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Original Article

Relationship between EGFR gene mutation and serum tumor biomarkers in advanced lung adenocarcinoma

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Abstract: Lung cancer has the highest morbidity and mortality in malignant tumor worldwide. EGFR-TKI targeted therapy is a type of treatment with low side effects. However, it needs to clarify EGFR mutation status. Limited to sampling, EGFR mutation test cannot be widely applied. Finding other simple and practical markers to predict EGFR mutation state is of great significance. This study aimed to evaluate the correlation between different tumor biomarkers and EGFR mutation in advanced lung adenocarcinoma patients for targeted therapy determination, including serum CEA, CA125, CA199, and CYFRA211. Venous blood and tumor tissue were collected from advanced primary lung adenocarcinoma patients in a Guangzhou tumor hospital. Electrochemiluminescence immunoassay was performed to detect serum CEA, CA125, CA199, and CYFRA211 levels. Sequencing was performed to test EGFR gene fragment after PCR amplification. SPSS13.0 software was used for statistical analysis. Among 312 cases of advanced lung adenocarcinoma patients, 163 cases were wild type while 149 cases were mutation, and the mutation rate was 47.76%. EGFR mutation rate in advanced lung adenocarcinoma patients was not related to gender and age, while it was high in smokers ($P < 0.05$). Patients with elevated serum CEA, CA125, and CA199 showed higher EGFR gene mutation rate ($P < 0.05$). Serum CYFRA211 level was not correlated to EGFR mutation rate ($P > 0.05$). Serum CEA, CA125, and CA199 levels could be used to predict EGFR gene mutation in advanced lung adenocarcinoma.

Keywords: Advanced lung adenocarcinoma, EGFR, gene mutation, tumor biomarker

Introduction

Lung cancer is one of the most common malignant tumors with increasing incidence. It has become the main cause of cancer deaths worldwide that seriously threats to human health [1]. Lung cancer includes non-small cell lung cancer (NSCLC) and small cell lung cancer, of which NSCLC is the most common type accounting for about 85% of the total number of lung cancer. NSCLC could be divided into squamous cell carcinoma, adenocarcinoma, undifferentiated carcinoma, bronchioloalveolar carcinoma, and large cell carcinoma according to the cell origin [2]. It can be classified into stage 0~IV according to the 2009 international standard for lung cancer staging. The patients in early and middle stages can receive surgical treatment, while patients in advanced stage can only choose chemotherapy or radiotherapy.

With development of lung cancer molecular biology, targeted therapy has become a research hotspot because of its little side effect. Epidermal Growth Factor Receptor (EGFR) signaling pathway tyrosine kinase inhibitor received the most in-depth investigation. EGFR expressed in the stroma, epithelium and neurogenic tissues that is of great importance to regulate normal cell differentiation and hyperplasia. It was found that EGFR targeted drug has selectivity. EGFR gene mutation can increase the sensitivity to small molecule tyrosine kinase inhibitor (TKI) [3]. The efficacy of TKI was associated with EGFR mutation [4], as TKI treatment effectiveness on NSCLC patients with EGFR mutation was significantly higher than patients without mutation [5-7]. These studies showed that EGFR gene mutation was a premise for NSCLC patient's targeted therapy, and also the biomarker of predicting TKI curative effect. Thus, EGFR mutation status should be tested

Table 1. EGFR gene mutation and related clinical characteristics in advanced lung adenocarcinoma patients

Clinical characteristics	Mutant type	Wild type	χ^2 value	*P* value
Gender				
Female	82	70	4.55	0.03
Male	67	93		
Age				
≤ 60 years old	72	75	0.17	0.68
> 60 years old	77	88		
Smoking history				
Yes	76	44	18.96	< 0.001
No	73	119		

before choosing EGFR targeted therapy, and it had been written to the NCCN guideline. However, because of unable to obtain tumor tissue and expensive testing, EGFR gene mutation detection has not been widely carried out. Therefore, searching simple and practical markers to predict EGFR mutation state is important.

Serum tumor biomarkers have the advantage of convenient detection that can help diagnosis. Studies had found that serum CEA level was associated with EGFR mutation rate in NSCLC patients [8, 9]. In addition, serum CEA and CA242 were also associated with EGFR mutation in lung adenocarcinoma patients [10, 11], suggesting that serum tumor marker can be used to evaluate EGFR mutations potentially. At present, related studies all focused on tumor marker CEA while the other serum biomarkers were still lack of research. Thus, we selected CA125, CA199, and CYFRA211 as the research target. Studies revealed that EGFR gene mutation rate was high in lung adenocarcinoma patients [12]. For advanced lung adenocarcinoma patients unable to undergo surgery treatment, targeted drugs was mainly used in these patients. Currently, there were still lack of report about the relationship between EGFR gene mutation and serum tumor markers in advanced lung adenocarcinoma patients. Through detecting EGFR mutation and serum CEA, CA125, CA199 and CYFRA211 levels, we aimed to evaluate the correlation between tumor markers and EGFR mutation. It may provide simple method to predict EGFR mutation, and searching potential feasible index for targeted therapy judgment.

Materials and methods

Main reagents and instruments

Electrochemical luminescence automatic immunity analyzer (Roche Elecsys 2010). CEA, CA125, CA199 and CYFRA211 detection kit (Roche). PCR kit (Takara, Japan). Microplate reader (Thermo Fisher Multiskan FC, USA).

Research objects

312 cases of primary lung adenocarcinoma needs surgery in Ruikang Hospital of Guangxi traditional Chinese Medicine University Affiliated between Jan 2011 and Jun 2015 were enrolled. No patients received chemotherapy or radiotherapy before surgery. All the subjects were diagnosed by pathology and staged in IIIB or IV according to TNM staging. There were 312 cases including 160 males and 152 females with mean aged 61.70 ± 8.68 (49-78) years old. All patients had signed the informed consent. Cancer tissue and fasting venous blood were collected for the experiment. Smoking was defined as at least one daily and sustained for more than one year.

Serum tumor biomarker concentration detection

3 ml fasting venous blood were collected to the heparin anticoagulant vacuum tube and let stand at room temperature for 20 min. It was then centrifuged at 3000 RPM for 10 min to separate serum. Electricity chemiluminescence immunoassay (ECLIA) method was performed to detect serum CEA, CA125, CA199 and CYFRA211 content according to the instrument and reagent kit instructions. The reference value of tumor markers in clinic was as follows: CEA < 5.0 ng/ml, CA125 < 35 U/ml, CA199 < 37 U/ml, CYFRA211 < 3.3 ng/ml.

EGFR gene mutation detection

Salt fraction was adopted to extract the whole genome DNA. DNA purity and content was determined by protein nucleic acid detector. The sample with absorbance A260/A280 between 1.8~2.0 can be used in the subsequent experiment. PCR amplification was used to detect EGFR gene 19 and 21 exon mutation. Amplification primers were referred to J. B. Pan's report [10]. Exon 19: forward, 5'-GCAATATCAGCCTTAGGTGCGGCGC-3', reverse, 5'-CA-

Table 2. Serum tumor marker comparison between EGFR gene mutation and wild type

Tumor marker	EGFR wild type	EGFR mutation type	T or t' value	*P* value
CEA (ng/ml)	16.38±7.56	50.27±30.28	13.04	< 0.001
CA125 (U/ml)	60.78±28.34	120.29±70.29	9.49	< 0.001
CA199 (U/ml)	46.25±12.35	100.89±40.54	15.52	< 0.001
CYFRA211 (ng/ml)	8.76±3.48	9.19±3.86	1.03	0.15

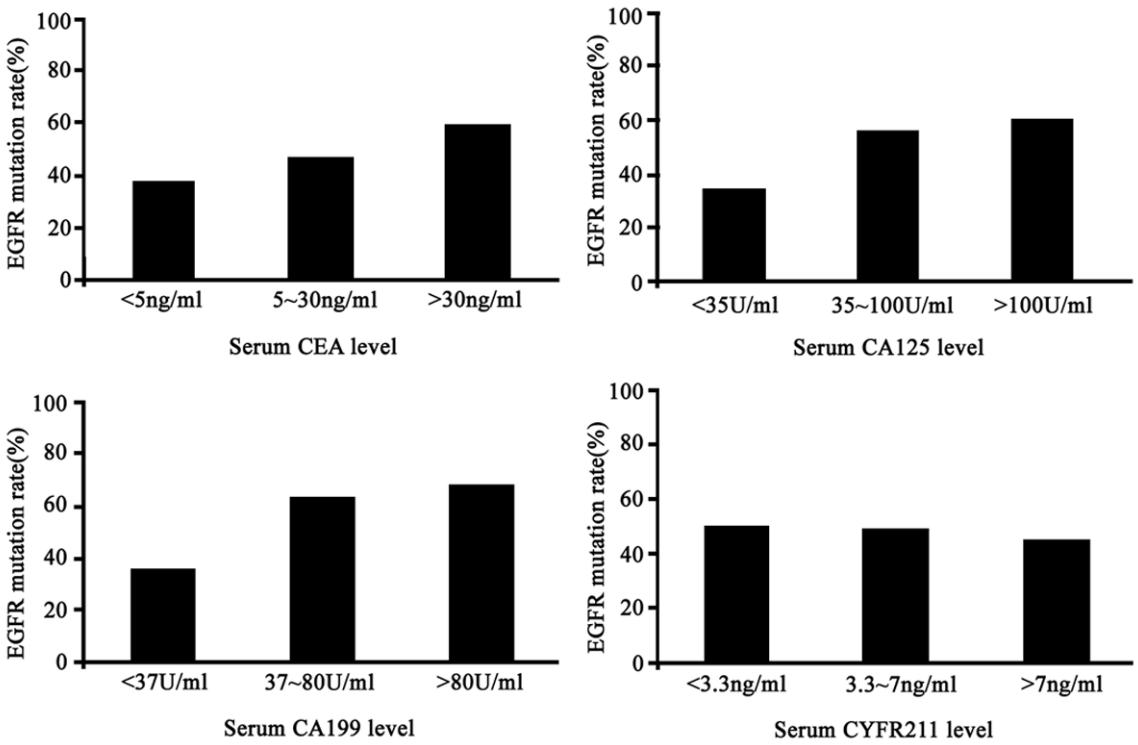

Figure 1. EGFR gene mutation rate in different concentration of tumor marker.

TAGAAAGTGAACATTTAGGATGTG-3'; Exon 21: forward: 5'-CTAACGTTCGCCAGCCATAAGTCC-3', reverse, 5'-GCTGCGAGCTCACCCAGAATGTCTGG-3'. PCR reaction system was prepared according to the instruction. The reaction condition was as follows: 95°C predegeneration for 10 min, continued by 30 cycles of 95°C degeneration for 15 s, 56°C anneal for 30 s, and 72°C extension for 30 s. 5 μl PCR products were identified by agarose gel electrophoresis. The product was sequenced by Genomics technology co., LTD.

Statistical analysis

All statistical analysis was performed on SPSS13.0 software. Chi-square test, t test, and logistic regression analysis were used for data comparison. $P < 0.05$ was considered as statistically significant.

Results

EGFR gene mutation results

All of the 312 cases received EGFR gene detection, 163 cases were wild type while 149 cases were mutation, and the mutation rate was 47.76%. Among them, 72 cases showed exon 21 mutation. The relationship between EGFR gene mutation and clinical characteristics were listed in **Table 1**. More EGFR gene mutation appeared in female and smokers ($P < 0.05$), but not related to age ($P > 0.05$).

Serum tumor marker comparison between EGFR gene mutation and wild type

T test was used to compare the serum tumor biomarker level in EGFR gene mutation and wild type advanced lung adenocarcinoma

Table 3. EGFR gene mutation rate comparison among different concentration of tumor marker

Tumor marker	n	Wild type	Mutation type	χ^2 value	*P* value
CEA					
< 5.0 ng/ml	101	63	38	9.68	0.008
5~30 ng/ml	108	58	50		
> 30 ng/ml	103	42	61		
CA125					
< 35 U/ml	172	114	58	18.58	< 0.001
35~100 U/ml	74	33	41		
> 100	66	26	40		
CA199					
< 37 U/ml	184	119	65	28.01	< 0.001
37~80 U/ml	60	22	38		
> 80	68	22	46		
CYFRA211					
< 3.3 ng/ml	121	61	60	0.52	0.77
3.3~7 ng/ml	80	41	39		
> 7 ng/ml	111	61	50		

Table 4. EGFR gene mutation logistic regression analysis

Influence factor	OR value	95% CI	*P* value
Gender	0.58	0.17~1.97	0.38
Age	1.03	0.96~1.11	0.39
Smoking history	5.26	1.46~18.97	0.01
CEA	0.85	0.79~0.91	< 0.001
CA125	0.96	0.94~0.98	< 0.001
CA199	0.92	0.87~0.95	< 0.001
CYFRA211	0.98	0.81~1.18	0.817

patients (**Table 2**). Serum CEA, CA125, and CA199 level showed significant difference between EGFR gene mutation and wild type patients. EGFR gene mutation patients presented obviously higher serum CEA, CA125, and CA199 levels than that of wild type patients (P < 0.001). CYFRA211 was lack of statistical difference between EGFR gene mutation and wild type patients (P > 0.05).

EGFR gene mutation rate comparison among different concentration of tumor marker

EGFR gene mutation rate with tumor markers in different concentration gradient was shown in **Figure 1**. Chi square test was performed for EGFR gene mutation rate comparison among different concentration gradient (**Table 3**). EGFR gene mutation rate showed markedly difference among CEA, CA125 and CA199 in different concentration gradient. It increased following concentration gradient elevation (P < 0.05). CYFRA211 in different concentration gradient showed no significant difference of EGFR mutation rate (P > 0.05).

EGFR gene mutation logistic regression analysis

The above results showed that EGFR mutation rate was affected by multiple factors. To exclude the confounding factor, EGFR gene mutation was set as dependent variable, while age, gender, smoking history, and serum tumor marker level were set as independent variable for logistic regression analysis. As shown in **Table 4**, smoking, CEA, CA125, and CA199 level increase EGFR gene mutation rate (P < 0.05).

Discussion

EGFR gene belongs to the type I growth factor family. Its encoded protein has the tyrosine kinase activity. Its tyrosine kinase functional domain is encoded by exon 18-24, and EGFR gene mutation can affect the enzyme activity. Clinical trials pointed out that EGFR gene mutation is an efficacy predictor of EGFR-TKI treatment in advanced NSCLC. EGFR mutation has been treated as the precondition of EGFR-TKI first line application [13, 14]. However, how to detect EGFR mutation is a problem to be solved. For the specimen is difficult to obtain, and studies found that some serum tumor markers have a close relationship with EGFR mutation [15], applying serum tumor markers detection instead of testing EGFR mutation directly became a good research direction. Current studies were still not enough to change EGFR gene mutation to tumor marker detection, and most focused on the relationship between serum CEA and EGFR mutation rate in NSCLC patients. EGFR mutation had a high incidence in lung adenocarcinoma [16]. Therefore, this study selected the advanced lung adenocarcinoma patients that most likely need EGFR-TKI. Meanwhile, we detected serum CEA, CA125, CA199 and CYFRA211 levels to clarify their relationships to the EGFR mutation rate.

For most EGFR mutations were concentrated in exon 19 and 21 [12, 17, 18], this study tested their mutations. It was found that the gene mutation rate of exon 19 and 21 was 47.76%. In 2010, the PIONEER study showed that the EGFR gene mutation rate in Chinese lung adenocarcinoma patients was 50.2%, which was slightly higher than our results. It might be caused that our study only detected two exons. Our results were in accordance with K. Wang's report as 48.6% [19]. In addition, it was found that smokers had higher EGFR gene mutation rate. Univariate analysis showed that EGFR gene mutation was related to gender, while logistic regression analysis presented they had no association. It might be caused that univariate analysis did not take into account the impact of smoking history in patients with different gender. In addition, age was also unrelated to EGFR mutation rate. C. D. Wang found that EGFR gene mutation showed no significant association with age, gender, or smoking history [20]. However, J. Gao revealed that EGFR gene mutation in Chinese NSCLC patients was related to gender and smoking history [21]. M. J. Fan demonstrated that female patients without smoking history had higher mutation rate [16]. Different conclusion may be caused by the different selected subjects and sample size. The impact of gender and smoking on EGFR gene mutation still needs further investigation.

Study about the relationship between serum tumor markers level and EGFR mutation rate showed that, EGFR gene mutation rate increased following the elevation of serum CEA, CA125, CA199 levels in advanced lung adenocarcinoma, but not associated with CYFRA211 level. Currently, there was still lack of reports about the relationship between serum tumor markers level and EGFR mutation rate in advanced lung adenocarcinoma. Z. M. Yang found that serum CEA level was associated with EGFR mutation rate in NSCLC patients [8]. B. Jin [11] discovered that EGFR mutation was related to CEA but not CA125 or CA199 in lung adenocarcinoma, which was similar to our results. It may be due to our study only selected the advanced lung adenocarcinoma patients, while J. B. Pan enrolled lung adenocarcinoma patients in stage II-IV.

To sum up, our study first found that CEA, CA125, and CA199 were related to EGFR mutation in advanced lung adenocarcinoma, suggesting that CEA, CA125, and CA199 can evaluate EGFR gene mutation in advanced lung adenocarcinoma to some extent. Serum CEA, CA125, and CA199 could be treated as the potential indicators of advanced lung adenocarcinoma targeted therapy, while its application in clinic still needs larger study.

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Disclosure of conflict of interest

None.

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References

[1] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62: 10-29.

[2] Brambilla E, Travis WD, Colby TV, Corrin B, Shimosato Y. The new World Health Organization classification of lung tumours. Eur Respir J 2001; 18: 1059-1068.

[3] Sirotnak FM, Zakowski MF, Miller VA, Scher HI, Kris MG. Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by coadministration of ZD1839 (Iressa), an inhibitor of EGFR tyrosine kinase. Clin Cancer Res 2000; 6: 4885-4892.

[4] Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350: 2129-2139.

[5] Han SW, Kim TY, Hwang PG, Jeong S, Kim J, Choi IS, Oh DY, Kim JH, Kim DW, Chung DH, Im SA, Kim YT, Lee JS, Heo DS, Bang YJ, Kim NK. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. J Clin Oncol 2005; 23: 2493-2501.

[6] Mu XL, Li LY, Zhang XT, Wang MZ, Feng RE, Cui QC, Zhou HS, Guo BQ. Gefitinib-sensitive mutations of the epidermal growth factor receptor tyrosine kinase domain in chinese patients with non-small cell lung cancer. Clin Cancer Res 2005; 11: 4289-4294.

[7] Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004; 304: 1497-1500.

[8] Yang ZM, Ding XP, Pen L, Mei L, Liu T. Analysis of CEA expression and EGFR mutation status in non-small cell lung cancers. Asian Pac J Cancer Prev 2014; 15: 3451-3455.

[9] Okamoto T, Nakamura T, Ikeda J, Maruyama R, Shoji F, Miyake T, Wataya H, Ichinose Y. Serum carcinoembryonic antigen as a predictive marker for sensitivity to gefitinib in advanced non-small cell lung cancer. Eur J Cancer 2005; 41: 1286-1290.

[10] Pan JB, Hou YH, Zhang GJ. Correlation between EGFR mutations and serum tumor markers in lung adenocarcinoma patients. Asian Pac J Cancer Prev 2013; 14: 695-700.

[11] Jin B, Dong Y, Wang HM, Huang JS, Han BH. Correlation between serum CEA levels and EGFR mutations in Chinese nonsmokers with lung adenocarcinoma. Acta Pharmacol Sin 2014; 35: 373-380.

[12] Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, Majem M, Lopez-Vivanco G, Isla D, Provencio M, Insa A, Massuti B, Gonzalez-Larriba JL, Paz-Ares L, Bover I, Garcia-Campelo R, Moreno MA, Catot S, Rolfo C, Reguart N, Palmero R, Sanchez JM, Bastus R, Mayo C, Bertran-Alamillo J, Molina MA, Sanchez JJ, Taron M; Spanish Lung Cancer Group. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009; 361: 958-967.

[13] Berg J, Fjellbirkeland L, Suhrke P, Jebsen P, Lund-Iversen M, Kleinberg L, Helgeland L, Brustugun OT, Helland A. EGFR mutation testing of lung cancer patients-Experiences from Vestfold Hospital Trust. Acta Oncol 2015; 1-7.

[14] Jotte RM, Spigel DR. Advances in molecular-based personalized non-small-cell lung cancer therapy: targeting epidermal growth factor receptor and mechanisms of resistance. Cancer Med 2015; 4: 1621-32.

[15] Abdurahman A, Anwar J, Turghun A, Niyaz M, Zhang L, Awut I. Epidermal growth factor receptor gene mutation status and its association with clinical characteristics and tumor markers in non-small-cell lung cancer patients in Northwest China. Mol Clin Oncol 2015; 3: 847-850.

[16] Fan MJ, Li HG, Lu ZQ, Zhang HZ, Shen XM. [Relationship of epidermal growth factor receptor gene mutations, clinicopathologic features and prognosis in patients with non-small cell lung cancer]. Zhonghua Bing Li Xue Za Zhi 2011; 40: 679-682.

[17] Mitsudomi T, Yatabe Y. Mutations of the epidermal growth factor receptor gene and related genes as determinants of epidermal growth factor receptor tyrosine kinase inhibitors sensitivity in lung cancer. Cancer Sci 2007; 98: 1817-1824.

[18] Chan SK, Gullick WJ, Hill ME. Mutations of the epidermal growth factor receptor in non-small cell lung cancer-search and destroy. Eur J Cancer 2006; 42: 17-23.

[19] Wang K, Gong H, Li X, Yang Z, Cao P, Wand C, Jiang Y, Wang H, Wang Y, Zhang G. [Relationship between histopathologic characteristics and epidermal growth factor receptor mutation in lung adenocarcinoma]. Zhonghua Bing Li Xue Za Zhi 2015; 44: 170-174.

[20] Wang CD, Wang XR, Wang CY, Tang YJ, Hao MW. Relevance of EGFR gene mutation with pathological features and prognosis in patients with non-small-cell lung carcinoma. Asian Pac J Trop Med 2015; 8: 249-252.

[21] Gao J, Chen JQ, Zhang L, Liang ZY. [Relationship between EGFR and KRAS mutations and prognosis in Chinese patients with non-small cell lung cancer: a mutation analysis with real-time polymerase chain reaction using scorpion amplification refractory mutation system]. Zhonghua Bing Li Xue Za Zhi 2012; 41: 652-656.