ALK and ROS1 concurrent with EGFR mutation in patients with lung adenocarcinoma

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Purpose: The purpose of this study was to explore the frequencies of ALK and ROS1 fusion genes in EGFR-mutant lung adenocarcinoma patients and examine the therapeutic efficacies of EGFR-tyrosine kinase inhibitors (TKIs).

Materials and methods: A total of 421 EGFR-mutated patients taking EGFR-TKIs were examined for ALK and ROS1 fusion genes based on reverse transcription-polymerase chain reaction (RT-PCR). Progression-free survival (PFS) and overall survival (OS) were evaluated by the Kaplan–Meier method and compared by the log-rank test.

Results: The mutations of ALK rearrangement (n=10) and ROS1 rearrangement (n=3) were detected. All the patients received EGFR-TKIs, and eight took subsequent ALK/ROS1 inhibitor. PFS was longer in single EGFR mutants (n=408) than in EGFR/ALK or EGFR/ROS1 counterparts (n=13; 10.7 vs 6.6 months, P=0.004). No difference in OS existed between single EGFR and EGFR/ALK or EGFR/ROS1 mutants (21.0 vs 23.0 months, P=0.196). The median PFS of eight patients treated with ALK/ROS1 inhibitor was 6.0 months.

Conclusion: Concomitant ALK/ROS1 fusion genes occurred in 3.1% EGFR-mutated lung adenocarcinoma patients. Concomitant ALK/ROS1–EGFR mutations may influence the therapeutic efficacy of EGFR-TKIs.

Keywords: epidermal growth factor receptor, ALK, ROS1, lung adenocarcinoma, efficacy

Introduction

It is well known that the mutations of EGFR act as one of the most frequent driver genes in non-small cell lung cancer (NSCLC) patients, especially among East Asian female lung adenocarcinoma patients.\(^1\)\(^2\) Previous studies have shown that EGFR-tyrosine kinase inhibitors (EGFR-TKIs) could achieve a high response rate and yield a promising efficacy for EGFR mutants.\(^1\)\(^-\)\(^6\)

Despite a response rate of 60%–70% in EGFR-mutated lung cancer patients treated with EGFR-TKIs, 10%–20% of individuals developed primary resistance.\(^4\)\(^-\)\(^6\) The mechanism is currently ill defined. Possible contributing factors included T790M mutation, MET amplification and PIK3CA mutation.\(^7\)\(^-\)\(^9\) Concomitant genetic alterations were found to be associated with primary resistance in EGFR-mutated NSCLC patients.\(^10\)\(^,\)\(^11\) However, the studies were somewhat limited, and only several case series were reported. Furthermore, the efficacies of EGFR-TKIs have remained elusive.

Here, ALK and ROS1 fusion gene panel was used for screening the patients harboring EGFR-mutated samples and further detecting the therapeutic efficacies of EGFR-TKIs.
Materials and methods

Patient samples

Consecutive EGFR-mutated patients receiving EGFR-TKIs for lung adenocarcinoma were screened at Hangzhou Cancer Hospital between 2009 and 2015. The inclusion criteria were as follows: 1) uses of EGFR-TKIs during advanced stage; 2) sufficient residual tissues for detecting ALK and ROS1 fusion genes and 3) confirmed as having sensitive EGFR mutation. Resistant mutations such as T790M, S768I and exon 20 insertions were excluded. The study protocol was approved by the ethics committee of Hangzhou Cancer Hospital, and written informed consents were obtained from all patients.

Detection of EGFR/ALK/ROS1 fusion gene

EGFR gene was detected by amplification refractory mutation system (ARMS)-based kit (Amoy, Xiamen, China). It was capable of detecting the following 23 mutations: three in exon 18 (G719A, G719C and G719S), 13 deletions in exon 19, two mutations in exon 20 (T790M and S768I), three insertions in exon 20 and two mutations in exon 21 (L858R and L861Q).

The ALK and ROS1 fusion mRNAs were detected by polymerase chain reaction (PCR) using fusion gene detection kit (Amoy). Briefly, total RNA was extracted using Qiagen (Dusseldorf, Germany) RNeasy FFPE kit. mRNA was reverse transcribed into cDNA for 1 h at 42°C. β-actin was used as an internal control. The conditions of reverse transcription-polymerase chain reaction (RT-PCR) were as follows: initial denaturation at 95°C for 5 min, followed by 95°C for 25 s, 64°C for 20 s and 72°C for 20 s for ensuring specificity and 31 cycles at 93°C for 25 s, 60°C for 35 s and 72°C for 20 s. Data collection and sensitivity analysis were detailed previously.12 The subtypes of EGFR/ALK/ROS1 genes are summarized in Table S1.

Evaluations of TKI treatment

Tumors were evaluated during the treatment with EGFR-TKIs or ALK/ROS1 inhibitor every 8 weeks. Objective tumor responses were evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1). Objective response rate (ORR) included complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD).

Statistical analyses

The Kaplan–Meier method was used for survival analysis. Progression-free survival (PFS) of TKIs was defined as the time from initiating TKI treatment to documented progression or mortality from any cause. Statistical analysis was performed using the SPSS 18 software (SPSS Inc., Chicago, IL, USA). The median follow-up period was 28.5 months (4.5–65 months). The last follow-up time was October 31, 2016.

Results

Patient characteristics

A total of 421 patients were enrolled, and their clinical characteristics are summarized in Table 1. Among the patients, 13 were confirmed as having concomitant ALK/ROS1 fusion gene. Their clinical characteristics are summarized in Table 2. There were six males and seven females with a median age of 55 years. One patient was a former smoker and 12 had never smoked. The comparisons of single EGFR versus concomitant gene mutations are listed in Table 1.

Gene profiles

The genetic details of 421 EGFR mutations are as follows: exon 19 deletions (n=217), L858R mutation in exon 21 (n=169), G719X in exon 18 (n=21) and L861Q (n=14). Concomitant gene fusions were identified in 13 patients (3.1%), including ALK (n=10, 2.4%) and ROS1 (n=3, 0.7%). Their profiles are detailed in Table 2.

Table 1 Characteristics of the study population and comparison (n=421)

| Characteristics            | Total (n=421) | Single EGFR mutation (n=408) | Concurrent gene (n=13) | P-value |
|----------------------------|--------------|-----------------------------|------------------------|---------|
| Gender                     | Male         | 245                         | 239                    | 6       |
|                           | Female       | 176                         | 169                    | 7       |
| Age, years                 | 0.04         |                             |                        |         |
| <60                        | 224          | 213                         | 11                     |
| ≥60                        | 197          | 195                         | 2                      |
| Smoking status             | 0.20         |                             |                        |         |
| Never                      | 305          | 295                         | 12                     |
| Former/current             | 116          | 113                         | 1                      |
| Stage at EGFR-TKI treatment| 0.83         |                             |                        |         |
| IIIB                       | 12           | 12                          | 0                      |
| IV                         | 409          | 396                         | 13                     |
| EGR mutation type          | 0.67         |                             |                        |         |
| Exon 19 deletion + exon 21 | 386          | 374                         | 12                     |
| L858R                      | 35           | 34                          | 1                      |
| Other types                | 0.70         |                             |                        |         |
| Performance score at       | 0.11         |                             |                        |         |
| EGFR-TKI treatment         | 0–1          | 356                         | 345                    |
|                           | 2–3          | 65                          | 63                     |

Abbreviation: TKi, tyrosine kinase inhibitor.
Efficacy of TKIs

All 421 patients received EGFR-TKIs. Among 13 patients with concomitant genes, eight switched to crizotinib after ineffective EGFR-TKIs. The agents of EGFR-TKIs included erlotinib (n=71), gefitinib (n=126) and icotinib (n=224). None of them received any second-generation EGFR-TKI since so far none have been approved in China.

The clinical efficacies of 408 single EGFR-mutated patients included CR (n=2, 0.5%), PR (n=249, 61.0%), SD (n=87, 21.3%) and PD (n=70, 17.2%). In concomitant ALK/ROS1 mutants, the outcomes of EGFR-TKIs were PR (n=6), SD (n=3) and PD (n=4). The efficacy comparisons between single EGFR and concomitant gene mutations are shown in Table 3.

The overall value of PFS was 10.7 months (95% CI, 10.0–11.4). The median values of PFS were 10.7 and 6.6 months in mutants of single EGFR and concomitant ALK/ROS1 fusion gene, respectively (Figure 1, P=0.004). For eight patients on crizotinib, the value of PFS was 6.0 months for concomitant ALK/ROS1 fusion gene mutations (95% CI, 3.2–8.8).

The overall median value of OS was 21.0 months (95% CI, 18.9–23.4). No survival difference existed between single EGFR mutants and those with concomitant ALK/ROS1 fusion gene mutations (21.0 vs 23.0 months, P=0.196; Figure 2).

Discussion

Our results showed that 3.1% of EGFR-mutated lung adenocarcinoma Chinese patients harbored ALK/ROS1 fusion genes. Concurrent ALK/ROS1 gene decreased the therapeutic efficacy of EGFR-TKIs. However, it had no impact on the overall survival (OS).

Although gene alterations were presumable mutually exclusive in lung adenocarcinoma, some reports

Table 2 Clinical characteristics of 13 patients with EGFR and ALK/ROS1 concurrent genes

| Case | Gender | Age (years) | Smoking history | EGFR type | EGFR-TKI PFS | Response | ALK/ROS1 Crizotinib Response | Post-TKI treatment | Response OS/month |
|------|--------|------------|----------------|-----------|-------------|---------|-------------------------|------------------|------------------|
| 1    | Female | 47         | No             | 19del     | 6.6         | PR      | EML4–ALK                | –                | Chemotherapy     | 23.0             |
| 2    | Male   | 55         | No             | G719X     | 2.0         | PD      | EML4–ALK                | 11.2              | Supportive care  | 21.0             |
| 3    | Female | 57         | No             | 19del     | 3.1         | SD      | EML4–ALK                | 6.0               | Supportive care  | 11.5             |
| 4    | Male   | 59         | No             | L858R     | 4.0         | SD      | EML4–ALK                | 5.0               | Chemotherapy     | 45.0             |
| 5    | Female | 65         | No             | 19del     | 17.5        | PR      | EML4–ALK                | –                | Supportive care  | 23.5             |
| 6    | Female | 62         | No             | L858R     | 12.0        | PR      | EML4–ALK                | –                | Chemotherapy     | 33.2             |
| 7    | Male   | 56         | Yes            | 19del     | 10.2        | PR      | EML4–ALK                | –                | Supportive care  | 24.0             |
| 8    | Female | 55         | No             | 19del     | 1.1         | PD      | CD74–ROS1               | 7.0               | Chemotherapy     | 15.4             |
| 9    | Male   | 45         | No             | 19del     | 2.0         | PD      | EZR–ROS1                | 23.0              | Chemotherapy     | 35.0             |
| 10   | Female | 49         | No             | L858R     | 1.3         | PD      | EML4–ALK                | –                | Supportive care  | 17.6             |
| 11   | Female | 38         | No             | 19del     | 9.0         | PR      | EML4–ALK                | 1.2               | Chemotherapy     | 21.5             |
| 12   | Male   | 59         | No             | L858R     | 7.6         | PD      | CD74–ROS1               | 2.0               | Chemotherapy     | 24               |
| 13   | Male   | 51         | No             | 19del     | 8.2         | SD      | EML4–ALK                | 7.5               | Chemotherapy     | 48               |

Abbreviations: TKI, tyrosine kinase inhibitor; PFS, progression-free survival; OS, overall survival; PR, partial response; SD, stable disease; PD, progressive disease.

Table 3 Clinical efficacy comparison of EGFR-TKI in single EGFR mutation and concurrent gene alterations

| Best response | Single EGFR mutation (n=408) | Concurrent gene (n=13) | P-value |
|---------------|-----------------------------|------------------------|---------|
| CR, n (%)     | 2 (0.5)                     | 0 (0.0)                | –       |
| PR, n (%)     | 249 (61.0)                  | 6 (46.2)               | –       |
| SD, n (%)     | 87 (21.3)                   | 3 (23.1)               | –       |
| PD, n (%)     | 70 (17.2)                   | 4 (30.8)               | –       |
| ORR (%)       | 61.5                        | 46.2                   | 0.26    |
| DCR (%)       | 82.8                        | 69.2                   | 0.37    |
| Median PFS    | 10.7                        | 6.6                    | 0.004   |
| Median OS     | 21.0                        | 23.0                   | 0.196   |

Abbreviations: TKI, tyrosine kinase inhibitor; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; PFS, progression-free survival; OS, overall survival.

Figure 1 Comparison of PFS between single EGFR and concomitant ALK/ROS1 gene mutants on EGFR-TKI (10.7 vs 6.6 months, P=0.004).

Abbreviations: PFS, progression-free survival; TKI, tyrosine kinase inhibitor.
revealed that genes might occur concomitantly.14,15 With the emerging of high-throughput sequencing, many concomitant genes have been detected. The frequency of concomitant EGFR/ALK or EGFR/ROS1 mutations was 3.1% in the present study, and this figure was consistent with previous reports.16–19

Owing to a low frequency of concomitant genes in EGFR-mutated lung cancer patients, the efficacy of EGFR-TKIs is largely unknown. The median PFS of first-generation EGFR-TKIs was 11.2 months in patients harboring concomitant EGFR/ALK genes in one report by Yang et al.20 For concomitant ALK/ROS1 and EGFR mutants, there was a practical dilemma of therapeutic sequence of EGFR-TKIs and ALK/ROS1 inhibitors. Relative levels of phospho-EGFR or ALK could predict the efficacy of targeted treatment in EGFR/ALK mutants in the study of Yang et al.20 In the present study, all patients took EGFR-TKIs initially and eight took subsequent ALK/ROS1 inhibitor. The median value of PFS was 6.6 months for 13 patients on EGFR-TKIs. However, among eight patients on crizotinib, six obtained disease control. Owing to efficacy difference for dosing order of targeted therapy, despite a low frequency, there is a future need of using a useful marker for selecting targeted treatment in patients with concomitant genes. Except at the level of phosphorylation, abundance of gene was identified previously as a predictor of targeted therapy;21 the abundance level of different genes in one sample should be detected for patients with concomitant genes.

A small sample size was a major drawback of the present study. Second, not all patients received crizotinib after ineffective EGFR-TKIs. As a result, the clinical efficacy of further treatment could not be fully evaluated. Third, only RT-PCR was used for ALK/ROS1 genes so that false positives might ensue. Moreover, RT-PCR could not provide convincing evidence for the dominance of expression for one oncogene aberration over another in the same samples. In addition, it was impossible to check whether individual tumor cells had these two mutations simultaneously or singly.22 However, our findings are meaningful for clinical practice.

**Conclusion**

A total of 3.1% of EGFR-mutated patients harbored ALK/ROS1 fusion genes. Concomitant genes of ALK/ROS1 might decrease the therapeutic efficacy of EGFR-TKIs.

**Disclosure**

The authors report no conflicts of interest in this work.

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**Figure 2** Comparison of OS between single EGFR and concomitant ALK/ROS1 gene mutants on EGFR-TKIs (21.0 vs 23.0 months, P=0.196). **Abbreviations:** OS, overall survival; TKI, tyrosine kinase inhibitor.
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## Table S1 The subtypes of EGFR/ALK/ROS1 genes

| Gene type | Subtype of gene |
|-----------|----------------|
| ALK fusion | EML4 exon 13; ALK exon 20 |
|           | EML4 exon 6 ins 33; ALK exon 20 |
|           | EML4 exon 20; ALK exon 20 |
|           | EML4 exon 18; ALK exon 20 |
|           | EML4 exon 2; ALK exon 20 |
| ROS1 fusion | SLC34A2 exon 4; ROS1 exon 32 |
|           | SLC34A2 exon 14 del; ROS1 exon 32 |
|           | CD74 exon 6; ROS1 exon 32 |
|           | SDC4 exon 2; ROS1 exon 32 |
|           | SDC4 exon 4; ROS1 exon 32 |
|           | SLC34A2 exon 4; ROS1 exon 34 |
|           | SLC34A2 exon 14 del; ROS1 exon 34 |
|           | CD74 exon 6; ROS1 exon 34 |
|           | SDC4 exon 4; ROS1 exon 34 |
|           | EZR exon 10; ROS1 exon 34 |
|           | TPM3 exon 8; ROS1 exon 35 |
|           | LRIG3 exon 16; ROS1 exon 35 |
|           | GOPC exon 8; ROS1 exon 35 |
|           | E746_A750del (1) |
|           | E746_A750del (2) |
|           | L747_P753>S |
|           | L747_751>T |
|           | L746_S752>V |
|           | L747_751>Q |
|           | L747_E749del |
|           | L747_S752del |
|           | L747_A750>P |
| EGFR mutation | L747_P753>Q |
|           | L747_T751del |
|           | L747_T751>P |
|           | S768I |
|           | L858R |
|           | G719A |
|           | G719C |
|           | G719S |
|           | T790M |
|           | L861Q |
|           | H773_V774insH |
|           | D770_N771insG |
|           | V769_D770insASV |