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Divergent epidermal growth factor receptor mutation patterns between smokers and non-smokers with lung adenocarcinoma

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ABSTRACT

Introduction: Smoking status is an important determinant of the prevalence of *epidermal growth factor receptor* (*EGFR*) mutations in lung cancer patients. However, it is unclear whether smoking status could also influence the spectrum of *EGFR* mutations.
Methods: We enrolled patients with lung adenocarcinoma from three medical centers in Taiwan. *EGFR* mutations were assessed by Sanger direct sequencing. The objective of this study was to evaluate the influence of smoking status on both the frequency and patterns of *EGFR* mutations.
Results: From 2001 to 2013, a total of 1175 patients with lung adenocarcinoma were enrolled for *EGFR* mutation analysis. The overall *EGFR* mutation rate was 59.6%, which was significantly higher in females than males (69.1% vs. 49.8%) and in non-smokers than current/former smokers (73.8% vs. 29.8%) (both $P < 0.001$). Among patients harboring *EGFR* mutations, smokers expressed L858R mutation less frequently (35.2% vs. 50.2%, $P = 0.005$) and exon 19 deletions more frequently (52.8% vs 38.8%, $P = 0.008$) than non-smokers. Smokers and non-smokers also had divergent exon 19 deletions subtypes (Del E746-A750 82.5% vs. 57.6%, respectively, $P < 0.001$). Among subgroup patients harboring the L858R mutation, smokers were associated with a higher rate of complex mutations than non-smokers (34.2% vs. 8.4%, $P < 0.001$).
Conclusions: Our results suggested that smoking status could influence not only the frequency but also the spectrum of *EGFR* mutations. These findings provide a clue for further investigation of *EGFR* mutagenesis.

1. Introduction

Epidermal growth factor receptor (*EGFR*), a member of the Erb B receptor family, plays a crucial role in cancer cell proliferation and survival and the mutation of *EGFR* gene is the most common genetic alteration in lung adenocarcinoma in Eastern Asians [1]. *EGFR* mutation is an important predictor of benefits with EGFR-tyrosine kinase inhibitors (TKIs) therapy. In recent years, several phase III studies demonstrated the significantly higher response

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rate and longer progression-free survival with EGFR-TKIs therapy than chemotherapy as the first line treatment in patients with *EGFR* mutant-non-small cell lung cancer (NSCLC) [2–6]. Moreover, the benefits of EGFR-TKIs are not confined to the first line therapy but can be also applied in subsequent treatment and in maintenance settings [7]. Therefore, a recently published guideline suggested routine testing of *EGFR* mutations in advanced NSCLC, especially those with adenocarcinoma histology [8].

Previous studies have identified several clinical features associated with the prevalence of *EGFR* mutations in lung cancers, including gender, histology, smoking status and ethnicity [9,10]. Regarding the smoking status, the *EGFR* mutations were significantly more frequent in non-smokers than current/former smokers. Study by Pham et al. further suggested that the number of smoking pack-years and smoke-free years could predict the frequency of *EGFR* mutations [11]. However, despite the well-documented association between the smoking status and the prevalence of *EGFR* mutations, many studies suggested that smokers should also undergo *EGFR* testing because a significant portion of them have been reported to harbor activating *EGFR* mutations [12] and would benefit from EGFR-TKIs therapy.

It is still unclear how smoking influences the *EGFR* mutagenesis. Furthermore, beyond the frequency of *EGFR* mutations, it is not known whether smoking status might influence the spectrum of *EGFR* mutations. We conducted this study to compare both the *EGFR* mutation frequency and patterns between non-smokers and smokers with lung adenocarcinoma.

2. Materials and methods

2.1. Patients

The study cohort consisted of 1175 patients with lung adenocarcinoma from three medical centers in Taiwan, including Taichung Veterans Hospital, Chang Gung Memorial Hospital and China Medical University Hospital, who were enrolled from 2001 to 2013. We included lung cancer patients with histologically or cytologically confirmed lung adenocarcinoma and with known *EGFR* mutation status determined by Sanger direct sequencing method. Patients were excluded if they had lung cancer other than adenocarcinoma histology, adenocarcinoma with doubtful lung origin or other active malignancy. Clinical data for analysis included patients' age, gender, smoking status and tumor stage. TNM (tumor, node, and metastases) staging was done according to the 7th edition of the American Joint Committee for Cancer (AJCC) staging system [13]. This study was approved by the institutional review boards of the participating institutions.

2.2. EGFR *mutation analysis*

EGFR mutational analysis using Sanger direct sequencing was performed in patients with adequate specimens. Tumor specimens were procured for *EGFR* mutational analysis as previously described [14]. Briefly, DNA was extracted from the tumors using a QIAamp DNA Mini kit (Qiagen, Valencia, CA) following the manufacturer's protocols. The tyrosine kinase domain of the *EGFR* coding sequence, exons 18–21, was amplified by polymerase chain reaction and sequenced bidirectionally with an ABI Prism 3730 DNA Analyzer following standard protocol. The sequences of primers for *EGFR* sequencing are shown in Supplementary Table 1.

2.3. Statistical methods

Univariate analysis by Fisher's exact test and Pearson's chi-square test was conducted on the frequency and spectrum of *EGFR* mutations to evaluate the effects of clinical factors relating to patient and disease characteristics. For further evaluation of the influence of smoking status on the *EGFR* mutation patterns, we analyzed patients with *EGFR*-mutant lung adenocarcinoma and divided them into a non-smokers' group and a smokers' group, which included current and former smokers. Non-smokers were defined as patients who had smoked less than 100 cigarettes in their lifetime and otherwise were defined as current or former smokers depending on whether they had stopped smoking for more than 1 year. *EGFR* mutation patterns were sorted as exon 19 deletions (19Del), exon 21 L858R and others. Complex mutations are defined as more than one different *EGFR* mutations are present in a single tumor specimen and complex mutations that involved 19Del and L858R were classified into the each group. We also evaluated if there was divergence in the 19Del and L858R mutation patterns among non-smokers and smokers. All statistical tests were done with SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Two-tailed tests and p values < 0.05 for significance were used.

Table 1
Patient characteristics and demographic data.

Characteristics	$n = 1175$
Age (years), median (range)	64 (25–98)
Gender, *n* (%)	
Male	580 (49.4)
Female	595 (50.6)
Smoking status, *n* (%)	
Non-smokers	800 (68.1)
Smokers	362 (30.8)
N/A	13 (1.1)
Stage, *n* (%)	
I	218 (18.6)
II	64 (5.4)
IIIa	105 (8.9)
IIIb	71 (6.0)
IV	716 (60.9)
N/A	1 (0.0)

N/A: not applicable.

3. Results

3.1. Patient characteristics

In total, we enrolled 1175 patients with lung adenocarcinoma from 3 hospitals for analysis. The baseline characteristics are shown in Table 1. The median age was 64 years (range: 25–98 years) and 580 patients (49.4%) were male. Seven hundreds and sixteen patients (60.9%) had metastatic lung cancer at baseline. Regarding the smoking status, 800 patients (68.1%) were non-smokers and 362 patients (30.8%) were current or former smokers. Thirteen patients (1.1%) had missing data on smoking status.

3.2. EGFR *mutation status and its association with clinical characteristics*

Of 1175 patients, the overall *EGFR* mutation rate was 59.6%. The spectrum of *EGFR* mutations is shown in Fig. 1 and Supplementary Table 2. Total 767 mutations were found in 700 *EGFR*-mutant patients (complex mutations are counted as separated mutation types here for full display of the *EGFR* mutation spectrum in our cohort). L858R (43.7%) and 19Del (37.4%) accounted for the major types of *EGFR* mutations. Five of 287 patients (1.7%) with 19Del and 38 of 335 patients (11.3%) with L858R harbored complex mutations. The frequencies of G719X, L861Q, E709X, exon 20 insertions and S768I were 2.9%, 2.9%, 2.5%, 1.7% and 1.0% respectively. Other rare mutation types were less than 1% frequency.

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