFR* mutation-positive NSCLC patients in the Chinese health care system (10).

Currently, more and more mutations are being found driving the mechanisms of NSCLC, especially in lung adenocarcinoma (ADC). Common mutations in the *EGFR* include *KRAS*, *HER2*, *PIK3CA*, *BRAF*, and *MET* genes, while gene rearrangements in *ALK*, *ROS1*, and *RET* are also common. Molecular-targeted drug therapy has been established as superior to chemotherapy in the treatment of gene mutations in NSCLC (6,11-14). Seventy percent of Chinese patients with ADC in the study harbored one of the four gene mutations (*EGFR*, *KRAS*, *MET*, and *ALK* mutations) (15). Therefore, for the diagnosis and treatment of NSCLC, the NCCN guidelines have recommended testing for the gene mutations of *KRAS*, *ALK*, *ROS1*, *EGFR*, *ERBB2*, *BRAF*, *RET*, and *MET* (16).

Previous studies have suggested that most cases of NSCLC patients only express one type of gene mutation, each gene mutation population has different clinicopathological features, and that each is expressed differently in different regions of the population (17). Sequist *et al.* found that 51% of the 552 smoking Caucasians diagnosed with NSCLC had a gene mutation, with the most common being *KRAS* (24%), *EGFR* (13%), *PIK3CA* (4%), and *ALK* (5%) (18). Additionally, Sun *et al.*'s study found that 90% of 52 non-smoking Chinese lung ADC patients had *EGFR*, *KRAS*, *ALK*, or *HER2* gene mutations (19). There are also differences in the types of *EGFR* gene mutations in different regions. For example, in China, the *EGFR* gene mutation in patients in Kunming is mainly exon 19, while in Shanghai, the *EGFR* gene mutation is mainly in exon 19 and exon 21 (20).

At present, there are few studies on multiple driver genes and clinicopathological features of the population in Hunan, China. In this study, we aimed to analyze multiple mutation genes and clinical features of NSCLC patients in this region in order to learn its relevance.

Methods

Study subjects

The cases of this study were collected from Xiangya

Hospital of Central South University. It is a large, comprehensive, tertiary first-class hospital with multiple departments including those in geriatrics, respiratory medicine, thoracic surgery, oncology, pathology, etc.

The inclusion criteria for subjects were as follows: (I) aged 18 or above; (II) pathologically confirmed as NSCLC, using bronchoscopy, thoracoscopy, biopsy, surgical resection or pleural effusion; (III) underwent next-generation sequencing (NGS) to complete the detection of eight genes (*EGFR/ALK/ROS1/MET/ERBB2/RET/BRAF/KRAS*); (IV) had complete medical records.

From February 2016 to December 2017, the Department of Geriatric Respiratory Medicine of Xiangya Hospital of Central South University diagnosed 113 cases of NSCLC, for which NGS genetic testing was performed, conforming to the inclusion criteria. Tumor staging was based on the 8th edition of TNM classification.

Clinical, pathological feature collection

Patient demographics and clinical characteristics including age, gender, smoking history, clinical diagnosis, pathology, TNM staging, and gene mutation information were collected from the database system of Xiangya Hospital of Central South University.

DNA preparation for NGS

According to the manufacturer's instructions, tissue DNA was extracted and circulating cell-free DNA was recovered by using the QIAamp DNA FFPE tissue kit and the QIAamp Circulating Nucleic Acid kit (Qiagen, Valencia, CA, USA) respectively. DNA quantification was conducted using the Qubit 2.0 fluorimeter (Thermo Fisher Scientific, Waltham, MA, USA).

NGS library preparation

DNA shearing was conducted by using the Covaris M220 Focused Ultrasonicator (Covaris, Inc., Woburn, MA, USA), with subsequent end repair, phosphorylation, and adaptor ligation. Sized 200–400 bp fragments were selected by using the Agencourt AMPure XP Kit (Beckman Coulter, Fullerton, CA, USA), then hybridized with capture probes baits, selected with magnetic beads, and amplified with PCR. Indexed samples were sequenced with paired-end reads by using a Nextseq500 sequencer (Illumina, Inc., San Diego, CA, USA). All sample genetic profiles were assessed by capture-based targeted deep sequencing using the 8-gene panel that contained the oncogenic driver mutations of *KRAS*, *ALK*, *ROS1*, *EGFR*, *ERBB2*, *BRAF*, *RET*, and *MET*.

Sequence data analysis

The sequence data analysis was performed as described previously (21,22). Sequence data were mapped to the human genome (hg19) by using Burrows-Wheeler Aligner. Local alignment optimization, mark duplication, and variant calling were conducted by using Genome Analysis Tool Kit, Picard, and VarScan. Gene rearrangements were called with Fusion And Chromosomal Translocation Enumeration and Recovery Algorithm, and copy number variations were analyzed according to sequencing depth. Variants were filtered by using the VarScan FP filter pipeline, with loci with depth less than 100 being filtered out. In both plasma and tissue samples, at least 5 supporting reads were needed for insertions and deletions, and 8 supporting reads were needed for single nucleotide variants. Variants with a population frequency over 0.1% were grouped as single nucleotide polymorphisms that were excluded from further analysis, while the remaining variants were annotated with SnpEff v3.6 and ANNOVAR.

Statistical analysis

Our data were analyzed by GraphPad Prism 6.01 statistical software. Chi-square test and Fisher's test were used to compare the proportions. When the two-tailed P value was less than 0.05, the difference was considered significant.

Results

Clinical features in patients with NSCLC

In this study, we included a total of 113 NSCLC cases, composed of 78 males and 35 females. In addition, there were 55 cases aged 60 or above and 58 cases aged lower than 60. According to the 8th edition of TNM classification, there were 95 patients of stage IV and 18 patients of non-stage IV. In the 113 cases, 67 of these people had a history of smoking. Histological distribution was characterized by ADC (78.76%) and squamous cell carcinoma (SCC, 21.24%). We found 71 cases of gene mutations. There was one concurrent mutation of *ALK* and *ROS1*, one concurrent mutation of *EGFR* and *BRAF*, one concurrent mutation of *EGFR* and *MET*, one concurrent mutation of *MET* and

Table 1 Basic information and gene mutations of 113 patients

Clinicopathological features	Classification	Number of cases	Rate (%)
Gender	Male	78	69.03
	Female	35	30.97
Age	≥60	55	48.67
	<60	58	51.33
TNM staging	IV	95	84.07
	Non-IV	18	15.93
Smoking history	Yes	67	59.29
	No	46	40.71
Pathological type	ADC	89	78.76
	SCC	24	21.24
Mutant genes	*EGFR*	49	62.82
	ALK	7	8.97
	ROS1	4	5.13
	MET	4	5.13
	ERBB2 (Her2)	4	5.13
	RET	0	0.00
	BRAF	2	2.56
	KRAS	8	10.26

ADC, adenocarcinoma; SCC, squamous cell carcinoma.

Table 2 Gender distribution of gene-mutated patients

Mutated genes	Male		Female	
	Cases	Rate (%)	Cases	Rate (%)
EGFR	25	51.02	24	48.98
ALK	6	85.71	1	14.29
ROS1	2	50.00	2	50.00
MET	3	75.00	1	25.00
ERBB2 (Her2)	2	50.00	2	50.00
BRAF	0	0.00	2	100.00
KRAS	7	87.50	1	12.50

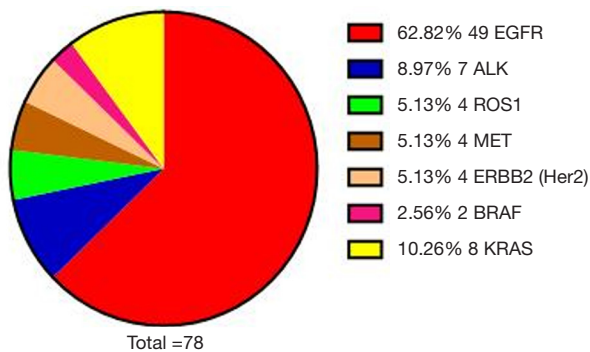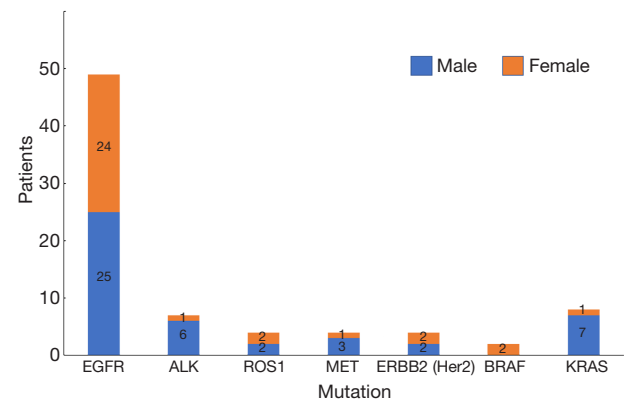**Figure 2** Gender distribution of gene-mutated patients.

Figure 1 Seventy-eight cases of gene mutation distribution.

BRAF, and one concurrent mutation of *EGFR* and *KRAS*; there were two cases for concurrent mutation of *EGFR* and *ERBB2*. The distribution of each of the mutated genes was as follows: *EGFR*, 62.82%; *ALK*, 8.97%; *ROS1*, 5.13%; *MET*, 5.13%; *ERBB2*, 5.13%; *RET*, 0.00%; *BRAF*, 2.56%; *KRAS*, 10.26% (*Table 1*, *Figure 1*).

Gender distribution of mutated NSCLC patients and gender differences in patients with mutated NSCLC

In our study, we found that 78 cases had genetic mutations, which included 45 males and 33 females. Among the patients with *EGFR* mutations, there were 25 males (51.02%) and 24 females (48.98%), in patients with *ALK* mutations there were 6 males (85.71%) and 1 female (14.29%), in patients with *ROS1* mutations there were 2 males (50.00%) and 2 females (50.00%), in patients with *MET* mutations there were 3 males (75.00%) and 1 female (25.00%), in patients with *ERBB2* (Her2) mutations there were 2 males (50.00%) and 2 females (50.00%), in patients with *BRAF* mutations there were 2 females (100.00%), and in patients with *KRAS* mutations there were 7 males (87.50%) and 1 female (12.50%) (*Table 2*, *Figure 2*). Our study showed that the total mutation rate was 53.85% in men and 82.86% in women (P<0.01), demonstrating that women are more susceptible to genetic mutations

Table 3 Gender differences in patients with mutations

Mutated genes	Male		Female		P value
	Cases	Mutation rate (%)	Cases	Mutation rate (%)	
Total	42	53.85	29	82.86	0.0061
EGFR	25	32.05	24	68.57	0.0003
ALK	6	7.69	1	2.86	0.4330
ROS1	2	2.60	2	5.71	0.5865
MET	3	3.85	1	2.86	1.0000
ERBB2 (Her2)	2	2.56	2	5.71	0.5865
BRAF	0	0	2	5.71	0.0940
KRAS	7	8.97	1	2.86	0.4313
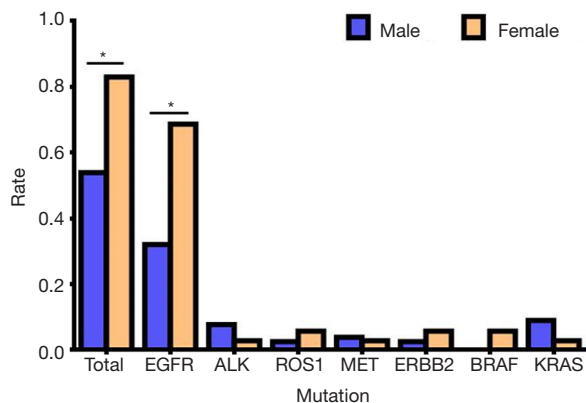
Table 4 Age distribution of gene-mutated patients

Mutated genes	≥60 years		<60 years	
	Cases	Rate (%)	Cases	Rate (%)
EGFR	22	44.90	27	55.10
ALK	2	28.57	5	71.43
ROS1	3	75.00	1	25.00
MET	2	50.00	2	50.00
ERBB2 (Her2)	1	25.00	3	75.00
BRAF	1	50.00	1	50.00
KRAS	5	62.50	3	37.50

Figure 3 Gender differences in patients with mutations. The difference of each ratio was tested with Chi-square or Fisher's; *, P<0.05 was considered statistically significant.
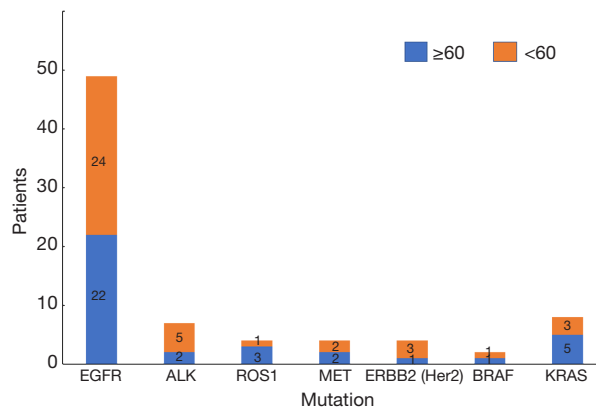

Figure 4 Age distribution of gene-mutated patients.

in NSCLC patients. Among the patients with *EGFR* mutations, the mutation rate was 32.05% in males and 68.57% in females (P<0.01), which proved that the female *EGFR* mutation rate was significantly higher than that of males, and there was a statistically significant difference. In the rest of the gene mutations, there was no significant difference in gender (P>0.05) (*Table 3*, *Figure 3*).

Age distribution in of mutated NSCLC Patients and age differences in patients with mutated NSCLC

Of the 113 total NSCLC cases, 78 people had genetic mutations, with 36 people aged 60 or above and 42 people aged lower than 60. Among patients with *EGFR* mutations,

there were 22 people aged 60 or above (44.90%) and 27 people less than 60 (55.10%); among the patients with *ALK* mutations, there were 2 people aged 60 or above (28.57%) and 5 patients aged lower than 60 (71.43%); among patients with *ROS1* mutations, there were 3 patients aged 60 or above (75.00%) and 1 patient aged lower than 60 (25.00%); among patients with *MET* mutations, there were 2 patients aged 60 or above (50.00%) and 2 patients aged lower than 60 (50.00%); among patients with *ERBB2* (Her2) mutations, there was 1 patient aged 60 or above (25.00%) and 1 patient aged lower than 60 (75.00%); among patients with *BRAF* mutations, there was 1 patient aged 60 or above (50.00%) and 1 patient aged lower than 60 (50.00%); among patients with *KRAS* mutations, there were 5 patients aged 60 or above (62.50%) and 3 patients aged lower than 60 (37.50%) (*Table 4*, *Figure 4*). Our data showed that the total mutation rate was 28.32% in patients aged 60 years and over, and the total mutation rate in patients under 60 years old was

Table 5 Age differences in patients with mutations

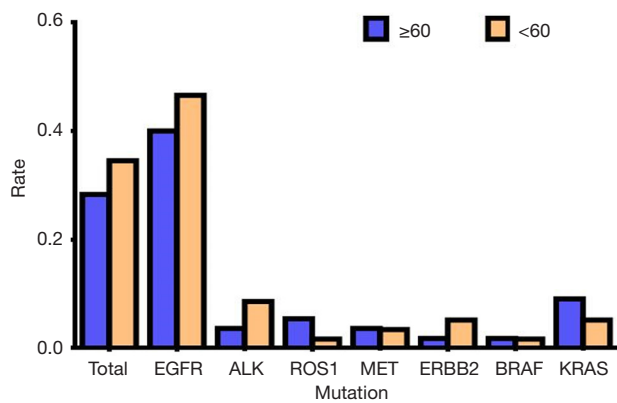| Mutated genes	≥60 years		<60 years		P value
	Cases	Mutation rate (%)	Cases	Mutation rate (%)	
Total	32	28.32	39	34.51	0.3192
EGFR	22	40.00	27	46.55	0.4824
ALK	2	3.64	5	8.62	0.4393
ROS1	3	5.45	1	1.72	0.3552
MET	2	3.64	2	3.45	1.0000
ERBB2 (Her2)	1	1.82	3	5.17	0.6188
BRAF	1	1.82	1	1.72	1.0000
KRAS	5	9.09	3	5.17	0.4823

Figure 5 Age differences in patients with mutations. The difference of each ratio was tested with Chi-square or Fisher's.

34.51% (P>0.05), suggesting that there was no significant correlation between the mutations detected and age. In addition, there was no significant correlation with age in *EGFR*, *ALK*, and *ROS1*, etc., mutations (P>0.05) (*Table 5*, *Figure 5*).

Pathological type distribution of mutated NSCLC Patients and pathological type differences in patients with mutated NSCLC

Of a total of 113 NSCLC cases, 78 people had genetic mutations, including 71 cases of ADC and 7 cases of SCC. In patients with *EGFR* mutations, there were 47 cases of ADC (95.92%) and 2 cases of SCC (4.08%); in patients with *ALK* mutations, there were 6 cases of ADC (85.71%) and 1 case of SCC (14.29%); in patients with *ROS1* mutations,

Table 6 Pathological type distribution in gene-mutated patients

Mutated genes	ADC		SCC	
	Cases	Rate (%)	Cases	Rate (%)
EGFR	47	95.92	2	4.08
ALK	6	85.71	1	14.29
ROS1	3	75.00	1	25.00
MET	3	75.00	1	25.00
ERBB2 (Her2)	4	100.00	0	0
BRAF	2	100.00	0	0
KRAS	6	75.00	2	25.00

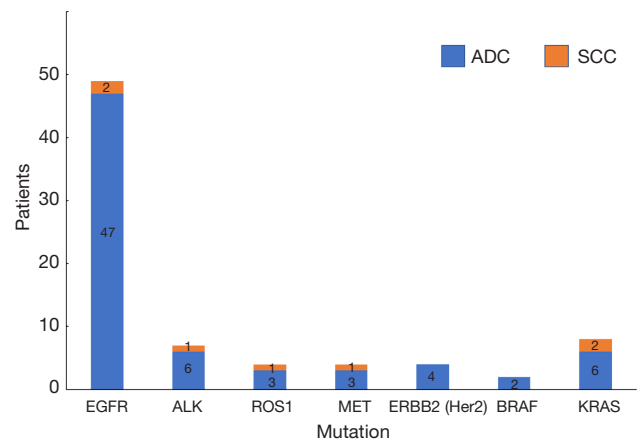ADC, adenocarcinoma; SCC, squamous cell carcinoma.

Figure 6 Pathological type distribution in gene-mutated patients. ADC, adenocarcinoma; SCC, squamous cell carcinoma.

there were 3 cases of ADC (75.00%) and 1 case of SCC (25.00%); in patients with *MET* mutations, there were 3 cases of ADC (75.00%) and 1 case of SCC (25.00%); in patients with *ERBB2* (Her2) mutations, there were 4 cases of ADC (100.00%); in patients with *BRAF* mutations, there were 2 cases of ADC (100.00%); in patients with *KRAS* mutations, there were 6 cases of ADC (75.00%) and 2 cases of SCC (25.00%) (*Table 6*, *Figure 6*). Our results showed that the total mutation rate of lung ADC was 57.52%, and the total mutation rate of lung SCC was 5.31% (P<0.01). The mutation rate of lung ADC proved to be significantly higher than in lung SCC. In patients with *EGFR* mutation, the mutation rate of ADC was 52.81%, the mutation rate of SCC was 8.33% (P<0.01). The mutation rate of *EGFR* in patients with lung ADC proved to be significantly higher than that found in patients with lung SCC. In other gene mutations, there was no significant difference in pathological types (P>0.05) (*Table 7*, *Figure 7*).

Table 7 Pathological type differences in patients with mutations

Mutated genes	ADC		SCC		P value
	Cases	Mutation rate (%)	Cases	Mutation rate (%)	
Total	65	57.52	6	5.31	<0.0001
EGFR	47	52.81	2	8.33	<0.0001
ALK	6	6.74	1	4.17	1.0000
ROS1	3	3.37	1	4.17	1.0000
MET	3	3.37	1	4.17	1.0000
ERBB2 (Her2)	4	4.49	0	0	0.5767
BRAF	2	2.25	0	0	1.0000
KRAS	6	6.74	2	8.33	0.6768

ADC, adenocarcinoma; SCC, squamous cell carcinoma.

Table 8 Smoking status in gene-mutated patients

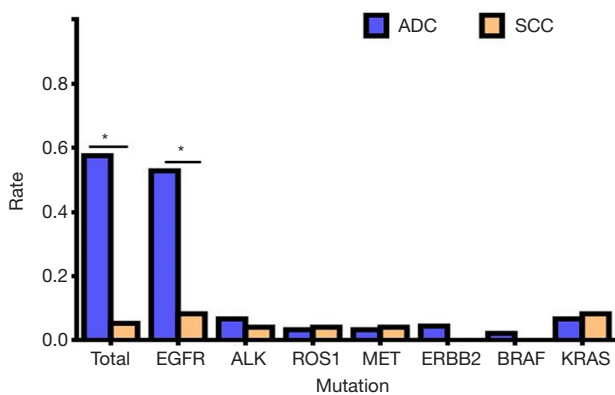| Mutated genes	Smoker		Non-smoker	
	Cases	Rate (%)	Cases	Rate (%)
EGFR	22	44.90	27	55.10
ALK	6	85.71	1	14.29
ROS1	1	25.00	3	75.00
MET	3	75.00	1	25.00
ERBB2 (Her2)	1	25.00	3	75.00
BRAF	0	0	2	100.00
KRAS	7	87.50	1	12.50

Figure 7 Pathological type differences in patients with mutations. The difference of each ratio was tested with Chi-square or Fisher's; *, P<0.05 was considered statistically significant. ADC, adenocarcinoma; SCC, squamous cell carcinoma.

Figure 8 Smoking status in gene-mutated patients.

Smoking status of mutated NSCLC Patients and smoking status differences in patients with mutated NSCLC

Of a total of 113 NSCLC cases, 78 people had genetic mutations including 40 smokers and 38 non-smokers. Among patients with *EGFR* mutations, there were 22 smokers (44.90%) and 27 non-smokers (55.10%); in patients with *ALK* mutations, there were 6 smokers (85.71%) and 1 non-smoker (14.29%); in patients with *ROS1* mutations, there was 1 smoker (25.00%) and 3 non-smokers (75.00%); in patients with *MET* mutations, there were 3 smokers (75.00%) and 1 non-smoker (25.00%); in patients with *ERBB2* (Her2) mutations, there was 1 smoker (25.00%) and 3 non-smokers (75.00%); in patients with *BRAF* mutations, there were 2

non-smokers (100.00%); in patients with *KRAS* mutations, there were 7 smokers (87.50%) and 1 non-smoker (12.50%) (*Table 8*, *Figure 8*). Our data analysis showed that the total mutation rate was 32.74% in smokers and 30.09% in non-smokers (P>0.05), indicating that the total incidence of gene mutations in NSCLC patients we detected was not significantly associated with smoking. However, among the patients with *EGFR* mutations, the mutation rate was 32.84% in smokers and 58.70% in non-smokers (P<0.01), demonstrating that non-smokers have significantly higher *EGFR* mutation rates than smokers. Among the other gene mutations, there was no significant difference between smokers and non-smokers (P>0.05) (*Table 9*, *Figure 9*).

Stage distribution of mutated NSCLC patients and stage differences in patients with mutated NSCLC

Of a total of 113 NSCLC cases, 78 people had genetic

Table 9 Smoking status differences in patients with mutations

Mutated genes	Smoker		Non-smoker		P value
	Cases	Mutation rate (%)	Cases	Mutation rate (%)	
Total	37	32.74	34	30.09	0.0685
EGFR	22	32.84	27	58.70	0.0064
ALK	6	8.96	1	2.17	0.2375
ROS1	1	1.49	3	6.52	0.3023
MET	3	4.48	1	2.17	0.6446
ERBB2 (Her2)	1	1.49	3	6.52	0.3023
BRAF	0	0	2	4.35	0.1636
KRAS	7	10.45	1	2.17	0.1389

Table 10 Stage distribution in gene-mutated NSCLC patients

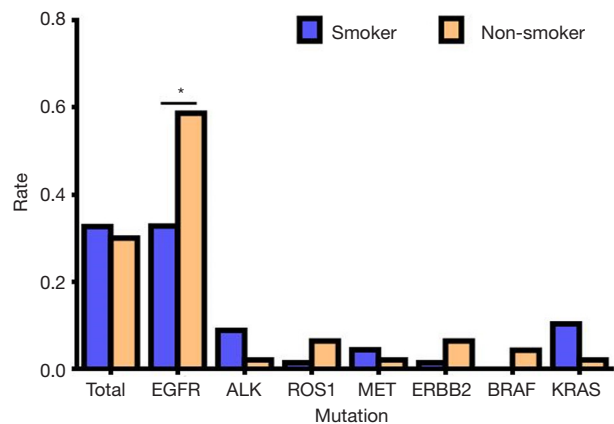| Mutated genes	IV		Non-IV	
	Cases	Rate (%)	Cases	Rate (%)
EGFR	45	91.84	4	8.16
ALK	7	100.00	0	0
ROS1	4	100.00	0	0
MET	2	50.00	2	50.00
ERBB2 (Her2)	4	100.00	0	0
BRAF	2	100.00	0	0
KRAS	7	87.50	1	12.50

Figure 9 Smoking status differences in patients with mutations. The difference of each ratio was tested with Chi-square or Fisher's; *, P<0.05 was considered statistically significant.
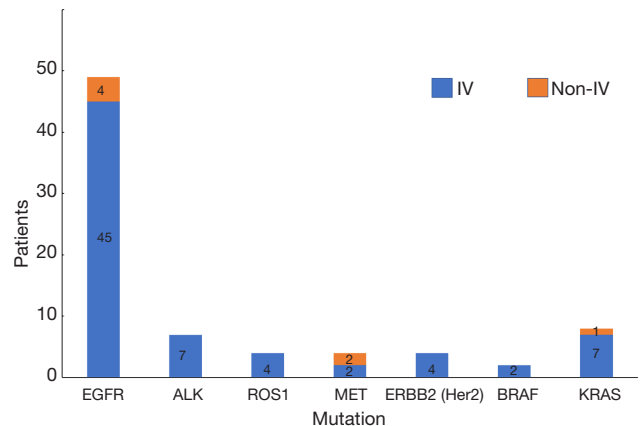

Figure 10 Stage distribution in gene-mutated NSCLC patients. NSCLC, non-small cell lung cancer.

Distribution of mutation types in NSCLC patients with EGFR gene mutation and association with clinical features

Of a total of 113 NSCLC cases, 78 cases had genetic mutations, with 49 of these being *EGFR* mutations. Among the 49 cases of *EGFR* mutation, there were 25 males (51.02%) and 24 females (48.98%). In addition, there were 22 people aged 60 or above (44.90%) and 27 people aged lower than 60 (55.10%). According to the 8th edition of TNM classification, there were 45 patients of stage IV (91.84%) and 4 patients of non-stage IV (8.16%). In 49 cases, 22 had a history of smoking. Histological distribution was characterized by 47 cases of ADC (95.92%) and 2 cases of SCC (4.08%). The distribution of each mutant type of *EGFR* mutation was as follows:

mutations, including 71 cases of stage IV and 7 cases of stage non-IV. In our study, the vast majorities of patients were advanced lung cancer cases, which might have been related to the source of the cases (*Table 10*, *Figure 10*). Our results showed that the total mutation rate was 57.52% in patients of stage IV and 5.31% in patients of the non-IV stage (P<0.05), demonstrating that the gene mutation rate in patients of stage IV was significantly higher than that in non-IV stage patients. In single gene mutations, there was no significant difference between stage IV and non-IV (P>0.05) (*Table 11*, *Figure 11*).

Table 11 Stage differences in patients with mutations

Mutated genes	IV		Non-IV		P value
	Cases	Mutation rate (%)	Cases	Mutation rate (%)	
Total	65	57.52	6	5.31	0.0105
EGFR	45	47.37	4	22.22	0.0864
ALK	7	7.37	0	0	0.5949
ROS1	4	4.21	0	0	1.0000
MET	2	2.11	2	11.11	0.1186
ERBB2 (Her2)	4	4.21	0	0	1.0000
BRAF	2	2.11	0	0	1.0000
KRAS	7	7.37	1	5.56	1.0000
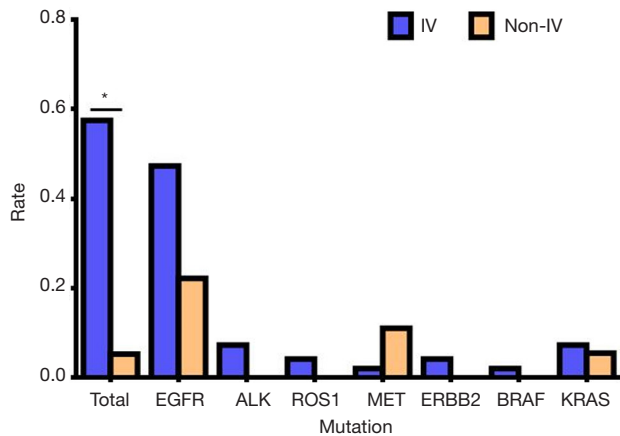
Figure 11 Stage differences in patients with mutations. The difference of each ratio was tested with Chi-square or Fisher's; *, P<0.05 was considered statistically significant.

Exon 2, 1.35%; exon 4, 1.35%; exon 6, 1.35%; exon 18, 1.35%; exon 19, 33.78%; exon 20, 16.22%; exon 21, 25.68%; exon 22, 1.35%; and *EGFR* amplification, 17.57%.

Clearly, mutations in *EGFR* are mainly concentrated in exons 19, 20, and 21. However, *EGFR* amplification cannot be ignored (*Table 12*, *Figure 12*).

Analysis of each EGFR mutation type

Since *EGFR* mutations are mainly concentrated in exons 19, 20, and 21 mutations and *EGFR* amplification, we mainly describe these types in detail. Of the exon 19 mutations of *EGFR*, males accounted for 13 of the total cases and females accounted for 12; there were 11 patients aged 60 or above

Table 12 Mutation types and basic information of *EGFR* gene mutations

Clinicopathological features	Classification	Cases	Rate (%)
Gender	Male	25	51.02
	Female	24	48.98
Age	≥60	22	44.90
	<60	27	55.10
TNM staging	IV	45	91.84
	Non-IV	4	8.16
Smoking history	Yes	22	44.90
	No	27	55.10
Pathological type	ADC	47	95.92
	SCC	2	4.08
EGFR gene mutations	Exon 2	1	1.35
	Exon 4	1	1.35
	Exon 6	1	1.35
	Exon 18	1	1.35
	Exon 19	25	33.78
	Exon 20	12	16.22
	Exon 21	19	25.68
	Exon 22	1	1.35
	EGFR amplification	13	17.57

ADC, adenocarcinoma; SCC, squamous cell carcinoma.
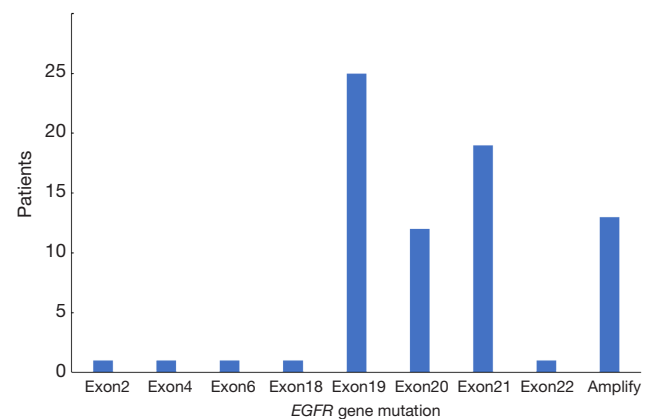
Figure 12 Mutation types of *EGFR* gene mutations.

and 14 patients aged lower than 60, and 25 patients were stage IV. Of the exon 19 mutations of *EGFR*, there were twelve patients who had a history of smoking, and all these

Table 13 Basic information of each mutation type of *EGFR*

Clinicopathological features	Classification	EGFR mutation types								
		Exon 2	Exon 4	Exon 6	Exon 18	Exon 19	Exon 20	Exon 21	Exon 22	Amplification
Gender	Male	1	1	0	1	13	6	9	1	7
	Female	0	0	1	0	12	6	10	0	6
Age	≥60	1	1	0	1	11	5	8	1	6
	<60	0	0	1	0	14	7	11	0	7
TNM staging	IV	1	1	1	0	25	12	16	1	11
	Non-IV	0	0	0	1	0	0	3	0	2
Smoking history	Yes	1	1	0	1	12	5	7	1	8
	No	0	0	1	0	13	7	12	0	5
Pathological type	ADC	1	1	1	0	25	12	19	1	12
	SCC	0	0	0	1	0	0	0	0	1

ADC, adenocarcinoma; SCC, squamous cell carcinoma.

cases were ADC. In exon 20 mutations of *EGFR*, males accounted for 6 of the total cases and females accounted for 6; there were 5 people aged 60 or above and 7 people aged lower than 60, and 12 patients of stage IV. In exon 20 mutations of *EGFR*, there were five patients who had a history of smoking, and all these cases were ADC. In exon 21 mutations of *EGFR*, males accounted for 9 of the total cases and females accounted for 10; there were 8 people aged 60 or above and 11 people aged lower than 60, and 16 patients of stage IV. In exon 21 mutations of *EGFR*, there were seven patients who had a history of smoking, and all these cases were ADC. In *EGFR* amplification mutations of *EGFR*, males accounted for 7 of the total cases, and females accounted for 6; there were 6 people aged 60 or above and 7 aged lower than 60, and 11 patients of stage IV. In *EGFR* amplification mutations of *EGFR*, there were eight patients who had a history of smoking, and only one case was SCC. According to the above data, we found that these types of mutations in *EGFR* had no apparent association with gender, age, and smoking history. Also, these types of mutations in *EGFR* are almost all advanced ADC (*Table 13*). Our results suggest that there is no statistically significant difference between exon 19, 20, and 21 mutations and *EGFR* gene amplification and clinical features (P>0.05) (*Tables 14-18*, *Figures 13-17*).

Discussion

Lung cancer is the most common malignancy in the world

and is the leading cause of cancer death (23). Eighty-five percent of all lung cancer cases are NSCLC (24). Most patients with NSCLC were diagnosed as an advanced stage and lost the opportunity for radical surgery, yet targeted therapy based on gene mutations has significantly improved progression-free survival in patients with advanced NSCLC (25). In recent years, the improvement of gene detection efficiency and the application of tyrosine kinases inhibitors (TKIs) have greatly improved the therapeutic effect of lung cancer (26). Previous studies have suggested that most cases of NSCLC patients only express one type of gene mutation, each gene mutation population has different clinicopathological features, and each is expressed differently in different regions of the population (17). This study analyzed multiple gene mutations and clinical features of NSCLC patients in the Hunan region to learn its relevance. In our study, we found that of a total of 113 NSCLC cases, 78 people had genetic mutations. The distribution of each of the mutated genes was as follows: *EGFR*, 62.82%; *ALK*, 8.97%; *ROS1*, 5.13%; *MET*, 5.13%; *ERBB2*, 5.13%; *RET*, 0.00%; *BRAF*, 2.56%; *KRAS*, 10.26%. It is clear from the data that *EGFR* mutations are the most common.

Xiao *et al.* found that the rate of *EGFR* mutations in women was significantly higher than that in men and that the *EGFR* mutation rate in ADC was significantly higher than that in SCC (27). Paez *et al.* analyzed the mutation and clinical features of 119 patients with NSCLC, finding that *EGFR* mutations in ADC were higher than in other

Table 14 Gender differences in *EGFR* gene mutation types

Gene mutation types	Male		Female		P value
	Cases	Mutation rate (%)	Cases	Mutation rate (%)	
Exon 19	13	52.00	12	50.00	0.8887
Exon 20	6	24.00	6	25.00	0.8019
Exon 21	9	36.00	10	41.67	0.9095
Amplification	7	28.00	6	25.00	0.9316

Table 15 Age differences in *EGFR* gene mutation types

Gene mutation types	≥60 years		<60 years		P value
	Cases	Mutation rate (%)	Cases	Mutation rate (%)	
Exon 19	11	50.00	14	51.85	0.8974
Exon 20	5	22.73	7	25.93	0.9402
Exon 21	8	36.36	11	40.74	0.9856
Amplification	6	27.27	7	25.93	0.8266

Table 16 Stage differences in *EGFR* gene mutation types

Gene mutation types	IV		Non-IV		P value
	Cases	Mutation rate (%)	Cases	Mutation rate (%)	
Exon 19	25	55.56	0	0	0.0502
Exon 20	12	26.67	0	0	0.5599
Exon 21	16	35.56	3	75.00	0.2848
Amplification	11	24.44	2	50.00	0.2839

Table 17 Smoking status differences in *EGFR* gene mutation types

Gene mutation types	Smoker		Non- smoker		P value
	Cases	Mutation rate (%)	Cases	Mutation rate (%)	
Exon 19	12	54.55	13	48.15	0.8742
Exon 20	5	22.72	7	25.93	0.9402
Exon 21	7	31.82	12	44.44	0.5435
Amplification	8	36.36	5	18.52	0.2792

Table 18 Pathological type differences in *EGFR* gene mutation types

Gene mutation types	ADC		SCC		P value
	Cases	Mutation rate (%)	Cases	Mutation rate (%)	
Exon 19	25	53.19	0	0	0.2347
Exon 20	12	25.53	0	0	1.0000
Exon 21	19	40.43	0	0	0.5153
Amplification	12	25.53	1	50.00	0.4643

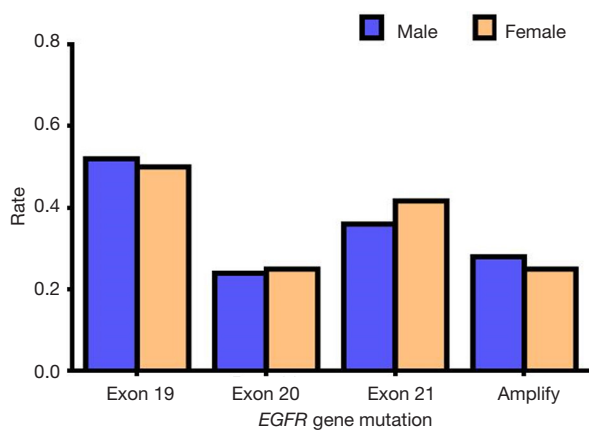ADC, adenocarcinoma; SCC, squamous cell carcinoma.

Figure 13 Gender differences in *EGFR* gene mutation types. The difference of each ratio was tested with Chi-square or Fisher's.
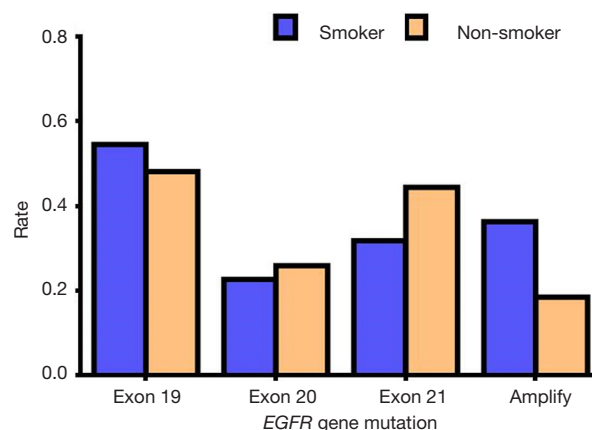

Figure 16 Smoking status differences in *EGFR* gene mutation types. The difference of each ratio was tested with Chi-square or Fisher's.
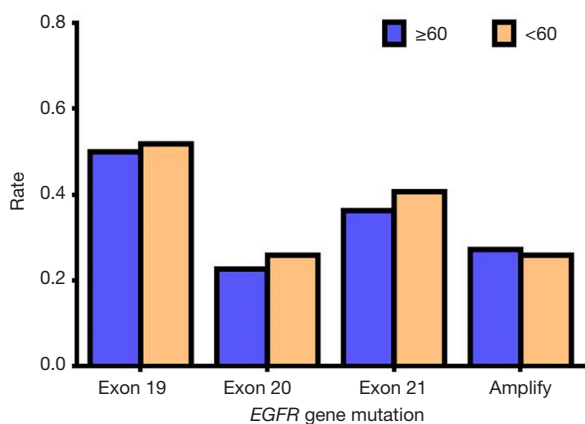

Figure 14 Age differences in *EGFR* gene mutation types. The difference of each ratio was tested with Chi-square or Fisher's.
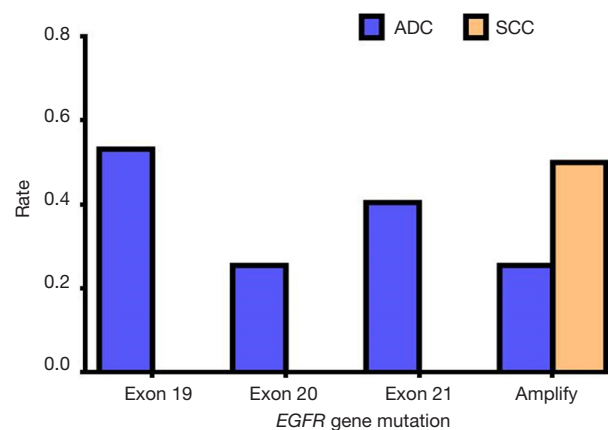

Figure 17 Pathological type differences in *EGFR* gene mutation types. The difference of each ratio was tested with Chi-square or Fisher's. ADC, adenocarcinoma; SCC, squamous cell carcinoma.
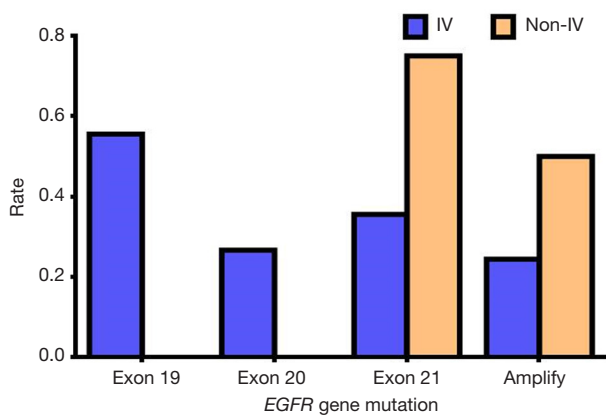

Figure 15 Stage differences in *EGFR* gene mutation types. The difference of each ratio was tested with Chi-square or Fisher's.

pathological types, with the chance of *EGFR* mutation is more frequent in women than in men (7). In addition, past studies have shown that in the factor analysis of smoking status, non-smokers *EGFR* mutation rate was 38.2%, which was far higher than the smokers mutation rate of 10.1%; *EGFR* mutation and the age of the patients with lung cancer and clinical stage had no obvious relation in these studies (28). As shown in our study, *EGFR* mutations were common in non-smoking women with ADC and had no obvious correlation with age. This is consistent with previous research showing that *EGFR* has the same mutation characteristics in different regions of the population. However, in our study, the mutation rate was higher in

Transl Cancer Res 2019;8(3):736-751 | http://dx.doi.org/10.21037/tcr.2019.04.10

patients with stage IV. This differs from previous studies and may be related to the source of the cases.

ALK-mutated NSCLC accounts for 2% to 5% of all NSCLC cases (29). Compared with NSCLC patients with no gene mutation, *ALK*-positive NSCLC patients tend to be mild or never-smokers, young, male, and in a more advanced stage or stage IV disease. In histology, the NSCLC cases with *ALK* mutations are more frequent in ADC (30-32). As shown in our study, *ALK* mutations were more likely to occur in young men with stage IV of ADC. These results are similar to previous findings, but there is no significant difference, which may be related to the small sample size. *ALK* mutation cases tended to be smokers, which is different from previous studies and may be related to the sample size and regional population.

The mutation rate of *ROS1* in NSCLC was 1.8% (33). *ROS1* mutation usually occurred in ADC and was mainly observed in young, non-smoking, advanced stage, female patients (33-35). Our results were similar with previous studies of *ROS1* mutations mainly observed in non-smoking, stage IV, female patients. However, in terms of age and organization type, our data demonstrated that more *ROS1* mutations were detected in patients who were older or had SCC. There is no significant difference in the above data, which may be related to the small number of *ROS1* mutation cases.

As for NSCLC, until 2007, there was only one report on the amplification of the *MET* gene (36). Recent studies have shown that NSCLC patients also report a similarly low incidence of *MET* gene amplification (1.4–11.1%) (37). However, a study from France showed that the incidence of the *MET* gene mutation was much higher (21%) (38). *MET* mutations are common in patients with advanced lung cancer. In another study, the *MET* mutations were found not to be significantly correlated with gender and age and were more prone to be SCC and smokers, although the differences were not significant (39). Similarly to this study, our results indicate that *MET* mutations are not significantly correlated with gender, age, pathological type, and smoking. This suggests that there may be no correlation between the *MET* mutations and these clinical features, or that the sample size of the *MET* mutation rate is low. The results of our study differ from previous studies in terms of the effects of staging on *MET* mutations, which may be related to our sample size.

Tomizawa *et al.* found that *HER2* mutations are more common in women, non-smokers, and ADC (40). And Sonobe *et al.* found that *ERBB2* mutations are more

common in older, non-smoking, female, ADC, and early-stage cases (41). Our results showed that *ERBB2* mutations were more common in women, ADC, and non-smokers, although there was no statistical difference. This is the same as the previous study. The lack of a significant difference can be attributed mainly to the low mutation rate of *ERBB2* and the small number of cases. In our results, the more frequent *ERBB2* mutations in young, late-stage lung cancer patients may be related to our small sample size or regional differences in population.

The frequency of *BRAF* mutations in NSCLC is about 4% (42,43). *BRAF* mutation is mainly found in ADC patients and was not significantly different in age, sex distribution, smoking history, and staging (44). Our results showed that *BRAF* mutation was not significantly correlated with gender, age, smoking history, and stage. This is the same as the previous study. The difference is that our study showed no significant difference in pathological types from *BRAF* mutations, but our two *BRAF* mutations are ADC cases and are considered to relate with a small number of cases. In short, it proves the clinical situation of *BRAF* mutation, and it proves the low mutation rate of *BRAF*.

Zhao *et al.* found that *KRAS* mutations were more common in men with ADC (45), while another study showed that *KRAS* mutations are more common in smokers (46). Our results showed that *KRAS* mutations are more common in men, smokers, older ages, SCC and advanced lung cancer cases, but none showed a statistical difference. Of these factors, male sex and smoking were consistent with other studies in their relation to *KRAS* mutation. In the type of pathology, age, and staging were different from other studies. It may be related to our study population comes from people who have already done genetic testing.

The most common *EGFR* mutations occur in exon 19 (about 46% of *EGFR* mutations), exon 20 (about 9% of *EGFR* mutations), and exon 21 (about 39% of *EGFR* mutations). The remaining 6% of the mutations occur mainly in exon 18 and other exons (47,48). *EGFR* amplification, as a kind of *EGFR* mutation, may be related to the progress of lung cancer (49). Soh *et al.* found that gene amplification may be related to the pathogenesis of advanced cancer (50). Zhang *et al.* found that the incidence of *EGFR* amplification in *EGFR* mutations was about 18%, and *EGFR* gene mutations occurred more frequently in samples that were accompanied by gene amplification than in those with high polysomy (51). Similar to previous studies, mutations in *EGFR* were mainly concentrated in exons 19, 20, and 21. Furthermore, *EGFR* amplification

accounting for 17.57% is a type of mutation that cannot be ignored. It can serve as an important target for targeted therapy for NSCLC. Finally, there was no statistically significant difference between the mutations of exon 19, 20, 21, gene amplification and clinical features.

Conclusions

(I) *EGFR* mutation was more common in non-smoking female patients with ADC and had no significant correlation with age and stage. (II) *EGFR* mutations were mainly concentrated in exon 19, 20, and 21 and *EGFR* amplification. There was no significant statistical difference between mutations in exons 19, 20, 21, *EGFR* gene amplification and clinical features. (III) There was no statistically significant difference in the *ALK/ROS1/MET/ERBB2/BRAF/KRAS* mutations with gender, age, tissue type, smoking history, and tumor stage.

Acknowledgments

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.04.10). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent from all patients and the approval from the Medical Ethics Committee of Xiangya Hospital of Central South University have been obtained (the ID number is 201512547).

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