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ORIGINAL ARTICLE

Associations Between Epidermal Growth Factor Receptor Gene Mutation and Serum Tumor Markers in Advanced Lung Adenocarcinomas: A Retrospective Study[△]

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Key words: advanced lung adenocarcinomas; epidermal growth factor receptor gene; mutation; epidermal growth factor receptor tyrosine kinase inhibitor; carcinoembryonic antigen

Objective To investigate the associations between epidermal growth factor receptor (EGFR) gene mutations and serum tumor markers in advanced lung adenocarcinomas.

Methods We investigated the association between EGFR gene mutations and clinical features, including serum tumor marker levels, in 97 advanced lung adenocarcinomas patients who did not undergo the treatment of EGFR tyrosine kinase inhibitors. EGFR gene mutation was detected by real-time PCR at exons 18, 19, 20, and 21. Serum tumor marker concentrations were analyzed by chemiluminescence assay kit at the same time.

Results EGFR gene mutations were detected in 42 (43%) advanced lung adenocarcinoma patients. Gender ($P=0.003$), smoking status ($P=0.001$), and abnormal serum status of carcinoembryonic antigen (CEA, $P=0.028$) were significantly associated with EGFR gene mutation incidence. Multivariate analysis showed the abnormal CEA level in serum was independently associated with the incidence of EGFR gene mutation ($P=0.046$) with an odds ratio of 2.613 (95% CI: 1.018-6.710). However, receiver operating characteristic (ROC) curve analysis revealed CEA was not an ideal predictive marker for EGFR gene mutation status in advanced lung adenocarcinoma (the area under the ROC curve was 0.608, $P=0.069$).

Conclusions EGFR gene mutation status is significantly associated with serum CEA status in advanced lung adenocarcinomas. However, serum CEA is not an ideal predictor for EGFR mutation.

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LUNG cancer remains the leading cause of cancer-related deaths worldwide and the total 5-year survival rate of lung cancer cases was less than 17%,¹ due to the lack of sensitive screening tests for early detection and ineffective treatment for advanced and metastatic disease.² Non-small-cell lung carcinoma (NSCLC) accounts for approximately 80% to 85% of all lung cancers. Epidermal growth factor receptor (EGFR) is a validated target in NSCLC. Two EGFR tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib have been approved for the treatment of advanced NSCLC by the U.S. Food and Drug administration (FDA) and the European Medicines Evaluation Agency (EMA).³ Good clinical responses were displayed in Asian, females, nonsmokers, and adenocarcinomas patients,⁴ which may related with the higher frequency of EGFR mutations within these subgroups.⁵ EGFR-TKIs are known to contribute to the extension of progression-free survival in EGFR-mutant NSCLC considerably and the EGFR mutations are widely accepted as indicators for the clinical efficacy of EGFR-TKIs in advanced adenocarcinomas patients.⁶

The detection of mutations in the EGFR gene of advanced NSCLC patients has some limitations. The best specimen for EGFR gene mutation detection is NSCLC tissues taken by surgery. However, 70%-80% NSCLC patients have difficulties for radical surgery at the time of diagnosis and are unable to obtain tissue samples for EGFR mutation testing. Another way to obtain tissue samples for EGFR mutation testing is tumor biopsy surgery which is also dangerous for the high risk of bleeding in advanced tumors. Meanwhile, the predictive value of peripheral blood for EGFR gene mutation tests is still controversial.⁷ So a considerable number of patients can not provide enough NSCLC samples for gene diagnosis.

Thus, we focused on a non-invasive and reliable method to predict the clinical efficiency of EGFR-TKIs for the treatment of advanced adenocarcinomas. Recently, several researches reported the serum level of tumor markers, such as carcinoembryonic antigen (CEA),⁸ cytokeratin 19 fragment (CYFRA21-1),⁹⁻¹¹ carbohydrate antigen 19-9 (CA19-9),¹² CA125,¹² polypeptide specific antigen¹³, and transforming growth factor- α ¹⁴ may predict the treatment efficiency of EGFR-TKIs for NSCLC patients. However, the mechanism was not clear yet. So we proposed these tumor markers may relate with frequency of EGFR gene mutations in advanced lung adenocarcinomas.

In this study, the correlations between mutations of EGFR gene and the serum levels of potential NSCLC biomarkers, CEA, CA125, CA19-9, neuron specific enolase

(NSE), and CYFRA21-1 were investigated in advanced lung adenocarcinoma patients retrospectively.

PATIENTS AND METHODS

Patients

From March 2012 to November 2013, 97 primary advanced adenocarcinoma patients who had undergone measurements of EGFR gene mutation and serum CEA, CA125, CA19-9, NSE, and CYFRA21-1 levels in Taizhou Hospital of Zhejiang Province were retrospectively recruited. None of them had received EGFR-TKI therapy before. All patients were pathologically or cytologically diagnosed. Histological diagnosis and tumor grade were in accordance with the World Health Organization International Histological Classification of Lung Tumors criteria. Patients' data were collected including age, gender, smoking history, clinical stage, EGFR gene mutations, and serum levels of CEA, CA125, CA19-9, NSE, and CYFRA21-1.

Detection of EGFR gene mutation

Genomic DNA was extracted from tumors tissue samples or pleural effusion sediment samples using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) according to manufacturer's protocols. Real-time PCR was performed using the AmoyDx™ human EGFR gene mutations fluorescence PCR diagnostic kit (Amoy Diagnostics, Xiamen, China) according to manufacturer's recommendations. A total of 29 mutations in exon 18, 19, 20, and 21 of EGFR gene were analyzed, including G719S/A/C mutations in exon 18, 19, deletion mutations in exon 19, T790M and S768I mutations in exon 20, and L858R/Q and L861Q mutations in exon 21. Positive, negative, and internal controls were included in each real-time PCR run. As defined by the manufacturer's instructions, quantitative real-time PCR assay for EGFR gene mutation was considered positive, if the sample Ct value <29.

Analysis of serum CEA, CA125, CA19-9, NSE, and CYFRA21-1 levels

Serums were obtained from peripheral fasting blood of all cases at the same time of EGFR gene mutation detection. Serum CEA, CA125, CA19-9, NSE, and CYFRA21-1 levels were detected with chemiluminescence assay kit according to the manufacturer's introductions (Roche, Shanghai, China). The normal range of serum NSCLC biomarkers were determined as CEA<5.0 ng/ml, CA125<35 U/ml, CA19-9<37 U/ml, NSE<13.0 ng/ml, and CYFRA21-1<3.3 ng/ml, respectively.

Statistical analysis

All statistical analyses were performed with SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA). Measurement data were described as mean±standard deviation and serum tumor marker levels between the EGFR gene mutation group and wild-type group were compared by using *t* test. Correlations between EGFR gene mutation status and clinical parameters were analyzed by using *Pearson* chi-square test and logistic regression models. The feasibility of using serum tumor marker as predictor for EGFR gene mutations in advanced lung adenocarcinomas was assessed using the receiver operating characteristic (ROC) curve analysis. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Patients' characteristics and EGFR gene mutation status

The profiles of these 97 patients and the status of CEA, CA125, CA19-9, NSEs and CYFRA21-1 are summarized in Table 1.

EGFR gene mutations had been detected in 42 (43%) advanced lung adenocarcinoma patients, which was predominant in female (74%) and non-smokers (76%), and the remaining 55 (57%) patients were wild-type EGFR. A deletion at exon 19 was detected in 15 (15%) patients. A point mutation of L858R was found in 21 (22%) patients, and L861Q was found in 4 (4%) patients in exon 21. Point mutation of G719A/S/C at exon 18 was found in 2 (2%) patients.

Correlation between EGFR gene mutations and serum tumor marker levels

The association between the serum CEA, CA125, CA19-9, NSE, and CYFRA21-1 levels and the incidence of EGFR gene mutations in advanced lung adenocarcinoma are shown in Table 2. No significant difference was found between these serum tumor markers levels and EGFR gene mutation incidence (all $P > 0.05$).

Association between EGFR gene mutation status and

clinical characteristics

Pearson chi-square test showed that the EGFR gene mutation status was correlated with gender ($P = 0.003$), smoking status ($P = 0.001$), and serum CEA status ($P = 0.028$) (Table 3). And other factors, including age, stage, CA125, CA19-9, NSE, and CYFRA21-1 had no association with the incidence of EGFR gene mutations (Table 3).

Table 1. Clinical characteristics of 97 patients with advanced lung adenocarcinoma

Characteristics	No. of patients (%)
Age (yrs)	
<64	47 (48)
≥64	50 (52)
Gender	
Male	42 (43)
Female	55 (57)
Smoking status	
Yes	41 (42)
No	56 (58)
Stage	
IIIB	28 (29)
IV	69 (71)
CEA	
<5 ng/ml	35 (36)
≥5 ng/ml	62 (64)
CA125	
<35 U/ml	48 (49)
≥35 U/ml	49 (51)
CA19-9	
<37 U/ml	65 (67)
≥37 U/ml	32 (33)
NSE	
< 13.0 ng/ml	40 (41)
≥13.0 ng/ml	57 (59)
CYFRA21-1	
<3.3 ng/ml	44 (45)
≥3.3 ng/ml	53 (55)

CEA: carcinoembryonic antigen; CA125: carbohydrate antigen 125; CA19-9: carbohydrate antigen 19-9; NSE: neuron specific enolase; CYFRA21-1: cytokeratin 19 fragment.

Table 2. Comparison of levels of serum tumor markers between the EGFR gene mutation group and wild-type group in advanced lung adenocarcinoma patients

Groups	<i>n</i>	CEA (ng/ml)	CA125 (U/ml)	CA19-9 (U/ml)	NSE (ng/ml)	CYFRA21-1 (ng/ml)
Wild-type EGFR group	55	129.5±366.1	125.9±231.9	98.7±206.0	17.7±11.1	6.5± 7.2
Mutant EGFR group	42	126.5±237.5	137.2±231.7	40.8± 80.0	20.1±18.4	8.6±13.3
<i>P</i> value		0.963	0.808	0.117	0.489	0.669

EGFR: epidermal growth factor receptor.

Table 3. Correlation between EGFR gene mutation status and clinical characteristics in advanced lung adenocarcinoma patients

Characteristics	Wild-type EGFR group (n=42) [No. (%)]	Mutant EGFR group (n=55) [No. (%)]	P value
Age (yrs)			
<64	25 (60)	23 (42)	0.084
≥64	17 (40)	32 (58)	
Gender			
Male	11 (26)	31 (56)	0.003
Female	31 (74)	24 (44)	
Smoking status			
Yes	10 (24)	31 (56)	0.001
No	32 (76)	24 (44)	
Stage			
IIIB	11 (26)	17 (31)	0.611
IV	31 (74)	38 (69)	
CEA			
<5 ng/ml	10 (24)	25 (46)	0.028
≥5 ng/ml	32 (76)	30 (55)	
CA125			
<35 U/ml	20 (48)	28 (51)	0.748
≥35 U/ml	22 (52)	27 (49)	
CA19-9			
<37 U/ml	30 (71)	35 (64)	0.419
≥37 U/ml	12 (29)	20 (36)	
NSE			
<13.0 ng/ml	17 (40)	23 (42)	0.894
≥13.0 ng/ml	25 (60)	32 (58)	
CYFRA21-1			
<3.3 ng/ml	18 (43)	26 (47)	0.665
≥3.3 ng/ml	24 (57)	29 (53)	

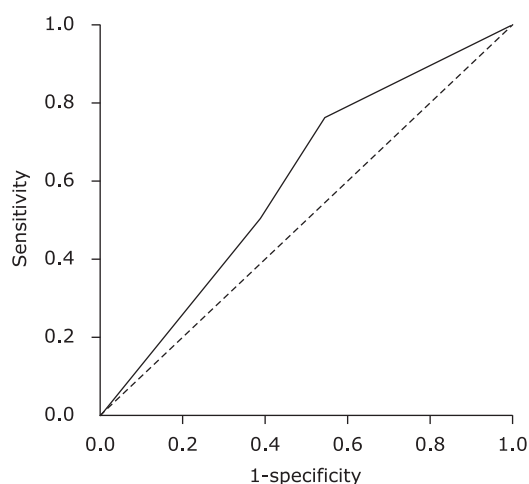
A multivariate logistic analysis showed elevated odd ratios were observed in gender (1.531, 95% *CI*: 0.215-10.883), smoking status (2.659, 95% *CI*: 0.368-19.222), and CEA level (2.613, 95% *CI*: 1.018-6.710), respectively (Table 4). However, the status of CEA was the only independent factor associated with EGFR mutation status in advanced lung adenocarcinoma patients ($P=0.046$, Table 4).

ROC curve analysis

ROC curve analysis showed the areas under the curve of CEA was 0.608 (95% *CI*: 0.496-0.721, $P=0.069$, Fig. 1). The sensitivity and specificity of CEA to predict EGFR gene mutations was 76% and 45% in our study respectively, and the positive predictive value and negative predictive value was 52% and 71%, respectively (Fig. 1).

Table 4. Results of multivariable analysis of the predictive factors for incidence of EGFR mutation using logistic regression models

Characteristics	Coefficient	SE	OR	95% CI	P value
Age (yrs)					
<64					0.123
≥64	-0.700	0.454	0.497	0.204-1.208	
Gender					
Male					0.670
Female	0.426	1.001	1.531	0.215-10.883	
Smoking status					
Yes					0.332
No	0.978	1.009	2.659	0.368-19.222	
CEA					
<5 ng/ml					0.046
≥5 ng/ml	0.961	0.481	2.613	1.018-6.710	

**Figure 1.** Receiver operating characteristic (ROC) curve analysis to assess the performance of CEA status in serum for differentiating mutant from wild-type EGFR gene patients with advanced lung adenocarcinoma. The area under the ROC curve is 0.608 ($P=0.069$).

DISCUSSION

EGFR is a member of ErbB receptor tyrosine kinase family and plays a critical role in the biology of many different tumors, implicating in tumor cell proliferation, invasion, angiogenesis, metastasis, protein translation, and cell metabolism.¹⁵ EGFR was found overexpressed or mutated in many tumors and is an attractive target for cancer therapy.^{16,17} Small molecules TKIs of EGFR, including gefitinib and erlotinib are validated therapeutic strategies for NSCLC. Good clinical responses were found in women, nonsmokers, adenocarcinomas, and Asians, which was related with EGFR gene mutations. Mutations in EGFR

exons 18 to 21 at EGFR intracellular tyrosine kinase catalytic domain, particularly deletions of exon 19 and point mutations of exon 21 in codon 858 can predict the response to EGFR-TKIs in lung adenocarcinoma.^{18, 19}

In lots of advanced NSCLC patients, samples for the detection of EGFR mutation are difficult to achieve and no other effective predictor for EGFR-TKI therapy has been found yet. Considering the EGFR mutation rates were extremely low in other histologic subtypes of lung cancer except adenocarcinoma, we chose only advanced lung adenocarcinoma patients in our study. In the current study, EGFR gene mutation rate was 43% in advanced lung adenocarcinoma patients, similar to several other reports with EGFR gene mutation rate ranging from 38% to 50% in Asian.^{20, 21} The most common two mutations are deletion at exon 19 and point mutation of L858R in exon 21, which is also in accordance with former reports.^{18, 19} As the *Pearson* chi-square test results shown, EGFR gene mutations were predominant in female ($P=0.001$) and non-smokers ($P=0.003$), but the logistic multivariate test suggested no difference in our study. Limited by the number of cases studied, most female patients in our cohort were nonsmokers, so these two factors may not be independent factors for EGFR gene mutations in our research.

Tumor markers, produced by tumor cells and released into body fluids, were reported to be useful tools for tumor diagnosis.²² Further researches also revealed tumor markers were associated with the development of tumor and can indicate the curative effects and prognosis in tumor.²³ Recently, there are several reports focused on the relationships between serum tumor makers and EGFR-TKIs treatment effect. Serum CEA, CA19-9, CA125, and CYFRA21-1 levels have been reported to relate with the survival of NSCLC patients treated with EGFR-TKIs.⁸⁻¹² Therefore, it is suggested that EGFR gene mutations may associated with the serum levels of these tumor markers. However, the relationships between these serum tumor markers with EGFR gene mutation status in advanced lung adenocarcinomas were unknown.

Shoji *et al*²⁰ reported that elevated serum CEA level was associated with higher EGFR gene mutation rates in recurrent lung adenocarcinomas after surgery ($n=48$). However, the samples for EGFR mutation test were primary surgery samples before disease recurrence and may not in accordance with all biological characteristic of recurrent tumor. Pan *et al*²¹ analyzed the relationships between EGFR mutations and serum levels of CA199, CEA, CA242, CA125, and CA153 in lung adenocarcinoma patients ($n=70$) and found that serum levels of CEA and CA242 can predict

EGFR gene mutation rate. In their research, the exon 19 and exon 21 mutations of EGFR were analyzed without exon 18 and exon 20, which may not represent all EGFR mutation status in all patients. In addition, both researches of Shoji and Pan analyzed all pathologic stages of lung adenocarcinoma patients. However, the serum levels of tumor markers are significantly higher in advanced tumor stages and rates of EGFR mutations were also significantly higher in stage III and IV than stage I and II. Analysis including all pathologic stages may not represent relationships between EGFR mutation status and serum tumor marker levels in advanced lung adenocarcinoma patients.

In our study, we analyzed the relationships between EGFR gene mutation rates with serum tumor markers CEA, CA125, CA19-9, NSE, and CYFRA21-1 in advanced lung adenocarcinoma patients. Both *Pearson* chi-square test and multivariate analysis showed that the serum CEA (cutoff value: 5 ng/ml) was the only independent tumor marker correlated with EGFR gene mutation status, which is in agreement with the results of Shoji and Pan's studies. However, *t* test showed no difference between serum tumor marker levels and EGFR mutation status, different with Pan *et al*'s reports. And the ROC curve revealed that CEA may not an ideal predictor for EGFR gene mutation in advanced lung adenocarcinoma patients (the area under the ROC curve was 0.608, $P=0.069$), which is also different to Pan *et al*'s observation. In Pan *et al*'s study, the higher serum levels of CEA and CA19-9 were significantly related with higher EGFR gene mutation rate and ROC curve revealed CEA can be used to forecast the EGFR gene mutation ($P<0.05$). These differences may due to the different stages of patients included. Serum tumor marker levels were extremely higher in advanced stages of lung adenocarcinoma patients and all stages of lung adenocarcinoma patients were cohorted in Pan *et al*'s study, which may not represent the relationship between serum tumor markers with EGFR mutations in advanced stages of lung adenocarcinomas.

In conclusion, this study demonstrated that EGFR gene mutation status was significantly associated with serum CEA status in advanced lung adenocarcinomas. However, serum CEA was not an ideal predictor for EGFR mutation. Due to the limited cases studied, further researches were needed to confirm our conclusion.

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