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Original Article

Correlation between serum CEA levels and EGFR mutations in Chinese nonsmokers with lung adenocarcinoma

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Aim: To evaluate the relationship between epidermal growth factor receptor (EGFR) mutations and serum carcinoembryonic antigen (CEA) levels in Chinese nonsmokers with pulmonary adenocarcinoma.
Methods: We sequenced exons 18–21 of the EGFR gene in 98 cases. The patients were divided into two groups based on their pre-treatment serum CEA levels (below or above 5 ng/mL) for analyzing the correlations with EGFR mutations.
Results: Sixty-seven cases harbored EGFR mutations. The rates of EGFR mutations and exon 19 mutations in the high-CEA group (78.2% and 49.1%, respectively) were significantly higher than those in the low-CEA group (55.8% and 20.9%, respectively). Serum CEA levels were found to be the only independent predictor of EGFR mutation (OR 2.837; 95% CI: 1.178–6.829) and exon 19 mutation (OR 3.618; 95% CI: 1.319–9.918). Furthermore, a higher serum CEA level was associated with a higher EGFR mutation rate and a higher exon 19 mutation rate: patients with serum CEA levels <5 ng/mL, ≥5 and <20 ng/mL, ≥20 ng/mL showed the EGFR mutation rate of 55.8%, 74.1%, 82.1%, respectively, and the exon 19 mutation rate of 20.9%, 40.7%, 57.1%, respectively. Patients with EGFR mutations displayed a significantly higher incidence of abnormal serum CEA levels (>5 ng/mL) than patients without EGFR mutations (64.2% *vs* 38.7%).
Conclusion: Elevated serum CEA levels predict the presence of EGFR gene mutations in Chinese nonsmokers with pulmonary adenocarcinoma.

Keywords: lung cancer; adenocarcinoma; biomarker; carcinoembryonic antigen; EGFR; mutation; exon 19 deletion; nonsmoker

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Introduction

With the advances in molecular biology research on tumors and an increased understanding of the pathogenesis of tumor formation, targeted molecular therapy for advanced and metastatic non-small cell lung cancer (NSCLC) patients has become an important treatment approach. As proven by many large-scale clinical trials, molecular targeted therapy, exemplified by treatments such as epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs), is highly effective in extending the life expectancy of NSCLC patients[1, 2]. Given the good response and low toxicity, treating NSCLC patients who harbor an EGFR mutation with an EGFR-TKI as the first-line treatment is recommended, replacing conventional cytotoxic chemotherapy. However, the treatment effect of EGFR-TKIs varies greatly between different populations. Many studies have shown that Asian nonsmokers with lung adenocarcinoma[3–5] and patients with EGFR mutation[6–8] are more responsive to EGFR-TKI treatment. Other researchers reported that Asian nonsmokers with lung adenocarcinoma displayed a high rate of EGFR mutation[6, 8, 9], indicating that mutations in the EGFR gene affect the treatment effects of EGFR-TKIs. It is therefore widely accepted that the presence of EGFR mutations is an indicator of the clinical efficacy of an EGFR-TKI in patients with NSCLC. Based on this concept, National Comprehensive Cancer Network (NCCN) clinical practice guidelines in oncology recommend measuring the local EGFR gene mutations of NSCLC patients before treatment.

Despite this development, it is still rare in current clinical practice to measure EGFR gene mutation. In the Iressa Pan-Asia Study[8], although 1038 patients (85.3%) consented to provide tumor specimens, specimens were obtained from 683 patients (56.1%) in the end. Additionally, only 437 patients (35.9%) provided specimens that qualified for EGFR mutation detection, mainly due to the difficulty of obtaining adequate tissue samples. Therefore, for those patients with various rea-

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sons for limited availability of biopsy samples to detect EGFR mutation, if an easily measurable biomarker that is predictive of EGFR mutation can be established and accepted, this marker will significantly simplify the process of identifying the patient population that is ideal for EGFR-TKI therapy.

Carcinoembryonic antigen (CEA) levels in the serum have been widely recognized as a diagnostic indicator of lung cancer, and particularly adenocarcinoma[10–16]. It has also been reported that the serum CEA level is closely related to the treatment outcome of EGFR-TKIs[17, 18]. These findings raise the question of whether there is any correlation between serum CEA levels and EGFR gene mutations. To answer this question, we designed and performed the current study, which measured serum CEA levels and EGFR gene mutations in histologically confirmed lung adenocarcinomas in Chinese patients without a smoking history. After dividing the patients into two groups by serum CEA level, we analyzed and compared the mutation rates of the EGFR gene (particularly at exon 19 and exon 21) between the two groups with different serum CEA levels. The current study investigated the correlation between serum CEA levels and EGFR gene mutation in Chinese nonsmokers with lung adenocarcinoma and offers to shed light on the potential to identify a patient population that is responsive to EGFR-TKI treatment in the absence of EGFR mutation detection due to limitations in the availability of biopsy samples.

Materials and methods

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and the guidelines set forth by the International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. The investigators obtained approval from the Institutional Review Board at the Shanghai Chest Hospital, Shanghai Jiao Tong University before initiating the study, and all patients provided written informed consent before any study-related procedures.

Study design

Patient inclusion criteria:

1. Diagnosis of lung adenocarcinoma per histology;
2. No history of smoking;
3. Chinese;
4. Never received any prior anti-cancer treatment.

Patient exclusion criteria:

1. No samples for EGFR mutation analysis;
2. Lung cancer identified as squamous cell carcinoma, small cell lung cancer, or large cell carcinoma;
3. History of smoking;
4. Non-Chinese;
5. Received prior anti-cancer treatment.

Clinicopathological characteristics, including gender, age, and the degree of differentiation, were recorded, and pretreatment serum was collected for chemiluminescent immunoassay measurement of CEA, cytokeratin 19 fragment antigen 21-1 (CYFRA21-1), neuron specific enolase (NSE), and CA125 levels. An amplification refractory mutation system (ARMS) was used to detect EGFR gene mutation at exons 18–21.

Given that sequencing is still the most accepted technology for the measurement of EGFR gene mutations, to confirm the reliability of ARMS, 20% of samples were randomly selected from samples that were detected by ARMS to have point mutations at exon 19 and exon 21 to undergo sequencing.

We analyzed and compared the correlation between clinicopathological characteristics and EGFR gene mutations (especially at exon 19 and exon 21, which are the most common). We divided patients into high-CEA and low-CEA groups. The mutation rates of the EGFR gene (especially exon 19 and exon 21) were compared between the two groups, and the correlation between serum CEA levels and EGFR gene (especially exon 19 and exon 21) mutation rates was analyzed. In addition, we explored the relationship of the mutant or wildtype EGFR gene with abnormal serum CEA levels.

Specimen collection

Formalin-fixed and paraffin-embedded (FFPE) specimens were collected by surgical biopsy of primary lung adenocarcinoma, bronchoscopy, or percutaneous needle biopsy. H&E staining was applied for the pathological assessment and diagnosis of adenocarcinoma, while ensuring sufficient tumor tissue or cells for mutation detection. Moreover, tumor-rich regions were chosen for gene mutation analysis. The minimum number of slices was 5 μm×4 pieces for surgical biopsy specimens and 5 μm×8 pieces for bronchoscopy or percutaneous needle biopsy specimens.

EGFR mutational analysis

A QIAamp™ DNA FFPE Tissue Kit (Qiagen, Germantown, MD, USA) was used to accomplish DNA extraction and quality control. EGFR gene mutations at exons 18–21 were detected by an ADx-EG01 ARMS™ EGFR 29 Mutations Detection Kit (Amoy Diagnostics Co, Ltd, Xiamen, China). The specific operations and data interpretations were performed as indicated in the kit manual.

To verify the reliability of the ARMS results, we performed direct sequencing on 20% of the samples that exhibited the two mutation types with the highest incidence in this study (exon 19 deletions and exon 21 point mutations).

See Table 1 for information on the PCR primers and reaction system.

The DNA amplified by PCR was sequenced and analyzed using an ABI 3730 XL DNA sequencer (Life Technologies, Grand Island, NY, USA) in both directions.

Statistical analysis

Using SPSS 11.5, the chi-square test was applied to assess the correlation between EGFR gene mutations and every factor involved. Logistic regression models were used to analyze multiple factors. We selected the Forward: LR method for including variables.

Table 1. PCR reaction.

			First round	Second round
PCR primer	Exon 19	Forward	5′-CCCAGCAATATCAGCCTTAGGTG-3′	5′-CCTTAGGTGCGGCTCCACAGC-3′
		Reverse	5′-CCACTAGAGCTAGAAAGGGAAAGA-3′	5′-CATTTAGGATGTGGAGATGAGC-3′
	Exon 21	Forward	5′-CTAACGTTCGCCAGCCATAAGTC-3′	5′-GCTCAGAGCCTGGCATGAA-3′
		Reverse	5′-GCTGCGAGCTCACCCAGAATGTCTGG-3′	5′-CATCCTCCCCTGCATGTGT-3′
The volume of PCR reaction system			20 μL	50 μL
Reaction conditions			Initial denaturation at 95 °C for 5 min 95 °C for 30 s 58 °C for 30 s Followed by 36 cycles 72 °C for 45 s One cycle of 72 °C for 7 min	Initial denaturation at 95 °C for 5 min 94 °C for 30 s 58 °C for 30 s Followed by 32 cycles 72 °C for 30 s One cycle of 72 °C for 7 min

Results

EGFR gene mutations

Mutations of the EGFR gene were detected in 67 (68.4%) of 98 patients. Among these mutations, 36 were deletions at exon 19, 26 were point mutations at exon 21 (23 were L858R, and 3 were L861Q), and 5 were double mutations (2 were exon 21 L858R with exon 20 mutation, 2 were exon 19 deletion with exon 20 mutation, and 1 was exon 19 deletion with exon 18 mutation). The distribution of the EGFR mutation status of all cases is shown in Figure 1.

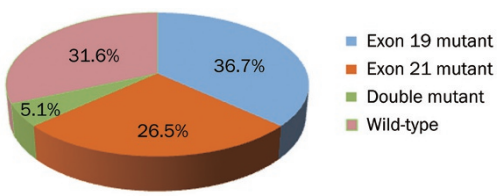

Figure 1. Constitutional diagram of EGFR gene mutations in Chinese nonsmokers with pulmonary adenocarcinoma. In 98 cases, 36.7% (36 of 98) harbored EGFR mutations at exon 19, 26.5% (26 of 98) harbored EGFR mutations at exon 21, 5.1% (5 of 98) harbored double exons EGFR mutations, 31.6% (31 of 98) were wild-type.

Direct sequencing analysis to verify ARMS results

Sequencing analysis was performed on 12 samples, which were selected from samples with the two types of mutations with the highest incidence (exon 19 deletions and exon 21 point mutations). Direct sequencing results revealed that 7 of these 12 cases were deletions at exon 19, and 5 were L858R point mutations at exon 21, which was the same as the ARMS results. Thus, the direct sequencing analysis confirmed the reliability an d accuracy of the ARMS method. The direct sequencing results of the 12 samples are shown in Figure 2.

Patient characteristics

This study included 98 patients, ranging in age from 38 to 76 years, with a median age of 57 years. The profiles of those patients are summarized in Table 2, including gender, age, tumor differentiation, serum CEA levels, and the expression level of other tumor biomarkers, such as CYFRA21-1, NSE, and CA125.

Table 2. Patient data.

Characteristic	No of patients (*n*=98)	%
Male	35	35.7
Female	63	64.3
Age		
<60	51	52.0
≥60	47	48.0
Differentiation		
Poor	27	27.6
Moderate	51	52.0
Well	20	20.4
CEA serum concentration		
Normal (<5 ng/mL)	43	43.9
Abnormal (≥5 ng/mL)	55	56.1
CYFRA21-1 serum concentration		
Normal (<5 ng/mL)	77	78.6
Abnormal (≥5 ng/mL)	21	21.4
NSE serum concentration		
Normal (<25 ng/mL)	85	86.7
Abnormal (≥25 ng/mL)	13	13.3
CA125 serum concentration		
Normal (<35 U/mL)	71	72.4
Abnormal (≥35 U/mL)	27	27.6
EGFR gene		
Mutant	67	68.4
Wild-type	31	31.6

Abbreviations: CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin 19 fragment antigen 21-1; NSE, neuron specific enolase; CA125, carbohydrate antigen-125; EGFR, epidermal growth factor receptor.

Clinicopathological characteristics and EGFR gene mutations

The distribution of EGFR gene mutations and their associa-

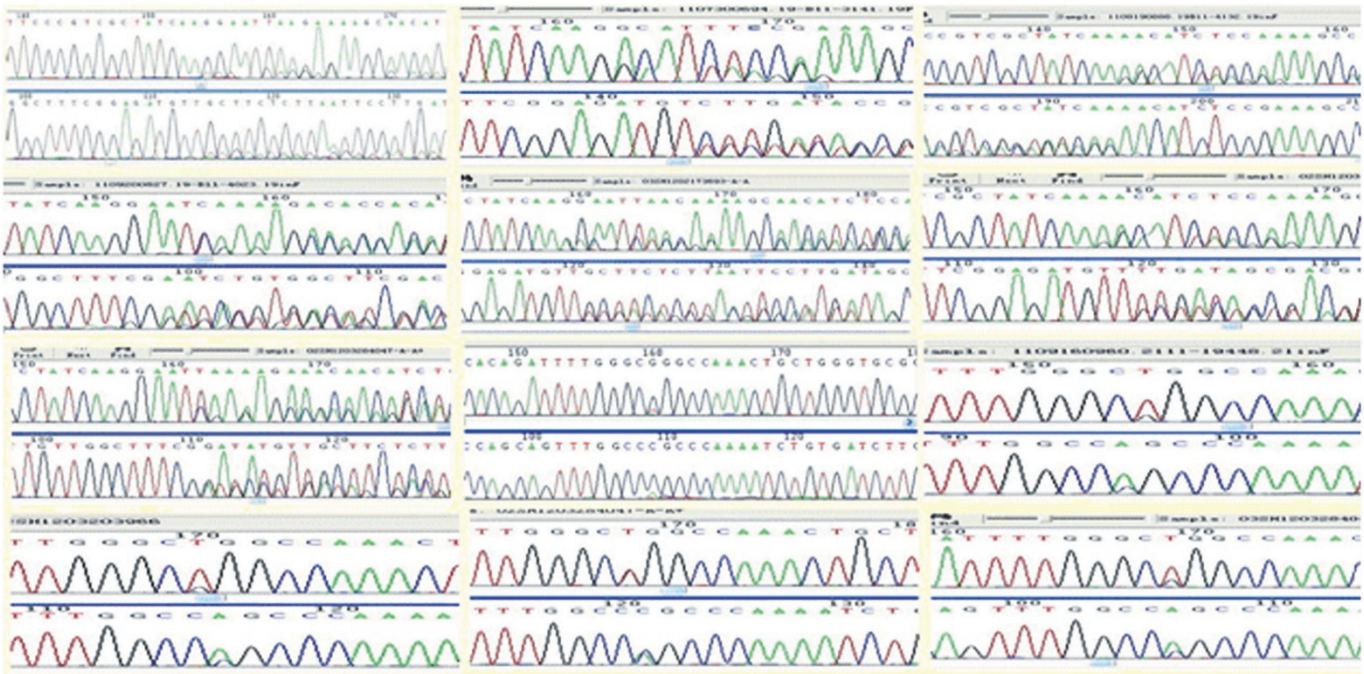

Figure 2. Gene sequence diagram of case 1–12. Note, direct sequencing on selected samples (20% of total) with exon 19 deletion and exon 21 point mutation was performed to verify the reliability of ARMS results.

tions (in particular, at exon 19 and exon 21) with every clinicopathological characteristic are listed in Table 3.

We found that EGFR gene mutation rates were significantly higher in patients with high serum CEA levels (≥5 ng/mL) than those in patients with low serum CEA levels (<5 ng/mL), with EGFR gene mutation rates at 78.2% and 55.8%, respectively (P=0.018). We also found that the mutation rates of the EGFR gene were positively correlated with the degree of tumor differentiation: the mutation rates were 51.9% in poorly differentiated tumors, 68.6% in moderately differentiated tumors, and 90.0% in well-differentiated tumors (P=0.021).

Mutation rates at EGFR exon 19 were significantly higher in the high-CEA group than those in the low-CEA group, at 49.1% and 20.9%, respectively (P=0.004).

No association was found between mutation at exon 21 and the analyzed clinicopathological characteristics.

Additionally, subgroup analysis (Table 4) indicated that whether male or female, patients with higher serum CEA levels had a much higher rate of EGFR mutation than patients with lower serum CEA levels had. For males, those with low serum CEA levels (<5 ng/mL) and high serum CEA levels (≥5 ng/mL) had EGFR mutation rates of 47.1% and 77.8%, respectively (P=0.05); exon 19 mutation rates of 17.6% and 44.4%, respectively (P=0.038); and exon 21 mutation rates of 17.6% and 33.3%, respectively (P=0.289). For females, those with low serum CEA levels (<5 ng/mL) and high serum CEA levels (≥5 ng/mL) had EGFR mutation rates of 61.5% and 78.4%, respectively (P=0.047); exon 19 mutation rates of 23.1% and 51.4%, respectively (P=0.024); and exon 21 mutation rates of 30.8% and 24.3%, respectively (P=0.570).

Logistic regression analysis of EGFR gene mutation

The results of logistic regression analysis (Table 5) showed that a high level of serum CEA was the only independent predictor of EGFR gene mutation (P=0.020, OR 2.837, 95% CI: 1.178–6.829). A high level of serum CEA was also the only independent predictor of exon 19 mutations (P=0.012, OR 3.618, 95% CI: 1.319–9.918).

Serum CEA levels and EGFR gene mutations

In this study, the mutation rates of the EGFR gene in patients with <5 ng/mL, 5–20 ng/mL, or ≥20 ng/mL serum CEA were 55.8%, 73.1%, and 82.8%, respectively (P=0.046) (Figure 3). The mutation rates of exon 19 in these three groups were 20.9%, 40.7%, and 57.1%, respectively (P=0.007) (Figure 4). The mutation rates at exon 21 showed no significant difference

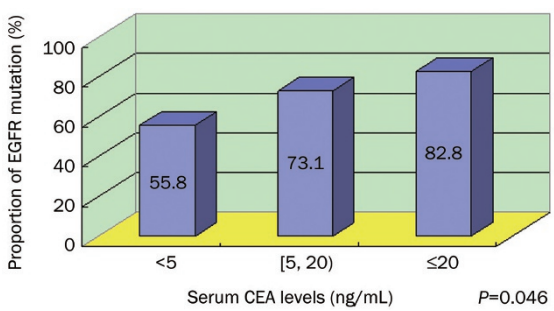

Figure 3. Proportion of patients with EGFR mutation in different serum CEA levels. 43 cases of serum CEA levels <5 ng/mL; 36 cases of serum CEA levels ≥20 ng/mL; 19 cases of serum CEA levels in between.

Table 3. Clinicopathologic features and EGFR mutation.

Characteristic	All patients No	EGFR mutation No	%	*P*	EGFR exon 19 mutation No	%	*P*	EGFR exon 21 mutation No	%	*P*
Gender										
Male	35	22	62.9	0.382	11	31.4	0.417	9	25.7	0.891
Female	63	45	71.4		25	39.7		17	27	
Age										
<60	51	34	66.7	0.706	18	35.3	0.758	14	27.5	0.830
≥60	47	33	70.2		18	38.3		12	25.5	
Differentiation										
Poor	27	14	51.9		5	18.5		5	18.5	
Moderate	51	35	68.6	0.021	21	41.2	0.055	13	25.5	0.249
Well	20	18	90		10	50		8	40	
CEA serum concentration										
Normal (<5 ng/mL)	43	24	55.8	0.018	9	20.9	0.004	11	25.6	0.851
Abnormal (≥5 ng/mL)	55	43	78.2		27	49.1		15	27.3	
CYFRA21-1 serum concentration										
Normal (<5 ng/mL)	77	52	67.5	0.734	26	33.8	0.243	21	27.3	0.750
Abnormal (≥5 ng/mL)	21	15	71.4		10	47.6		5	23.8	
NSE serum concentration										
Normal (<25 ng/mL)	85	59	69.4	0.570	30	35.3	0.449	24	28.2	0.328
Abnormal (≥25 ng/mL)	13	8	61.5		6	46.2		2	15.4	
CA125 serum concentration										
Normal (<35 U/mL)	71	48	67.6	0.793	24	33.8	0.329	20	28.2	0.551
Abnormal (≥35 U/mL)	27	19	70.4		12	44.4		6	22.2	

Abbreviations: EGFR, epidermal growth factor receptor; CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin 19 fragment antigen 21-1; NSE, neuron specific enolase; CA125, carbohydrate antigen-125.

Table 4. Subgroup analysis of gender.

	All patients	EGFR mutation			EGFR exon 19 mutation			EGFR exon 21 mutation		
CEA serum concentration (Male)										
Normal (<5 ng/mL)	17	8	47.1	0.050	3	17.6	0.038	3	17.6	0.289
Abnormal (≥5 ng/mL)	18	14	77.8		8	44.4		6	33.3	
CEA serum concentration (Female)										
Normal (<5 ng/mL)	26	16	61.5	0.047	6	23.1	0.024	8	30.8	0.570
Abnormal (≥5 ng/mL)	37	29	78.4		19	51.4		9	24.3	

Abbreviations: EGFR, epidermal growth factor receptor; CEA, carcinoembryonic antigen.

among the three groups (25.6%, 26.9%, and 27.6%, respectively, P=0.981). These results suggested that serum CEA levels were positively correlated with histological EGFR gene mutation rates, especially for mutations at exon 19.

EGFR gene types and serum CEA levels

The incidence of abnormal serum CEA levels (≥5 ng/mL) differed between patients with different types of EGFR gene mutations (Figure 5). Patients with a mutant EGFR gene displayed significantly higher CEA levels than patients with a wildtype EGFR gene. Whereas only 38.7% of wildtype patients were found to have an abnormal CEA level, in their mutated counterparts, the incidence was 64.2% (P=0.018). Specifically, 75.0% (P=0.004) of patients with exon 19 mutations and 57.7% (P=0.851) of patients with exon 21 mutations were observed to have abnormal serum CEA levels.

Table 5. Multivariable analysis of the predictive factors for EGFR mutation.

	EGFR mutation				EGFR exon 19 mutation			
	SE	OR	95% CI	*P*	SE	OR	95% CI	*P*
Gender								
Male				0.437				0.658
Female	0.487	1.46	0.562–3.789		0.485	1.239	0.479–3.209	
Age								
<60				0.506				0.624
≥60	0.467	1.365	0.546–3.410		0.458	1.251	0.510–3.067	
Differentiation								
Poor								
Moderate				0.212				0.155
Well	0.861	0.301	0.056–1.625		0.706	2.694	0.675–10.742	
CEA serum concentration								
Normal (<5 ng/mL)				0.02				0.012
Abnormal (≥5 ng/mL)	0.448	2.837	1.178–6.829		0.515	3.618	1.319–9.918	

Abbreviations: EGFR, epidermal growth factor receptor; SE, standard error; OR, odds ratio; 95% CI, 95% confidence interval; CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin 19 fragment antigen 21-1; NSE, neuron specific enolase; CA125, carbohydrate antigen-125.

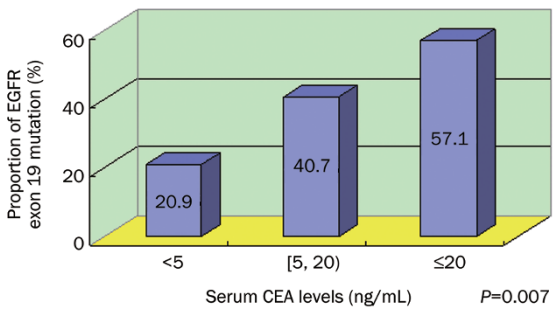

Figure 4. Proportion of patients with EGFR exon 19 mutation in different serum CEA levels. 9 cases of serum CEA levels <5 ng/mL; 16 cases of serum CEA levels ≥20 ng/mL; 11 cases of serum CEA levels in between.

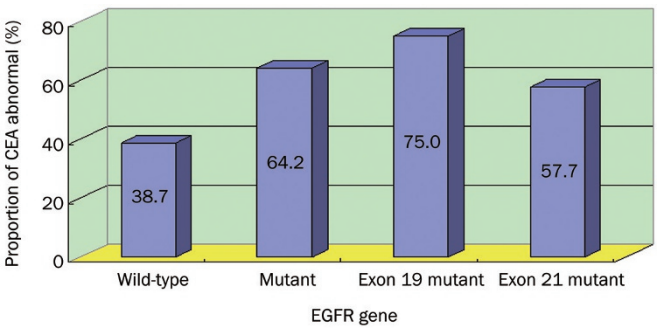

Figure 5. Proportion of patients with CEA abnormal in wild-type or mutant EGFR genes. 31 cases of wild-type; 67 cases of total EGFR mutation with 36 cases of exon 19 mutation and 26 cases of exon 21 mutation.

Discussion

It is well known that the type of EGFR gene is closely associated with EGFR-TKI treatment effects. The Iressa Pan-Asia Study has demonstrated that the presence of a mutation of the EGFR gene in a tumor is a strong predictor of a better outcome when gefitinib is used as the first-line treatment[8]. The WJTOG3405 study conducted by Mitsudomi *et al* also proved the importance of EGFR mutation measurement[6]. It is now well accepted that patients with EGFR gene mutations are more sensitive to EGFR-TKI treatment, with a better treatment outcome[6–8]. It has also been reported that the pre-treatment serum CEA level is associated with EGFR-TKI treatment efficacy; patients with a higher level of pre-treatment serum CEA are more sensitive than patients with normal serum CEA levels[19–21]. Therefore, is there any correlation between serum CEA levels and EGFR gene mutation?

Shoji *et al* reported that serum CEA levels correlated with EGFR gene mutation in 48 cases with postoperative recurrence of lung adenocarcinomas[22]. Additionally, researchers found that 59.7%–75.3% Asian nonsmokers with lung adenocarcinomas harbored EGFR gene mutations[6, 8, 9]. Thus, is it more relevant to apply serum CEA levels as a predictive biomarker in Asian nonsmokers with lung adenocarcinomas to assess the EGFR-TKI treatment outcome?

To answer this question, we focused on nonsmokers with lung adenocarcinomas, which is a population with a high incidence of EGFR gene mutation, and demonstrated that CEA levels were positively correlated with histological EGFR gene mutations and mutations at exon 19. Moreover, logistic regression analysis showed that a high level of serum CEA was the only independent predictor of EGFR gene mutations, and especially mutations at exon 19. More importantly,

our results showed that patients with serum CEA levels ≥20 ng/mL had an EGFR gene mutation rate as high as 82.8%. Therefore, we conclude that in the population of Chinese nonsmokers with pulmonary adenocarcinoma, a high serum CEA level is an indicator of EGFR mutation.

EGFR gene mutation detection is still based on tissue sample analysis. In most Phase 3 trials for EGFR-TKIs, biomarker detection also requires tissue biopsy samples. Because the majority of NSCLC patients have no operative indications at the time of diagnosis, no surgical specimens would be available for the detection of gene mutation in these patients. In patients whose biopsy samples are obtained by bronchoscopy or CT-guided percutaneous lung biopsy, the sample volume limitation often does not allow gene mutation measurement. In addition, many patients with a poor performance status (PS) could not endure invasive biopsy examinations. These constraints ultimately limit the application of EGFR-TKIs to treat NSCLC patients.

CEA was first identified in rectal adenocarcinoma in 1965[23]. The diagnostic value of serum CEA levels in NSCLC, and particularly adenocarcinoma patients, has already been widely accepted and utilized[10-16]. In certain reports, including those of our previous research, it has been found that changes in serum CEA levels are closely associated with chemotherapeutic efficacy and prognosis[24, 25]. Notably, researchers have also found that serum CEA levels could be applied as a predictive biomarker for EGFR-TKI treatment effects[17, 18]. Those previous studies suggest that serum CEA levels possess a certain value in clinical application for the prediction of treatment efficacy and prognosis.

Serum CEA is frequently found to be highly expressed in NSCLC, and especially in adenocarcinoma patients[26, 27]. Additionally, adenocarcinoma patients display significantly higher mutation rates of the EGFR gene than do non-adenocarcinoma patients[28]. Shoji *et al* reported that in lung adenocarcinoma patients with post-operative recurrence, serum CEA levels are positively correlated with EGFR mutation rates[22]. However, our study results clearly demonstrated that Chinese nonsmokers with pulmonary adenocarcinoma, whether male or female, who had serum CEA levels ≥20 ng/mL are the ideal patient population to be targeted for EGFR-TKI therapy. As we known, patients with exon 19 mutations usually have longer PFS and OS. More interestingly, the mutation rate of exon 19 is more closely related to serum CEA levels. Regarding the exon 21 mutation rate, it also appears to correlate with serum CEA levels, but the correlation did not show statistical significance, highlighting the exon 19 mutation rate as a more important factor related to serum CEA levels.

Sordella *et al* reported that the mutated EGFR gene could abnormally activate the downstream signal transduction pathway and induce transcription factor expression and activation, thus initiating the anti-apoptotic pathway and accelerating cell proliferation, which play an important role in the tumorigenesis of lung cancer[29]. The mechanism by which EGFR-TKIs inhibit tumor development is the disruption of the EGFR mutation-induced abnormal downstream signaling pathway to induce the apoptosis of tumor cells. CEA is an adhesion protein, and its expression may be upregulated by the EGFR downstream signaling pathway. Wirth *et al* and Ordonez *et al* reported that CEA overexpression could inhibit the apoptosis of tumor cells[30, 31]. Based on these studies, we could conclude that CEA might be an anti-apoptotic factor related to EGFR gene mutations, and particularly exon 19 mutation. This hypothesis still needs to be verified by further basic cancer research.

Although the mechanism that underlies the positive correlation between serum CEA levels and EGFR gene mutation remains unclear, serum CEA level determination could still serve as a straightforward indicator in patients who are unable to provide sufficient tissue specimens for EGFR gene mutation detection and could guide individualized treatment strategies. Furthermore, as several studies have noted, the serum CEA level is closely associated with EGFR-TKI treatment efficacy, so this level may also be used as a potential indicator of follow-up and prognosis, especially when the treatment effect is difficult to measure. We are taking this direction in future research on this topic.

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Author contribution

Bao-hui HAN designed the research; Bo JIN, Yu DONG, Hui-min WANG, and Jin-su HUANG performed the research; Bo JIN and Yu DONG analyzed the data; and Bo JIN wrote the paper.

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