Original Article

Clinicopathological features of Chinese lung cancer patients with *epidermal growth factor receptor* mutation

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Background: *Epidermal growth factor receptor* (*EGFR*) gene was the major causative gene of lung cancer and also the specific treatment target. It is necessary to analyze the genotype and phenotype characters of patients.

Methods: We investigated 1,034 lung cancer patients in this study. The collected clinicopathological parameters included gender, age at diagnosis, smoking status, pathological TNM stage, tumor morphology and location, visceral pleural invasion as well as histological type.

Results: Almost 50% participants had *EGFR* mutations. L858R in exon 21 was the most common type. Concomitant mutation, 19 del and L858R, were detected in 20 patients. Compared to patients with exon 19 del or L858R mutations solely, they were inclined to have small size adenocarcinomas which occurred in bilateral and invaded the visceral pleura. The tyrosine kinases inhibitors (TKIs)-resistant mutation, insertions in exon 20, was detected in 11 patients.

Conclusions: The summarized clinicopathological features will help clinicians to implement the feasible treatment plan.

Keywords: Lung cancer; *epidermal growth factor receptor* (*EGFR*) gene; mutation; phenotype; genotype

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Introduction

Epidermal growth factor receptor (*EGFR*) gene locates at chromosome 7 and has 28 exons. Mutations in *EGFR* gene are associated with different kinds of tumor including lung cancer. The EGFR protein which is a member of the protein kinase superfamily is a transmembrane glycoprotein. It is a cell surface protein receptor that binds to epidermal growth factor (EGF). The ligand and receptor binding induces dimerization and tyrosine autophosphorylation of EGFR (1). EGFR regulates cellular signaling pathways, promotes tumor cell differentiation, proliferation, maintenance, invasion and metastasis (2,3).

EGFR contains extracellular domain, transmembrane domain and intracellular domain which had tyrosine kinases (TK) domain and autophosphorylation domain. The TK domain which is the functional core of the protein consists of a smaller N-terminal and a larger C-terminal lobe (4). It stretches from exon 18 to exon 24. In lung cancer, the *EGFR* mutation sites center on exons 18–21 (5). Increasing catalytic activity of TK domain caused by *EGFR* mutation results in greatly over-expressed EGFR (3,6). Meanwhile

it also provides a specific therapeutic strategy. Tyrosine kinases inhibitors (TKIs) targeted to TK domain have been approved for the treatment of NSCLC (7,8). Several studies suggest that the application of TKIs improved response rates and progression-free survival of lung cancer patients with *EGFR* mutations (9,10). The sensitivity of lung cancer patients to TKIs is associated with the mutation type. Patients with deletions in exon 19 and L858R in exon 21 responded positively. In this respect, the detection of *EGFR* mutations is the premise to the treatment of lung cancer patients. But in clinic the quantity of biopsy samples were not enough to fulfill the entire mutation screening. The phenotypic traits summary could help clinicians make judgement beforehand. Furthermore, most previous studies on *EGFR* mutations mainly focused on lung adenocarcinoma, few studies have evaluated the *EGFR* mutations in other lung cancer type in large scale. In the current study, we analyzed the *EGFR* mutation spectrum in Chinese lung cancer patients and summarized the clinicopathological characters of patients with *EGFR* gene mutations.

Methods

Ethical approval

This study was approved by the Institutional Review Board (IRB) of Shanghai Pulmonary Hospital affiliated Tongji University (No. 2014-016). Written informed consents were obtained from all participants. The methods were carried out in accordance with the approved guidelines.

Patients and specimen collection

The consecutive primary lung cancer patients who were admitted into the Shanghai Pulmonary Hospital affiliated Tongji University from Jun. 2014 to Oct. 2015 were recruited. No choose or correct was performed on patients' collection. None of these patients received any anticancer therapies prior to surgery. The recurrent or metastatic patients were excluded. The samples which contained more than 50% tumor cells were qualified. Fresh primary tumor tissues were collected during the surgery.

Clinical and pathological data which was gathered for analysis included gender, age at diagnosis, pathological TNM stage, histological type, tumor morphology and location, visceral pleural invasion as well as smoking status. Tumors were staged pathologically according to the Union International Contre le Cancer (UICC-7) staging system for lung cancer (11).

Candidate gene mutation analysis

According to the manufacturer's instruction, genomic DNA and total RNA were extracted from fresh tumor tissues using QIAamp DNA Tissue Kit and RNeasy Kit (Qiagen, Germany) respectively. *EGFR* mutations were detected by Amoy Diagnostics kits (Xiamen, China) which were based on amplification refractory mutation system (ARMS) real-time PCR. Twenty-nine mutations in exons 18–21 of *EGFR* gene were detected including T790M, L858R, L861Q, S768I, G719S, G719A, G719C, three types of insertions in exon 20, and 19 kinds of deletions in exon 19.

Statistical analysis

χ^2 test was used to analyze the association between the *EGFR* mutation type and other clinicopathology data. All data were analyzed by the SPSS package for Windows (Version 18.0, Chicago, IL). P value <0.05 was considered statistically significant.

Results

Mutation spectrum

In total, 1,034 lung cancer patients were recruited in this study (*Table 1*), 515 of them had the *EGFR* gene mutations, 51.26% of them had L858R mutation and 39.61% had deletions in exon 19. G719X and L861Q were detected in less than 2% patients respectively. Besides, it was noteworthy that 20 patients had complex mutation, 19 del and L858R together.

The phenotype and genotype associations

Compared to the wild-type *EGFR* patients, *EGFR* L858R mutation patients showed evident differences in the tumor site, pathological stage and type, tobacco using status, tumor size and visceral pleura invasion status (*Table 2*). Most tumors with *EGFR* L858R mutation located on the right side while tumors with non-*EGFR* mutation were on the left sides. Although the age profile looked similar between these two groups, it still had a little difference if it was analyzed by gender. L858R mutation female patients were elder than that of non-*EGFR* mutation.

Table 1 The demography information of 1,034 Chinese lung cancer patients

Item	Female	Male	Total
Case	342	692	1,034
Age			
<30	0.00%	0.14%	0.10%
31–40	2.34%	1.73%	1.93%
41–50	16.96%	9.68%	12.09%
51–60	33.63%	32.37%	32.79%
61–70	35.38%	38.44%	37.43%
>70	11.70%	17.63%	15.67%
Smoking			
Never smoked	92.40%*	29.62%*	50.39%
<100	0.29%	0.29%	0.29%
101–500	4.97%*	24.28%*	17.89%
501–1000	2.05%*	36.85%*	25.34%
>1000	0.29%*	8.96%*	6.09%
Pathological type			
Lung adenocarcinomas	96.20%*	69.51%*	78.34%
Squamous carcinoma	1.17%*	20.38%*	14.02%
Large cell carcinoma	0.29%*	4.62%*	3.19%
Small cell carcinoma	0.00%	1.88%	1.26%
Other	1.17%*	20.38%*	14.02%

*, P<0.01.

Tumors with *EGFR* 19 del mutation were also inclined to occur on the right side. This group had more advanced tumor (stage III) compared to the *EGFR* L858R mutation and wild-type patients. The invasion to visceral pleura was also common in this group.

Discussion

Lung cancer with the highest prevalence around the world caused great damage to patients. The burden of it was more and more serious. An effective treatment was urgently needed. In the past few years, the application of TKIs had greatly improved the treatment effect of lung cancer (9). But not all lung cancer patients were sensitive to TKIs. Previous reports suggested Asian female adenocarcinoma patients with no-smoking were sensible to TKIs (12,13). But it is still controversial (9). Therefore patient stratification is critical for successful application of TKIs. Accumulated evidences had suggested that the identification of *EGFR* mutations in lung adenocarcinomas was essential before administering TKIs (10).

EGFR is the major causative gene of lung cancer. But the mutation frequency is highly dependent on ethnicity. Patients with Asian origin have a higher prevalence compared with Caucasian. It was reported that *EGFR* mutation was detected in 34.3% of lung patients in Hong Kong (14) and 18.9% in South Korea (15). It was only 8–15% in patients from western European countries and 12–16% in American patients (16). In this study, we found the *EGFR* mutation rate was around 50% in our patients which was higher than the previous reported prevalence.

The most common *EGFR* mutations with lung cancer are 19 del and L858R mutation. Mutant EGFR protein results in constitutive activation of EGFR and promotes EGFR-mediated pro-survival and anti-apoptotic signal pathways (17). In our study, L858R was the most common type of *EGFR* mutation which was consistent with the previous report (18). But exon 19 del mutation was the dominant type in other reports (19). The inconsistent results might be caused by ethnic variations.

The concomitant mutation was reported occasionally. We found 1.93% investigated patients had both exon 19 del and L858R mutations simultaneously. Compared to patients with exon 19 del or L858R mutations solely, they were inclined to have small size (1–20 mm) adenocarcinomas which occurred in bilateral and invaded the visceral pleura.

The most serious issue with the TKIs treatment was drug resistant. Part of patients who had sensitive mutations will inevitably develop disease progression (20). This is frequently due to the development of a second-site mutation in the *EGFR* gene (21,22). Exon 20 insertion and T790M was confirmed as two major TKIs resistant mutations which account for up to 50% of acquired resistance (23,24). EGFR with T790M lose the binding ability through steric hindrance to TKIs (25). Most T790M mutations were developed after TKI using and the *de novo* T790M mutation was rare. In this study, we found 11 patients with exon 20 ins. Most of them were 51–70 years old. Right side, stage I lung adenocarcinoma was the character of tumor in this group. But our sample size was too small. Further large scale studies are needed to validate our results.

Tobacco using is one of the major aetiological factors of lung cancer and affects the *EGFR* mutation frequency. The *EGFR* mutation prevalence was 42.5 % *vs.* 8.5 % in never

Table 2 The clinical features comparison of lung cancer patients with non-*EGFR* mutation and *EGFR* mutation

Items	WT		L858R		19del		19del + L858R	
	Female	Male	Female	Male	Female	Male	Female	Male
Site								
Left	56.83%	55.53%	40.54%	37.93%	54.76%	42.59%	25.00%	25.00%
Right	40.29%	43.16%	56.08%	57.76%	45.24%	57.41%	75.00%	68.75%
Bilateral	2.88%	1.32%	3.38%	4.31%	0.00%	0.00%	0.00%	6.25%
TNM stage								
Stage I	72.66%	57.11%	79.05%	70.69%	57.14%	66.67%	75.00%	56.25%
Stage II*	10.79%	18.16%	2.70%	2.59%	14.29%	0.00%	0.00%	6.25%
Stage III*	10.79%	21.32%	12.84%	23.28%	14.29%	33.33%	25.00%	25.00%
Stage IV*	5.76%	3.16%	5.41%	3.45%	14.29%	0.00%	0.00%	12.50%
Stage T0	0.00%	0.26%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Smoking								
Never smoked	92.09%	31.32%	93.24%	46.55%	90.48%	11.11%	100.00%	56.25%
<100	0.00%	0.53%	0.68%	0.00%	0.00%	0.00%	0.00%	0.00%
101–500*	5.04%	22.37%	3.38%	28.45%	9.52%	25.93%	0.00%	25.00%
501–1,000*	2.16%	36.58%	2.70%	21.55%	0.00%	50.00%	0.00%	18.75%
>1,000*	0.72%	9.21%	0.00%	3.45%	0.00%	12.96%	0.00%	0.00%
Tumor size (mm)								
1–10*	22.30%	14.21%	16.22%	12.07%	7.14%	9.88%	0.00%	12.50%
11–20	30.94%	23.42%	29.73%	27.59%	23.81%	22.22%	25.00%	31.25%
21–30*	17.99%	22.89%	36.49%	33.62%	11.90%	30.86%	50.00%	18.75%
31–40	14.39%	14.74%	14.19%	16.38%	28.57%	7.41%	0.00%	12.50%
41–50	3.60%	11.58%	1.35%	5.17%	0.00%	12.35%	0.00%	12.50%
51–60*	7.19%	7.11%	2.03%	1.72%	14.29%	12.35%	25.00%	6.25%
61–70	1.44%	3.16%	0.00%	1.72%	0.00%	4.94%	0.00%	0.00%
71–80	0.00%	1.05%	0.00%	1.72%	0.00%	0.00%	0.00%	6.25%
81–90*	0.72%	0.53%	0.00%	0.00%	7.14%	0.00%	0.00%	0.00%
91–100*	0.72%	1.05%	0.00%	0.00%	7.14%	0.00%	0.00%	0.00%
>100	0.72%	0.26%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

*, there are significantly statistic differences among patients with non-*EGFR* mutation, with *EGFR* L858R mutation, with *EGFR* 19del mutation and with *EGFR* 19del + L858R mutation.

smoked lung cancer patients and patients with more than 26 pack-years of smoking in Americans (16). Similarly, in our study, 47.59% patients with wild-type *EGFR* never smoked, while more than 70% patients with different kinds of *EGFR* mutations were non-smokers, especially for patients with both exon 19 del and L858R mutation.

The major limitations of our study were the small sample size and short observation time. We would continue to follow up these patients and report the treatment response of them.

In a word, *EGFR* gene was also a major causative gene for Chinese lung cancer patients. L858R in exon 21 were the most common types. Patients with bilateral, small sized (1–20 mm) adenocarcinomas accompanied by the visceral pleura invasion were more likely to have both exon 19 del and L858R mutation. The clinicopathological characters summary will be helpful for clinicians to make treatment plan.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This study was approved by the Institutional Review Board (IRB) of Shanghai Pulmonary Hospital affiliated Tongji University (No. 2014-016). Written informed consents were obtained from all participants.

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