Clinical characteristics of non-small cell lung cancer patients with EGFR mutations and ALK&ROS1 fusions

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Abstract

Background

The purpose of this study was to study the relationship between ALK&ROS1 gene rearrangement, EGFR mutation, non-ALK&ROS1/EGFR and their clinical characteristics in non-small cell lung cancer (NSCLC) patients to distinguish these different types.

Methods

Both ALK&ROS1 gene rearrangements and EGFR mutations testing were performed in 461 NSCLC patients. The clinical characteristics and associated pulmonary abnormalities were investigated.

Results

Of the 461 patients studied, we identified 175 cases with EGFR mutations (38.0%, 175/461). ALK gene fusions were identified in 33 cases (33/461, 7.2%) and ROS1 gene fusions were identified in 9 cases (9/461, 1.9%). In the EGFR-positive group, the majority were female (50.9%, P < 0.001), non-smoking (76.6%, P < 0.001), adenocarcinoma (90.9%, P < 0.001) patients. Compared with the non-ALK&ROS1/EGFR group, the significant differences in ALK&ROS1-positive group were: younger (P < 0.001), the majority were female (52.4%, P < 0.001), non-smoking (66.7%, P = 0.001), adenocarcinoma (90.5%, P = 0.002) patients, appeared lymphangitic was more (23.8%, P = 0.044) and emphysema was less (9.5%, P < 0.001). The NSCLC patients with exon 19 deletion (74.7%) and L858R (86.4%) most were non-smokers, while patients with L861Q (62.5%) and G719x (66.7%) most were smokers.

Conclusions

NSCLC with EGFR mutation and ALK&ROS1 gene rearrangement were more likely to occur in young, non-smoking women. Patients with ALK&ROS1 gene rearrangements and EGFR mutations were less likely to develop emphysema.

Introduction

With the increasing morbidity and mortality, lung cancer has become the first malignant tumor. The morbidity and mortality of male lung cancer rank first in China[1–4]. The morbidity of lung cancer among women in China ranks second and the mortality rate of lung cancer among women is the first[5]. More than 80% of lung cancer which is non-small cell lung cancer (NSCLC)[6, 7].

In recent years, personalized molecular targeted therapy as the core has become a research hotspot in the treatment of lung cancer[8–10]. Currently, the most commonly used treatment of NSCLC is molecular targeted drugs targeting at mutations of epidermic growth factor receptor (EGFR), also known as small-molecule tyrosine kinase inhibitors (TKIs), which have obvious clinical efficacy on NSCLC patients with EGFR mutations. Echinoderm microtubule associated protein-like 4 (EML4) and anaplastic lymphomakinase (ALK) fusion gene EML4-ALK and proto-oncogene protein tyrosine kinase ROS (ROS1) were found after EGFR mutations in NSCLC[11–13]. For patients with ALK gene fusion and ROS1 gene fusion, targeted therapy with crizotinib could achieve better efficacy. Therefore, the detection of EGFR, ALK and ROS1(ALK&ROS1) gene mutations before targeted therapy is of great significance for the prediction of the efficacy of targeted therapy and the appropriate patient screening.

The purpose of this study was to evaluate the clinical characteristics of patients with NSCLC in order to distinguish ALK&ROS1 gene rearrangement, EGFR mutation and non-ALK&ROS1/EGFR (no mutations and rearrangements), so as to distinguish these different types.
Materials And Methods

Specimen collection
Test specimens were collected from the Meizhou People's Hospital (Huangtang Hospital) (Meizhou, Guangdong, China) between April 2018 and August 2019 for the pathological diagnosis of NSCLC. All protocols were approved by the Human Ethics Committees of Meizhou People's Hospital, Meizhou Academy of Medical Sciences, Meizhou Hospital Affiliated to Sun Yat-sen University (Guangdong, China). Written informed consent was obtained from all patients. The medical records of each patient were reviewed and the corresponding clinical characteristics were extracted.

DNA And RNA Extraction
Ten pieces of formalin-fixed and paraffin-embedded (FFPE) slices (5 µm thick per slice) were placed into a 1.5 ml EP tube. After FFPE slices were deparaffinized, DNA and RNA were extracted by AmoyDx® Tissue DNA/RNA Co-separation Kit (Spin Column) (Amoy Diagnostics, Xiamen, China), following the manufacturers’ instructions and the quantity and quality of extracted DNA and RNA were evaluated.

Detection of EGFR gene mutations by ARMS PCR
EGFR gene mutations were detected by real-time amplification refractory mutation system (ARMS)-PCR and the 29 mutation hotspots from exon 18 to 21 in this gene were covered with the EGFR Gene Mutations Fluorescence Polymerase Chain Reaction Diagnostic Kit (Amoy Diagnostics, Xiamen, China). PCR was performed with initial denaturation at 95°C for 5 minutes, followed by 15 cycles of first amplification (at 95°C for 25 seconds, 64°C for 20 seconds and 72°C for 20 seconds) and 31 cycles of second amplification (at 95°C for 25 seconds, 60°C for 35 seconds and 72°C for 20 seconds). Positive results were defined as Ct (sample)-Ct (control) < Ct (cut-off) according to the criteria defined by the manufacturer’s instructions.

Detection of ALK and ROS1 gene fusions by RT-PCR
The fusion genes of ALK and ROS1 were analyzed by RT-PCR according to the manufacturer’s protocol for AmoyDx® ALK Gene Fusions and ROS1 Gene Fusions Detection Kit (Amoy Diagnostics, Xiamen, China) with the LightCycler 480 real-time PCR system. The detection range included the fusions of ALK gene with EML4, KIF5B, TFG and KLC1 genes, and the fusions of ROS1 gene with SLC34A2, CD74, SDC4, EZR, TPM3, LRIG3 and GOPC genes. RT reaction system: reverse transcriptase 0.5 µl, RNA template 6 µl (total RNA 0.5 ~ 5.0 µg), 42°C for 1 h and 95°C for 5 min, to obtain cDNA. PCR amplification: 1.5 µl ALK&ROS1 mixed enzyme was respectively taken to ALK cDNA and ROS1 cDNA of the samples to be tested, 5 µl was successively transferred to the eight-tube PCR reaction system, and negative and positive controls were set up. The detection instrument and circulating conditions were the same as EGFR mutation detection, and Ct values were also interpreted.

Statistical analysis
All analysis was conducted using SPSS statistical software version 21.0. Fisher’s exact test and the Student’s t-test were performed in this study. EGFR-positive group, ALK&ROS1-positive group and non-ALK&ROS1/EGFR group, pairwise comparisons were performed. The relationship between EGFR, ALK and ROS1 genes mutations and clinical characteristics, various types of mutations in the EGFR gene and clinical characteristics were analyzed. P < 0.05 is considered statistically significant.

Results

Population characteristics
This study involved 461 Chinese NSCLC patients who performed with EGFR mutation and ALK&ROS1 fusion test.
Three hundred and eleven were male and 150 were female. There were 349 (349/461, 75.7%) were adenocarcinoma, 106 (106/461, 23.0%) were squamous and 6 (6/461, 1.3%) were adenosquamous, respectively. HE staining and immunohistochemical staining for squamous cell carcinoma and lung adenocarcinoma are shown in Fig. 1. Most of these patients, 255 were nonsmokers (255/461, 55.3%) and 272 were older than 60 years old (272/461, 59.0%). Thirty-four (7.4%), 18 (3.9%), 90 (19.5%), and 319 (69.2%) patients were in stage I, II, III and IV, respectively. The clinical characteristics of patients are shown in Table 1.
Table 1
Characteristics of patients with lung cancer included in this study.

| Parameter              | n (%)          |
|------------------------|----------------|
| Age (years)            |                |
| ≤ 60                   | 189(41.0)      |
| >60                    | 272(59.0)      |
| Mean ± SD              | 61.76 ± 10.34  |
| Range                  | 27–87          |
| Gender                 |                |
| Male                   | 311(67.5)      |
| Female                 | 150(32.5)      |
| Smoking status         |                |
| Never smoked           | 255(55.3)      |
| Smoking                | 206(44.7)      |
| Pathology              |                |
| Adenocarcinoma         | 349(75.7)      |
| Squamous               | 106(23.0)      |
| Adenosquamous          | 6(1.3)         |
| Disease stage          |                |
| I                      | 34(7.4)        |
| II                     | 18(3.9)        |
| III                    | 90(19.5)       |
| IV                     | 319(69.2)      |
Comparisons of characteristics between EGFR-positive and ALK&ROS1-positive cases, ALK&ROS1-positive and non-ALK&ROS1/EGFR cases, and EGFR-positive and non-ALK&ROS1/EGFR cases in NSCLC patients

Of the 461 patients studied, we identified 175 cases of EGFR mutations in exons 18, 19, 20, or 21 (38.0%, 175/461). The G719X mutation (in exon 18) were identified in 3 cases (1.7%); exon 19 deletion were identified in 95 cases (54.3%), exon 20 insertion were detected in 2 cases (1.1%) and S768I mutation (in exon 20) was detected in 1 case (0.6%), L858R mutation (in exon 21) were detected in 66 cases (37.7%) and L861Q mutation (in exon 21) were detected in 8 cases (4.6%), respectively. ALK gene fusions were identified in 33 cases (33/461, 7.2%) and ROS1 gene fusions were identified in 9 cases (9/461, 1.9%).

Compared with the EGFR-positive group, the significant differences in ALK&ROS1-positive group was younger (P < 0.001). There were no significant differences in gender, smoking history, pathology, clinical stage, computed tomography characteristics (lymphangitic, lymphadenopathy, emphysema, fibrosis and pleural effusion).

Comparisons of characteristics between EGFR-positive and non- ALK&ROS1/EGFR cases in NSCLC patients. In the EGFR-positive group, the majority were female (50.9%, P < 0.001), non-smoking (76.6%, P < 0.001), adenocarcinoma (90.9%, P < 0.001) patients. In addition, there were significant difference in clinical stage (P = 0.002), emphysema (P < 0.001) and fibrosis (P = 0.021). There were no significant differences in age, lymphangitic, lymphadenopathy and pleural effusion.

Compared with the non-ALK&ROS1/EGFR cases in NSCLC patients, the ALK&ROS1-positive group that significantly differed from the non-ALK&ROS1/EGFR group were: younger (P < 0.001), the majority were female (52.4%, P < 0.001), non-smoking (66.7%, P = 0.001), adenocarcinoma (90.5%, P = 0.002) patients, appeared lymphangitic was more (23.8%, P = 0.044) and emphysema was less (9.5%, P < 0.001) (Table 2).

Table 2

| Patient characteristic | EGFR (+) (n = 175) | ALK&ROS1 fusion (+) (n = 42) | Non-ALK&ROS1/EGFR (n = 246) | P value | EGFR (+) vs ALK&ROS1 fusion (+) | EGFR (+) vs Non-ALK&ROS1/EGFR | ALK fusion (+) vs Non-ALK&ROS1/EGFR |
|-----------------------|-------------------|-----------------------------|-----------------------------|---------|--------------------------------|--------------------------------|---------------------------------|
| No. (Total 461)       | 175(38.0)         | 42(9.1)                     |                             |         |                               |                                |                                 |
| Age                   |                   |                             |                             | <0.001  | 0.477 (χ² test)                | <0.001                        |                                 |
| ≤ 60                  | 70(40.0)          | 31(73.8)                    | 90(36.6)                    |         |                               |                                |                                 |
| > 60                  | 105(60.0)         | 11(26.2)                    | 156(63.4)                   |         |                               |                                |                                 |
| Mean ± SD             | 61.74 ± 9.82      | 54.90 ± 13.03               | 62.87 ± 9.78                | <0.001  | 0.245 (t test)                 | <0.001                        |                                 |
| Range                 | 27–86             | 30–85                       | 28–87                       |         |                               |                                |                                 |
| Gender                |                   |                             |                             | 0.859   | <0.001                        | <0.001                        |                                 |
| Male                  | 86(49.1)          | 20(47.6)                    | 207(84.1)                   |         |                               |                                |                                 |
The association between EGFR, ALK&ROS1 genes status and clinical characteristics

Compared with EGFR-negative cases, in the EGFR-positive group, the most were female (50.9% vs 21.3%, \( P < 0.001 \)), non-smoking (76.6% vs 42.3%, \( P < 0.001 \)), adenocarcinoma (90.9% vs 66.4%, \( P < 0.001 \)) patients. In addition, there were significant difference in clinical stage (\( P = 0.002 \)) and emphysema (\( P < 0.001 \)). There were no significant difference in age, lymphangitic, lymphadenopathy, fibrosis and pleural effusion.

Compared with ALK&ROS1-negative group, the ALK&ROS1-positive group was younger than ALK&ROS1-negative group (73.8% vs 37.7% in \( \leq 60 \) years, \( P < 0.001 \)). About the ALK&ROS1-positive group, the most were female.

|                | EGFR-negative | EGFR-positive | ALK&ROS1-negative | ALK&ROS1-positive |
|----------------|---------------|---------------|-------------------|-------------------|
| Female         | 89(50.9)      | 22(52.4)      | 39(15.9)          |                   |
| Smoking        |               |               |                   |                   |
| Never          | 134(76.6)     | 28(66.7)      | 95(38.6)          |                   |
| Smoking        | 41(23.4)      | 14(33.3)      | 151(61.4)         |                   |
| Pathology      |               |               |                   |                   |
| Adenocarcinoma | 159(90.9)     | 38(90.5)      | 154(62.6)         |                   |
| Squamous       | 13(7.4)       | 4(9.5)        | 89(36.2)          |                   |
| Adenosquamous  | 3(1.7)        | 0(0)          | 3(1.2)            |                   |
| Disease stage  |               |               |                   |                   |
| I              | 17(9.7)       | 4(9.5)        | 13(5.3)           |                   |
| II             | 10(5.7)       | 0(0)          | 8(3.3)            |                   |
| III            | 20(11.4)      | 8(19.0)       | 62(25.2)          |                   |
| IV             | 128(73.1)     | 30(71.4)      | 163(66.3)         |                   |
| Lymphangitic   | 29(16.6)      | 10(23.8)      | 30(12.2)          |                   |
| Lymphadenopathy| 123(70.3)     | 30(71.4)      | 186(75.6)         |                   |
| Emphysema      | 26(14.9)      | 4(9.5)        | 103(41.9)         |                   |
| Fibrosis       | 15(8.6)       | 2(4.8)        | 40(16.3)          |                   |
| Pleural effusion| 73(41.7)     | 16(38.1)      | 91(37.0)          |                   |

|                | EGFR-negative | EGFR-positive | ALK&ROS1-negative | ALK&ROS1-positive |
|----------------|---------------|---------------|-------------------|-------------------|
|                |               |               |                   |                   |
| Smoking        |               |               |                   |                   |
| Never          | 134(76.6)     | 28(66.7)      | 95(38.6)          |                   |
| Smoking        | 41(23.4)      | 14(33.3)      | 151(61.4)         |                   |
| Pathology      |               |               |                   |                   |
| Adenocarcinoma | 159(90.9)     | 38(90.5)      | 154(62.6)         |                   |
| Squamous       | 13(7.4)       | 4(9.5)        | 89(36.2)          |                   |
| Adenosquamous  | 3(1.7)        | 0(0)          | 3(1.2)            |                   |
| Disease stage  |               |               |                   |                   |
| I              | 17(9.7)       | 4(9.5)        | 13(5.3)           |                   |
| II             | 10(5.7)       | 0(0)          | 8(3.3)            |                   |
| III            | 20(11.4)      | 8(19.0)       | 62(25.2)          |                   |
| IV             | 128(73.1)     | 30(71.4)      | 163(66.3)         |                   |
| Lymphangitic   | 29(16.6)      | 10(23.8)      | 30(12.2)          |                   |
| Lymphadenopathy| 123(70.3)     | 30(71.4)      | 186(75.6)         |                   |
| Emphysema      | 26(14.9)      | 4(9.5)        | 103(41.9)         |                   |
| Fibrosis       | 15(8.6)       | 2(4.8)        | 40(16.3)          |                   |
| Pleural effusion| 73(41.7)     | 16(38.1)      | 91(37.0)          |                   |
(52.4% vs 30.5%, \( P = 0.004 \)) patients, and emphysema was less (9.5% vs 30.8%, \( P = 0.004 \)). There were no significant differences in smoking history, pathology, clinical stage, lymphangitic, lymphadenopathy, fibrosis and pleural effusion (Table 3).

Table 3
Analysis of the relationship between \( \text{EGFR} \) and \( \text{ALK&ROS1} \) genes status and clinical characteristics.

| Characteristic       | \( \text{EGFR} \) mutation | \( \text{ALK&ROS1} \) fusion | \( P \) value |
|----------------------|-----------------------------|--------------------------------|--------------|
| No. (Total 461)      | 175(38.0)                   | 286(62.0)                      | 286(62.0)    |
| Age                  |                            | 0.733                          |              |
| ≤ 60                 | 70(40.0)                    | 119(41.6)                      | 70(40.0)     |
| >60                  | 105(60.0)                   | 167(58.4)                      | 105(60.0)    |
| Gender               |                            | < 0.001                         | 0.004        |
| Male                 | 86(49.1)                    | 225(78.7)                      | 86(49.1)     |
| Female               | 89(50.9)                    | 61(21.3)                       | 89(50.9)     |
| Smoking              |                            | < 0.001                         | 0.121        |
| Never                | 134(76.6)                   | 121(42.3)                      | 134(76.6)    |
| Smoking              | 41(23.4)                    | 165(57.7)                      | 41(23.4)     |
| Pathology            |                            | < 0.001                         | 0.061        |
| Adenocarcinoma       | 159(90.9)                   | 190(66.4)                      | 159(90.9)    |
| Squamous             | 13(7.4)                     | 93(32.5)                       | 13(7.4)      |
| Adenosquamous        | 3(1.7)                      | 3(1.0)                         | 3(1.7)       |
| Disease stage        |                            | 0.002                          | 0.545        |
| I                    | 17(9.7)                     | 17(5.9)                        | 17(9.7)      |
| II                   | 10(5.7)                     | 8(2.8)                         | 10(5.7)      |
| III                  | 20(11.4)                    | 70(24.5)                       | 20(11.4)     |
| Type               | Column 1 (IV) | Column 2 (191) | Column 3 (30) | Column 4 (289) | Column 5 |
|-------------------|--------------|----------------|--------------|---------------|----------|
| Lymphangitic      | 29 (16.6%)   | 40 (14.0%)     | 10 (23.8%)   | 59 (14.1%)    | 0.092    |
| Lymphadenopathy   | 123 (70.3%)  | 214 (74.8%)    | 30 (71.4%)   | 307 (73.3%)   | 0.798    |
| Emphysema         | 26 (14.9%)   | 107 (37.4%)    | < 0.001      | 129 (30.8%)   | 0.004    |
| Fibrosis          | 15 (8.6%)    | 42 (14.7%)     | 2 (4.8%)     | 55 (13.1%)    | 0.116    |
| Pleural effusion  | 73 (41.7%)   | 106 (37.1%)    | 16 (38.1%)   | 163 (38.9%)   | 0.919    |

**The relationship between various types of mutations in the EGFR gene and clinical characteristics**

Among the exon 19 deletion, L858R, L861Q, G719X, exon 20 insertion and S768I mutations in *EGFR*, there were no significant differences in age and gender. The NSCLC patients with exon 19 deletion (74.7%) and L858R (86.4%) most were non-smokers, while patients with L861Q (62.5%) and G719 × (66.7%) most were smokers (Table 4). But the sample size of patients with L861Q, G719X, exon 20 insertion and S768I in our study is relatively small, and this result cannot represent the actual situation and we need a large sample size to analyze this problem.
Table 4
Analysis of the relationship between various types of mutations in the *EGFR* gene and clinical characteristics.

| Characteristic | Exon 19 deletion | L858R | L861Q | G719X | Exon 20 insertion | S768I | P value |
|----------------|------------------|-------|-------|-------|------------------|-------|---------|
| No. (Total 175) | 95(54.3)         | 66(37.7) | 8(4.6) | 3(1.7) | 2(1.1)          | 1(0.6) |         |
| Age             |                  |        |       |       |                  |       | 0.059   |
| ≤ 60            | 47(49.5)         | 19(28.8) | 2(25.0) | 1(33.3) | 0(100)          | 1(100) |         |
| >60             | 48(50.5)         | 47(71.2) | 6(75.0) | 2(66.7) | 2(100)          | 0(0)   |         |
| Gender          |                  |        |       |       |                  |       | 0.199   |
| Male            | 47(49.5)         | 28(42.4) | 7(87.5) | 2(66.7) | 1(50.0)         | 1(100) |         |
| Female          | 48(50.5)         | 38(57.6) | 1(12.5) | 1(33.3) | 1(50.0)         | 0(0)   |         |
| Smoking         |                  |        |       |       |                  |       | 0.012   |
| Never           | 71(74.7)         | 57(86.4) | 3(37.5) | 1(33.3) | 1(50.0)         | 1(100) |         |
| Smoking         | 24(25.3)         | 9(13.6)  | 5(62.5) | 2(66.7) | 1(50.0)         | 0(0)   |         |

Discussion

The number of lung cancer patients and death cases in China has ranked first among all malignant tumors[3, 14, 15]. In the past decade, as the development of tumor molecular diagnosis and the continuous discovery of targeted drugs, the treatment of NSCLC has entered an era of individualized molecular targeted therapy. With the discovery of therapeutic targets *EGFR*, *ALK* and *ROS1* and the advent of corresponding targeted drugs, targeted therapy has become a very effective way to treat NSCLC clinically[16]. Studies have shown that NSCLC patients with *EGFR* sensitive mutations and *ALK*&*ROS1* gene fusions can benefit from corresponding targeted therapy[10, 17, 18]. Therefore, detection of *EGFR*, *ALK*&*ROS1* gene mutations has important clinical significance in screening patients suitable for targeted therapy. In present study, we investigated the clinical characteristics of NSCLC with *ALK*&*ROS1* gene rearrangement, *EGFR* mutations. Distinguishing the clinical characteristics of different molecular subtypes will be beneficial to the diagnosis and treatment of lung cancer.

*EGFR* is a transmembrane receptor tyrosine kinase, and the activation or phosphorylation of this region is of great significance for the signaling of proliferation and growth of cancer cells. *EGFR* mutation mainly includes 4 types: deletion mutations in exon 19, point mutations in exon 21, point mutations in exon 18 and insertion mutations in exon 20, among which exon 19 deletion mutation and L858R are the most common mutations sensitive to EGFR-TKI therapy. In concordance with previous reports, *EGFR* mutations were mainly 19 exon deletion mutations and L858R mutation in 461 NSCLC patients of this study. A number of researches have shown that the incidence of *EGFR* mutation is higher in women, non-smokers and adenocarcinoma[19, 20]. This study found that the incidence of *EGFR* mutation in women, adenocarcinoma and non-smokers is significantly higher than that in men, squamous cell carcinoma and smokers, and the difference between these groups is statistically
Since Soda et al. [21] first discovered a new EML4-ALK gene fusion in NSCLC patients in 2007, many scholars have conducted studies on it. Regarding the mutation rate of EML4-ALK in NSCLC, different literatures reported slight differences. Domestic and foreign research data showed that the incidence of ALK gene fusion in NSCLC patients was 3%-7% [22-24]. The positive rate of ROS1 gene fusion in NSCLC was 1.0%-3.4% [25], and the clinical characteristics of ALK and ROS1 gene fusion lung cancer were also very similar. The results of this study showed that the positive rate of ALK and ROS1 genes fusions was 9.1%, and the incidence of ALK and ROS1 gene fusions were relatively high in women and those less than 60 years old, and the difference was statistically significant. ALK gene fusions were identified in 33 cases (33/461, 7.2%) and ROS1 gene fusions were identified in 9 cases (9/461, 1.9%). Our work also confirms the low incidence of the ALK&ROS1 fusion among unselected NSCLC patients.

In this study, comparisons with non-ALK&ROS1/EGFR mutations NSCLC patients, patients with ALK&ROS1 gene rearrangement and EGFR mutation had a lower incidence of pulmonary emphysema. And it reflected that most of NSCLC patients with ALK&ROS1 gene rearrangement and EGFR mutation had a history of non-smoking. A plausible reason for this is that ALK&ROS1 gene rearrangement and EGFR mutation status have a stronger association with non-emphysema status.

Among the deletions in exon 19, L858R, L861Q, G719X, insertions in exon 20 and S768I mutations in EGFR, there were no significant difference in age and gender. The NSCLC patients with deletions in exon 19 (74.7%) and L858R (86.4%) most were non-smokers, while patients with L861Q (62.5%) and G719X (66.7%) most were smokers. But the sample size of patients with L861Q, G719X, insertions in exon 20 and S768I in our study is relatively small, and this result cannot represent the actual situation and we need a large sample size to analyze this problem.

According to reports, the incidence of ALK gene rearrangement in NSCLC patients is about 3%-7% [10], while the incidence of EGFR mutation is 40–80% [26], the sample size of patients with ALK&ROS1 gene rearrangement is small in our study. This is one of the limitations in this study. The purpose of this study was to evaluate the clinical characteristics of NSCLC patients to distinguish between ALK&ROS1 gene rearrangement, EGFR mutation, and non-ALK&ROS1/EGFR mutation. These results may assist clinicians to assess the NSCLC patients with these genetic abnormalities.

**Abbreviations**

NSCLC

non-small cell lung cancer

EGFR

epidermic growth factor receptor

EML4

echinoderm microtubule associated protein-like 4

ALK

anaplastic lymphomakinase

ROS1

proto-oncogene protein tyrosine kinase ROS

ARMS
amplification refractory mutation system

Declarations

Ethics approval and consent to participate

This study was conducted on the basis of the Declaration of Helsinki, and was supported by the Ethics Committee of the Meizhou People's Hospital.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Qinghua Liu, Heming Wu and Zhixiong Zhong designed the study. Heming Wu, Hailing Wu, Qingyan Huang and Hui Rao performed the experiments. Qinghua Liu, Hailing Wu and Hui Rao recruited subjects and collected clinical data. Hailing Wu and Zhikang Yu helped to analyze the data. Heming Wu prepared the manuscript. All authors were responsible for critical revisions, and all authors read and approved the final version of this work.

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Figure 1

Pathological features of non-small cell lung cancer. A, HE staining of squamous cell carcinoma; B, HE staining of adenocarcinoma; C, immunohistochemical staining of P63 expression in squamous cell carcinoma; D, immunohistochemical staining of NapsinA expression in adenocarcinoma; scale bar, 100μm.