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Original Article

Association between *BIM* deletion polymorphism and the risk of non-small cell lung cancer in Chinese

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Abstract: The incidence rate of lung cancer is increasing rapidly recent years. Except smoking, air-pollution and other environmental cancer-related issues, genetic risk sites contribute to lung cancer in another aspect. Previous studies demonstrated 2903-bp deletion polymorphism of *BIM* was critical in resistance of tyrosine kinase inhibitors (TKI) therapy. Besides, the expression level of *BIM* played an important role in cancers. The deletion polymorphism could lead to similar effect as low expression level of *BIM*. Hence, we aimed to study the potential association between the deletion polymorphism and the risk of non-small cell lung cancer (NSCLC). We enrolled 6858 participants in present study. χ^2 test or fisher's exact test were utilized in the association analysis between the deletion polymorphism and participants' characteristics. The associations between the deletion polymorphism and lung cancer was analyzed with logistic regression model. In present study, no significant association between *BIM* polymorphism and risk of cancer was observed neither in combined cohort nor sub-groups. However, the people carried the deletion polymorphism showed a trend toward risk of squamous cell carcinoma in elderly people (P=0.08). In addition, carriers of deletion polymorphism had lower risk of thrombocytopenia after chemotherapy (P=0.048, OR=0.58; 95% CI, 0.34-0.99). In conclusion, no evidence of association between the deletion polymorphism of *BIM* and the risk of NSCLC was observed in present study.

Keywords: BIM, 2903-bp deletion polymorphism, BH3 domain, Bcl-2 family, NSCLC

Introduction

Lung cancer, which has a rather low cure rate, is the leading cause of cancer-related death, 80% of which is caused by non-small cell lung cancer (NSCLC) [1]. The incidence rate of lung cancer is increasing rapidly recent years [2]. Except smoking, air-pollution and other environmental cancer-related issues, genetic risk sites contribute to lung cancer in another aspect.

Traditional chemotherapy could kill uncontrollably dividing tumor cells, but normal cells might be damaged inevitably [3]. Hence, due to the efficiency and low toxicity of normal cells, target therapy is a better choice than traditional chemotherapy for lung cancer treatment [4-7]. The epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitors (TKI), such as gefitinib and erlotinib, were most frequently used in target therapy of lung cancer [4, 5]. Recent studies indicated *BIM* was essential for apoptosis triggered by *EGFR*-TKI in lung cancer [8-10]. Nevertheless, rare study focused on the association between *BIM* and the risk of NSCLC.

BIM (*BCL2L11*) encodes a BH3-only pro-apoptotic factor of Bcl-2 family. Bcl-2 family includes anti-apoptotic factors (e.g. Bcl-2 and Mcl-1), multidomain pro-apoptotic factors (e.g. Bak and Bax), and BH3-only pro-apoptotic factors (e.g. Bim, Bid and Noxa). Interactions between anti- and pro-apoptotic proteins of Bcl-2 family are key determinants to cell death triggers [11, 12]. Recent study discovered that the 2903-bp deletion polymorphism switched *BIM* splicing from exon 4 to exon 3, which leaded to expression of *BIM* isoforms lacking the pro-apoptotic BH3 domain and further mediated intrinsic

resistance to TKI [10]. As a pro-apoptotic factor, Bim promotes cell apoptosis by opposing the anti-apoptotic factors, such as Bcl-2 and Mcl-1, or interacting with other pro-apoptotic members, such as Bax and Bak, and directly activating the apoptosis [13]. Previous studies had indicated those anti- or pro-apoptotic factors were correlated with the risk of lung cancer. BH3 is a critical domain in the interaction between Bim and other anti- or pro-apoptotic factors [14-16]. Furthermore, previous studies had demonstrated the expression level of *BIM* play a critical role in carcinomas [17, 18]. The isoforms lacking BH3 domains due to deletion polymorphism could lead to similar effect as low expression level of *BIM*. Hence, we hypothesis the deletion polymorphism might be a potential marker for detecting the susceptibility of NSCLC. Although a very recent study had reported no association between 2903-bp deletion and lung cancer risk in Japanese [19], we aim to study the relationship between deletion polymorphism and NSCLC in Chinese. Considering *EGFR* participates in the regulation of MAPK signaling, which is upstream and regulates the expression of *BIM* [20], we also analyzed the relationship between the deletion polymorphism and the risk of NSCLC with or without *EGFR* mutations. In addition, as platinum chemotherapy still the first-line treatment for those patients without positive biomarkers [21, 22], we further analyzed the relationship between the deletion polymorphism and treatment response or side-effect of platinum-based chemotherapy.

Methods and materials

Study population

A total of 6858 participants were enrolled in present study, including 2583 patients were histologically confirmed as having NSCLC (383 paraffin embedded tumor issues which were utilized for genotyping of *EGFR* mutation). 4275 of total participants were healthy people who were free of cancer. All the participants were unrelated ethnic Han Chinese. Personal information, including age at diagnosis, gender, smoking status and pack-year, family and personal history of disease, was recorded from patients' self-report. Clinical index involved in the analysis was gathered from clinical laboratory reports and pathological reports. Patients' responses to platinum chemotherapy were determined by the WHO criteria. Term effect was assessed after two cycles of treatment. Gastrointestinal and hematological toxicity incidence was assessed twice a week during the whole first-line treatment, according to the National Cancer Institute Common Toxicity Criteria. All patients consented to participate in the study and to allow their biological samples to be genetically analyzed accordance with the process approved by the Ethical Committee of Shanghai Chest Hospital.

Specimen preparation

For each participant, 2 ml peripheral blood was collected with EDTA-anticoagulant tubes. Genomic DNA was extracted from the blood, using the QIAamp DNA MAX Kit (Qiagen, Hilden, Germany), according to the manufacture.

Genotype of the 2903-bp deletion

Touchdowm PCR on Roche LightCycler 480 (Roche, Inc.) was utilized to identify the deletion polymorphism. See Supplementary for details (Table S1).

Genotype of the somatic mutation in EGFR

Considered the potential epistasis effect of *EGFR*, we genotyped the somatic mutation of *EGFR* in tumor issues of 195 patients. Human *EGFR* Gene Mutations Fluorescence Polymerase Chain Reaction (PCR) Diagnostic Kit (Amoy Dx, Inc.) was utilized to identify 29 common somatic mutations in *EGFR* exon 18-21. See Supplementary for details (Table S2).

Statistical analysis

Nonparametric χ^2 test or fisher's exact test were utilized for the test of Hardy-Weinberg equilibrium and the association analysis between the deletion polymorphism and participants' characteristics. The deletion polymorphism was further examined by stratified analysis in sub-groups, which were grouped with sex, age, smoking status or tumor types. Logistic regression model was utilized to study the associations between the deletion polymorphism and the risk of NSCLC or TNM stage, after the adjustment of age, gender and smoking status. The potential associations between the deletion polymorphism and treatment response or severe toxicity were also analyzed with logistic model, after the adjustment of sex,

Table 1. Clinical characteristics of participants

Participants' characteristics	Combined, n (%)	Cancer, n (%)	Control, n (%)
Total no. participants = 6858		2583 (37.7)	4275 (62.3)
Median age in years	65	59	69
Gender			
Male	3746 (56.1)	1598 (66.7)	2148 (50.2)
Female	2926 (43.9)	799 (33.3)	2127 (49.8)
Smoke status			
Never	3939 (66.8)	1096 (50.2)	2843 (76.6)
Previous	364 (6.2)	83 (3.8)	281 (7.6)
Current	1593 (27.0)	1006 (46.0)	587 (15.8)
Drink			
No	4611 (91.3)	1931 (91.4)	2680 (91.2)
Yes	442 (8.7)	182 (8.6)	260 (8.8)
Tumor			
Adenocarcinoma	1800 (26.2)	1800 (69.7)	-
Squamous cell carcinoma	527 (7.7)	527 (20.4)	-
Others	256 (3.7)	256 (9.9)	-
Cancer-free	4275 (62.3)	-	4275 (100)
Stage			
Ia	32 (2.4)	32 (2.4)	-
Ib	80 (5.9)	80 (5.9)	-
IIa	56 (4.1)	56 (4.1)	-
IIb	45 (3.3)	45 (3.3)	-
IIIa	208 (15.3)	208 (15.3)	-
IIIb	175 (12.9)	175 (12.9)	-
IV	765 (56.2)	765 (56.2)	-
2903-bp deletion*			
II	5796 (84.5)	2168 (83.9)	3628 (84.9)
ID	949 (13.8)	343 (13.3)	606 (14.2)
DD	34 (0.5)	18 (0.7)	16 (0.4)
NA	79 (1.2)	54 (2.1)	25 (0.6)

*The distribution of 2903-bp deletion showed no significant differences between patients and control (P>0.05).

performance status and treatment regimen. A value of P<0.05 was considered statistically significant. All analyses were carried out with R 3.1.2.

Results

Participants characteristics

6858 participants were finally enrolled in present study, including 2583 cancer patients and 4275 healthy participants. As **Table 1** showed, the median age was 65 years old for combined cohort, while 59 for cancer patients and 69 for control cohort. The median age was utilized for age stratification analysis. A total of 3746 males and 2926 females were enrolled in this study. About a third of people were smokers in combined cohort, but this proportion reached 49.8% in cancer patients. The proportion of alcohol abusers was less than 10%, which was similar in cancer patients and control cohort. The cancer patients consisted of 1800 adenocarcinomas, 527 squamous cell carcinomas and 256 unclassified NSCLC patients. The frequency of deletion polymorphism of *BIM* was about 7.5% in combined cohort, while the genotype frequencies of II, ID and DD were 83.9%, 13.3% and 0.7% respectively. The genotype distributions of deletion polymorphism were similar in control cohort and cancer patients. The genotype distribution of deletion polymorphism was in Hardy-Weinberg equilibrium (P>0.05). The genotyping success rate was over 98%.

Association between the deletion polymorphism and characteristics

As shown in **Table 2**, we did not discover any significant association between the genotype distribution of deletion polymorphism and gender in all of three cohorts. Nevertheless, smokers showed an increased trend of deletion polymorphism in combined cohort (P=0.069). Different subtypes of NSCLC showed no difference in the genotype distributions of deletion polymorphism. However, logistic regression analysis discovered a trend toward significant association between the 2903-bp deletion and TNM stage of NSCLC patients (P=0.062).

Association between the deletion polymorphism and the risk of NSCLC

Adjusted by age, gender and smoking status, we first analyzed the relationship between the deletion polymorphism and the risk of all

Table 2. Association between 2903-bp deletion and participants' characteristics

	Combined							Control							Cancer					
	II, n (%)	ID, n (%)	DD, n (%)	*P*	ID+DD, n (%)	*P*		II, n (%)	ID, n (%)	DD, n (%)	*P*	ID+DD, n (%)	*P*		II, n (%)	ID, n (%)	DD, n (%)	*P*	ID+DD, n (%)	*P*
Gender							Gender							Gender						
Male	3142 (85.1)	534 (14.5)	18 (0.5)	0.614	552 (14.9)	0.325	Male	1818 (85.1)	311 (14.6)	7 (0.3)	0.742	318 (14.9)	0.664	Male	1324 (85.0)	223 (14.3)	11 (0.7)	0.533	234 (15.0)	0.263
Female	2491 (85.9)	394 (13.6)	14 (0.5)		408 (14.1)		Female	1810 (85.6)	295 (14.0)	9 (0.4)		304 (14.4)		Female	681 (86.8)	99 (12.6)	5 (0.6)		104 (13.2)	
Smoke Status							Smoke Status							Smoke Status						
Never	3353 (86.2)	517 (13.3)	22 (0.6)	0.258	539 (13.8)	0.179	Never	2427 (85.9)	385 (13.6)	12 (0.4)	0.374	397 (14.1)	0.231	Never	926 (86.7)	132 (12.4)	10 (0.9)	0.444	142 (13.3)	0.471
Previous	301 (84.1)	56 (15.6)	1 (0.3)		57 (15.9)		Previous	231 (83.4)	45 (16.2)	1 (0.4)		46 (16.6)		Previous	70 (86.4)	11 (13.6)	0 (0)		11 (13.6)	
Current	1323 (84.4)	238 (15.2)	6 (0.4)		244 (15.6)		Current	490 (83.8)	94 (16.1)	1 (0.2)		95 (16.2)		Current	833 (84.8)	144 (14.7)	5 (0.5)		149 (15.2)	
Positive	1624 (84.4)	294 (15.3)	7 (0.4)	0.079	301 (15.6)	0.069	Positive	721 (83.6)	139 (16.1)	2 (0.2)	0.153	141 (16.4)	0.098	Positive	903 (84.9)	155 (14.6)	5 (0.5)	0.148	160 (15.1)	0.264
Tumor							Tumor							Tumor						
Adenocarcinoma	1513 (85.8)	236 (13.4)	14 (0.8)	0.147	250 (14.2)	0.345	Adeno-carcinoma	-	-	-	-	-	-	Adeno-carcinoma	1513 (85.8)	236 (13.4)	14 (0.8)	0.147	250 (14.2)	0.345
Squamous cell	432 (84.2)	80 (15.6)	1 (0.2)		81 (15.8)		Squa-mous cell	-	-	-		-		Squa-mous cell	432 (84.2)	80 (15.6)	1 (0.2)		81 (15.8)	
Others	223 (88.1)	27 (10.7)	3 (0.1)		30 (10.8)		Others	-	-	-		-		Others	223 (88.1)	27 (10.7)	3 (0.1)		30 (10.8)	

Nonparametric χ^2 test or fisher's exact test were utilized for the association analysis between the deletion polymorphism and participants' characteristics.

Table 3. Stratification analysis of association between 2903-bp deletion and NSCLC risk by gender, smoke or age

	Smoker						Nonsmoker						Combined					
	II, n (%)	ID, n (%)	DD, n (%)	*P*	ID+DD, n (%)	*P*	II, n (%)	ID, n (%)	DD, n (%)	*P*	ID+DD, n (%)	*P*	II, n (%)	ID, n (%)	DD, n (%)	*P*	ID+DD, n (%)	*P*
Control	721 (83.6)	139 (16.1)	2 (0.2)	-	141 (16.4)	-	2427 (85.9)	385 (13.6)	12 (0.4)	-	397 (14.1)	-	3628 (85.4)	606 (14.3)	16 (0.4)	-	622 (14.6)	-
Tumor	903 (84.9)	155 (14.6)	5 (0.5)	0.657	160 (15.1)	0.776	926 (86.7)	132 (12.4)	10 (0.9)	0.909	142 (13.3)	0.741	2168 (85.7)	343 (13.6)	18 (0.7)	0.745	361 (14.3)	0.91
Adenocarcinoma	536 (85.1)	90 (14.3)	4 (0.6)	0.691	94 (14.9)	0.868	786 (86.3)	116 (12.7)	9 (1.0)	0.643	125 (13.7)	0.981	1513 (85.8)	236 (13.4)	14 (0.8)	0.555	250 (14.2)	0.918
Squamous cell	294 (83.5)	58 (16.5)	0 (0)	0.644	58 (16.5)	0.588	105 (87.5)	14 (11.7)	1 (0.8)	0.94	15 (12.5)	0.793	432 (84.2)	80 (15.6)	1 (0.2)	0.762	81 (15.8)	0.789
	Male						Female											
	II, n (%)	ID, n (%)	DD, n (%)	*P*	ID+DD, n (%)	*P*	II, n (%)	ID, n (%)	DD, n (%)	*P*	ID+DD, n (%)	*P*						
Control	1818 (85.1)	311 (14.6)	7 (0.3)	-	318 (14.9)	-	1810 (85.6)	295 (14.0)	9 (0.4)	-	304 (14.4)	-						
Tumor	1324 (85.0)	223 (14.3)	11 (0.7)	0.365	234 (15.0)	0.593	681 (86.8)	99 (12.6)	5 (0.6)	0.449	104 (13.2)	0.297						
Adenocarcinoma	822 (84.8)	138 (14.2)	9 (0.9)	0.219	147 (15.2)	0.423	638 (86.7)	93 (12.6)	5 (0.7)	0.511	98 (13.3)	0.34						
Squamous cell	400 (83.9)	76 (15.9)	1 (0.2)	0.594	77 (16.1)	0.624	25 (86.2)	4 (13.8)	0 (0)	0.434	4 (13.8)	0.442						
	Age≤65 years-old						Age>65 years-old											
	II, n (%)	ID, n (%)	DD, n (%)	*P*	ID+DD, n (%)	*P*	II, n (%)	ID, n (%)	DD, n (%)	*P*	ID+DD, n (%)	*P*						
Control	1308 (86.3)	202 (13.3)	5 (0.3)	-	207 (13.7)	-	2316 (84.8)	404 (14.8)	11 (0.4)	-	415 (15.2)	-						
Tumor	1574 (86.4)	233 (12.8)	15 (0.8)	0.981	248 (13.6)	0.669	431 (82.7)	89 (17.1)	1 (0.2)	0.483	90 (17.3)	0.478						
Adenocarcinoma	1155 (86.1)	174 (13.0)	13 (1.0)	0.623	187 (13.9)	0.999	305 (84.0)	57 (15.7)	1 (0.3)	0.713	58 (16.0)	0.725						
Squamous cell	330 (86.4)	51 (13.4)	1 (0.3)	0.344	52 (13.6)	0.323	95 (76.6)	29 (23.4)	0 (0)	0.087	29 (23.4)	0.08						

Data were calculated by logistic regression, after the adjustment of age, gender and smoking.

Table 4. Association between 2903-bp deletion and NSCLC risk under different backgrounds of EGFR somatic mutations

	Control	EGFR (-)	EGFR (+)	EGFR-Exon19 (-)	EGFR-Exon19 (+)	EGFR-Exon21 (-)	EGFR-Exon21 (+)
II	3628 (85.4)	103 (82.4)	92 (83.6)	151 (82.1)	44 (86.3)	147 (83.1)	48 (82.8)
ID	606 (14.3)	22 (17.6)	17 (15.5)	33 (17.9)	6 (11.8)	29 (16.4)	10 (17.2)
DD	16 (0.4)	0 (0)	1(0.9)	0 (0)	1 (2.0)	1 (0.6)	0 (0)
P-value	-	0.455	0.273	0.197	0.746	0.326	0.369
ID+DD	622 (14.6)	22 (17.6)	18 (16.4)	33 (17.9)	7 (13.7)	30 (16.9)	10 (17.2)
P-value	-	0.392	0.355	0.152	0.967	0.375	0.323

Data were calculated by logistic regression, after the adjustment of age and gender.

Table 5. Response and toxicity outcomes

	n (%)
Response (n=664)	
Complete response (CR) or partial response (PR)	70 (10.5)
Progressive disease (PD) or stable disease (SD)	594 (89.5)
Toxicity outcomes	
Any grade 3 or 4 gastrointestinal toxicity (n=132)	12 (9.1)
Any grade 3 or 4 hematologic toxicity (n=327)	143 (43.7)
Anemia (n=67)	7 (10.4)
Agranulocytosis (n=101)	48 (47.5)
Leukocytopenia (n=286)	83 (29.0)
Thrombocytopenia (n=120)	37 (30.8)

tumors, but no significant result was observed. Hence, we further analyzed the relationship between the deletion polymorphism and different types of tumor in sub-groups, which grouped by smoking status, gender and age (**Table 3**). Still no significant correlation was found between the deletion polymorphism and the risk of NSCLC in all three cohorts. However, the carriers of deletion polymorphism showed a trend toward significant correlation with squamous cell carcinoma risk in elderly people (*P*=0.08). Considering the potential epistasis effect of *EGFR*, we further genotyped the somatic mutations of *EGFR* in 195 patients. As most mutations of *EGFR* were located in exon19 or exon 21, we divided those patients into 6 groups (*EGFR*+: with mutations in any exons of *EGFR*; *EGFR*-: without mutations of *EGFR*; *EGFR*-exon19+: with mutations in exon19 of *EGFR*; *EGFR*-exon19-: without mutations of *EGFR* exon 19; *EGFR*-exon21+: with mutations in exon21 of *EGFR*; *EGFR*-exon21-: without mutations of *EGFR* exon 21; **Table 4**). As **Table 4** showed, the genotype of deletion polymorphism were comparable among *EGFR* mutate patients and controls, suggesting lack of association between the deletion polymorphism and the risk of NSCLC under different backgrounds of *EGFR* mutations.

Association between the deletion polymorphism and treatment response or severe toxicity

We combined complete response (CR) partial response (PR) and stable disease (SD) as responders, progressive disease (PD) as non-responders. Toxicities included gastrointestinal and homological toxicity. Severe homological toxicity consisted of anemia, agranulocytosis, leukocytopenia and thrombocytopenia (**Table 5**).

We did not discover any significant association between the deletion polymorphism and response. No significance was observed between the deletion polymorphism and gastrointestinal toxicity, either. However, the deletion polymorphism was significantly correlated with thrombocytopenia, after the adjustment of performance status and treatment regimen (*P*=0.048, OR=0.58; 95% CI, 0.34-0.99). Carriers of deletion polymorphism had lower risk of thrombocytopenia after chemotherapy.

Discussion

In present study, we analyzed the association between the deletion polymorphism of *BIM* and the risk of NSCLC in Chinese population. We discovered an increasing trend of deletion polymorphism in smokers, which might implicate that the wild-type of *BIM* might mutate to deletion polymorphism, under the effects of environmental factors, such as smoking. Nevertheless, this speculation needs further

study to confirm. In present study, the 2903-bp deletion was found to be associated with TNM stage of patients, which might be caused by its role in NSCLC treatment.

Previous studies had indicated the deletion polymorphism of *BIM* played a key role in TKI target therapy of NSCLC [8-10]. NSCLC consists of adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma and large cell lung cancer. In present study, we identified a potential risk of squamous cell carcinoma in elderly people who carried *BIM* deletion polymorphism. Previous study had identified Mcl-1 as a dominant survival factor in squamous cell carcinoma [23]. The stability and degradation of Mcl-1 was regulated by proteins which contain BH3 domains [24-26]. The deletion polymorphism leads to expression of *BIM* isoforms lacking BH3 domain [10], results in Mcl-1 stabilization and thereby protects tumor cells against death signaling [23, 27, 28]. In addition, other pro-apoptotic factors of Bcl-2 family might play a similar role in the regulation of Mcl-1 and therefore circumvent the effect of deletion polymorphism [29, 30]. Hence, the mutation in other pro-apoptotic factors of Bcl-2 family and reduction of *BIM* level with age might be a possible explanation why the trend toward significant association was observed in elderly people [31], but it needs further evidence.

Furthermore, epistasis interaction between *EGFR* and *BIM* was not observed. Under different background of *EGFR* mutations, no evidence of association between the deletion polymorphism and the risk of NSCLC was found.

We indicated the deletion polymorphism might be associated with thrombocytopenia after chemotherapy in present study. Bim could bind with Bcl-xL through BH3 domain, and Bcl-xL is critical for the survival of platelets [32, 33]. Therefore, that is the possible reason that the carriers of deletion polymorphism of *BIM* showed lower risk of thrombocytopenia after chemotherapy.

In summary, no significant association between the *BIM* polymorphism and the risk of NSCLC was observed in present study. However, the carriers of deletion polymorphism had lower risk of thrombocytopenia after chemotherapy. Present study provides reference for future studies. Nevertheless, Bcl-2 family members construct a complex network to regulate the cell apoptosis. Due to the limitation of present study, we look forward to further studies that include more members of Bcl-2 family.

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Disclosure of conflict of interest

None.

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Genotype of the 2903-bp deletion

The reaction contained LightCycler® 480 High Resolution Melting Master 5 μl, genomic DNA 5 ng, $MgCl_2$ 0.3 mM and primers 0.2 μM (Table S1). The reaction started with pre-denature at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 65~55°C for 15 s, 72°C for 20 s. The renaturation temperature of the initial 20 cycles was reduced from 65°C to 55°C with rate of 0.5°C per cycle and holding at 55°C in the last 25 cycles. The melting curve program contained 95°C for 15 s, 40°C for 1 min, 65°C for 1 s, and then read the fluorescence from 65°C to 95°C at every 0.05°C increase. The amplicon length was 113 bp (non-deletion) and 85 bp (deletion), respectively. Hence, the 2903-bp deletion polymorphism could be identified on the basis of melting curve.

Genotype of the somatic mutation in *EGFR*

Human EGFR Gene Mutations Fluorescence Polymerase Chain Reaction (PCR) Diagnostic Kit (AmoyDx, Inc.) was utilized to indentify 29 common somatic mutations in EGFR exon 18-21 (Table S2) according to the manufacture. The enrichment reaction started with pre-denature at 95°C for 1 min, followed by 15 cycles of 95°C for 25 s, 64°C for 20 s, 72°C for 20 s. The data collection program contained 35 cycles of 93°C for 25 s, 60°C for 35 s, 72°C for 20 s.

Table S1. Primer sequence for 2903-bp deletion genotyping

	Sequence
Upper	5'-ATACCATCCAGCTCTGTCTTCATAG-3'
Downstream-1	5'-CCCAACCTCTGACAAGTGACC-3'
Downstream-2	5'-TTGGTGGGAATGTAAAATGGC-3'

Table S2. The target sites for EGFR somatic mutation genotyping

ID	Protein change	Exon	Nucleic acid change	Cosmic ID
Ex18-mutant-1	G719A	18	2156G>C	6239
Ex18-mutant-2	G719S	18	2155G>A	6252
Ex18-mutant-3	G719C	18	2155G>T	6253
Ex19-mutant-1	E746_A750del	19	2235_2249del15	6223
Ex19-mutant-2	E746_A750del	19	2236_2250del15	6225
Ex19-mutant-3	L747_P753>S	19	2240_2257del18	12370
Ex19-mutant-4	E746_T751>I	19	2235_2252>AAT (complex)	13551
Ex19-mutant-5	E746_T751del	19	2236_2253del18	12728
Ex19-mutant-6	E746_T751>A	19	2237_2251del15	12678
Ex19-mutant-7	E746_S752>A	19	2237_2254del18	12367
Ex19-mutant-8	E746_S752>V	19	2237_2255>T (complex)	12384
Ex19-mutant-9	E746_S752>D	19	2238_2255del18	6220
Ex19-mutant-10	L747_A750>P	19	2238_2248>GC (complex)	12422
Ex19-mutant-11	L747_T751>Q	19	2238_2252>GCA (complex)	12419
Ex19-mutant-12	L747_E749del	19	2239_2247del9	6218
Ex19-mutant-13	L747_T751del	19	2239_2253del15	6254
Ex19-mutant-14	L747_S752del	19	2239_2256del18	6255
Ex19-mutant-15	L747_A750>P	19	2239_2248TTAAGAGAAG>C (complex)	12382
Ex19-mutant-16	L747_P753>Q	19	2239_2258>CA (complex)	12387
Ex19-mutant-17	L747_T751>S	19	2240_2251del12	6210
Ex19-mutant-18	L747_T751del	19	2240_2254del15	12369
Ex19-mutant-19	L747_T751>P	19	2239_2251>C (complex)	12383
Ex20-mutant-1	T790M	20	2369C>T	6240
Ex20-mutant-2	S768I	20	2303G>T	6241
Ex20-mutant-3	H773_V774insH	20	2319_2320insCAC	12377
Ex20-mutant-4	D770_N771insG	20	2310_2311insGGT	12378
Ex20-mutant-5	V769_D770insASV	20	2307_2308insgccagcgtg	12376
Ex21-mutant-1	L858R	21	2573T>G	6224
Ex21-mutant-2	L861Q	21	2582T>A	6213