

## RESEARCH ARTICLE

# Prevalence and Clinical Profile of EGFR Mutation In Non-Small-Cell Lung Carcinoma Patients in Southwest China

Juan Zhou, Xing-Bo Song, He He, Yi Zhou, Xiao-Jun Lu\*, Bin-Wu Ying\*

## Abstract

**Aims:** To investigate the distribution of epidermal growth factor receptor (EGFR) mutations, and explore any relationships with clinical characteristics in non-small-cell lung carcinoma (NSCLC) patients. **Materials and Methods:** EGFR mutations were assessed by ADx-ARMS in 261 NSCLC patients from West China Hospital of Sichuan University. Relationships between EGFR mutation and clinical characteristics were analyzed by SPSS. **Results:** The EGFR mutation rate was 48.7% (127/261), 19-del and L858R mutations occurred predominantly, accounting for 33.1% and 40.9%, respectively, in mutated cases. Moreover, 10.2% patients were found to carry double mutations. EGFR mutations occurred more frequently in women (57.5%) than in men (41.8%) ( $P=0.01$ ), and were more frequent in non-smokers (61.2%) than in former or current smokers (31.2%) ( $P<0.00$ ). In addition, they were more common in adenocarcinomas (52.8%) and adenosquamous carcinomas (42.8%) than in squamous cell carcinomas (14.8%) ( $p<0.00$ ). However, only smoking history and pathological types, rather than gender, proved to be associated with EGFR mutations on multivariate logistic regression analysis. No significant differences in pathological stage and metastasis status were found between EGFR wild-type and mutated cases, although EGFR mutation type was related to pathological type ( $p=0.00$ ) - 19-del, L858R and other mutation types respectively occurred in 34.2%, 42.5% and 23.3% of adenocarcinomas, but in 14.3%, 0% and 85.7% of non-adenocarcinomas. **Conclusions:** The EGFR mutation rate was 48.7% in NSCLCs in Southwest China, so that nearly 40% patients might benefit from targeted therapies. Smoking status and pathological types were independent predictors of EGFR mutation, while EGFR mutation type was related to only pathological type, rather than smoking status.

**Keywords:** Non-small-cell lung carcinoma - mutation - epidermal growth factor receptor - Southwest China

*Asian Pac J Cancer Prev*, 17 (3), 965-971

## Introduction

Lung cancer is one of the most common cancers in the world, with sharply increasing morbidity and mortality in the last decades. It was estimated that about 1.8 million new lung cancer cases occurred in 2012 worldwide by GLOBOCAN, occupied 13% of all cancers, with only 15% five-year survival rate. It has become the leading cause of cancer death among males in the whole world, and has surpassed breast cancer as the leading cause of cancer death among females in more developed countries (Torre et al., 2015). Even worse, the morbidity and mortality were both higher in China (National Office for Cancer Prevention and Control, 2010; She et al., 2013). According to WHO classification of lung cancer, non-small-cell lung cancer (NSCLC) is the main type of lung cancer, occupied 80-85%, including Adenocarcinoma, squamous carcinoma and adenosquamous carcinoma, et al. NSCLC is a cancer with high malignancy, 30-40% cases are diagnosed at the advanced stage, without the opportunity of operation, and the effect of chemotherapy

is limited. With the development of molecular mechanism research in lung cancer, some gene mutations and genetic recombinations were found to be involved in NSCLC pathogenesis, including KRAS, EGFR, ALK, ROS1, et al, which provided new treatment protocols, pushing NSCLC into a new era of targeted therapy (Pao and Hutchinson, 2012).

EGFR has become a hotspot target in NSCLC therapy development in recent years. EGFR gene locates in the 7p12-14 region, encoding a member of the receptor tyrosine kinase (RTK) family, which forms receptor heterologous or homologous dimers on cell surface after combination with corresponding ligands, leading to specific tyrosine residues phosphorylation, to regulate PI3K/AKT, ERK/MAPK and STAT pathways, and finally participate in cell proliferation, apoptosis and angiogenesis (Eck and Yun, 2010; Yasuda et al., 2012). Over expression of EGFR or EGFR ligand, activation by EGFR mutation were reported to induce carcinogenesis, and the latter is the major cause of abnormal biological behavior of tumor cells, which could enhance the effect of receptor and

Department of Laboratory Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan Province, China \*For correspondence: [bwydoc@163.com](mailto:bwydoc@163.com)

prolong the function duration (Lynch et al., 2004). EGFR tyrosine kinase inhibitors (TKIs) such as Gefitinib and Erlotinib, could competitive inhibit combination of ATP with kinase catalytic site in EGFR intracellular region, thus inhibit EGFR tyrosine kinase activity, blocking EGFR signal pathway, and finally target cancer cells. Previous researches indicated that EGFR-TKIs could significantly prolong PFS and OS in patients with EGFR mutation (Shepherd et al., 2005; Mok et al., 2009). IPASS (Iressa Pan-Asian Study) reported that the response rate in the patients with gefitinib was much higher than those with carboplatin–paclitaxel (71.2% versus 47.3%) in the mutation-positive subgroup, while much lower (1.1% versus 23.5%) in the mutation-negative subgroup (Mok et al., 2009). In summary, it was commonly recognized that only patients with EGFR mutation would benefit from EGFR-TKIs.

EGFR gene contains 28 exons, mutations often occur in the EGFR tyrosine kinase encoding region, focused on exons 18-21, account for more than 90% of all mutation types, and deletions in exon 19 and L858R in exon 21 are most common, which are considered as sensitive mutations. However, patients with different mutation type obtained different efficacy, for example, patients with deletions in exon 19 achieved higher effective rate and longer survival time than patients with L858R mutation after erlotinib or gefitinib therapy (Mitsudomi et al., 2005); D770-N771 mutation in exon 20 was reported to be less sensitive to EGFR-TKIs (Kobayashi et al., 2005); T790M mutation in exon 20 could change the structure of ATP-binding pocket, generating steric effect when TKI bind with kinase region, thus leads to TKI resistance (Doss et al., 2014). So, knowledge of EGFR mutation type is as essential as EGFR mutation status in the NSCLC individualized treatment.

EGFR mutation rate varies in different districts worldwide, from 10% to 20% in west world, while from 30% to 50% in Asia. However, there were few reports about EGFR mutation profile of NSCLC patients in Southwest China. Besides, relationship between EGFR mutation and clinical characteristics of NSCLC remains controversial (Gu et al., 2007; Wu et al., 2007; Liam et al., 2014; Shi et al., 2014; Tian et al., 2014; Zhao et al., 2014; Zheng et al., 2014). So, this study was conducted to reveal the EGFR mutation profile of NSCLC patients in Southwest China, and explore the relationship between EGFR mutation and clinical characteristics, including age, gender, smoking history, pathological types, and pathological stage.

## Materials and Methods

### Subjects and samples

261 cases with pathologically confirmed NSCLC patients were enrolled from West China Hospital, including 148 males and 113 females, the average age was 58.3±11.5 years. Pathological types and pathological stage (pTNM) were determined according to WHO tumor classification and diagnostic criteria. Biopsy or pleural fluid samples were collected, and detected by a pathologist to have enough cancer cells. Written informed consents

were obtained from all included individuals and approval for this study was obtained from the ethical committee of West China Hospital, Sichuan University.

### EGFR mutation detection

DNA was extracted by QIAamp DNA FFPE Tissue kit (QIAGEN) according to the instructions, and then the DNA purity and concentration were detected by NanoDrop 2000 spectrophotometer (Thermo Fisher), with A260/A280 limited to 1.8-2.0, and then adjusted the concentration to 1.5-3ng/μl.

Amplification refractory mutation system (ARMS) was used to detect EGFR mutation, with ADx-ARMS® EGFR mutation detection kit (AmoyDx) following instructions, simultaneously detecting 29 mutations of EGFR, including 19-del (19 deletions in exon 19), 20-ins (3 insertions in exon 20), G719X (including G719S, G719A, G719C), L858R, L861Q, S768I, and T790M. External control in every sample and internal control in every tube were used to avoid effect of DNA insufficiency or PCR inhibitors.

### Statistics

Means Testing was used to compare age of EGFR mutation group with wild-type group. Pearson Chi-square or Fisher exact test were used to analyze the relationship between EGFR mutation and characteristics of NSCLC, including gender, smoking history, pathological types, and pathological stage. Furthermore, logistic stepwise regression was used to rule out the impact of confounding factors. All the statistics were performed by SPSS 17.0, *P*<0.05 was defined as significant with two-sided test.

## Results

### EGFR mutation distribution of NSCLC patients in Southwest China

EGFR mutations were found in 127 cases out of 261 NSCLC patients, accounted for 48.7% mutation rate. L858R and 19-del were the major types, accounted for 40.9% (52 cases) and 33.1% (42 cases) of all the mutated cases respectively; T790M mutation took the third place, accounted for 6.3% (8 cases); The rest mutation types were rare, accounted for 9.4% (12 cases), including 20-INS, G719C, G719S, L861Q and S768I, as shown in Table 1. Moreover, sensitive mutations accounted for

**Table 1. EGFR Mutation Distribution of NSCLC Patients in Southwest China**

Mutation type	Cases	Mutation rate
Sensitive mutations	102	39.1%(102/261)
19-del	42	33.1%(42/127)
L858R	52	40.9%(52/127)
G719C or G719S	2	0.9%(2/127)
L861Q or S768I	6	4.7%(6/127)
Resistant mutations	12	4.6%(12/261)
T790M	8	6.3%(8/127)
20-INS	4	3.1%(4/127)
Double mutations	13	10.2%(13/127)
Total	127	48.7%(127/261)

**Table 2. Mutation Patterns of 13 Cases with Double Mutations**

Patient	19-del	L858R	T790M	20-INS	G719C or G719S	L861Q or S768I	Gender*	Smoking history**	Pathological types***	TNM stage	Pathological stage
1	+	-	+	-	-	-	F	N	A	T2N2M1	IV
2	+	-	-	-	+	-	F	N	A	T3N1M1	IV
3	+	-	-	-	-	+	F	N	A	T2N2M0	I
4	-	+	+	-	-	-	F	N	S	T3N2M0	III
5	-	+	+	-	-	-	F	N	A	T2N2M0	III
6	-	+	+	-	-	-	M	N	A	T2N3MX	III
7	-	+	+	-	-	-	M	Y	A	T4N2M0	III
8	-	+	+	-	-	-	M	N	S	T4N2M1	IV
9	-	+	+	-	-	-	M	Y	A	T4N3M1	IV
10	-	+	-	-	-	+	F	N	A	T3N1M0	III
11	-	-	+	+	-	-	M	Y	A	T4N3M1	IV
12	-	-	+	-	-	+	M	Y	A	T2N0M0	I
13	-	-	+	-	-	+	M	N	A	T1N1M1	IV

PS:\* F: female, M: male; \*\* N: no, no smoker; Y: yes, former of current smoker; \*\*\* A: adenocarcinoma; S: squamous carcinoma

39.1% (102/261) of all the patients, including 19-del, L858R, G719C, G719S, L861Q and S768I, while resistant mutations accounted for 4.6% (12/261), including T790M and 20-INS.

#### *Mutation patterns and clinical characteristics of 13 cases with double mutations*

13 cases (10.2%) were found to contain double mutations, as shown in table 2, F/M was 6/7, smoking rate was 30.8% (4/13), adenocarcinoma patients accounted for 84.6% (11/13), pathological stage I, III, IV accounted for 15.4%, 38.5%, 46.2% respectively, similar to the whole group (as shown in Table 3. Line "Total"). 7 mutation patterns were found, of which L858R combined with T790M occurred most common, accounted for 46.2% (6 cases). Interestingly, mutation pattern carrying T790M accounted for 76.9% (10/13), which was recognized as a common secondary drug-resistant mutation.

#### *Correlation analysis of EGFR mutation status and characteristics of NSCLC patients*

We compared age, gender, smoking history, pathological types, and pathological stage between EGFR mutated and wild-type patients, EGFR mutation rate was found to be related with gender, smoking history, and pathological types, as shown in Table 3.

No significant difference of average age was found between patients with EGFR mutation (58.8±10.6 years) and those without EGFR mutation (57.8±12.2 years) ( $p=0.49$ ); EGFR mutation rate was higher in females (57.5%, 65/113) than in males (41.9%, 62/148) ( $p=0.01$ ); It was also higher in patients without smoking history (61.2%, 93/152) than patients with smoking history (31.2%, 34/109) ( $p<0.00$ ); Fisher exact testing revealed significant difference of EGFR mutation rate among subgroups classified by pathological types ( $p<0.00$ ), EGFR mutation rate of adenocarcinoma patients was highest, accounted for 52.8% (120/227), mutation rate of adenosquamous carcinoma patients took the second place (42.8%, 3/7), and mutation rate of squamous carcinoma

patients was lowest (14.8%, 4/27).

Correlation analysis of EGFR mutation status with pathological stage showed no significant difference of EGFR mutation rate between subgroups classified by either  $P$  stage or TNM stage ( $p>0.05$ ). Meanwhile, we could find that EGFR mutation rates were similar in patients with or without lymphatic metastasis (N0 vs N1+N2+N3) ( $\chi^2=0.15$ ,  $P=0.69$ ), which accounted for 48.0% (94/196) and 50.8% (33/65) respectively; Distant metastasis was uncorrelated with EGFR mutation status either (M0 vs M1) ( $\chi^2=0.01$ ,  $P=0.94$ ).

However, we found that smoking history was closely related with gender ( $P<0.00$ ), smoking history occurred more often in males than in females (68.2% vs 7.1%). To rule out the impact of confounding factors in correlation analysis, Logistic stepwise regression was performed, using EGFR mutation status as dependent variable, factors including age, gender, smoking history, pathological types (set dummy variable, adenocarcinoma as reference), pathological stage and gender\*smoking (interaction effect) as independent variables. Results showed that only smoking history and pathological types were correlated with EGFR mutation status. EGFR mutation occurred less frequent in patients with smoking history, odds ratio was 0.26 (95%CI: 0.15-0.45); EGFR mutation rate was lower in squamous carcinoma patients than in adenocarcinoma patients, odds ratio was 0.15 (95%CI: 0.05-0.47), as shown in Table 4.

#### *Correlation analysis between EGFR mutation types and NSCLC characteristics*

Moreover, we analyzed the correlation between EGFR mutation types and NSCLC characteristics in EGFR mutated patients. Since sample size of mutation types except for 19-del and L858R was small, they were merged into one group—"others". As shown in Table 3, only pathological type was found relative with EGFR mutation types ( $P=0.02$ ). No significant difference was found among the three groups: 19-del, L858R and others, with respect to age, gender, smoking history and pathological stages

**Table 3. EGFR mutation in Subgroups Classified by NSCLC Characteristics**

	All NSCLC patients			P	EGFR mutated NSCLC patients			p
	Total	EGFR mutated	EGFR wild-type		19-del	L858R	Others*	
Age (year, Mean±SD)	58.31±11.45	58.82±10.64	57.83±12.18	0.486	56.61±12.53	60.65±8.62	59.18±10.56	0.144
Gender (N, %)								
Female	113(43.3%)	65(57.5%)	48(42.5%)	0.012	23(35.4%)	30(46.2%)	12(18.5%)	0.089
Male	148(56.7%)	62(41.9%)	86(58.1%)		19(30.6%)	21(33.9%)	22(35.5%)	
Smoking history (N, %)								
Former or current smoker	109(41.8%)	33(30.3%)	76(69.7%)	<0.001	8(24.2%)	13(39.4%)	12(36.4%)	0.274
No smoker	152(58.2%)	94(61.8%)	58(38.2%)		34(36.2%)	38(40.4%)	22(23.4%)	
Pathological types (N, %)								
Adenocarcinoma	227(87.0%)	120(52.9%)	107(47.1%)		41(34.2%)	51(42.5%)	28(23.3%)	
Squamous carcinoma	27(10.3%)	4(14.8%)	23(85.2%)	<0.001	1(25.0%)	0(0.0%)	3(75.0%)	0.002
Adenosquamous carcinoma	7(2.7%)	3(42.9%)	4(57.1%)		0(0.0%)	0(0.0%)	3(100.0%)	
Pathological stage (N, %)								
P stage								
I	25(9.6%)	13(52.0%)	12(48.0%)		5(38.5%)	5(38.5%)	3(23.1%)	
II	21(8.0%)	10(47.6%)	11(52.4%)	0.981	2(20.0%)	6(60.0%)	2(20.0%)	0.182
III	74(28.4%)	35(47.3%)	39(52.7%)		6(17.1%)	16(45.7%)	13(37.1%)	
IV	141(54.0%)	69(48.9%)	72(51.1%)		29(42.0%)	24(34.8%)	16(23.2%)	
T stage								
T1	51(19.5%)	24(47.1%)	27(52.9%)		8(33.3%)	10(41.7%)	6(25.0%)	
T2	92(35.2%)	44(47.8%)	48(52.2%)	0.23	15(34.1%)	17(38.6%)	12(27.3%)	0.906
T3	53(20.3%)	21(39.6%)	32(60.4%)		6(28.6%)	7(33.3%)	8(38.1%)	
T4	65(24.9%)	38(58.5%)	27(41.5%)		13(34.2%)	17(44.7%)	8(21.1%)	
N stage								
N0	65(24.9%)	33(50.8%)	32(49.2%)		13(39.4%)	14(42.4%)	6(18.2%)	
N1	29(11.1%)	13(44.8%)	16(55.2%)	0.951	4(30.8%)	5(38.5%)	4(30.8%)	0.908
N2	119(45.6%)	57(47.9%)	62(52.1%)		18(31.6%)	23(40.4%)	16(28.1%)	
N3	48(18.4%)	24(50.0%)	24(50.0%)		7(29.2%)	9(37.5%)	8(33.3%)	
M stage								
M0	126(48.3%)	61(48.4%)	65(51.6%)	0.939	15(24.6%)	28(45.9%)	18(29.5%)	0.146
M1	135(51.7%)	66(48.9%)	69(51.1%)		27(40.9%)	23(34.8%)	16(24.2%)	

PS: \* Represent other mutations, including 20-ins (3 insertions in exon 20), G719X (including G719S, G719A, G719C), L861Q, S7681, T790M and double mutation

**Table 4. Logistic Stepwise Regression Analysis of EGFR Mutation and NSCLC Characteristics**

Independent variable	B	S.E	Wals	df	Sig.	Exp (B) (95%CI)
Smoking history	-1.331	0.275	23.459	1	0.000	0.264 (0.154-0.453)
Pathological types			11.07	2	0.004	
Squamous carcinoma	-1.877	0.574	10.701	1	0.001	0.153 (0.050-0.471)
Adenosquamous carcinoma	-0.612	0.807	0.575	1	0.448	0.542 (0.111-2.638)

**Table 5. EGFR Mutation Types and Pathological Types of EGFR Mutated NSCLC Patients**

EGFR mutation type	adenocarcinoma	Non-adenocarcinoma	P	OR(95%CI)
19-del	41(34.2%)	1(14.3%)	0.452*	/
L858R	51(42.5%)	0(0.0%)	0.003**	1.214 (1.040-1.418)
others	28(23.3%)	6(85.7%)	0.041***	/
Total	120	7	0.001	

PS: \*19-del vs L858R; \*\* L858R vs others; \*\*\* others vs 19-del;  $\alpha'=\alpha/3=0.05/3=0.0167$  after bonferroni correction



(All  $p > 0.05$ ). Meanwhile, from the pathological stage analysis, we could also find that lymphatic metastasis (N0 vs N1+N2+N3) ( $\chi^2=1.8$ ,  $P=0.40$ ) and distant metastasis (M0 vs M1) ( $\chi^2=3.8$ ,  $P=0.15$ ) were irrelative with EGFR mutation types either.

Squamous carcinoma and adenosquamous carcinoma were merged into one group called non-adenocarcinoma due to the limited sample size. It showed that EGFR mutation type distribution was different between adenocarcinoma and non-adenocarcinoma ( $p=0.00$ ), 19-del and L858R occurred more often in adenocarcinoma patients, while other mutations occurred more in non-adenocarcinoma patients. Pairwise comparison showed that frequencies of L858R and other mutation between adenocarcinoma and non-adenocarcinoma patients were statistically different ( $p=0.00$ , OR=1.2, 95%CI: 1.0-1.4), after bonferroni correction, as shown in Table 5. Of the 6 non-adenocarcinoma patients, 3 cases with T790M alone were all Adenosquamous carcinoma, while 2 cases with L858R combining T790M and 1 case with L861Q or S768I were squamous carcinoma patients.

## Discussion

EGFR mutation status and mutation type are essential for EGFR-TKIs application in NSCLC patients. In this study, we investigated the EGFR mutation profile of NSCLC patients in Southwest China. Nearly half of the patients were EGFR mutated, with L858R and 19-del as dominating types. Smoking history and pathological type were independent predictors of EGFR mutation, while only pathological type was related with specific EGFR mutation type.

EGFR mutation rate varies in different countries, for instance, 22% in USA (Sholl et al., 2015), 11.9% in Lebanon (Naderi et al., 2015), 9.6% in Germany (Gahr et al., 2013), 9.6% in France (Mansuet-Lupo et al., 2014), 16.6% in Spain (Rosell et al., 2009), 53.9% in Japan (Yoshizawa et al., 2013), 36.1% in Korea (Koo et al., 2015), 37.7% in South India (Matam et al., 2015), range from 10% to 20% in western world, while 30%-50% in Asia. EGFR mutation rate was much higher in Asian people (about 30%) than in Caucasian people (about 10%) (Zhou and Christiani, 2011). China is a state with vast territory and multi-nations, EGFR mutation rate varies in different regions, range from 30% to 50%. For example, EGFR mutation rate was 35.3% (Zhao et al., 2014) to 45.8% (Zheng et al., 2014) in Hunan province, 38.2% in Ningbo city (Tian et al., 2014), 32.4% in Shanghai, Hangzhou and Kunming cities (Dong et al., 2006), 45.8% in Hunan province, 50.2% in 17 hospitals in mainland China (Shi et al., 2014). The mutation rate in Southwest China was 48.7% in this study. The discrepancy may be caused by reasons such as region divergence, inclusion criteria, and detection method, especially the last one. Studies presented by Zhao Jing (Zhao et al., 2014), Tian Hui (Tian et al., 2014) and Dong Gangqiang (Dong et al., 2006) were performed using Sanger sequencing method, while ARMS method was used in studies conducted by Zheng et al. (2014), Shi et al. (2014) and us, we could easily conclude that mutation rate was much

higher in studies performed by ARMS than sequencing. The sensitivity of ARMS was much higher than direct sequencing, which could detect mutation as low as 0.1-1.0%, and the results from ARMS were more consistent with EGFR-TKIs treatment response (Shaozhang et al., 2014). As tumor cells account for only a small part of pathological tissue, especially the pleural fluid sample, and only part of the tumor cells were EGFR mutated, choosing a hypersensitive method is essential, to detect more people who would benefit from EGFR-TKIs.

Our study showed that 19-del and L858R were the dominating mutation types, consistent with previous studies. Mutation rate of 19-del was reported higher than L858R in foreign researches (Gahr et al., 2013; Yoshizawa et al., 2013; Naderi et al., 2015), while in China, the proportion of the two mutations was controversial, some reported 19-del occurred more often, (Dong et al., 2006; Zhao et al., 2014) some showed similar occurrence rate (Tang et al., 2014; Zheng et al., 2014), and a report in Sichuan showed L858R mutation rate was higher (Dong et al., 2011). In our study, L858R mutation was found most frequently, accounted for 40.9%, slightly higher than 19-del (33.1%).

EGFR double mutation is not rare in Asia, and it may resulted in completely different bio effects due to conformation change on the previous mutant (Doss et al., 2014; Lowder et al., 2015), thus need to pay more attention. 6.9% (7/102) double mutation rate of all mutated patients in Japan was reported (Yokoyama et al., 2006), of which patterns carrying mutations in exon 20 accounted for 57.1% (4/7); A research in China discovered 5 double mutations in 145 NSCLC patients, which were all L858R/delE746-A750 (Zhang, 2007); 7.1% double mutation rate of all mutated patients was found in another study (Lin et al., 2014), mutation in exon 21 was more likely to be concurrent with exon 20 mutation (resistant mutation) than 19-del (4/11 vs 1/11). In our study, double mutation rate was 10.2% (13 cases), of which L858R combined with T790M occurred most often. The mutation pattern differed from one another in above researches, maybe caused by limited sample size of double mutation, however, we could find that L858R concurrent with other mutations were more common in above studies, it might indicate that patients with exon 21 mutation was more likely to induce other mutations. It's worth mentioning that mutation pattern carrying T790M accounted for 76.9% (10/13), the combination of T790M mutation might change the structure of cytoplasmic juxtamenbrane segment (JM) or make the ligand escape from the binding pocket, leading to extending oncogenic activity and sharply sensitivity reduction (Doss et al., 2014; Lowder et al., 2015).

It was found that females, no smoker, adenocarcinoma patients had greater EGFR mutation rate, consistent with most previous studies. However, in our study, logistic stepwise regression analysis showed that only smoking history and pathological type were independent predictor of EGFR mutation, rather than gender, consistent with studies conducted by Wu YL and Shi Y (Wu et al., 2007; Shi et al., 2014), while inconsistent with Liam et al.' study, which regarded only smoking status as independent predictor (Liam et al., 2014). The difference of EGFR

mutation rate between females and males may be caused by higher smoking rate in males. In addition, lymph nodes involvement was reported with EGFR mutation in some studies (Shi et al., 2014; Zheng et al., 2014), P and T stages were also reported correlated with EGFR mutation (Tomita et al., 2014), while our studies indicated no association of EGFR mutation with pathological stage, lymph nodes and distant metastasis, consistent with other studies (Sahoo et al., 2011; Zhang et al., 2013; Tian et al., 2014; Zhao et al., 2014; Zheng et al., 2014), suggesting constant impact of EGFR mutation during the whole pathogenic process from the very beginning.

The mutation pattern distribution was found no significant difference with respect to age, gender, and smoking history, consistent with previous study (Gu et al., 2007), indicating that EGFR mutations in smokers carried no signatures of mutagens in cigarette smoke. Furthermore, Gu et al. reported no association of mutation pattern with tumor histology (Gu et al., 2007), however, in our study, 19-del and L858R were found to occur more often in adenocarcinoma patients while other mutations except for 19-del and L858R tended to occur in non-adenocarcinoma patients. Of the 6 non-adenocarcinoma patients, 3 cases with T790M alone were all adenosquamous carcinoma patients, while 2 cases with L858R combining T790M and 1 case with L861Q or S768I were squamous carcinoma patients, suggested that different mutation pattern may participate in particular pathogenesis of different NSCLC pathological types. Other mutations except for 19-del and L858R, especially the resistant mutation T790M, should be brought to attention in non-adenocarcinoma patients.

In summary, EGFR mutation rate in Southwest China was 48.7%, consistent with most Asian regions, and nearly 40% patients carried sensitive mutations, who could benefit from EGFR-TKIs targeting therapy. EGFR mutation has been tested in only 9.6% patients in China, and EGFR-TKIs were used more as salvage (14.8%) rather than upfront therapy (5.3%) (Xue et al., 2012). Therefore, promoting EGFR mutation detection is of great value in NSCLC treatment. Furthermore, we found that only smoking history and pathological type were independent predictors for EGFR mutation. 19-del and L858R were the dominating mutation types, tending to occur more frequently in adenocarcinoma patients, while non-adenocarcinoma patients were tend to be other mutation types, especially mutation patterns containing T790M.

## Acknowledgements

**Funding:** This study was supported by grants from the National Natural Science Foundation of China (NSFC-81401727 and NSFC-81472026). The fund had no role in the study design, data collection and analysis, decision to publish, or the preparation of the manuscript.

## References

Dong DD, Tang Y, Zou Y, et al (2011). Study of exons 19 and 21 mutations of epidermal growth factor receptor gene in patients with lung adenocarcinoma of Sichuan Province. *Chinese J Clin Experimental Pathol*, **27**, 1306-9.

- Dong QG, Han BH, Huang JS, et al (2006). Analysis of EGFR mutations in 176 cases of non-small cell lung cancer. *Chinese J Oncol*, **28**, 686-90.
- Doss GP, Rajith B, Chakraborty C, et al (2014). Structural signature of the G719S-T790M double mutation in the EGFR kinase domain and its response to inhibitors. *Sci Rep*, **4**, 5868.
- Eck MJ, Yun CH (2010). Structural and mechanistic underpinnings of the differential drug sensitivity of EGFR mutations in non-small cell lung cancer. *Biochim Biophys Acta*, **1804**, 559-66.
- Gahr S, Stoehr R, Geissinger E, et al (2013). EGFR mutational status in a large series of Caucasian European NSCLC patients: data from daily practice. *Br J Cancer*, **109**, 1821-8.
- Gu D, Scaringe WA, Li K, et al (2007). Database of somatic mutations in EGFR with analyses revealing indel hotspots but no smoking-associated signature. *Hum Mutat*, **28**, 760-70.
- Kobayashi S, Boggon TJ, Dayaram T, et al (2005). EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med*, **352**, 786-92.
- Koo DH, Kim KP, Choi CM, et al (2015). EGFR-TKI is effective regardless of treatment timing in pulmonary adenocarcinoma with EGFR mutation. *Cancer Chemother Pharmacol*, **75**, 197-206.
- Liam CK, Leow HR, How SH, et al (2014). Epidermal growth factor receptor mutations in non-small cell lung cancers in a multiethnic Malaysian patient population. *Asian Pac J Cancer Prev*, **15**, 321-6.
- Lin LA, Liu ZY, Yang JS, et al (2014). Distribution and clinical significance of EGFR mutations in phase I non-small-cell lung cancer. *Chinese J Thoracic Cardiovascular Surgery*, **30**.
- Lowder MA, Doerner AE, Schepartz A (2015). Structural Differences between Wild-Type and Double Mutant EGFR Modulated by Third-Generation Kinase Inhibitors. *J Am Chem Soc*, **137**, 6456-9.
- Lynch TJ, Bell DW, Sordella R, et al (2004). Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*, **350**, 2129-39.
- Mansuet-Lupo A, Bobbio A, Blons H, et al (2014). The new histologic classification of lung primary adenocarcinoma subtypes is a reliable prognostic marker and identifies tumors with different mutation status: the experience of a French cohort. *Chest*, **146**, 633-43.
- Matam K, Goud I, Lakshmi MA, et al (2015). Correlation between EGFR Gene Mutations and Lung Cancer: a Hospital-Based Study. *Asian Pac J Cancer Prev*, **16**, 7071-6.
- Mitsudomi T, Kosaka T, Endoh H, et al (2005). Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. *J Clin Oncol*, **23**, 2513-20.
- Mok TS, Wu YL, Thongprasert S, et al (2009). Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med*, **361**, 947-57.
- Naderi S, Ghorra C, Haddad F, et al (2015). EGFR mutation status in Middle Eastern patients with non-squamous non-small cell lung carcinoma: A single institution experience. *Cancer Epidemiol*, **39**, 1099-102.
- National Office for Cancer Prevention and Control NCfCR, Disease Prevention and Control Bureau, Ministry of Health National Office for Cancer Prevention and Control, National Center for Cancer Registry, Disease Prevention and Control Bureau, Ministry of Health (2010). Chinese cancer mortality report: Third national retrospect spot-check of death-causation. Beijing, China: People's Medical

- Publishing House, **35**.
- Pao W, Hutchinson KE (2012). Chipping away at the lung cancer genome. *Nat Med*, **18**, 349-51.
- Rosell R, Moran T, Queralt C, et al (2009). Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med*, **361**, 958-67.
- Sahoo R, Harini VV, Babu VC, et al (2011). Screening for EGFR mutations in lung cancer, a report from India. *Lung Cancer*, **73**, 316-9.
- Shaozhang Z, Ming Z, Haiyan P, et al (2014). Comparison of ARMS and direct sequencing for detection of EGFR mutation and prediction of EGFR-TKI efficacy between surgery and biopsy tumor tissues in NSCLC patients. *Med Oncol*, **31**, 926.
- She J, Yang P, Hong Q, et al (2013). Lung cancer in China: challenges and interventions. *Chest*, **143**, 1117-26.
- Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al (2005). Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med*, **353**, 123-32.
- Shi Y, Au JS, Thongprasert S, et al (2014). A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). *J Thorac Oncol*, **9**, 154-62.
- Sholl LM, Aisner DL, Varella-Garcia M, et al (2015). Multi-institutional Oncogenic Driver Mutation Analysis in Lung Adenocarcinoma: The Lung Cancer Mutation Consortium Experience. *J Thorac Oncol*, **10**, 768-77.
- Tang Y, Zhang L, Tan X, et al (2014). Analysis of EGFR mutations in non-small cell lung cancer in Nanning Guangxi China. *J Modern Oncol*.
- Tian H, Kaizhong YU, Mao Z, et al (2014). Study on mutation of EGFR gene in 314 cases of non-small cell lung cancers. *J Shanxi Medical University*.
- Tomita M, Ayabe T, Chosa E, et al (2014). Epidermal growth factor receptor mutations in Japanese men with lung adenocarcinomas. *Asian Pac J Cancer Prev*, **15**, 10627-30.
- Torre LA, Bray F, Siegel RL, et al (2015). Global cancer statistics, 2012. *CA Cancer J Clin*, **65**, 87-108.
- Wu YL, Zhong WZ, Li LY, et al (2007). Epidermal growth factor receptor mutations and their correlation with gefitinib therapy in patients with non-small cell lung cancer: a meta-analysis based on updated individual patient data from six medical centers in mainland China. *J Thorac Oncol*, **2**, 430-9.
- Xue C, Hu Z, Jiang W, et al (2012). National survey of the medical treatment status for non-small cell lung cancer (NSCLC) in China. *Lung Cancer*, **77**, 371-5.
- Yasuda H, Kobayashi S, Costa DB (2012). EGFR exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications. *Lancet Oncol*, **13**, 23-31.
- Yokoyama T, Kondo M, Goto Y, et al (2006). EGFR point mutation in non-small cell lung cancer is occasionally accompanied by a second mutation or amplification. *Cancer Sci*, **97**, 753-9.
- Yoshizawa A, Sumiyoshi S, Sonobe M, et al (2013). Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: analysis of 440 Japanese patients. *J Thorac Oncol*, **8**, 52-61.
- Zhang G (2007). Molecular epidemiologic investigation of EGFR double mutations in Chinese non-small-cell lung cancer population and related studies on biological properties. *Doctoral Dissertation*, **2007**, 3.
- Zhang Y, Wang Q, Han ZG, et al (2013). Differences in epidermal growth factor receptor gene mutations and relationship with clinicopathological features in NSCLC between Uygur and Han ethnic groups. *Asian Pac J Cancer Prev*, **14**, 2879-83.
- Zhao J, Tian Q, Huang Y, et al (2014). An Analysis of the EGFR gene mutation in 238 patients with non-small cell lung cancer in hunan province. *Anti-tumor Pharmacy*.
- Zheng J, Xie G, Jiao LI, et al (2014). Clinical significance of EGFR mutations in non-small cell lung cancer. *Chinese J Clin Oncol*, **41**, 904-7.
- Zhou W, Christiani DC (2011). East meets West: ethnic differences in epidemiology and clinical behaviors of lung cancer between East Asians and Caucasians. *Chin J Cancer*, **30**, 287-92.