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ORIGINAL ARTICLE

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

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Keywords
EGFR mutation; lung adenocarcinoma; mutation frequency; mutation type; staging.

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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 35% 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 ADJUVENT, 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-naïve patients treated at the Gongdong 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-naïve) 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 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 IIB) to 60.2% (171/284, stage IA). The *EGFR*

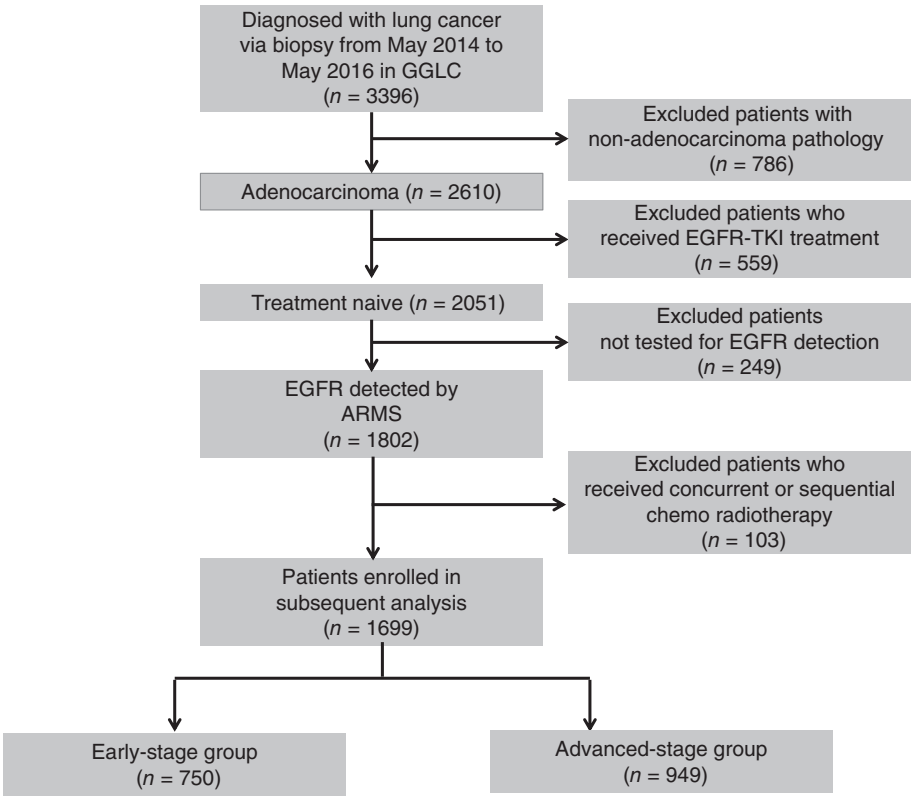

Figure 1 Flow chart of study enrolment. ARMS, amplification refractory mutation system.

Table 1 Baseline patient characteristics

Characteristic	Number		*P*
	Early-stage (*n* = 750)	Advanced-stage (*n* = 949)	
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%)	347 (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%)	*P* = 0.379

mutation status was significantly higher in stage IA than in stage IIB ($P = 0.002$) and stage IIIA ($P < 0.001$), but there was no difference between stage IA and IB ($P = 0.256$) or stage IIA ($P = 0.107$) 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.[16] 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%,

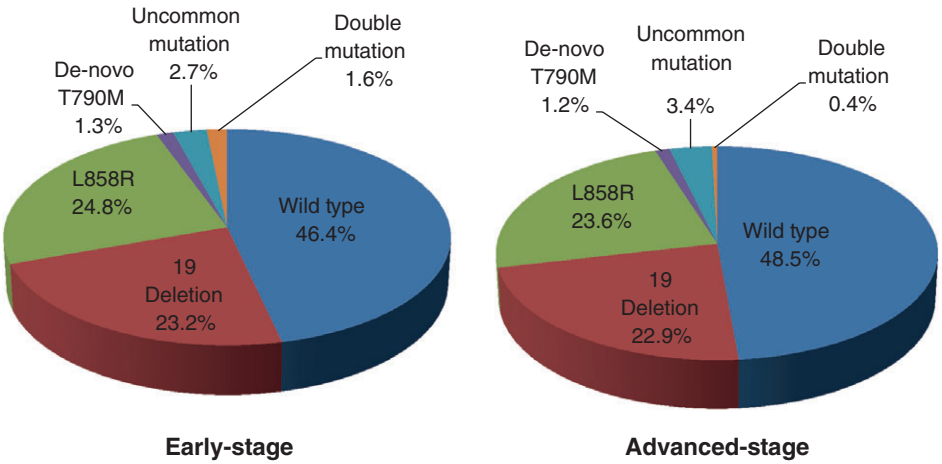

Figure 2 *EGFR* mutation types and rates in early-stage and advanced-stage groups.

Table 2 *EGFR* mutation rate at different stages

Clinical stage	*EGFR* mutation	*EGFR* wild type	Mutation rate
IA	171	113	60.2%
IB	105	87	54.7%
IIA	35	36	49.3%
IIB	12	25	32.4%
IIIA	67	93	41.9%
Total (IIA–IIIA)	114	154	42.5%
Lymph node metastasis			
N0	288	234	55.2%
N1	35	42	45.5%
N2	65	80	44.8%

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 *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 *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 *EGFR* mutations, with 3382 advanced NSCLC patients from Asia-Pacific and Russia enrolled.[17] The *EGFR* mutation rate was 49.3% in adenocarcinoma patients. A high *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 *EGFR* mutation rate was 51.4%, consistent with the *EGFR* mutation rate in advanced adenocarcinoma in our study. Previous studies have reported varying *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 *EGFR* mutation rate was only 28.3%.[19] Another study enrolled 230 patients with stage I–III NSCLC, and the *EGFR* mutation rate was only 16.9% (39/230).[20] Similarly, an *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 *EGFR* mutation rate of 34.5% in 754 patients with stage I–III NSCLC; according to subgroup analysis, the *EGFR* mutation rate was 38.7% in patients with adenocarcinoma.[22] However, in our study, the *EGFR* mutation rate in stage I–IIIA lung adenocarcinoma was 53.6%. The difference in our *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 *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 *EGFR* detection may also have affected the results.

In our study, we used ARMS to detect *EGFR* mutations. ARMS can be used to detect *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 *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

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 Informationmay 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.

