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Original Article

Predictive value of serum markers for targeted treatment in advanced lung adenocarcinoma

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Abstract: Lung cancer seriously threats human health with the highest morbidity and mortality worldwide. EGFR-TKI targeted therapy presents low side effects, but the premise is to definitude EGFR mutation status. Limited to sampling, EGFR mutation cannot be widely tested. Searching for other simple and utility markers to predict EGFR mutation state can help guide EGFR-TKI. This study intended to investigate the correlation among different tumor biomarkers, EGFR mutation, and EGFR-TKIs curative effect in advanced lung adenocarcinoma patients, including serum CEA, CA199, and CA242. Venous blood and tumor tissue were gathered from advanced primary lung adenocarcinoma patients aiming to receive EGFR-TKIs treatment. Electrochemiluminescence immunoassay was used to detect serum CEA, CA199, and CA242 levels. Sequencing was applied to detect EGFR gene fragment after PCR amplification. SPSS13.0 was used for statistical analysis. EGFR-TKIs curative effect in lower PS patients was better than that in higher PS patients ($P < 0.05$). EGFR-TKIs curative effect was better in patients with higher serum CEA, CA199, and CA242 levels ($P < 0.05$). EGFR mutation patients showed significantly higher level of serum CEA, CA199, and CA242 ($P < 0.05$). On the other hand, patients with higher serum CEA, CA199, and CA242 levels presented statistically higher EGFR mutation rate ($P < 0.05$). Serum CEA, CA199, and CA242 levels can be used to evaluate EGFR gene mutation in advanced lung adenocarcinoma to some extent, and are potential index for EGFR-TKIs curative effect.

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

Introduction

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer accounting for about 80% of the total number. It is the leading cause of malignant cancer death [1]. NSCLC could be classified into squamous carcinoma, adenocarcinoma, undifferentiated carcinoma, bronchioloalveolar carcinoma, and large cell carcinoma based on cell origin [2].

Following the development of lung cancer research, targeted therapy has been adopted in clinic widely. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) had been recommended as the first line choice for EGFR mutant NSCLC patients by NCCN in 2011. However, targeted therapy is closely associated with EGFR gene mutation [3]. EGFR gene mutation can increase EGFR-TKIs sensitivity [4], and the drug efficacy is related to EGFR gene mutation [5]. Numerous studies revealed that EGFR-TKIs curative effect is obviously higher for NSCLC patients with EGFR mutation compared with wild type [6-8]. Therefore, definitude EGFR mutation is of great significance to improve EGFR-TKIs efficacy. However, limited to high test cost, long detection period, and difficult tumor tissue obtain, EGFR gene detection cannot be easily generally carried out.

Serum tumor biomarkers have been widely used for lung cancer detection and diagnosis [9]. In addition, serum CEA and CA242 have been found associated with EGFR mutation in lung adenocarcinoma patients [10, 11], while serum CA199 level was related to EGFR-TKIs curative effect [12], indicating that serum tumor marker can be treated as potential target to guide EGFR-TKIs therapy. Studies showed that

Table 1. The relationship between EGFR-TKIs first line therapy effect and clinical features

Clinical feature	Cases	Relationship to ORR			Relationship to DCR		
		ORR (n)	χ^2 value	*P* value	DCR (n)	χ^2 value	*P* value
Gender							
Female	50	27	0.35	0.55	35	0.93	0.34
Male	62	30			38		
Age							
≤ 60	48	25	0.05	0.83	32	0.08	0.77
> 60	64	32			41		
Smoking history							
With	56	28	0.04	0.85	36	0.04	0.84
Without	56	29			37		
PS score							
0~1	58	37	8.01	0.005	44	6.05	0.01
2~3	54	20			29		

*P < 0.05.

Table 2. The relationship between serum tumor marker level and EGFR-TKIs short-term effect

Tumor marker	Cases	Relationship to ORR			Relationship to DCR		
		ORR (n)	χ^2 value	ORR (n)	χ^2 value	ORR (n)	χ^2 value
CEA							
< 5 ng/ml	44	8	31.03	< 0.001*	17	22.49	< 0.001*
≥ 5 ng/ml	68	49			56		
CA199							
< 37 U/ml	66	19	31.42	< 0.001*	34	13.22	< 0.001*
≥ 37 U/ml	46	38			39		
CA242							
< 20 U/ml	74	24	29.74	< 0.001*	39	14.96	< 0.001*
≥ 20 U/ml	38	33			34		

*P < 0.05.

Table 3. Logistic multivariate analysis of serum tumor marker and EGFR-TKIs effect

Tumor marker	Relationship to ORR			Relationship to DCR		
	OR	95% CI	*P* value	OR	95% CI	*P* value
CEA	1.06	1.04~1.08	< 0.001*	1.04	1.02~1.06	< 0.001*
CA199	1.03	1.02~1.04	< 0.001*	1.02	1.01~1.03	0.001*
CA242	1.04	1.02~1.06	< 0.001*	1.03	1.01~1.05	< 0.001*

*P < 0.05.

lung adenocarcinoma patients presented high EGFR mutation rate [13-15]. Since advanced lung adenocarcinoma patients cannot receive surgery, targeted therapy is mainly applied in advanced lung adenocarcinoma patients with EGFR mutation. Thus, this research aimed to clarify the relationship between tumor markers and EGFR-TKIs efficacy by selecting advanced lung adenocarcinoma patients received EGFR-TKIs treatment as objects and detecting serum CEA, CA199, and CA242 levels. Meanwhile, we further detected EGFR gene mutation condition to confirm whether EGFR-TKIs efficacy was affected by EGFR mutation.

Materials and methods

Main reagents and instruments

Electrochemical luminescence automatic immunity analyzer (Roche Elecsys 2010). CEA, CA199, and CA242 detection kit (Roche). PCR kit (Takara, Japan). Microplate reader (Thermo Fisher Multiskan FC, USA).

Research objects

Primary lung adenocarcinoma patientsaiming to receive EGFR-TKIs first line therapy in the First Affiliated Hospital of Xinxiang Medical University between Jan 2011 and Jun 2014 were enrolled. All the patients were diagnosed by pathology and staged in IIIB or IV according to TNM staging. There were 112 cases including 60 males and 52 females with mean aged 62.86 ± 8.52 (50-77) years old. All patients had signed the informed consent. Fasting venous blood was collected for the following experiment. 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. All the objects received performance status (PS) grading. The criteria was as follows: 0, normal activity; 1, slight symptom, comfortable life, light physical activity; 2, tolerate to tumor symptom, life-independent, time in bed during the day

Table 4. EGFR gene mutation and clinical features in advanced lung adenocarcinoma

Clinical feature	Mutation type	Wild type	χ^2 value	*P* value
Gender				
Female	31	19	1.82	0.18
Male	30	31		
Age				
≤ 60	25	23	0.19	0.66
> 60	36	28		
Smoking history				
Yes	28	28	0.90	0.34
No	33	23		
PS score				
0~1	34	24	0.84	0.36
2~3	27	27		

Table 5. Serum tumor markers levels comparison between EGFR mutation and wild type

Tumor marker	EGFR wild type	EGFR mutation	T or t' value	*P* value
CEA (ng/ml)	20.06±23.99	35.20±26.13	3.17	0.002
CA199 (U/ml)	38.71±46.46	67.61±58.96	2.84	0.005
CA242 (U/ml)	23.33±29.87	40.39±39.05	2.62	0.010

time no more than 50%; 3, severe tumor symptom, time in bed during the day time more than 50%, can stand up, part of life-independent; 4, remain in bed; 5, death. All of the objects PS score at 0-3.

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 used to detect serum CEA, CA199, and CA242 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, CA199 < 37 U/ml, CA242 < 20 U/ml.

EGFR gene mutation detection

Tumor tissue diagnosed by pathology was collected. 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: foward, 5'-GCAATATCAGCCTTAGGTGCGGCGC-3', reverse, 5'-CATAGAAAGTGAACATTTAGGATGTG-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.

Therapeutic effect evaluation

Solid tumor curative effect evaluation standard was applied to evaluate the curative effect as follows: complete remission (CR), partial remission (PR), stable diseases (SD), and progressive diseases (PD). Disease control rate (DCR) = (CR + PR + SD)/total treatment cases ×100%, objective response rate (ORR) = (CR + PR)/total treatment cases ×100%.

Statistical analysis

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

Results

The relationship between EGFR-TKIs first line therapy effect and clinical features

All of the 112 patients received curative effect evaluation, including 6 cases in CR (5.36%), 51 cases in PR (45.54%), 16 cases in SD (14.29%), and 39 cases in PD (34.82%). ORR was 50.89%, while the DCR was 65.18%. **Table 1** showed the relationship between EGFR-TKIs first line treating advanced lung adenocarcinoma and clinical features. Univariate analysis revealed that EGFR-TKIs has better effect in patients at PS 0-1 than that in smoking patients at PS 2-3 (P <

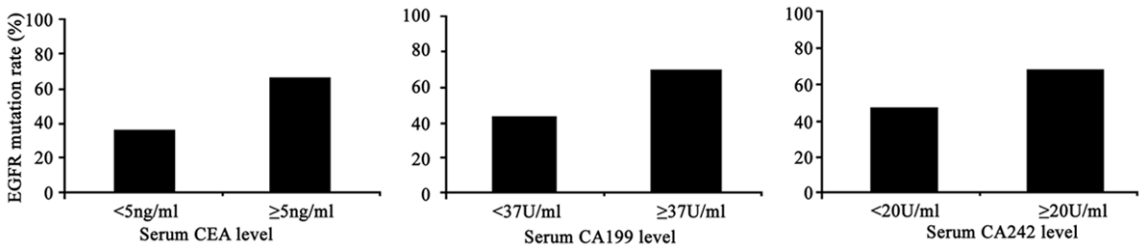

Figure 1. EGFR mutation rate in different tumor marker concentration.

Table 6. The correlation between serum tumor marker concentration and EGFR mutation rate

Tumor marker	n	Wild type	Mutation	χ^2 value	*P* value
CEA					
< 5.0 ng/ml	44	28	16	9.57	0.002
≥ 5.0 ng/ml	68	23	45		
CA199					
< 37 U/ml	66	37	29	7.18	0.007
≥ 37 U/ml	46	14	32		
CA242					
< 20 U/ml	74	39	35	4.52	0.03
≥ 20 U/ml	38	12	26		

0.05), whereas the efficacy was not related to gender, age, and smoking history.

The relationship between serum tumor marker level and EGFR-TKIs short-term effect

Chi-square analysis was applied to compare EGFR-TKIs short-term effect in patients with different serum tumor markers level. As shown in **Table 2**, EGFR-TKIs curative effect was better in patients with high CEA, CA199, and CA242 levels than that with low levels ($P < 0.001$).

To rule out the possible confounding factors, logistic regression analysis was used to perform multivariate analysis by setting tumor markers, gender, age, smoking history, PS score as independent variables, and ORR or DCR as dependent variable. It was found that the higher tumor markers CEA, CA199, and CA242 levels, the better the EGFR-TKIs effect (**Table 3**).

EGFR gene mutation

To further clarify whether the impact of serum tumor markers on EGFR-TKIs effect was associated with EGFR mutation, this study further examined EGFR gene mutation in patients with advanced lung adenocarcinoma. There were 61 gene mutation cases (54.46%) and 51 wild type cases (45.53%). The relationship between EGFR mutation and clinical features were listed in **Table 4**. It was found that EGFR gene mutation showed no significant correlation with patient's gender, age, smoking history and PS score ($P > 0.05$).

Serum tumor markers levels comparison between EGFR mutation and wild type

T test was used to compare serum tumor markers levels between advanced lung adenocarcinoma patients with EGFR gene wild type and mutation (**Table 5**). EGFR mutation patients showed significantly higher levels of serum CEA, CA199, and CA242 compared with wild type patients ($P < 0.05$). To exclude possible confounding factors, we performed multiple linear regressions by selecting CEA, CA199, and CA242 as dependent variables, and choosing tumor markers, gender, age, smoking history, and PS score as independent variables. The results showed that serum CEA, CA199, and CA242 concentration in wild type patients were statistically different from that in EGFR mutation patients ($t = 3.16$, $P = 0.002$; $t = 2.85$, $P = 0.005$; $t = 2.61$, $P = 0.011$).

The correlation between serum tumor marker concentration and EGFR mutation rate

Chi-square test was applied to determine EGFR mutation rate in different tumor marker concentration (**Figure 1**; **Table 6**). Patients with higher serum CEA, CA199, and CA242 levels presented obviously higher EGFR mutation rate ($P < 0.05$).

Discussion

EGFR encoded protein has tyrosine kinase activity, while gene mutation can influence the enzyme activity. Many large scale clinical trials showed that EGFR-TKIs targeted therapy can

extend NSCLC patients survival. EGFR mutation status has become a predicator of EGFR-TKIs in treating NSCLC, and also an important condition for EGFR-TKIs first line therapy [16, 17]. However, limited by tissue samples, many researchers were looking for new index to substitute EGFR gene mutation for guiding EGFR-TKIs medication or prediction. Serum tumor markers received much attention because of its convenient detection, and some researches also confirmed that part of the serum tumor markers showed close relationship with EGFR mutation [18]. Up to now, most studies focused on the relationship between serum tumor markers and EGFR mutations, whereas fewer researches considered tumor markers as EGFR-TKIs efficacy predictor. Therefore, this study selected advanced lung adenocarcinoma patients to clarify the relationship between EGFR-TKIs curative effect and serum CEA, CA199, and CA242 levels.

Our result demonstrated that the advanced lung adenocarcinoma patients with high CEA, CA199, and CA242 levels gained better EGFR-TKIs short-term effects, suggesting that serum CEA, CA199, and CA242 detection was potential predicators for EGFR-TKIs treating advanced lung adenocarcinoma. J. b. Pan, et al. also reported similar results about CEA and CA199 with us [12]. However, there was still lack of investigation about the relationship between CA242 and EGFR-TKIs treatment.

To clarify whether serum tumor marker level impact on EGFR-TKIs curative effects was related to EGFR mutation, we further detected EGFR gene mutation status. It was found that CEA, CA199, and CA242 levels were higher in EGFR mutant patients, and patients with high CEA, CA199, and CA242 levels showed higher EGFR mutation rate, indicating that CEA, CA199, and CA242 levels can predict EGFR gene mutation to a certain extent. Our results also revealed that EGFR-TKIs curative effect was better in EGFR mutant patients. To sum up, it was speculated that patients with high CEA, CA199, and CA242 levels showed higher EGFR mutation rate, and better EGFR-TKIs curative effect. Currently, there was still no report about the relationship between serum tumor marker level in advanced lung adenocarcinoma patients and EGFR mutation rate. Z. M. Yang found that serum CEA level was correlated with EGFR mutation rate in NSCLC patients [19]. W. T. Wang discovered that CEA level was associated with EGFR mutation in lung adenocarcinoma patients [20]. J. B. Pan detected that CEA and CA242 levels were not related to EGFR mutation rate, which was similar with our results [10]. There was still lack of investigation about the relationship between CA199 level and EGFR mutation.

In conclusion, this study first time found that serum CEA, CA199, and CA242 levels in advanced lung adenocarcinoma patients were associated with EGFR mutation rate and EGFR-TKIs efficacy, suggesting that CEA, CA199, and CA242 levels can predict EGFR mutation and EGFR-TKIs curative effect. Limited to sample scale, further investigation was needed for clinical application.

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Disclosure of conflict of interest

None.

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