Observational Study

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Association between advanced NSCLC T790 M EGFR-TKI secondary resistance and prognosis

A observational study

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Abstract

Epidermal growth factor receptor (EGFR) mutations for EGFR-tyrosine kinase inhibitors (EGFR-TKI) in non-small cell lung cancer (NSCLC) patients are with clinical benefits. Nevertheless, eventual resistance to EGFR-TKI is almost inevitable. In about 50% patients, EGFR-TKI develops a secondary mutation, which is often the T790 M mutation. We aimed to investigate the relationship between EGFR gene status in the peripheral blood and prognosis (progression-free survival [PFS] and overall survival [OS]) in advanced lung adenocarcinoma patients and the 20 exon 790 site mutation (T790 M) and acquired resistance to EGFR-TKI.

A total of 49 patients with EGFR-TKI resistance and advanced lung cancer who visited the Shihezi University School of Medicine between 12/2013 and 12/2014 were enrolled in this study. Peripheral blood plasma DNA was isolated after EGFR-TKI resistance and the EGFR exon 20 T790 M mutation was detected using the probe amplification refractory mutation system method.

The T790 M mutation rate was 30.6% (15/49). There was no association between T790 M mutation and age, gender, smoking, clinical stage, Eastern Cooperative Oncology Group rating, initial EGFR mutation, and EGFR-TKI drugs, but EGFR-TKI resistance was associated with progression ($P = .009$). Median progression-free survival (PFS) of patients with T790 M mutation was 9.6 months and median overall survival (OS) was 17.6 months, compared to 6.8 and 12.7 months in controls ($P = .018$ and $P = .027$). Multivariate analysis showed that T790 M mutations independently affected the PFS (risk ratio, RR = 0.653, 95% confidence interval, CI: 0.069–0.886, $P = .032$) and OS (RR = 0.847, 95% CI: 0.208–2.696, $P = .008$).

T790 M mutation and EGFR-TKI resistance are independent factors to affect PFS and OS of non-small cell lung cancer patients.

Abbreviations: ABI7500 = Applied Biosystems7500, ADx-EGFR = Automatic Data Exchange System-EGFR, AJCC = American Joint Committee on Cancer, ARMS = Amplified Refractory Mutation System, ARQ197 = mesenchymal epithelial transition factor inhibitor, BIM = BCL2-like 11, cfDNA = cell-free DNA, CR = complete response, Ct = cyclic threshold, DNA = deoxyribonucleic acid, ECOG = Eastern Cooperative Oncology Group, EGFR = epidermal growth factor receptor, EGFR-TKI = epidermal growth factor receptor tyrosine kinase inhibitor, IBM = International Business Machines Corporation, IQR = interquartile range, NCCN = National Comprehensive Cancer Network, NSCLC = non-small cell lung cancer, OS = overall survival, PCR = polymerase chain reaction, PD = progressive disease, PFS = progression-free survival, PIK3CA = phosphatidylino-sitol 3-kinases, PR = partial response, RECIST = response evaluation criteria in solid tumor, SD = stable disease, SPSS = Statistical Package for Social Sciences, T790 M = 20 exon 790 site mutation, TNM = tumor node metastasis.

Keywords: epidermal growth factor, free DNA, non-small cell lung cancer, prognosis

1. Introduction

Lung cancer is one of the most common cancers worldwide, and over 80% of them was non-small cell lung cancer (NSCLC).[1] Surgery and combined therapy are mainly used for early lung cancer treatment while radiotherapy and chemotherapy are the main treatments for advanced lung cancer with 15% to 40% efficiency[2–4] and the median survival of 9 months.[5] The guidelines of National Comprehensive Cancer Network (NCCN) pointed out that epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (EGFR-TKI) can be selected as the first-line treatment for advanced NSCLC when EGFR mutation is detected. EGFR-TKI treatment in NSCLC patients with EGFR mutations showed clinical benefit rates around 70%, progression-free survival (PFS) of 6 to 12 months, and overall survival (OS) of 2 to 24 months.[6,7] It is currently widely accepted that EGFR gene mutations are a key predictor for the efficacy of EGFR-TKI, and it is necessary to detect the EGFR mutations before treatment planning. Indeed, some evidence suggests that EGFR-TKI are not effective for all patients with NSCLC and EGFR mutations.[8–13] Wrong treatment might lead to treatment delays, making the patients lose the best treatment timing and suffer economic losses. Therefore, how to further improve the prediction of EGFR-TKI efficacy has become an urgent problem to be solved.

Resistance to EGFR-TKI treatment is almost inevitable.[11,14] At present, the mechanisms of EGFR-TKI resistance include the EGFR 20 exon 790 site mutation (T790 M), c-Met amplification, and phosphatidylino-sitol 3-kinases (PIK3CA) mutation.[15,16]

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These mutations can be overcome by additional drugs, but with additional toxicities and financial burden. For example, mesenchymal epithelial transition factor inhibitor (ARQ197) was developed to overcome the amplification of c-Met gene leading to EGFR-TKI resistance. A phase II clinical trial in 2011 investigated the efficacy of erlotinib ± ARQ197 in advanced NSCLC patients with failed previous treatment, and confirmed that the combination significantly reduced the risk of disease progression, but without significant improvement of median PFS.[17] Another drug, afatinib, interferes with EGFR autophosphorylation and inhibits the growth of EGFR-TKI resistant NSCLC.[18] It is also effective in the presence of the T790 M mutation.[19] Nevertheless, EGFR mutations detecting is needed to prescribe the best treatment.

For EGFR gene mutations screening, a certain amount of fresh tumor tissues is required, which is not always possible. Under this circumstance, the screening for EGFR gene mutations becomes even more difficult. Indeed, most patients with NSCLC at an advanced stage are treated with chemotherapy, targeted therapy, and radiation therapy, without surgery. Therefore, fresh tumor samples are not obtained. The use of blood samples could solve this issue. Circulating tumor deoxyribonucleic acid (DNA) can be found in peripheral blood,[9,20–22] allowing for the determination of the EGFR gene status and guiding treatment. The non-invasive detection of the EGFR T790 M mutation in peripheral blood is feasible in cases where tumor biopsy is difficult to obtain. It has been shown that the agreement between the EGFR status in the tumor and the blood reached 80% to 90%.[6,7,11]

In order to analyze its association with clinical features, therapy response, and prognosis, free tumor DNA from the peripheral blood using the Amplified Refractory Mutation System (ARMS) assay to determine the presence of the T790 M mutation in patients with EGFR-TKI resistant advanced NSCLC. The results could help to explain the causes of EGFR-TKI resistance and provide some basis for subsequent selection of drug treatment options.

2. Materials and methods

2.1. Specimen source

A total of 114 patients with advanced NSCLC receiving EGFR-TKI therapy (all patients had indications for EGFR-TKI therapy) at the First Affiliated Hospital of Shihezi University School of Medicine between December 2013 and December 2014, and 60 patients were with EGFR-TKI resistance (either primary or acquired). Primary resistance was defined as the absence of any curative effect with EGFR-TKI. Acquired resistance was defined as an initial response to the EGFR-TKI, but that progressed to resistance after at least 6 months.[15]

The inclusion criteria were: histological or cytological stage IIIB/IV NSCLC; the patient accepted voluntary EGFR-TKI targeted therapy and treatment before EGFR genetic testing, specifically for EGFR gene mutation, and were able to comply with the study and follow-up; the patient underwent CT imaging every 2 months after starting targeted therapy until disease progression, which was defined as a relative increase of at least 20% of the index lesion (minimum of 5 mm); and blood samples were collected (n = 49 patients). The exclusion criteria were: Eastern Cooperative Oncology Group (ECOG) score >3 points; or unknown EGFR gene status.

This study was approved (AF/SC-08/01.0) by the Ethics Committee of Shihezi University School of Medicine at the First Affiliated Hospital (Reference number: 2015–129–01). All patients signed informed consent before blood collection.

2.2. Sample collection, separation, and processing

Fasting blood samples (5 mL) were collected from all patients with disease progression. The blood sample was added to a centrifuge tube (radius of 8 cm) with a serum separation gel and then centrifuged at 3000 rpm for 10 minutes. The upper serum layer was collected. Free DNA was extracted from peripheral blood within 4 to 5 hours using the QIAamp Blood Mini kit (Qiagen, Venlo, the Netherlands) and stored at −80°C. The concentration and purity of the DNA samples were tested at A260/280 nm with a UV spectrophotometer.

Using a probe amplification refractory mutation system (Amplification Refractory Mutation System, ARMS) method, the patient's peripheral blood plasma DNA was tested with the human ADx-EGFR mutation detection kit (Xiamen Eide Biological Pharmaceutical Co. Ltd., Beijing, China). Test sample DNA concentration was adjusted to 1 to 3 ng/μL. The PCR was conducted by an ABI7500 real-time PCR instrument (Applied Biosystems, Foster City, CA). PCR cycling parameters were shown as follows: 95°C denaturation for 5 minutes, 1 cycle; 95°C denaturation for 25 s, 64°C annealing for 20 seconds, and 72°C extension for 20 seconds, for a total of 15 cycles; 93°C denaturation for 25 seconds, 60°C annealing for 35 seconds, and 72°C extension for 20 seconds, for 31 cycles.

The EGFR gene mutation status was considered positive when the mutation Ct values was <28 and the T790 M ΔCt value mutation was ≤7. Otherwise it was considered negative or below the detection limit of the kit.

2.3. Follow-up

After starting treatment, CT imaging was conducted every 2 months. Response evaluation criteria in solid tumor (RECIST) version 1.1 was used to determine complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). CR + PR were used to determine the response rate. PFS was defined as the first day of starting EGFR-TKI treatment to disease progression, death, or last follow-up. OS was defined as the first day of starting EGFR-TKI therapy to death or last follow-up. All 49 patients were followed up by telephone and outpatient visit. Follow-up was censored on June 20, 2015.

2.4. Statistical analysis

Normality of the distribution of continuous variables was determined using the Kolmogorov–Smirnov test. Continuous data were presented as mean ± SD or median (range or interquartile range, IQR), and analyzed using the student *t* test or the Wilcoxon test, as appropriate. Categorical data were presented as n (%) and analyzed using the Fisher exact test. The SPSS 17.0 software (IBM, Armonk, NY) was used. PFS and OS were analyzed using the Kaplan–Meier method and the log-rank test. Factors independently associated with survival were analyzed using the Cox multivariate regression analysis (backward). Two-sided *P*-values $<.05$ were considered statistically significant.

3. Results

3.1. Characteristics of the patients

A total of 49 patients (28 males and 21 females), aged 39 to 78 years (median age: 64 years) met the inclusion criteria. Eighteen

Table 1

The correlation between T790M mutation and basic features in NSCLC patients.

Clinical features	n	T790M Mutant	T790M mutation negative	*P*
Gender				
Male	28	6	22	.788
Female	21	9	12	
Age, ys				
<60	26	5	21	.066
≥60	23	10	13	
Smoking status				
Never-smoker	31	8	23	.109
Current/former smoker	18	7	11	
Stage				
IIIB	9	2	7	.546
IV	40	13	27	
ECOG score				
0–1	38	11	27	.638
2–3	11	4	7	
Initially EGFR mutations				
19-Del	29	9	20	.938
21-L858R	20	6	14	

ECOG = Eastern Cooperative Oncology Group, EGFR = epidermal growth factor receptor, EGFR-TKI = epidermal growth factor receptor inhibitor, 19-Del = 19 exon deletion mutation, 21-L858R = 21 exon mutation, NSCLC = non-small cell lung cancer.

patients had a history of smoking. ECOG score was 0/1 in 38 patients and 2/3 in 11 patients. According to the American Joint Committee on Cancer tumor node metastasis (AJCC TNM) staging, 8th edition, nine patients were stage IIIB and 40 were stage IV. All 49 patients were tested positive for EGFR mutation before EGFR-TKI treatment; the 19-Del mutation was observed in 29 patients and the 21-L858R mutation in 20 patients; the T790 M mutation was not observed before treatments. EGFR-TKIs given as second-line therapy and above accounted for 65.3% (32/49) of the patients. Initial EGFR-TKI treatment median PFS was 11.3 months.

3.2. T790M resistance mutation status analysis after EGFR-TKI treatment

DNA test results from serum samples of the 49 secondary-resistant patients showed that the EGFR gene mutation rate was 46.9% (23/49). The T790 M mutation rate was 30.6% (15/49, Supplemental Figure 1, http://links.lww.com/MD/C323), the 19-Del mutation rate was 6.1% (3/49, Supplemental Figure 2, http://links.lww.com/MD/C323), the 21-L858R mutation rate was 10.2% (5/49, Supplemental Figure 3, http://links.lww.com/MD/C323), including three cases of 21-L858R + T790 M mutation (Supplemental Figure 4, http://links.lww.com/MD/C323), and the EGFR mutation-negative rate was 53.1% (26/34, Supplemental Figure 5, http://links.lww.com/MD/C323). As shown in Table 1, the T790 M mutation did not correlate with advanced age, gender, smoking status, clinical stage, ECOG score, initial EGFR mutation, and EGFR-TKI drugs, while the association between EGFR-TKI resistance and progression was significant ($P = .009$).

3.3. T790M mutation relationship status and the efficacy of EGFR-TKI

Among the 49 patients with secondary EGFR-TKIs resistance, the response rate was 55.1% (27/49). There was no difference in the frequency of the T790 M mutation between patients with CR +PR compared with patients with SD+PD ($P = .647$).

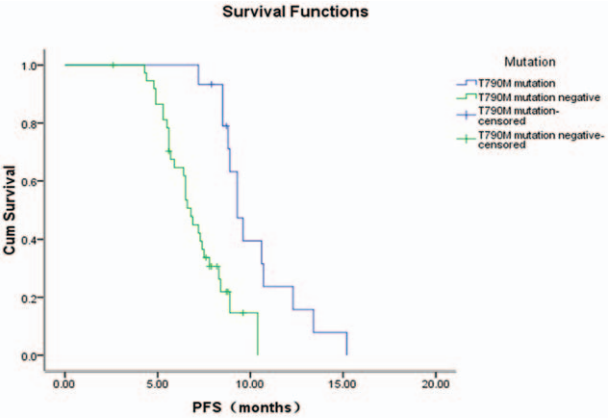

Figure 1. Comparison of progression-free survival between patients with and without the T790 M mutation.

3.4. Relationship between PFS and T790M mutation

Among the patients with acquired resistance to EGFR-TKI, the median PFS was 8.2 months and the median OS was 15.4 months. The patients with the T790 M mutation had a median PFS of 9.6 months and median OS of 17.6 months, compared to 6.8 and 12.7 months in patients without the mutation ($P = .018$ and $P = .027$) (Figs. 1 and 2). The impact of gender, age, smoking status, clinical stage, ECOG score of PFS, and OS was not statistically significant. The Cox multivariate analysis showed that T790 M mutations were independent factors for PFS and OS ($P = .032$ and $P = .008$) (Table 2).

4. Discussion

As we know, circulating tumor DNA (ctDNA) is released by tumor cells, and could effectively monitor tumor volume and cellular turnover increase 1. ctDNA is highly degraded (~166 bp) and firstly released to the circulation, plasma and then other body fluids, such as cerebrospinal fluid, saliva and urine 2 to 5. However, because of the kidney barrier filtration, urine trans-renal DNA (tr-DNA) is in the form of more shorter fragments (less than 100 bp) than plasma ctDNA, which bring out technical

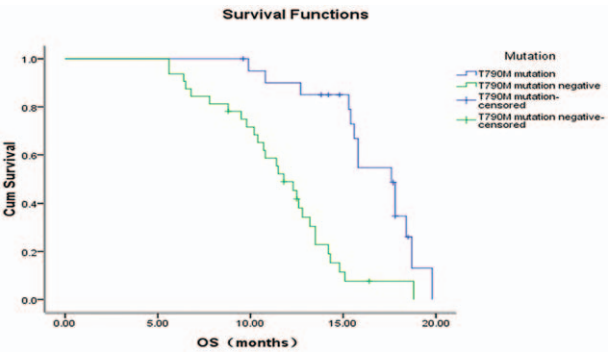

Figure 2. Comparison of overall survival between patients with and without the T790 M mutation.

Table 2

PFS and OS of 49 advanced NSCLC patients with acquired resistance to EGFR-TKI by multivariate cox model analysis.

	PFS			OS		
Clinical features	**RR**	**95% CI**	***P***	**RR**	**95% CI**	***P***
Gender	0.421	0.283–2.145	.056	1.438	0.899–2.302	.783
Age	1.895	0.317–2.282	.749	1.947	1.024–3.812	2.334
Smoking status	0.247	0.179–1.302	.130	0.482	0.258–2.073	.943
Stage	0.752	0.941–8.521	.064	0.981	0.732–2.527	1.088
ECOG score	0.851	0.054–3.802	.073	2.832	0.983–3.654	.067
T790 M mutation status	0.653	0.069–0.886	.032	0.847	0.208–2.696	.008

ECOG = Eastern Cooperative Oncology Group, EGFR-TKI = epidermal growth factor receptor inhibitor, NSCLC = non-small cell lung cancer, OS = overall survival, PFS = progression-free survival, T790 M = 20 exon 790 site mutation.

difficulties to detect the highly fragmented and low abundant tumor-specific DNA in urine 6. Therefore, so far clinical liquid biopsies are most commonly applied to plasma-derived ctDNA.

In this study, the T790 M mutation in exon 20 of the EGFR gene in serum free DNA of patients with advanced NSCLC and EGFR-TKI resistance was detected using the ARMS method after the occurrence of drug resistance. Ma et al[23]suggested that EGFR mutation status tested by ARMS in plasma cannot replace a tumor tissue biopsy though positive EGFR mutation results detected in plasma are fairly reliable. However tumor biopsy also has its limitations due to heterogeneity of the tumor cells.[24] Therefore the ARMS method, as a non-invasive method to detect EGFR mutations may have its clinical advantages. The relationship of the T790 M mutation with the efficacy and prognosis of targeted therapy was investigated. The results showed that the T790 M mutation rate was 30.6% (15/49) in 49 patients with secondary drug resistance, which was lower than that reported by Kuang et al,[25] who reported 45.2% (19/42) positivity in peripheral blood specimens from patients receiving EGFR-TKIs and using the Scorpions Amplification Refractory Mutation System (Scorpions ARMS) whole-genome amplification approach. This difference might be due to the different detection methods and to the study population. Because of the high cost of this method, it is not widely used in the clinical setting in China. The serum-free DNA in blood samples might be contaminated with mutated and wild type DNA from cancer cells, as well as with wild type DNA from normal tissues. In addition, there is genetic heterogeneity between tumor cells. Therefore, how to obtain reliable cfDNA for the detection of T790 M mutation in blood samples remains to be studied further. The T790 M mutation is the main factor for EGFR-TKI resistance in patients with EGFR sensitive mutation. Therefore, it is clinically significant to detect and investigate the T790 M mutation in patients with NSCLC. Bai et al[26] detected the EGFR gene in serum and tissue samples, and they showed that serum free DNA could replace tissue DNA for EGFR gene mutation detection. With the continuous improvement of the T790 M mutation detection methods, the sensitivity and specificity of detection were greatly improved.[27] The ARMS method has the advantages of convenient operation and good repeatability, and has been applied in the clinical setting.[21] Therefore, in this study, we detected the T790 M mutation in patients with EGFR-TKI resistance using ARMS. The results of the present study indicated that the T790 M mutation was not associated with age, gender, smoking, clinical stage, ECOG score, initial EGFR mutation types, and EGFR-TKI types in patients with advanced NSCLC, but was associated with the prognosis after EGFR-TKI resistance ($P = .032$).

Oxnard et al[10] reported that EGFR-sensitive patient plasma showed continuous relationship between T790 M mutation and radiological tumor changes within 16 weeks before radiographic progression of the disease, suggesting that the dynamic monitoring of T790 M gene status during EGFR-TKI treatment may better predict drug resistance than the RECIST evaluation, which could facilitate individualized treatment of patients with NSCLC, and also help to identify the T790 M mutation TKI resistance induction mechanism and heterogeneity.[28]

The present study showed that the PFS and OS of patients with the T790 M mutation were higher than those of patients without the T790 M mutation. The Cox multivariate analysis showed that the T790 M mutation was independently associated with PFS and OS. These results were consistent with the studies by Kuiper et al[29] and Li et al.[30] A retrospective study in Korea observed that the patients with the T790 M mutation had a better prognosis than those without.[16] Uramoto et al[13] found that the 5-year survival rate was 86.7% in patients with the T790 M mutation and 13.3% in those without. Therefore, in patients resistant to EGFR-TKI, the T790 M mutation demonstrates a relatively good prognosis. This could be due to the presence of the T790 M indicating an inert tumor, the inactivation of the other mechanisms of resistance (K-ras gene mutation, C-met gene amplification, BRAF gene mutation, BIM deletion polymorphism, and so on), or to a better EGFR-TKI efficacy.[10] A recent study showed a TKI effectiveness rate of 53% and disease control rate of 82% in T790 M mutation-positive patients, with a clear association between the T790 M mutation in EGFR-TKI therapy and three generations.[12]

The limitations of this study include small sample size, absence of dynamic and continuous detection of T790 M mutation, and subsequent clinical monitoring of blood to determine TKI resistance. In addition, the number of patients with the T790 M mutation was small, preventing subgroup analyses. Finally, tumor tissue was not available for most patients and no agreement between the tumor and the circulating tumor DNA could be made. Thereby, larger sample pool will be collected for further study, following clinical monitoring will be processed, and further sampling, sequencing and analysis would be performed.

In summary, the T790 M mutation detection has tremendous potential clinical value in patients with lung cancer. The T790 M mutation can facilitate timely assessment of treatment efficacy, patient prognosis, and individualized treatment plans.

Author contributions

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