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Epidermal Growth Factor Receptor (EGFR) Mutations and Anaplastic Lymphoma Kinase/Oncogene or C-Ros Oncogene 1 (ALK/ROS1) Fusions Inflict Non-Small Cell Lung Cancer (NSCLC) Female Patients Older Than 60 Years of Age

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Background: Lung cancer has become a leading disease for the tumor-induced mortality. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancers. The present research aimed to evaluate the correlation between the anaplastic lymphoma kinase/oncogene or c-ros oncogene 1 (ALK/ROS1) fusions or mutations of epidermal growth factor receptor (EGFR) and ages or gender of patients.

Material/Methods: Among 1449 NSCLC patients, 457 patients who were diagnosed as consecutive EGFR mutations or ALK/ROS1 fusions between November 2016 and February 2018 were involved in the present study. EGFR genes or ALK/ROS1 mutations were detected by using DNA sequencing technique and amplification-refractory mutation system (ARMS). The mRNAs of ROS1 and ALK fusion were examined by using polymerase chain reaction technique and fusion gene detection kit.

Results: Females were more often inflicted by the EGFR mutations, especially for the exon 19 deletion and L858R mutation. There were significantly more ALK/ROS1 fusions in females compared to males ($P<0.05$) and significantly more ALK/ROS1 fusions in <60 years of age patients compared to patients older than 60 years of age ($P<0.05$). Exon 21 L858R and L861Q dominantly occurred in patients ≥60 years of age and exon 19 deletion in patients <60 years of age. EML-ALK-1 mainly existed in the female NSCLC patients.

Conclusions: EGFR mutations and ALK/ROS1 fusions mainly occurred in the NSCLC female patients who were older than 60 years of age.

MeSH Keywords: 7-Alkoxycoumarin O-Dealkylase • Artificial Gene Fusion • Carcinoma, Non-Small-Cell Lung • Genes, erbB-1

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Background

Nowadays, lung cancer has been considered a leading reason for tumor-induced mortality worldwide [1]. Among lung cancers, non-small cell lung cancer (NSCLC) accounts for approximately 80% [2]. In clinical settings, the conventional chemotherapeutic strategy only improves the clinical outcomes for the advanced stages of NSCLC, with a shorter survival time of even less than one-year post diagnosis [3]. A previous study [4] reported that the chromosomal-alteration or the protein-kinase-activation might be a well-known mechanism for tumorigenesis. Until now, a large number of molecule mutations in NSCLS have been discovered, such as fusion of oncogene or c-ros oncogene 1 (ROS1) or fusion of anaplastic lymphoma kinase (ALK) and the mutation of epidermal growth factor receptor (EGFR), [5,6].

The mutation of EGFR acts as the most critical driver-gene of NSCLC patients, especially for the east Asian patients [7,8]. Meanwhile, the EGFR-tyrosine kinase inhibitor could be considered a potential and effective biomarker for the EGFR mutation and frequent induction for NSCLC [9]. The ALK is the first discovered targetable fusion oncokinase; it was identified in only 4% to 6% of NSCLC patients. The most frequent fusion of ALK is the EML4-AKL fusion in clinical settings [10,11]. ROS1 fusion gene is considered a potential driver for NSCLC and can activate several different downstream signaling pathways. Previous studies reported that the prevalence of ROS1 fusions in NSCLC ranges from 0.9% to 3.7% in clinical settings [12,13].

Although the previous studies have described many EGFR mutations, ALK fusions or ROS1 fusions in clinical, there were no investigations for clarifying the distribution of these mutations or fusions in NSCLC patients with different gender and ages. We believed that it was important to further discover the distribution of EGFR mutations or ALK/ROS1 fusions for exploring the mechanism of NSCLC. Therefore, this study involved a total of 1449 NSCLC patients and selected 457 patient with EGFR mutations or ALK/ROS1 fusions for further study.

Material and Methods

Patients and samples

A total of 1449 patients diagnosed as NSCLC at The First Affiliated Hospital of University of Science and Technology of China, Hefei City, P.R, China, between November 2016 and February 2018 were involved in the present research. Among all of these NSCLC patients, the consecutive EGFR mutations or ALK/ROS1 fusions characterized patients (457 cases) were selected for further investigation. The clinical characteristics or parameters for NSCLC patients are illustrated in the Table 1.

Table 1. Characteristics and comparison of the population with EGFR mutations.

Characteristics	EGFR mutation (n=417)	*p* Value
Gender		
Male	168	0.036
Female	249	
Age, years		0.072
<60	212	
≥60	205	
Single EGFR mutation		0.029
Exon 19 deletion	187	
Exon 21 L858R	189	
Exon 20 insertion	11	
Exon 21 L861Q	14	
Other types	7	
Double EGFR mutation		0.082
L858R+T790M	5	
L858R+S768I	1	
G719X+S768I	2	
19del+T790M	1	

All of the patients provided written informed consents and approved their participation in this study. The present research was approved by Ethics Committee of The First Affiliated Hospital of University of Science and Technology of China, Hefei City, P.R, China.

EGFR mutations evaluation

The EGFR genes or mutations were detected using the DNA sequencing technique and the amplification-refractory mutation system (ARMS) commercial regent (Amoy, Xiamen, China) according to the manufacturer's construction. In this study, the following mutations were detected: single mutation of exon 19 deletion, exon 20 insertion, exon 21 L858R, exon 21 L861Q, exon 18 G719X, 2 mutations in exon 20 (L858R+T790M, L858R+S768I), 2 mutations in exon 18 and 20 (G719X+S768I), and exon 19 deletion combining the T790M mutation.

Examination of the ALK/ROS1 fusions

The mRNAs of ALK and ROS1 fusion were examined by using the polymerase chain reaction (PCR) technique and fusion gene

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detection kit (Amoy, Xiamen, China). In brief, total RNAs were extracted with the Qiagen RNeasy kit (Qiagen, Hilden, Germany) and were transcribed into the cDNA by using the Reverse Transcription kit (Invitrogen/Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The conditions for PCR were the followings: denaturation for 5 minutes at 95°C, followed by 94°C for 25 seconds, 65°C for 20 seconds and 72°C for 20 seconds, and for 35 cycles. The data collection and sensitivity analysis were conducted as described in a previous study [14].

Statistical analysis

SPSS software (version 20.0, SPSS, Chicago, IL, USA) was used for all statistical analyses in this study. The quantitative data were recorded as mean values ± standard deviation (SD) and analyzed by using the Student's *t*-test for analysis between 2 groups. Meanwhile, Tukey's post hoc test was used to validate ANOVA for comparing measurement data among groups. The categorical variables (recorded as percentage) were analyzed and compared by employing the chi-square test. $P<0.05$ was assigned as a statistical significance.

Results

Characteristics of patients with EGFR mutation

In summary, 417 NSCLC patients were involved and selected for the EGFR mutation analysis and the characteristics are listed in Table 1. Among these patients, there were 168 males and 249 females (Table 1). The results showed that the females suffered from EGFR mutations more frequently. Meanwhile, there were no significant differences for the EGFR mutations between patients younger than 60 years old and older than 60 years old (Table 1). For the single EGFR mutations, there were 187 exon 19 deletion cases, 189 exon 21 L858R mutation cases, 11 exon 20 insertion cases, and 14 exon 21 L861Q mutation cases (Table 1). Meanwhile, the results showed that the differences of EGFR gene mutations among single mutation types were significant (Table 1, $P=0.029$). For the double EGFR mutations, there were 5 cases of L858R+T790M, 1 case of L858R+S768I, 2 cases of G719X+S768I, and 1 case of 19del+T790M, among which no significant differences appeared (Table 1, $P=0.082$).

Characteristics of patients with ALK/ROS1 fusion

In this study, 40 NSCLC patients were involved and selected for the ALK/ROS1 fusion analysis; the characteristics are listed in Table 2. Among these patients, there were 18 males and 22 females, and there were significant differences (Table 1, $P=0.041$). The data illustrated that the females were for frequently found to have the ALK/ROS1 fusions. Meanwhile, the occurrence of ALK/ROS1 fusion was more frequent in <60 years of age patients compared to patients older than 60 years of age (Table 1, $P=0.024$). For the ALK fusions, there were 19 EML4-ALK-1 fusion cases, 7 ALK-1 mutation cases, 2 EML4-ALK-3 fusion cases, and 2 ALK mutation cases (Table 2). There were significant differences for the ALK fusions among different fusion types (Table 2, $P=0.013$). For the ROS1 fusion, there were 4 cases of ROS1-1, 5 case of ROS1-2, and 1 case of ROS1-1+ROS1-2, among which no significant differences appeared (Table 2, $P=0.059$).

Table 2. Characteristics and comparison of patients with ALK/ROS fusion.

Characteristics	ALK/ROS (n=40)	*p* Value
Gender		
Male	18	0.041
Female	22	
Age, years		0.024
<60	25	
≥60	15	
ALK fusion		0.013
EML4-ALK-1	19	
ALK-1 mutation	7	
EML4-ALK-3	2	
ALK mutation	2	
ROS fusion		0.059
ROS1-1	4	
ROS1-2	5	
ROS1-1+ROS1-2	1	

Female NSCLC patients were more frequently found to have EGFR gene mutations

To identify the distribution of the EGFR gene mutations by gender, the exon 19 deletion, exon 20 insertion, exon 21 L858R, exon 21 L861Q, exon 18 G719X, L858R+T790M, L858R+S768I, G719X+S7687I, and 19del+T790M were detected. The results showed that 82 out of 187 patients suffered from exon 19 deletion; however, 105 out of 187 patients suffered from exon 19 deletion (Table 3). The occurrence rate of exon 19 deletion in females was significantly higher compared to males (Table 3, $P=0.031$). Meanwhile, the amount of exon 21 L858R mutations in females (120 cases) was significantly more compared to males (69 cases) (Table 3, $P=0.012$). Furthermore,

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Table 3. Comparison for the distribution of EGFR mutations between males and females.

Characteristics	Total (n)	Male	Female	*p* Value
Single EGFR mutation				
Exon 19 deletion	187	82	105	0.031
Exon 21 L858R	189	69	120	0.012
Exon 20 insertion	11	5	6	0.074
Exon 21 L861Q	14	7	7	0.131
Exon 18 G719X	5	3	2	NS
Double EGFR mutation				
L858R+T790M	5	1	4	NS
L858R+S768I	1	1	0	NS
G719X+S768I	2	0	2	NS
19del+T790M	2	0	2	NS

Table 4. Comparison for the distribution of *EGFR* mutations between difference ages.

Characteristics	Total (n)	<60	≥60	*p* Value
Single EGFR mutation				
Exon 19 deletion	187	100	87	0.042
Exon 21 L858R	189	82	107	0.033
Exon 20 insertion	11	2	9	0.045
Exon 21 L861Q	14	2	12	0.011
Exon 18 G719X	5	1	4	NS
Double EGFR mutation				
L858R+T790M	5	2	3	NS
L858R+S768I	1	1	0	NS
G719X+S768I	2	1	1	NS
19del+T790M	2	0	2	NS

no significant differences were discovered for the exon 20 insertion, exon 21 L861Q, and exon 18 G719X between males and females (Table 3, $P>0.05$). Also, we did not find a different distribution of double EGFR mutations between the males and females (Table 3).

Exon 21 L858R and L861Q dominantly occurred in patients ≥60 years of age and exon 19 deletion in patients <60 years of age

In order to clarify the distribution for the EGFR gene mutations in different ages in NSCLC patients, the mutations of EGFR genes were examined in both ≥60 years old patients and <60 years old patients. The results indicated that there were more cases of exon 19 deletion in patients <60 years old (100 cases) compared to patients ≥60 years old (87 cases) (Table 4, $P=0.042$). Meanwhile, more cases of exon 21 L858R and exon 21 L861Q appeared in the patients ≥60 years of age compared to patients <60 years of age (Table 4, $P=0.033$ and 0.011, respectively). Moreover, there were no significant differences for other single EGFR mutations and double EGFR mutations between patients ≥60 years old and patients <60 years old (Table 4).

Comparison for distribution of ALK/ROS1 in different genders

To evaluate the distribution of ALK/ROS1 fusions in different genders, the ALK fusion (EML4-AKL-1, EML4-ALK-1 mutation, EML4-ALK-3, and EML4-ALK mutation) and ROS1 fusion

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Table 5. Comparison for the distribution of ALK/ROS fusion between males and females.

ALK/ROS fusion	Total (n)	<60	≥60	*p* Value
EML4-ALK-1	19	10	9	0.093
EML4-ALK-1 mutation	7	4	3	NS
EML4-ALK-3	2	0	2	NS
EML4-ALK mutation	2	1	1	NS
CD74-ROS1-1	5	2	3	NS
EZR-ROS1-2	6	2	4	NS

Table 6. Comparison for the distribution of AKL/ROS fusion between difference ages.

ALK/ROS fusion	Total (n)	<60	≥60	*p* Value
EML4-ALK-1	19	12	7	0.039
EML4-ALK-1 mutation	7	4	3	NS
EML4-ALK-3	2	1	1	NS
EML4-ALK mutation	2	1	1	NS
CD74-ROS1-1	5	3	2	NS
EZR-ROS1-2	6	4	2	NS

(CD74-ROS1-1 and EZR-ROS1-2), were detected. The results showed that there were no significant differences for these fusions between males and females (Table 5, *P*>0.05).

EML-ALK-1 existed in the females of NSCLC patients

The analysis of ALK fusions showed that the EML4-ALK-1 fusion (12 cases) mainly was distributed in patients <60 years of age. However, there were only 7 cases of EML4-ALK-1 fusion in patients ≥60 years of age, which was significantly fewer compared to patients <60 years of age (Table 6, *P*=0.039). Moreover, there were no significant difference for the EML4-ALK-1 mutation, EML4-ALK-3 mutation, EML4-ALK mutation, CD74-ROS1-1 and EZR-ROS1-2 fusions between patients ≥60 years of age and patients <60 years of age (Table 6).

Discussion

The EGFR mutations are critical due to their predictive capabilities for targeting therapy in NSCLC [15]. ALK rearrangements or fusions have been discovered in the samples of NSCLC patients and are also the targeting site for cancer therapy [10]. Therefore, in cases of advanced NSCLC, detecting EGFR mutations and ALK/ROS1 fusions strongly suggests conduct targeting therapy [16]. Therefore, the EGFR mutations and ALK/ROS1 fusions are important drivers for the initial evaluation of the NSCLC.

In the present study, EGFR gene mutations were examined in 417 NSCLC patients; and ALK/ROS1 fusions were detected in 40 NSCLC patients. The EGFR mutations frequency in female patients were significantly higher compared to that in male patients, and the frequency of the ALK/ROS1 fusions in females were also significantly higher compared to that in males. The EGFR mutations and ALK fusions have not, to our knowledge, been investigated or clarified. However, for the ROS1 fusions, a previous study [17] reported that the frequency of ROS1 in women was higher than that in men, which is consistent with our results of ROS1 fusions. Meanwhile, cases of ALK/ROS1 fusions in patients <60 years of age were significantly higher compared to patients ≥60 years of age. However, there were no significant differences for the EGFR mutations between patients <60 years of age and patients ≥60 years of age. These results suggested that the EGFR mutations and the ALK/ROS1 fusions were closely correlated with the pathogenesis of NSCLC in clinical settings these findings were in accord with the speculation of the previous study described [18,19].

Until now, studies [20,21] have reported several EGFR gene mutations, such as exon 19 deletion, exon 21 L858R, exon 20 insertion, exon 21 L861Q, and exon 18G719X. However, EGFR gene mutations distribution in NSCLC patients with different ages or genders, has not been clarified. In this study, the occurrence of exon 19 deletion frequency and L858R frequency in females was significantly higher compared to that in males. Meanwhile, the exon 21 L858R and L861Q dominantly occurred in patients ≥60 years of age and exon 19 deletion in patients

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<60 years of age. These results suggested that female NSCLC patients and patients older than 60 years of age, it was relatively common to be inflicted by the EGFR mutations, as suggested by previous studies [22].

The ALK/ROS1 fusion genes have been used for detecting NSCLC patients harboring EGFR mutation samples and applied to therapeutic efficacy [23]. The fusion of ALK was found in a relatively small number of patients with NSCLC [24], however, little is known about the ALK fusion distribution in different ages and genders. In addition, many gene fusion partners were discovered, including EZR, CD74, SLC34A2, TPM3, and LRIG3 [12], all of which could fuse with the ALK or ROS1. In this study, there were no significant differences for all of the aforementioned fusions between males and females. The EML4-ALK-1 fusion (12 cases) was mainly distributed in patients <60 year of age, and was more significant compared to patients ≥60 years of age. These findings hint that the EML4-ALK-1 fusion mainly distributed in the NSCLC patients <60 year of age.

Conclusions

Females were often inflicted by the EGFR mutations, especially for the exon 19 deletion and L858R mutation. There were significantly more ALK/ROS1 fusions in females compared to that in males and significantly more ALK/ROS1 fusions in patients <60 years old compared to patients older than 60 years of age. Exon 21 L858R and L861Q dominantly occurred in patients ≥60 years of age and exon 19 deletion in patients <60 years of age. EML-ALK-1 mainly existed in female NSCLC patients. Therefore, EGFR mutations and ALK/ROS1 fusions mainly were found in NSCLC female patients who were older than 60 years of age.

Conflict of interests

None.

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