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Relevance of *EGFR* gene mutation with pathological features and prognosis in patients with non-small-cell lung carcinoma

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ABSTRACT

Objective: To study the relevance of *EGFR* gene mutation with pathological features and prognosis in patients with non-small-cell lung carcinoma. **Methods:** A total of 297 patients from July 2009 to May 2013 were chosen as objects. *EGFR* gene mutation were detected with fluorescence quantitative PCR. Relevance of *EGFR* gene mutation with clinical and pathological features was analyzed, and the prognosis of EGFR- mutant-patients and that of EGFR- wide type-patients was compared. **Results:** In 297 patients, 136 (45.79%) showed *EGFR* gene mutation. *EGFR* gene mutation had no significant relevance with age, gender, smoking history, family history of cancer and clinical stage ($P>0.05$); there was significant relevance between *EGFR* gene mutation and blood type, pathologic types, differentiation and diameter of cancer ($P<0.05$). The difference between prognosis of EGFR- mutant-patients and that of EGFR- wide type-patients was statistical significance ($P<0.05$). **Conclusions:** *EGFR* gene mutation has significant relevance with pathological features, the prognosis of EGFR-mutant-patients is better than that of EGFR- wide type-patients.

1. Introduction

In recent 50 years, many countries have reported that the incidence and mortality of lung cancer have been significantly increased. The mortality of lung cancer is highest in malignant tumors, which seriously jeopardize people's health[1]. And non-small-cell lung cancer (NSCLC) accounts for the vast majority of lung cancer (approximately 80%)[2]. Studies have showed that there has been gene mutation of epidermal growth factor receptor (EGFR) in about 30% of NSCLC patients. Therefore EGFR gene mutation is the crucial factor of effective treatment in NSCLC patients, and closely related to the prognosis of them[3,4]. In this study, 297 patients were selected as objects, and the correlation between EGFR mutation, clinical characteristics and pathological features were analyzed.

2. Materials and methods

2.1. Materials

A total of 297 NSCLC patients with confirmed diagnosis and complete information, admitted by our hospital during July 2009 to June 2010 were selected as research subjects. Then a retrospective analysis was taken on the clinical information of these patients. Among them, 204 men and 39 women aged between 35 and 78 years, averagely aged (59.2±15.1) years. All patients met the following criteria: (1) There were measurable lesions in the lungs of all patients; (2) Expected survival were longer than 6 months;

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(3) The case which complied with any one of the following need to be excluded: ① The patients who received pre-chemotherapy or other systemic anti-tumor therapy; ② The patients who suffered with other lung diseases, such as radioactive pulmonary disease, interstitial pneumonia; ③ Absolute neutrophil count<1.5×10^9/L, platelets<100×10^9/L; serum bilirubin>1.5× more the upper limit one of normal value (ULN); creatinine clearance<45 mL/min; ④ Either the patients who did not appear liver metastasis of lung cancer, and their alanine aminotransferase or aspartate aminotransferase> 2.5× more the upper one limit of normal value (ULN). Or the patients who appeared liver metastasis of lung cancer, their alanine aminotransferase or aspartate aminotransferase> 5× more ULN; ⑤ Pregnant or lactating women;(4)All the patients participated in the study signed informed consent. And the study met the medical ethics criteria.

2.2. Methods

2.2.1. Data collection

Clinical features included age, sex, smoking history (lifetime smoking quantity≥100 branches), family history of cancer (In two-line three generations, someone suffered from certain malignant tumor) and clinical staging. Pathological features of patients included pathological type, degree of differentiation and tumor diameter. The prognosis included[5] tumor progression-free survival (PFS) and overall survival (OS). Follow-up endpoint was death. All follow-up were finished on October 2014.

2.2.2. EGFR gene mutation detection

Fluorescence quantitative PCR was used to detect *EGFR* gene mutation of 297 patients. A total of 182 specimens were resected from surgery, 115 specimens were from bronchoscopic biopsy or percutaneous lung biopsy. Due to different sources of specimens, they were classified as paraffin section or non-paraffin section samples. The latter included fresh tissue of lesions, frozen sections of pathological diagnosis, non-heparin anticoagulated plasma, serum, non-heparin anticoagulated whole blood. Recommended DNA concentration of paraffin section samples was 2 ng/μL-3 ng/μL. A total of 9.4 ng-14.1 ng DNA was added into a single reaction tube as. According to the age limits of paraffin samples' preservation, 9.4 ng DNA was added into a single reaction tube whose age limit of preservation was less than three years. 14.1 ng DNA was added into a single reaction tube whose age limit of preservation was longer than three years. Recommended that DNA concentration of non-paraffin section samples was 0.4 ng/μL-1 ng/μL. A total of 1.88 ng-4.7 ng DNA was added into a single reaction tube. The mixed DNA samples were orderly added into 8-pore PCR reaction header pipets, covered with lids carefully, then centrifugated or lightly swung reaction in header pipets.

Patients with drug susceptibility mutation caused by 19 deletions in exon 18 (G719S, G719A, G719C), exons 21 (L858R, L861Q), exons 20 (S768I) , exon 19 were selected as mutation group. Patients absent with mutation and with drug-resistant mutation caused by exon 20 (T790M, 20-ins) were selected as wild group. The correlation between clinical features and pathological features in patients with EGFR mutations was analyzed, and the prognoses of patients in two groups was compared.

2.2.3. Statistical analysis

The data of study were processed and analyzed by using SPSS16.0 software. Measurement data were analyzed by t test and represented as mean ± sd. Count data were analyzed by χ^2 test. Multivariate logistic regression analysis was used in correlation analysis. $P<0.05$ was considered as statistically significant different.

3. Results

3.1. EGFR mutation test results

There were 136 cases with mutation in all 297 patients. Among them, there were 8 cases of exon 18 mutation, 82 cases of exon 19 mutation, 46 cases of exon 21 mutation, 2 cases of exon 18 and 19 mutations, 4 cases of exon 19 and 21 mutations. There were 15 cases of exon 20 mutation in patients of wild type (Figure 1&2).

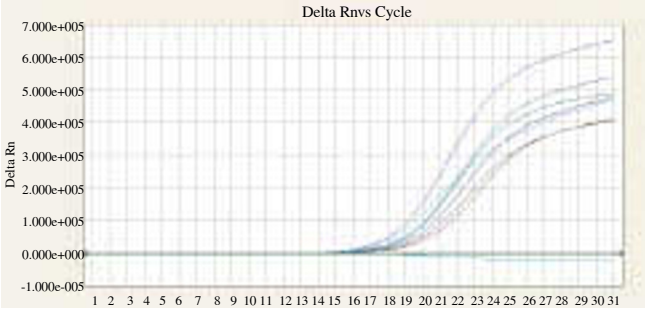

Figure 1. Curve of internal control reagent curve (VIC).

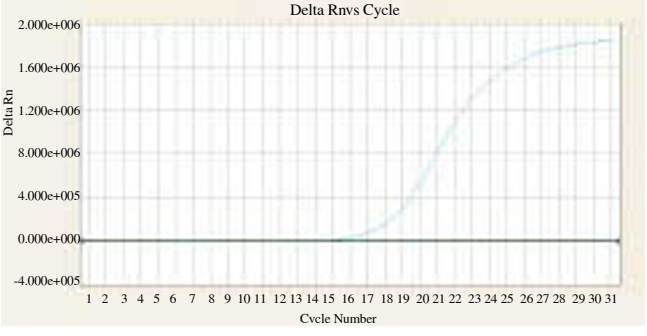

Figure 2. Amplification curve of mutation sample (FAM).

Table 1
Multivariate analysis of *EGFR* gene mutation.

Variable	*OR* (95% *CI*)	*P*
Age (≤60/>60)	0.785 (0.323-1.231)	>0.05
Sex (male/female)	1.317 (0.973-1.587)	>0.05
Smoking history (Y/N)	1.231 (0.876-1.374)	>0.05
Family history of tumor (Y/N)	1.414 (0.932-1.463)	>0.05
Clinical stage (stage Ⅰ-Ⅱ/stage Ⅲ-Ⅳ)		
0.654 (0.345-1.043)	>0.05	
Blood type (AB/A,B, O)	0.147 (0.023-0.354)	<0.05
Pathological type (squamous cell carcinoma、 adenocarcinoma /others)	2.643 (2.112-3.344)	<0.05
Degree of differentiation (well/intermediate, poorly)	0.221 (0.032-0.412)	<0.05
Tumor diameter (<3/≥3)	0.196 (0.027-0.386)	<0.05

3.2. Multivariate analysis of EGFR gene mutation

There were 59 patients with history of smoking and 45 patients with family history of tumor in 297 patients. There were 103 patients at clinical stages Ⅰ-Ⅱ, 194 patients at clinical stages Ⅲ-Ⅳ; 47 patients of pathological type AB, 87 of type A, 84 of type B, 79 of type O. There were 89 patients suffered from squamous cell carcinoma, 172 patients suffered from adenocarcinoma, and 36 cases with other types. There were 94 patients with well-differentiation, 135 with intermediate differentiation, 68 with poorly differentiated. Tumor diameters of 69 patients were <3 cm , the ones of 118 patients were 3-8 cm, the ones of 110 patients were>8 cm.

Clinical data, case data and *EFGR* gene mutations of patients were analysed by logistic regression analysis. There was no significant correlation with *EFGR* gene mutation and patients' age, sex, smoking history, family history of tumor, clinical stage (P>0.05). There were significant correlation with the patients' blood type, pathological type of tumor, degree of differentiation, tumor diameter and EGFR mutations (P <0.05) (Table 1).

3.3. EGFR gene mutation and prognosis of patients

According to the results of genetic mutations, the patients were divided into mutation group and wild type group. PFS of patients in mutation group were (23.6 ± 5.4) months, OS were (29.6 ± 6.3) months. PFS of patients in wild type group were (17.8 ± 4.9) months, OS were (24.2 ± 5.7) months, the difference was statistically significant (P <0.05) (Figure 3).

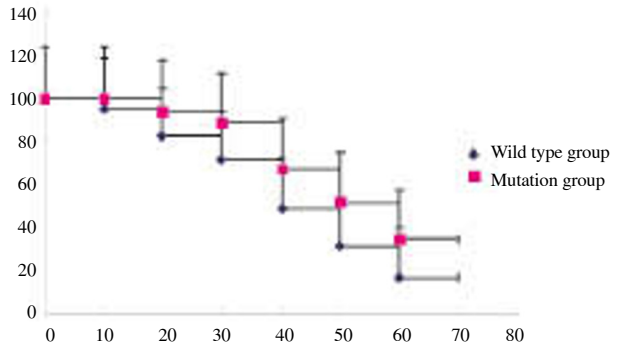

Figure 3. Comparison of survival curves in the two groups of patients.

4. Discussion

China's annual emerging cases of tumor and deaths will reach 2.68 million and 1.97 million in 2010. The number will be further increased to 3.49 million and 2.63 million[7]. Lung cancer is the top killer of malignant tumor, which has been a serious threat to the health of the population in this region[8]. Studies have shown that there are epidermal growth factor receptor gene mutations in 30% of NSCLC patients. And the mutation rate in women and non-smoking adenocarcinoma patients are even as high as 70%[9,10].

EGFR gene mutations of 297 patients treated with ARMS were detected. Among them, there were 136 cases with mutation type (45.79%). Mutation was located on exon 18, exon 19 and exon 21. *EFGR* gene mutation, clinical features and pathological features of patients were analyzed by correlation analysis. There was no significant correlation with *EFGR* gene mutation and patients' age, sex, smoking history, family history of tumor, clinical stage. There were significant correlation with the patients' pathological type of tumor, degree of differentiation, tumor diameter and EGFR mutations. Gene mutation rates of patients with adenocarcinoma were up to 60.47% (104/172), significantly higher than ones in other types of tumors. The higher was the degree of tumor differentiation, the more increased was *EGFR* gene mutation. *EGFR* gene mutation reduced as patients' tumor' diameters increased.

EGFR, a kind of transmembrane protein widely distributed on the cell surface, which belongs to receptor tyrosine kinase (TK) erbB / HER family, hold activity of tyrosine kinase[11]. EGFR can be combined with epidermal growth factor and then activate intracellular tyrosine kinase domain. It also can implement functions by PI3k-Akt and SOS-RAS-RAF-MEK-MAPK pathways. Meanwhile, EGFR plays an important role in division, proliferation, differentiation, migration, adhesion, anti-apoptosis, chemotherapy resistance and other processes in tumor cells[12,13]. The study found that *EGFR* gene mutations and targeted therapies drug sensitivity NSCLC patients showed had correlation[14]. In this study, PFS and OS of patients with EGFR mutations were significantly longer than

the ones of wild-type patients', the prognosis of former were better.

Study results announced by 2012 American Society of Clinical Oncology (ASCO) showed that EGFR mutation of NSCLC patients is one of the key factors in effective treatment and is closely related to the prognosis of patients[15]. At present, some EGFR-targeted chemotherapy drugs have emerged clinically, such as gefitinib. It is one kind of EGFR tyrosine kinase inhibitor (TKI) clinically often applied. It can be competitive binding EGFR and prevent phosphorylation of tyrosine residues, then promote tumor cell apoptosis, and further prevent the proliferation and spread of tumors by signaling transduction pathway[16,17]. The study found the *EGFR* mutations rate in female patients and patients with no previous history of smoking were higher and these patients could get more benefits from chemotherapy with EGFR-TKI.

The study found that *EGFR* gene mutation was common in patients who were female, non-smoking, in earlier tumor stage, with smaller size and higher degree of differentiation tumor. It was more common in patients with adenocarcinoma. And pathological features of patients were independent predictors of EGFR mutations. The prognosis of patients with EGFR mutation was significantly better than the one of wild-type patients.

Conflict of interest statement

We declare that we have no conflict of interest.

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