**ORIGINAL ARTICLE**

**EGFR mutations in early-stage and advanced-stage lung adenocarcinoma: Analysis based on large-scale data from China**

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**Abstract**

**Background:** EGFR-tyrosine kinase inhibitors play an important role in the treatment of advanced non-small cell lung cancer (NSCLC). EGFR mutations in advanced NSCLC occur in approximately 30% of Asian patients and 60% of patients with adenocarcinoma. However, the frequency and type of EGFR mutations in early-stage lung adenocarcinoma remain unclear.

**Methods:** We retrospectively collected data on patients diagnosed with lung adenocarcinoma tested for EGFR mutation. Early stage was defined as pathological stage IA–IIIA after radical lung cancer surgery, and advanced stage was defined as clinical stage IIIB without the opportunity for curative treatment or stage IV according to the American Joint Committee on Cancer Staging Manual, 7th edition.

**Results:** A total of 1699 patients were enrolled in this study from May 2014 to May 2016; 750 were assigned to the early-stage and 949 to the advanced-stage group. Baseline characteristics of the two groups were balanced, except that there were more smokers in the advanced-stage group ($P < 0.001$). The total EGFR mutation rate in the early-stage group was similar to that in the advanced-stage group (53.6% vs. 51.4%, respectively; $P = 0.379$). There was no significant difference in EGFR mutation type between the two groups. In subgroup analysis of smoking history, there was no difference in EGFR mutation frequency or type between the early-stage and advanced-stage groups.

**Conclusion:** Early-stage and advanced-stage groups exhibited the same EGFR mutation frequencies and types.

**Introduction**

With the development of precision medicine, targeted therapies are playing an increasingly significant role in advanced non-small cell lung cancer (NSCLC). EGFR is the most important driver gene in NSCLC, especially in Asians. As the first-line therapy for advanced EGFR-mutant NSCLC, EGFR-tyrosine kinase inhibitors (TKIs), including gefitinib, erlotinib, afatinib, and osimertinib, prolong progression-free survival (PFS) to 9–18 months and have become standard first-line treatment.1–7

In addition to advanced-stage NSCLC, several studies have indicated that EGFR-TKIs play a role in early-stage NSCLC. Two recent clinical trials, SELECT and ADJUVANT, demonstrated that adjuvant EGFR-TKI treatment is feasible in patients with EGFR-mutant early-stage NSCLC.8,9 EGFR mutation status can predict the effects of EGFR-TKIs.10,11 EGFR mutations in advanced NSCLC occur in approximately 30% of Asian patients and 60% of female non-smokers with adenocarcinoma.12–14 However, the frequency and type of EGFR mutations in early-stage lung adenocarcinoma remain unclear. In this study, we
retrospectively reviewed the clinical characteristics and EGFR status of patients with lung adenocarcinoma to evaluate the differences in EGFR mutation rates and subtypes between early-stage and advanced-stage lung adenocarcinoma.

Methods

Patients and study design

All treatment-naive patients treated at the Guangdong Lung Cancer Institute/Guangdong General Hospital over the last 10 years signed informed consent permitting a query of their clinical information for the purpose of research.

We retrospectively collected data on patients diagnosed with adenocarcinoma (treatment-naive) and tested for EGFR mutations from May 2014 to May 2016 at Guangdong General Hospital. Patients with non-adenocarcinoma NSCLC, those without EGFR mutations, and those who previously received anti-tumor treatment or underwent re-biopsy were excluded. The patients were divided into two groups: early-stage, defined as pathological stage IA–IIIA (pT1-3N0-2M0 or T4N0-1M0) after radical lung cancer surgery; and advanced-stage, defined as stage IIIB without the opportunity for curative treatment or stage IV by clinical examination. Tumor stage was categorized according to the American Joint Committee on Cancer (AJCC) Staging Manual, 7th edition. Patients who received concurrent or sequential chemoradiotherapy were excluded.

The EGFR mutation type was categorized into five subgroups: exon 19 deletion, exon 21 L858R mutation, de novo exon 20 T790M mutation, compound mutations, and uncommon mutations (including G719X, L861Q, S768I, and 20 insertions). The definition of a compound mutation was two coexisting EGFR-sensitive mutations, including exon 19 deletion, L858R, S768I, L861Q, and G719X, in the same patient.

Data collection

The baseline characteristics of all patients, including age, gender, smoking history, pathology, EGFR mutation type, and clinical or pathological stage, were collected from the electronic medical record system of the Gongdong Lung Cancer Institute. In early-stage NSCLC, T and N staging was based on the results of surgical resection, and in advanced-stage NSCLC, tumor node metastasis (TNM) staging was based on comprehensive imaging results. EGFR mutations were detected using an amplification refractory mutation system (ARMS) (AmoyDx, XiaMen, China), as previously described.15

Statistical analysis

Differences among subgroups stratified by gender, age, and smoking status were analyzed by chi-square or Fisher’s exact tests, where appropriate. All analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Two-sided P values of < 0.05 were considered statistically significant.

Results

Patient characteristics

A flow chart of patient enrolment into the study is shown in Figure 1. A total of 3396 patients underwent EGFR mutation screening. Reasons for exclusion from the study were as follows: non-adenocarcinoma pathology (n = 786), history of EGFR-TKI treatment (n = 559), and no EGFR mutation screening (n = 249). Thus, a total of 1699 patients were included in the subsequent analyses. Of the 1699 patients, 750 were assigned to the early-stage and 949 to the advanced-stage group. The baseline characteristics of all patients are listed in Table 1. There were more smokers in the advanced-stage group (P < 0.001), but there was no difference in gender or age.

Comparison of the EGFR mutation rate between early-stage and advanced-stage adenocarcinoma

The EGFR mutation rate was 53.6% (402/750) in the early-stage and 51.4% (488/949) in the advanced-stage (P = 0.379) group. The mutation subtypes and rates in the early-stage group were: exon 19 deletion (23.2%), L858R mutation (24.8%), uncommon mutation (1.6%), de novo mutation (24.8%), T790M mutation (1.3%), and compound mutations (1.6%). There were no significant differences in EGFR mutation subtypes between the two groups, except for compound mutations (early-stage 1.6% vs. advanced-stage 0.4%; P = 0.02). The rates of the different EGFR mutation subtypes are listed in Figure 2. We further compared the differences in EGFR mutations between ever-smokers and never-smokers within the early-stage and advanced-stage groups. No significant differences in EGFR mutation frequency or subtype between the groups were found (Tables S1 and S2).

EGFR mutation in early-stage lung adenocarcinoma

The EGFR mutation status at each NSCLC stage is listed in Table 2. The mutation rate ranged from 32.4% (12/37, stage IIIB) to 60.2% (171/284, stage IA). The EGFR
Diagnosed with lung cancer via biopsy from May 2014 to May 2016 in GGLC (n = 3396)

Adenocarcinoma (n = 2610)

Treatment naive (n = 2051)

EGFR detected by ARMS (n = 1802)

Patients enrolled in subsequent analysis (n = 1699)

Table 1 Baseline patient characteristics

| Characteristic          | Early-stage (n = 750) | Advanced-stage (n = 949) | P   |
|-------------------------|-----------------------|--------------------------|-----|
| Gender                  |                        |                          |     |
| Male                    | 406 (54.1%)           | 557 (58.7%)              | 0.061|
| Female                  | 344 (45.9%)           | 392 (41.3%)              |     |
| Age                     |                        |                          |     |
| ≤ 60 years              | 434 (57.9%)           | 527 (55.5%)              | 0.349|
| > 60 years              | 316 (42.1%)           | 422 (44.5%)              |     |
| Smoking                 |                        |                          |     |
| Never-smoker            | 527 (70.3%)           | 402 (53.7%)              | < 0.001|
| Ever-smoker             | 223 (29.7%)           | 447 (46.3%)              |     |
| EGFR mutation type      |                        |                          |     |
| 19DEL                   | 174 (23.2%)           | 217 (22.9%)              | 0.908|
| L858R                   | 186 (24.8%)           | 224 (23.6%)              | 0.569|
| De novoT790M            | 10 (1.3%)             | 11 (1.2%)                | 0.826|
| Double mutation         | 12 (1.6%)             | 4 (0.4%)                 | 0.02 |
| Uncommon mutation       | 20 (2.7%)             | 32 (3.4%)                | 0.479|
| G719X                   | 9                     | 12                       |     |
| S768I                   | 1                     | 1                        |     |
| L861Q                   | 3                     | 3                        |     |
| 20 insertion            | 7                     | 16                       |     |
| Total                   | 402 (53.6%)           | 488 (51.4%)              | 0.379|

The EGFR mutation rate in patients with stage IIA–IIIA in the potential adjuvant targeted therapy population was 42.5% (114/268). We further explored the EGFR mutation rates according to lymph node metastasis status (N0, N1 and N2). The EGFR mutation rate was similar among N0, N1, and N2 NSCLC patients (N0: 55.2%, N1: 45.5%, N2: 44.8%; P = 0.391).

**Discussion**

Research on the differences in EGFR mutation status between early-stage and advanced-stage NSCLC is lacking. Herein, we retrospectively analyzed the records of 750 early-stage and 949 advanced-stage patients diagnosed with lung adenocarcinoma who received EGFR mutation screening at Guangdong General Hospital from May 2014 to May 2016. The clinical characteristics and EGFR mutation rates and types of these patients were compared. There were no significant differences in EGFR mutation frequency or subtype between early-stage and advanced-stage lung adenocarcinoma.

Previous research indicated that EGFR mutation is an “early event,” occurring during the initiation of lung cancer. Our research suggests that the EGFR mutation rate and type are similar between early-stage and advanced-stage adenocarcinoma patients (53.6% vs. 51.4%).
respectively; \( P = 0.379 \)). There were more never-smokers in the early-stage than in the advanced-stage group \( (P < 0.001) \). To eliminate any error caused by smoking status, we compared the never-smoker and ever-smoker subgroups within the early-stage and advanced-stage groups, respectively. The \( \text{EGFR} \) mutation rate and type were similar between the never-smoker and ever-smoker subgroups. When considering the whole population, the rate of compound mutations differed significantly between the early-stage and advanced-stage groups \( (P = 0.02) \). However, in the never-smoker and ever-smoker subgroups, the compound mutation rate did not differ significantly between the early-stage and advanced-stage groups. This result may have been caused by the small sample size of the compound mutation subgroup. Thus, our results indicate that \( \text{EGFR} \) mutations detected during the early stage of tumor growth may be an important treatment target, similar to advanced-stage NSCLC.

The IGNITE study is the largest analysis of real-world \( \text{EGFR} \) mutations, with 3382 advanced NSCLC patients from Asia-Pacific and Russia enrolled.\(^{17} \) The \( \text{EGFR} \) mutation rate was 49.3% in adenocarcinoma patients. A high \( \text{EGFR} \) mutation rate in tumors in Asian patients with adenocarcinoma was also reported in the PIONEER prospective study.\(^{18} \) In total, 1482 patients from seven Asian regions were enrolled, and the \( \text{EGFR} \) mutation rate was 51.4%, consistent with the \( \text{EGFR} \) mutation rate in advanced adenocarcinoma in our study. Previous studies have reported varying \( \text{EGFR} \) mutation rates in early-stage NSCLC patients. A retrospective study enrolled 311 patients with resected lung adenocarcinoma (high-risk stage IB–IIIA), and the \( \text{EGFR} \) mutation rate was only 28.3%.\(^{19} \) Another study enrolled 230 patients with stage I–III NSCLC, and the \( \text{EGFR} \) mutation rate was only 16.9% (39/230).\(^{20} \) Similarly, an \( \text{EGFR} \) mutation rate of only 20% was detected among 1118 patients with stage I–III lung adenocarcinoma, enrolled from 2002 to 2009.\(^{21} \) Yet another study reported an \( \text{EGFR} \) mutation rate of 34.5% in 754 patients with stage I–III NSCLC; according to subgroup analysis, the \( \text{EGFR} \) mutation rate was 38.7% in patients with adenocarcinoma.\(^{22} \) However, in our study, the \( \text{EGFR} \) mutation rate in stage I–IIIA lung adenocarcinoma was 53.6%. The difference in our \( \text{EGFR} \) mutation rate from those of previous studies may be explained by the following. First, the study population in the majority of previous studies was non-Asian, and the \( \text{EGFR} \) mutation rate is significantly higher in Asians than non-Asians. Additionally, we focused exclusively on patients with adenocarcinoma. Second, most of the previous studies used a small sample size. Finally, the method of \( \text{EGFR} \) detection may also have affected the results.

In our study, we used ARMS to detect \( \text{EGFR} \) mutations. ARMS can be used to detect \( \text{EGFR} \) mutations in tumor tissue at a frequency as low as 0.1%.\(^{23} \) Other methods, such as direct DNA sequencing, COBAS, and droplet digital PCR are also commonly used worldwide; however, ARMS is the only method approved by the China Food and Drug Administration for \( \text{EGFR} \) detection in tumor tissue.

Our study has a few limitations. First, this was a respective, single-center study and thus may not be representative of the general population. Data from multiple centers

### Table 2 $\text{EGFR}$ mutation rate at different stages

| Clinical stage | $\text{EGFR}$ mutation | $\text{EGFR}$ wild type | Mutation rate |
|----------------|------------------------|-------------------------|--------------|
| IA             | 171                    | 113                     | 60.2%        |
| IB             | 105                    | 87                      | 54.7%        |
| II A           | 35                     | 36                      | 49.3%        |
| II B           | 12                     | 25                      | 32.4%        |
| III A          | 67                     | 93                      | 41.9%        |
| Total (II A–III A) | 114                 | 154                     | 42.5%        |

| Lymph node metastasis | $\text{EGFR}$ mutation | $\text{EGFR}$ wild type | Mutation rate |
|-----------------------|------------------------|-------------------------|--------------|
| N0                    | 288                    | 234                     | 55.2%        |
| N1                    | 35                     | 42                      | 45.5%        |
| N2                    | 65                     | 80                      | 44.8%        |
would be more comprehensive. Second, the number of patients who underwent EGFR mutation screening during the early stages of their disease, especially stage IIA and IIB, was small, which may have affected our results. Finally, we only analyzed EGFR mutation status, while other driver genes such as ALK, ROS1, BRAF, and MET may also be adjuvant treatment targets.

In our study, early-stage and advanced-stage groups exhibited the same EGFR mutation frequencies and types.

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Disclosure

No authors report any conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Table S1. Baseline characteristics of never-smokers in early-stage and advanced-stage groups.

Table S2. Baseline characteristics of ever-smokers in early-stage and advanced-stage groups.