

# The co-mutation of *EGFR* and tumor-related genes leads to a worse prognosis and a higher level of tumor mutational burden in Chinese non-small cell lung cancer patients

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**Background:** Lung cancer is the leading cause of cancer mortality in China. The clinicopathologic features and genetic profile of Chinese lung cancer patients need to be investigated. This study evaluated the gene mutation profile, analyzed the frequency of concurrent genes in epidermal growth factor receptor (EGFR)-mutant non-small cell lung cancer (NSCLC) patients, and determined its prognostic value.

**Methods:** We collected the clinical data from 151 initially diagnosed patients NSCLC. Tumor samples underwent targeted next-generation sequencing (NGS). Progression-free survival (PFS) and overall survival (OS) were analyzed using the Kaplan-Meier method.

**Results:** Among the 151 participants, the mutational profile revealed alterations in 29 genes, where *TP53* (37.09%) and *EGFR* (30.46%) exhibited the highest mutation rates. Mutations in the *EGFR* gene were most prevalent (40%) in adenocarcinoma (LUAD) and were only present in 8.8% of participants with squamous cell carcinoma (LUSC). The most frequently mutated genes in LUAD patients were *TP53* (47%), followed by *KRAS* (11.7%). In all 39 participants with *EGFR* mutations, *TP53*, *KRAS*, *PIK3CA*, *APC*, and *FBXW*? were also mutated. Those with only *EGFR* mutation appeared to have a better prognosis; however, the difference was not statistically significant. Tumor mutational burden (TMB) was roughly significantly increased in patients who harbored *EGFR* and other mutant driver genes, compared with only *EGFR* mutant patients. The TMB value was significantly higher in those with *P53* mutation than in *P53* wild-type patients. **Conclusions:** We described the genetic profiles of NSCLC and compared the difference in genetic profiles between LUAD and LUSC. The concomitant genetic alterations might be a poor prognostic factor for patients with *EGFR* mutation, and TMB might be prognostically related to the co-mutations of *EGFR* and other genes.

**Keywords:** Non-small cell lung cancer (NSCLC); tumor mutational burden (TMB); epidermal growth factor receptor (*EGFR*); prognosis

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# Introduction

Lung cancer is the main cause of cancer deaths globally and in China (1). Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, comprising approximately 80% (2,3). Adenocarcinoma (about half of cases) and Squamous cell carcinoma (about a quarter of cases) are the main histological subtypes of NSCLC (4). The treatment of NSCLC includes chemoradiotherapy, surgery, targeted therapy, and immunotherapy (5).

With the accumulation of mutations, if the key genes that control cell survival and proliferation undergo activating mutations, their continuous activation of signal pathways related to cell proliferation will lead to malignant transformation of cells and obtain the characteristics of a series of malignant tumors such as immortal proliferation and metastasis (6,7), so this type of mutation is also called a driver mutation (8). Previous study demonstrated that nextgeneration sequencing (NGS) technologies were performed to identify the genetic characterizations of NSCLC and determine targets of tyrosine kinase inhibitors (TKIs) (9). The most frequent somatic changes were detected in certain genes, such as EGFR, TP53, FGFR1, KRAS, PIK3CA, ERBB2 (HER2), BRAF, ALK, ROS1, MAP2K1/MEK1, RET, NRAS, and AKT1 (10).

Each histological subtype of cancer has its own unique genetic mutational profile, based on different genetic and environmental risk factors. Genetic profiling of the individual tumor could facilitate understanding of the distinct molecular mechanism that regulates cancer progression and reveal potential therapeutic targets. Lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) should have different genetic profiles, responsible for their different responses to therapies.

Epidermal growth factor receptor (*EGFR*) and tumor suppressor *TP53* genes have been shown to be the 2 most commonly mutated genes in patients with NSCLC. Deletion of *EGFR* exon19 and exon21 L858R represent 85– 90% *EGFR* activation mutations in lung cancer, and they are sensitive to approved EGFR-TKIs such as erlotinib, gefitinib, and afatinib (11-13). Exon18 and 20 mutations are uncommon mutations and less sensitive or completely insensitive to TKIs (14). The tumor protein p53 (*TP53*) is a tumor suppressor gene and the most commonly mutated gene in human cancers (15). Patients who carry *TP53* mutations generally might experience more aggressive disease, increased rates of resistance to chemotherapy, and shorter survival (16,17). It has been reported that patients with both *EGFR* and *TP53* mutations might have decreased responsiveness to EGFR-TKIs (18,19). Barnet *et al.* reported that PIK3CA and EGFR co-mutation provide clinically meaningful information predicting a poor response (20). This study analyzed the prognostic implication of co-mutations of other cancer-related genes in LUAD patients with EGFR mutation.

Tumor mutational burden (TMB) is a hallmark of cancer, which has been associated with microsatellite instability, defective DNA replication/repair, and response to PD-1 and PD-L1 blockade immunotherapy (21). The TMB can be calculated from somatic mutation counts in the coding genome using NGS data. The determination of TMB is of great value not only in the characteristics of tumors but also in predicting the clinical efficacy of immunotherapy. Elevated TMB has been related to the efficacy of immune checkpoint blockade (ICB) response in lung cancers and other tumor types (22-24).

Our study aimed to determine the clinicopathologic features and genetic profile of Chinese NSCLC patients. We attempted to elucidate the prognostic impact of comutations in other cancer-related genes on survival in patients with NSCLC harboring *EGFR* mutations. We present the following article in accordance with the REMARK reporting checklist (available at https://jtd. amegroups.com/article/view/10.21037/jtd-21-1921/rc).

# Methods

#### Patients and samples

Matched pairs of tumor and normal tissue samples of 151 Chinese patients with NSCLC were collected in Zhongshan Hospital of Xiamen University. Basic and clinicopathological information from all participants was included and all samples were formalin-fixed and paraffin-embedded. This study was approved by the Ethics Committee of Zhongshan Hospital of Xiamen University (No. 2021-172). All procedures performed in this study involving human participants were under the Declaration of Helsinki (revised in 2013). Written informed consent was obtained from all study participants. We extracted DNA using a formalin-fixed paraffin-embedded whole-genome extraction kit (cat. No. 180134, Qiagen, Hilden, Germany) according to the manufacturer's protocol.

#### Targeted NGS

The DNeasy Blood & Tissue Kit (69504, QIAGEN, Venlo, Netherlands) was used to extract total DNA by strictly

#### Journal of Thoracic Disease, Vol 14, No 1 January 2022

Table 1	Basic clinica	characteristics of lu	ng cancer patients
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Characteristics	N=151
Age (years)	65.76±10.74
Gender (%)	
Male	64.24
Female	35.76
Histology (%)	
Adenocarcinoma	65.56
Squamous cell carcinoma	22.52
Unclear	11.92
Stage (%)	
1	0.66
2	3.31
3	17.22
4	65.56
Unknown	13.25
Smoking history (%)	55.41 (82/148)
Metastasis (%)	
Brain metastasis	2.95 (13/132)
Bone metastasis	9.84 (39/132)

follow the procedures. Agilent's Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) was used to assess the amount of DNA. The NGS was performed by five different lung cancer-related gene panels and whole-exome sequencing (WES).

#### Mutation calling and analysis

The sequencing data were compared and sequenced by Burrow-Wheeler Aligner (BWA) (25) with the human reference genome (NCBI Build 37; https://www.ncbi.nlm. nih.gov/genome/guide/human/). The duplicate polymerase chain reaction (PCR) data was removed through SAMtools (http://www.htslib.org/) (26). Vardict is based on the compilation and detection algorithm of heuristic algorithm to realize the mutation extraction of SNP and INDEL, and judge the authenticity of the site through mutation reads and database information. Annovar can determine whether SNP or CNV causes protein coding changes and affected amino acids, and can autonomously select gene definition systems (such as RefSeq/UCSC/Ensemble, etc.), and can also identify variants of specific genomic regions, or link other databases to variants Make comments (e.g., dbSNP/1000 Genome Project). The variation call files generated by VarDict (27) were exegesis with ANNOVAR (28) and then modified to MAF files using maftools (29). After removing all synonymous mutations from the MAF file, maftools was used to calculate the variance of each participant. Only non-synonymous mutations were included in the TMB (mutations/MB) calculation.

#### Statistical analysis

Continuous variables were expressed as medians and interquartile ranges (IQR) and categorical variables were presented as percentages, using analysis of variance (ANOVA) test and chi-square test for comparison of their differences, respectively. These statistical analyses were performed by SPSS version 26.0 (IBM Corp., Chicago, IL, USA). Overall survival (OS) was analyzed using the Kaplan-Meier method and the log-rank test was used to compare survival curves, which were performed by Graphpad Prism version 8 (GraphPad Software, San Diego, CA, USA).

#### **Results**

#### **Clinical characteristics**

A total of 151 NSCLC samples underwent molecular testing at initial diagnosis, among which 142 were detected by different cancer-related gene panels, and the others were detected by WES. The cohort average age was 65.76±10.74 years. There were 97 (97/151, 64.24%) male and 54 (54/151, 35.76%) female patients. There were 99 (99/151, 65.56%) cases of LUAD and 34 (34/151, 22.52%) cases of LUSC. Slightly more than half of the participants (82/148, 55.41%) had a history of smoking. Most participants had stage III (17.22%, n=26) or IV (65.56%, n=99) disease at the time of initial diagnosis; 9.84% (13/132) patients had brain metastasis, and 2.95% (39/132) of patients had bone metastasis (*Table 1*).

The distribution of mutated genes is shown in Figure 1A. The top three frequent mutated genes were TP53, EGFR, and KRAS. We found a distinct difference of mutation pattern between LUAD and LUSC from the somatic mutational profile of the patients. As shown in Figure 1B,1C, EGFR mutations were most prevalent in LUAD, and approximately 40% of participants harbored somatic EGFR mutations. Unlike LUAD, only 8.8% of LUSC patients carried somatic mutations of EGFR. The



Figure 1 Mutational profiling of Chinese NSCLC patients. (A) Genetic alteration frequency identified by NGS in all 151 samples; (B,C) genetic alteration frequency identified by NGS in LUAD (B) and LUSC (C) patients. NSCLC, non-small-cell lung cancer; NGS, next-generation sequencing; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

most frequent mutant genes in LUSC participants were *TP53* (47%), followed by *KRAS* (11.7%).

There were 39 LUAD and 3 LUSC participants identified with somatic *EGFR* mutations. Of these NSCLC cases, 85.7% (36/42) patients [84.6% (33/39) LUAD and 100% (3/3) LUSC patients] had only one type *EGFR* mutation, while the other 14.3% had compound *EGFR* mutations (with 2 or more *EGFR* mutations). Due to the low incidence of EGFR in LUSC patients (only three patients), we show the statistical results of EGFR mutation types and mutation frequencies in LUAD patients in *Figure 2*. The patients with only *EGFR* mutations were distributed in exon18 (2.6%), exon19 (30.8%), exon20 (12.8%), and exon21 (38.4%). Compound *EGFR* mutations were located in exon19 del + CNV (5.1%), L858R + CNV (5.1%), G719C + p.S768I + CNV (2.6%), and E709K + p.L858R (2.6%).

# Prognostic implications of co-mutations with EGFR mutation in LUAD patients

Among the 39 EGFR-mutated LUAD patients, 20 patients were concomitant to other somatic cancer-related gene

mutations; that is, the incidence of cancer-related gene co-mutation in patients with lung adenocarcinoma in NSCLC was 20.2% (20/99). Mutations occurring alongside EGFR mutations included those of TP53, KRAS, PIK3CA, APC, and FBXW7. Among these 5 genes, TP53 was the most frequently co-mutated (Figure 3A). We analyzed the prognostic implication of co-mutations of cancerrelated genes in LUAD patients with EGFR mutation by comparing the OS and progression-free survival (PFS) between the two groups (Figure 3B-3E). The participants with EGFR mutation only appeared to have a better prognosis (*Figure 3B,3C*); however, the difference was not statistically significant (P=0.25 for PFS; P=0.34 for OS). Among participants with co-mutations of EGFR and other genes, there were 17 patients with TP53 gene mutations (Figure 3D, 3E); however, there was no significant difference in the prognosis between participants with EGFR and TP53 co-mutation and those with only EGFR mutation.

#### TMB of LUAD patients with EGFR mutations

The TMB values were calculated from the mutation data of 23 LUAD patients. As shown in *Figure 4A*, among the 10



Figure 2 Frequency and distribution of EGFR mutations in LUAD patients. EGFR, epidermal growth factor receptor; LUAD, lung adenocarcinoma.

of 23 LUAD participants with EGFR mutation, the TMB value was not significant difference between EGFR wildtype and EGFR mutation patients (P=0.57). The TMB value was roughly significantly increased in patients who harbored EGFR and other driver genes co-mutations, compared with only EGFR mutant patients (Figure 4B, P=0.14). Furthermore, the TMB value was significantly higher in participants with P53 mutation than in P53 wildtype participants (Figure 4C, P=0.002). However, in the patients with EGFR mutation, the TMB values were not significantly different between P53 mutation and P53 wildtype (Figure 4D, P=0.25).

# Discussion

Lung cancer is the leading cause of death among men and the second leading cause among women. A previous study revealed the differences in genetic profiles of tumor tissues between LUAD and LUSC: Ding *et al.* analyzed the mutational profile of 318 Chinese NSCLC patients and found that more than half of LUAD patients had *EGFR* mutation, and a total of 90% of the LUSC patients carried the *TP53* gene mutation (30). In this study, we described the somatic mutation profile of NGS in Chinese NSCLC patients, assessed the impact of co-mutation of *EGFR* and other cancer-related genes on the prognosis, and the differences in TMB between these groups. We found that *EGFR* is the most frequently mutated gene in LUAD patients. The most commonly mutated gene in LUSC patients is TP53. Patients with LUAD who carry *EGFR* mutations and without other cancer-related gene mutations seem to have longer PFS and OS. In patients with *EGFR* mutations, *TP53* wild-type and *TP53* mutation do not affect the prognosis. We found that the TMB of patients with *EGFR* single mutation was significantly lower than patients with co-mutation of *EGFR* and cancer-related genes.

Almost all of the driver mutations currently found in non-small cell lung cancer occur in key kinases that control cell survival and proliferation signaling pathways, including members of the receptor tyrosine family expressed on cell membranes (such as EGFR, HER2, FGFR, DDR2, and MET), as well as some kinases in the cell membrane (such as KRAS, BRAF, AKT1, P13KCA, and MEK1) (31,32). Some previous studies have suggested that the differences in prognosis for NSCLC patients was due to mutations in EGFR. For instance, clinical data has shown that the benefit of EGFR-TKIs was greater for patients with exon19 deletions than exon21 L858R substitutions (33,34). Patients with exon21 L858R substitutions had significantly longer PFS compared to those with exon19 deletions who underwent chemotherapy (35). The differences in cooccurring molecular events may partly explain the clinical heterogeneity in patients with EGFR-mutant NSCLC. Tang et al. reported several types of research about the prognosis of EGFR-mutant patients with concomitant genetic alterations (36). In our study, the top co-mutated

#### Li et al. Co-mutation worsens Chinese NSCLC patients



**Figure 3** The differences of prognosis between single EGFR mutation and concurrent gene alterations patients. (A) Co-occurring genetic alterations of LUAD EGFR-mutant patients. (B,C) Kaplan-Meier analyses of PFS (B, HR 0.20; 95% CI: 0.019–2.17) and OS (C, HR 0.25; 95% CI: 0.014–4.43), respectively, between EGFR-only mutation and co-mutation with other genes. (D,E) Kaplan-Meier estimates the differences of progression-free survival (D, HR 1.07; 95% CI: 0.09–12.31) and overall survival (E, HR 0.89; 95% CI: 0.07–11.36) between EGFR and TP53 co-mutation patients and EGFR mutation with TP53 wild-type patients. EGFR, epidermal growth factor receptor; LUAD, lung adenocarcinoma; PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.



**Figure 4** The TMB values of LUAD patients. (A) EGFR-mutant versus EGFR wild-type patients; (B) EGFR-Only mutant versus comutant patients. (C) P53 wild-type *vs.* P53 mutant patients. (D) EGFR and P53 co-mutation *vs.* EGFR mutation with TP53 wild-type patients. \*\*, 0.001<P<0.01. TMB, tumor mutational burden; EGFR, epidermal growth factor receptor.

190

#### Journal of Thoracic Disease, Vol 14, No 1 January 2022

genes with *EGFR* in NSCLC patients were *TP53*, *KRAS*, and *PIK3CA*. Eng *et al.* showed that concurrent *PIK3CA* mutation did not decrease the efficacy of EGFR-TKIs in patients who had *EGFR/PIK3CA* co-mutation (37). In a Chinese cohort, NSCLC patients without co-mutation genes had a significantly longer PFS with EGFR-TKIs treatment and no significant OS difference between patients with single *EGFR* mutation and co-mutation genes (38). In our study, the patients with *EGFR* mutation only seemed to have longer PFS and OS, but without statistical significance, which might be due to the small sample size.

Multiple somatic mutations in the same tumor are associated with poorer outcomes in resected NSCLC (39). Hence, several studies have focused on the prognostic effect of co-mutated TP53 and EGFR genes. Labbe et al. demonstrated that the coexistence of a TP53 mutation had no influence on relapse-free survival (RFS) or OS after primary surgical resection, and PFS while on first-line EGFR-TKI therapy was significantly shorter for patients with dual mutations when comparing only TP53 missense mutations to TP53 wild-type (40). Jiao et al. reported that TP53 mutation was not conducive to OS in metastatic NSCLC patients, and the estimated survival time for wildtype TP53 was significantly longer than mutated TP53 (41). In this study, the prognosis was no different between TP53 WT and TP53 mutant patients who carried EGFR mutation. Maybe because the participants were divided into subgroups according to different treatment strategies, the number of participants that underwent prognosis assessment was limited.

A previous study revealed that the TMB was lower in *EGFR* mutant patients than *EGFR* wild-type patients in lung cancer, which may play a negative role in the efficacy of EGFR-TKI therapy in lung cancer patients who carry the *EGFR*-mutant (42). In our study, we found that TMB was significantly lower in *EGFR* only mutation participants than those with *EGFR* co-mutation. We speculate that TMB is a helpful marker for predicting the efficacy of targeted therapy. However, whether TMB is associated with clinical outcomes remains to be elucidated.

# Conclusions

This study revealed the genetic profiles of NSCLC and compared the difference in genetic profiles between LUAD and LUSC. Concomitant genetic alterations might be a poor prognostic factor for patients with *EGFR* mutation, and TMB might be related to the co-mutations of *EGFR*  191

and other genes in terms of prognosis.

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# Footnote

*Reporting Checklist:* The authors have completed the REMARK reporting checklist. Available at https://jtd. amegroups.com/article/view/10.21037/jtd-21-1921/rc

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Ethics Committee of Zhongshan Hospital of Xiamen University (No. 2021-172). All procedures performed in this study involving human participants were under the Declaration of Helsinki (revised in 2013). Written informed consent was obtained from all study participants.

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