RESEARCH ARTICLE

Analysis of CEA Expression and EGFR Mutation Status in Non-small Cell Lung Cancers

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Abstract

Background: The serum carcinoembryonic antigen (CEA) level can reflect tumor growth, recurrence and metastasis. It has been reported that epidermal growth factor receptor (EGFR) mutations in exons 19 and 21may have an important relationship with tumor cell sensitivity to EGFR -TKI therapy. In this study, we investigated the clinical value of EGFR mutations and serum CEA in patients with non-small cell lung cancer (NSCLC). Materials and Methods: The presence of mutations in EGFR exons 19 and 21 in the tissue samples of 315 patients with NSCLC was detected with real-time fluorescent PCR technology, while the serum CEA level in cases who had not yet undergone surgery, radiotherapy, chemotherapy and targeted therapy were assessed by electrochemical luminescence. Results: The mutation rates in EGFR exons 19 and 21 were 23.2% and 14.9%, respectively, with the two combined in 3.81%. Measured prior to the start of surgery, radiotherapy, chemotherapy and targeted treatment, serum CEA levels were abnormally high in 54.3% of the patients. In those with a serum CEA level <5 ng/mL, the EGFR mutation rate was 18.8%, while with 5~19 ng/mL and ≥20 ng/mL, the rates were 36.4% and 62.5%. In addition, in the cohort of patients with the CEA level being 20~49 ng/mL, the EGFR mutation rate was 85.7%, while in those with the CEA level ≥50 ng/mL, the EGFR mutation rate was only 20.0%, approximately the same as in cases with the CEA level<5 ng/mL. Conclusions: There is a positive correlation between serum CEA expression level and EGFR mutation status in NSCLC patients, namely the EGFR mutation-positive rate increases as the serum CEA expression level rises within a certain range (≥20 ng/mL, especially 20~49 ng/mL). If patient samples are not suitable for EGFR mutation testing, or cannot be obtained at all, testing serum CEA levels might be a simple and easy screening method. Hence, for the NSCLC patients with high serum CEA level (≥20 ng/mL, especially 20~49 ng/mL), it is worthy of attempting EGFR-TKI treatment, which may achieve better clinical efficacy and quality of life.

Keywords: Carcinoembryonic antigen - EGF receptor - non-small cell lung cancer - tyrosine kinase inhibitor

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Introduction

Epidemiological data suggest that the incidence of non-small cell lung cancer (NSCLC) is rapidly rising, and smoking is the leading cause of lung cancer in the world. The traditional therapies for NSCLC mainly include surgery, radiotherapy, chemotherapy etc (Liu et al., 2013; Lu et al., 2013; Yan et al., 2013; Ji et al., 2014; Cui et al., 2014). However, these therapeutic interventions often bring about strong adverse reactions. Thus, molecular targeted therapy versus specific targeted sites has become a research hotspot in recent years.

Epidermal growth factor receptor (EGFR) is a type of receptor tyrosine kinase, and it has been reported that EGFR mutations are mainly concentrated in exons 19 and 21 (Ji et al., 2006; Lim et al., 2014; Liam et al, 2014). After epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) treatment, the signaling through this pathway is blocked, leading to tumor cell necrosis. Some studies have shown that NSCLC patients with EGFR mutations are particularly suited to EGFR-TKI treatment, with a curative effect more than 70% (Mok et al., 2009; Fukuoka et al., 2011). Thus, EGFR mutation status in lung cancer tissue is an effective means of judging whether NSCLC patients will respond well to EGFR-TKI therapy. However, application of tumor tissue samples is problematic, as some tissue specimens are unsuitable for detection, and in some patients, the tissue samples are not easy to obtain, so it is important to discover a simple and easy clinical screening method.

Serum carcinoembryonic antigen (CEA), a common lung cancer tumor marker (TM), is a cell adhesion molecule in the immunoglobulin super family with the roles in homophilic and heterophilic adhesions (McFarlane et al., 2013). Excessive CEA expression leads to aberrant cell adhesion and inhibits cell apoptosis in the cancer

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cell nest, consequently making the cell escape apoptosis and increasing the metastatic opportunity of tumor cells (Ordoñez et al., 2000). CEA level can reflect the tumor growth, recurrence and metastasis, and its serum specimens are easy to obtain from patients. At present, it is still not fully understood whether there is an inherent relationship between serum CEA expression level and EGFR mutation rate or not, particularly whether the former can be used to predict the outcome of EGFR-TKI therapy for NSCLC patients. Hence, the clinical value of EGFR mutations and serum CEA expression level in NSCLC patients was investigated in this study so as to provide some help for those that cannot undergo EGFR detection and need to accept EGFR-TKI treatment.

Materials and Methods

Materials

Field study area: This study was carried out in Chengdu, Sichuan province, which was situated in the southwest of China. The several selected hospitals were all in Chengdu with subtropical climate where it is cold in winter and moderate in summer, one of which was Pengzhou Hospital of Tradition Chinese Medicine.

Patient data: A total of 315 NSCLC patients confirmed by CT or MRI were selected, and the biopsy, surgical removal and pathology inspection were all conducted from Jan, 2009 to Feb, 2014. There were respectively 165 males and 150 females, aging 31~82 (median age 66.87), in which 147 cases were less than 65 years old and 168 more than 65 years old. There were 174 smokers (smoking index ≥100) and 141 non-smokers enrolled in the study. According to the international lung cancer TNM staging established in 2009, there were 15 cases in phase I, 87 phase II, 135 phase III and 78 phase IV. For all cases, the serum specimens were collected before surgery, radiotherapy, chemotherapy and targeted therapy, and the tissue samples were acquired by surgical resection, fiberoptic bronchoscopy or CT-guided puncture, fixed by 10% neutral buffered formalin and paraffin embedded.

The study was approved by the Ethics Committee of College of Life Science, Sichuan University and Pengzhou Hospital of Traditional Chinese Medicine. Each patient enrolled in this study had signed the informed consent.

Experimental methods

Serum CEA detection: All NSCLC patients were extracted 5 mL of venous blood early in the morning, and all specimens were then promptly sent to the clinical laboratory in 30 min. Serum CEA level was detected by electrochemiluminescence immunoassay method after serum separation. The Roche E170 electrochemiluminescence instrument and an electrochemical luminescence kit (Elecsys CEA assay) were used, with the operation strictly carried out according to the manufacturers' instructions. The CEA level <5 ng/mL was considered as the normal, while ≥5 ng/mL as the high.

EGFR detection of tissue specimens: DNA extraction and polymerase chain reaction (PCR) amplification

After histopathology wax block was sliced and slices were placed in Eppendorf tubes, the appropriate amount of xylene was added to get the wax off. A suitable amount of alcohol was added to elute xylene, and necrotic tissue was resected after the specimen was thoroughly dry. Cracking buffer (300 μL) and proteinase K (300 μL, 200 μg/mL) were added to the tubes before they were incubated in a 60°C water bath for 8 h. Following this step, tubes were centrifuged for 15 min at 12 000 rpm and the supernatant was absorbed. Phenol/chloroform (1:1) and anhydrous ethanol were added, the solutions were mixed, and DNA was allowed to precipitate for 2 h at -20°C. Samples were dried in vacuum before being centrifuged for another 20 min; the supernatant was removed and a suitable amount of 70% alcohol was added to wash the precipitate. After being dissolved in ddH2O, DNA concentration and purity were detected by 1 μL aliquot of the liquid, and the rest was saved in a -80°C freezer for future application. PCR was performed in a pipe by 20 μL system, with the final volume of 25 μL. Fifty nanograms of template DNA and 1.0 U Taq DNA polymerase were used in reactions. The primer sequences used to amplify EGFR exons were as follows: exon 19 forward (attcgtggagcccaacag), exon 19 reverse (gccagtaattgcctgtttcc), exon 21 forward (gtcagcagcgggttcatct), and exon 21 reverse (aagcagctctggctcacact). The PCR conditions were as follows: initial melting at 95°C for 15 min; 10 cycles of melting at 94°C for 30 s, annealing at 65°C for 30 s and extension at 72°C for 1 min; 30 cycles of melting at 94°C for 50 s, annealing at 57°C for 1 min and extension at 72°C for 1 min.

Sequence determination: EGFR exons 19 and 21 mutation status was detected by Real-time PCR technology based on TaqMan test probes. The PCR product was purified with a DNA gel recovery kit according to the manufacturer's instructions. PCR purification products were sequenced with a sequencing instrument, and a comparison of the sequence diagram with the standard sequence was made for some results.

Statistical data analysis

SPSS13.0 statistical software package was used. All experimental data results were presented with ($\chi \pm s$). The enumeration data was analyzed with x^2 test, while the measurement data with t test. Differences were considered statistically significant when the *P* value was less than 0.05.

Results

Analysis of EGFR mutation in NSCLC specimens

EGFR exon 19 mutations were observed in 66 cases, with the mutation rate of 23.17% (73/315); EGFR exon 21 mutations were found in 42 cases, with the mutation rate of 14.92% (47/315); both exons 19 and 21 mutations were found in 12 cases, with the mutation rate of 3.81% (12/315) (Table 1, Figure 1).

In addition, the clinical features of 315 NSCLC cases were examined. It was found that the presence of EGFR mutations in NSCLC patients had nothing to do with tumor staging and age. However, there was a relationship between EGFR mutation status and the gender, smoking

Table 1. Mutations of EGFR Exons 19 and 21 in Patients with NSCLC

EGFR	Mutation type (n)	Wild type (n)	Mutation rates (%)
Exon19	73	242	23.17% (73/315)
Exon21	47	268	14.92% (47/315)

Table 2. Relationship Between Clinical Features and EGFR Mutations in Patients with NSCLC

Clinical features	n	EGFR +(n)	EGFR -(n)	Mutation rates (%)	*P*
Age					
<65 years	147	45	102	30.61	*P*>0.05
≥65 years	168	63	105	37.50	
Gender					
Male	165	27	138	16.36	**P*<0.01
Female	150	81	69	54.00	
Smoking status					
smoking	174	36	138	20.69	**P*<0.01
non-smoking	141	72	69	51.06	
Histology					
Adenocarcinoma	177	81	96	45.76	**P*<0.01
Squamous cell carcinoma	138	27	111	19.57	
Staging					
I+II	102	33	69	32.35	*P*>0.05
III+IV	213	75	138	35.21	

Note: The mutation rates of EGFR were 34.29% (108/315). Compared the males with females, smoking with non-smoking and squamous cell carcinoma with adenocarcinoma, **P*<0.01

status and pathological types. EGFR mutations were found more frequently in women than men, in non-smokers than smokers, and in patients with lung adenocarcinoma than those with lung squamous carcinoma. The EGFR mutation rate was 54.00% for females and only 16.36% for males ($P<0.01$), 51.06% for non-smokers and 20.69% for smokers ($P<0.01$), 45.76% for the patients with lung adenocarcinoma and 19.57% for those with lung squamous carcinoma ($P<0.01$) (Table 2).

Analysis of serum CEA expression in NSCLC

Serum CEA levels were measured in 315 NSCLC patients prior to surgery, radiotherapy, chemotherapy and targeted therapy. It was found that in 171 cases, the serum CEA level was ≥5 ng/mL, thus, the abnormal CEA expression rate was 54.29%. In 144 cases, the serum CEA level was <5 ng/mL, thus, the normal CEA expression rate was 45.71%. Compared with different histological types regarding the serum CEA concentration, the CEA positive rate was 66.10% for adenocarcinoma and 39.13% for squamous cell carcinoma ($P<0.01$) (Table 3).

Relationship between EGFR mutation and serum CEA expression level

The serum CEA expression level and EGFR mutation status in 315 NSCLC patients before surgery, radiotherapy, chemotherapy and targeted treatment were analyzed. It was found that the EGFR mutation rate was only 18.75% in the patients with serum CEA level <5 ng/mL, 36.36% in those with serum CEA being 5~19 ng/mL and 62.50% in those with serum CEA level ≥20 ng/mL, in which all the

Table 3. Serum CEA Levels in Patients with NSCLC ($\bar{\chi}\pm s$)

Histology	n	CEA (ng/mL)	CEA+ (n)	Positive rate (%)
Squamous cell carcinoma	138	9.06±16.06	54	39.13
Adenocarcinoma	177	24.26±35.69**	117	66.10**

Compared with squamous cell carcinoma, **P*<0.01

Table 4. Relationship Between Serum CEA Expression Level and EGFR Mutation Status [n (%)]

CEA (ng/mL)	n	Mutation types	Wild types	Mutation rate (%)
<5	144	27 (18.75)	117 (81.25)	18.75
≥5	171	81 (47.37)	90 (52.63)	47.37
5~19	99	36 (36.36)	63 (63.64)	36.36
≥20	72	45 (62.50)	27 (37.50)	62.50
20~49	42	36 (85.71)	6 (14.29)	85.71
≥50	30	6 (20.00)	24 (80.00)	20.00

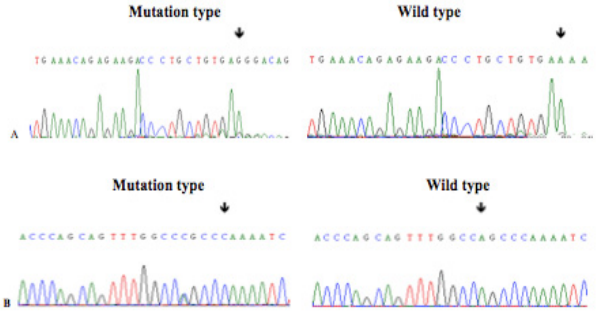

Figure 1. Mutation Sites in EGFR Exons 19 and 21 in Patients with NSCLC: A. EGFR Exons 19; B. EGFR Exons 21

differences were statistically significant ($P<0.05$). These results indicated that the EGFR mutation rate went up as serum CEA level increased, showing a linear relationship. The EGFR mutation rate came up to 85.71% when the serum CEA level reached 20~49 ng/mL, whereas it was only 20% when ≥50 ng/mL. Besides, in the patients with the CEA level <5 ng/mL, the EGFR mutation rate was 18.75% (Table 4).

Discussion

EGFR is a transmembrane receptor that is normally present on the cell surface and often overexpressed in NSCLC patients. It has been reported that EGFR mutations mainly occur in exons 19 and 21, and these mutations have an important relationship with tumor cell sensitivity to EGFR-TKI therapy (Sequist et al., 2007; Hsiao et al., 2013; Tsai et al., 2013). Studies have shown that EGFR mutation rate has regional differences, as it is roughly 10% in Western countries but about 50% in Asian countries. Further, in non-smokers, women and the patients with gland cancer, its incidence is higher (Sequist et al., 2007). The results in this study revealed that among 315 NSCLC patients, exons 19 and 21 mutation rates were 23.17% and 14.92%, respectively. Additionally, the EGFR mutation rate varied depending on the patient groups (adenocarcinoma group, 45.76% vs. squamous cell carcinoma group, 19.57%; women, 54.00% vs. men,

16.36%; smokers, 20.69% vs. non-smokers, 51.06%). Similar to previous reports, these results suggest that EGFR mutations are closely related to the gender, smoking status and pathological types.

EGFR mutation status and CEA expression level can have important effects on the complex network of signal transduction pathways regulating cell apoptosis. EGFR mutations can lead to abnormal EGFR activation, which in turn leads to the aberrant activation of Akt and STAT3/5, which are the downstream of EGFR signal transduction and play important roles in preventing the induction of apoptosis (Sordella et al., 2004; Sisto et al., 2014). Abnormal activation of EGFR signaling leads to the abnormal activation of various pathways, which in turn leads to related transcription factors being synthesized and activated. These changes eventually cause enhanced cell proliferation. CEA is a type of adhesion protein whose expression can be activated and adjusted by EGFR signaling, which may be one of the reasons that CEA expression appears to be up-regulated following EGFR mutation (Kobayashi et al., 2008). Previous research has demonstrated that some TMs are valuable not only because of their functions in cancer and differential diagnosis, but also because TM expression level changes significantly before and after treatment in different pathological types, they can be used for dynamic monitoring of tumor recurrence and metastasis as well as clinical efficacy (Duffy et al., 2003; Figueredo et al 2003). CEA is a commonly used TM in lung cancer, as it is related to tumor metastasis and recurrence, and plays a major role in the prognostic evaluation (Zheng et al., 2013). In this study, it was found that the abnormal CEA expression rate was 54.29%. However, it was 66.10% in the patients with adenocarcinoma, while being 39.13% in those with squamous cell carcinoma. Thus, more patients with adenocarcinoma have elevated serum CEA level compared with those with squamous cell carcinoma, which is in accordance with the fact that EGFR mutations are more frequent in adenocarcinoma than in squamous cell carcinoma.

Although EGFR and CEA belong to different protein super families, the studies in recent years have reported a correlation between CEA expression level and EGFR mutations. A Japanese study investigated the relationship between EGFR mutation status and serum CEA expression level at different stages of NSCLC; it was found that EGFR mutations in lung cancer were related to the serum CEA expression level in recurrent tumors, but not the preoperative serum CEA expression level (Shoji et al., 2007). A domestic study regarding the relationship between CEA expression level in NSCLC surgical specimens and EGFR mutation status demonstrated that NSCLC patients with abnormally elevated CEA protein expression were prone to suffer from EGFR mutations, and this correlation was statistically significant (Kappers et al., 2010; Li et al., 2012). Wang et al found that within a certain range, the EGFR gene mutation rate would increase with increasing preoperative serum CEA expression level (Wang et al., 2012). In this study, it was found that the EGFR mutation rate was only 18.7% in the patients with the serum CEA level <5 ng/mL before surgery, radiotherapy, chemotherapy and targeted treatment. As the serum CEA level increased, so did the EGFR mutation rate: 5~19 ng/mL CEA, mutation rate 36.36%; ≥20 ng/mL CEA, mutation rate 62.50%; 20~49 ng/mL CEA, mutation rate 85.71%; ≥50 ng/mL CEA, mutation rate 20.00% only, similar to that <5 ng/mL, suggesting that the serum CEA expression level before surgery, radiotherapy, chemotherapy and targeted treatment is related to pathological types, as more patients with adenocarcinoma have elevated serum CEA level compared with those with squamous cell carcinoma. Within the range of statistical significance, the serum CEA expression level is positively correlated with EGFR mutations, namely as EGFR mutations occur, the serum CEA expression level rises.

Epidemiological surveys have confirmed that lung cancer is one cause of the most common cancer-related deaths all over the world (Fei, et al., 2013). The traditional therapies for lung cancer are mainly surgery, radiotherapy and chemotherapy. However, the outcome of these therapeutic interventions is not satisfactory, with the main reason being that while patients are diagnosed and accept treatment, the tumors are far advanced (Bordi et al., 2014; Janssens et al., 2014). Previous data show that the overall 5-year survival rate of patients with lung cancer is only 15% in United States (Herbst et al., 2008), much lower in our country.

Because traditional treatments for lung cancer often bring about strong adverse reactions, EGFR-TKI therapy targeting specifically at EGFR mutants in recent years has been developed. It was reported in the literature that the mutations of EGFR exons 19 and 21 did not predict the survival and prognosis of patients with NSCLC (Tsao, et al., 2005). However, a large number of studies have confirmed that the serum CEA expression level can be employed to assess tumor prognosis and existing researches also suggest that EGFR mutations can predict the clinical efficacy of patients receiving EGFR-TKI treatment (Miller et al., 2008; Sequist et al., 2008). Thus, these measurements can complement each other, and by EGFR mutation combined with serum CEA expression level, a clinician can not only evaluate the prognosis of patients with NSCLC, but also predict the clinical efficacy.

In conclusion, EGFR mutation rate in NSCLC patients is closely related to the gender, smoking status and pathological types, with EGFR mutations more frequently in women, non-smokers and patients with adenocarcinoma. More patients with adenocarcinoma have elevated serum CEA level compared with those with squamous cell carcinoma. Besides, a positive correlation between serum CEA expression level and EGFR mutation rate is observed, namely the EGFR mutation-positive rate increases as the serum CEA expression level rises within a certain range (≥20 ng/mL, especially 20~49 ng/mL). If the patient samples are not suitable for EGFR mutation testing, or cannot be obtained at all, to test serum CEA expression level is a simple and easy screening method to estimate EGFR mutation. Hence, for the NSCLC patients with high serum CEA level (≥20 ng/mL, especially 20~49 ng/mL), it is worthy of attempting EGFR-TKI treatment, which may be achieve the better clinical efficacy and quality of life.

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